Degradation of Three Trishaloalkyl Phosphates under Anoxic Condition in the Presence of Reduced Sulfur Species

by

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Abstract

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Advisor: Professor Urs Jans

Tris(haloalkyl)phosphates are widely used flame retardant in the U.S. They have recently been identified as one of the most frequently detected contaminants in U.S. streams. These contaminants are of toxicological concern in sensitive coastal ecosystems such as estuaries and salt marshes. It is likely that reactions with reduced sulfur species such as polysulfides (S_n^{2-}), bisulfide (HS^-), and thiophenolate (PhS^-) present in anoxic subregions of coastal water bodies could have a significant impact on rates of removal of such contaminants. The kinetics of reactions of reduced sulfur species with three tris(haloalkyl)phosphates have been determined in well-defined aqueous solutions under anoxic conditions. Reactions were monitored at varying concentrations of reduced sulfur species to obtain second-order rate constants from the observed pseudo-first order rate constants. The reactivity of S_n^{2-}, PhS^-, and HS^-, were compared in this work. The degradation products of hydrolysis and the reactions with polysulfides, thiophenolate, and bisulfide with tris(2-chloroethyl)phosphate (TCEP) were studied with GC-FID and LC-MS-MS and were quantified. In addition, the degradation products of hydrolysis and the reactions with polysulfides and thiophenolate, with tris(2-chloroisopropyl)phosphate (TCPP) and tris(1,3-dichloro-2-propyl)phosphate (TDCP) were quantified as well.

Dedicated to those who made this thesis possible:

My family and my friends

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Chapter 1

Introduction

1.1 Definition of flame retardants

Flame retardants are chemicals added to polymeric materials, both natural and synthetic, to enhance flame-retardance properties. Flame retardant chemicals are most often used to improve the fire performance of low-to-moderate cost commodity polymers. Those flame retardants may be physically blended with or chemically bonded to the host polymers. They generally have either lower ignition susceptibility or lower flame spread once ignition has occurred. Some polymers are inherently less flammable due to more stable polymeric structures; these are usually higher priced engineering plastics such as polyimides, polybenzimidazoles and polyetherketones [1].

1.2 Types of flame retardants

A distinction is made between reactive and additive flame retardant. Reactive flame retardants are reactive components chemically built into a polymer molecule. Additive flame retardants are incorporated into the polymer either prior to, during or (most frequently) following polymerization.

There are three main families of flame retardant chemicals [2-7]: inorganic flame retardants, halogenated products and organophosphorus products.

1.2.1 Inorganic flame retardants

Antimony compounds are used as synergistic co-additives in combination with halogen compounds, facilitating the reduction in total flame retardant levels needed to achieve a desired level of flame retardancy. To a limited extent, compounds of other metals also act as synergists with halogen compounds. They may be used alone but are most commonly used with antimony trioxide to enhance other characteristics, for example, smoke reduction or afterglow suppression. Ionic compounds have a very long history as flame retardants for wool- or cellulose-based products. Inorganic phosphorus compounds are primarily used in polyamides and phenolic resins, or as components in intumescent formulations.

1.2.1.1 Metal hydroxides

Metal hydroxides function in both the condensed and gas phases of a fire by absorbing heat and decomposing to release their water of hydration. This process cools both the polymer and the flame and dilutes the flammable gas mixture. The very high concentration (50 to 80%) required to impart flame retardancy often adversely affect the mechanical properties of the polymer into which they are incorporated. Aluminum hydroxide, also known as alumina trihydrate (ATH) decomposes when exposed to temperature over 200 ^{o}C, which limits the polymer in which it can be incorporated. Magnesium hydroxide is stable to temperature above 300 ^{o}C and can be processed into several polymers.

1.2.1.2 Antimony compounds

Antimony trioxide is not a flame retardant, per se, but it is used as synergist. It is utilized in plastics, rubbers, textiles, paper and paints, typically 2-10% by weight, with organochlorine and organobromine compounds to diminish the flammability of a wide range of plastics and textiles [8].

Antimony oxides and antimonates must be converted to volatile species. This is usually accomplished by the release of halogen acids at fire temperatures. The halogen acids react with the antimony-containing materials to form antimony trihalide and/or antimony halide oxide. These materials act both in the substrate (condensed phase) and in the flame to suppress flame propagation. In the condense phase, they promote char formation, which acts as a physical barrier to flame and inhibits the volatilization of flammable materials. In the flame, the antimony halides and halide oxides, generated in sufficient volume, provide an inert gas blanket over the substrate, thus excluding oxygen and preventing flame spread. These compounds alter the chemical reactions occurring at fire temperature in the flame, thus reducing the ease with which oxygen can combine with the volatile products. It is also suggested that antimony oxychloride or trichloride products reduce the rate at which the halogen leaves the flame zone, thus increasing the probability of reaction with the reactive species. Antimony trichloride probably evolves heavy vapors that form a layer over the condensed phase, stop oxygen attack and thus choke the flame. It is also assumed that the liquid and solid antimony trichloride particles contained in the gas phase reduce the energy content of the flame by wall or surface effects [2].

Other antimony compounds include antimony pentoxide, available primarily as a stable colloid or as a redispersible powder. It is designed primarily for highly specialized applications, although manufacturers suggest it has potential use in fiber and fabric treatment. Sodium antimonite is recommended for formulations in which deep colors are required trioxide may promote unwanted chemical reactions.

1.2.1.3 Boron compounds

Within the class of boron compounds, by far the most widely used is boric acid. Boric acid and sodium borate (borax) are the two flames retardants with the longest history, and are used primarily with cellulosic material, e.g. cotton and paper. Both products are effective, but their use is limited to products for which non-durable flame retardancy is acceptable since both are very water-soluble.

Zinc borate, however, is water-insoluble and is mostly used in plastics and rubber products. It is used either as a complete or partial replacement for antimony oxide in polyvinyl chloride (PVC), nylon, polyolefin, epoxy, ethylene propylene rubber (EPDM), etc. In most systems, it displays synergism with antimony oxide. Zinc borate can function as a flame retardant, smoke suppressant and anti-arcing agent in condensed phase. Recently, zinc borate has also been used in halogen-free, fire-retardant polymers.

1.2.1.4 Other metal compounds

Molybdenum compounds have been used as flame retardants in cellulosic materials for many years and more recently with other polymers, mainly as smoke suppressants [2]. They appear to function as condensed-phase flame retardants [9]. Titanium and zirconium compounds are used for textiles, especially wool [10]. Zinc compounds, such as zinc stannate and zinc hydroxy-stannate are also used as synergists and as partial replacements for antimony trioxide.

1.2.1.5 Phosphorus compounds

Red phosphorus and ammonium polyphosphate (APP) are used in various plastics. Red phosphorus was first investigated in polyurethane foams and found to be very effective. It is now used particularly for polyamides and phenolic applications. The flame-retarding effect is due, in all probability, to the oxidation of elemental phosphorus during the combustion process to

phosphoric acid or phosphorus pentoxide. The latter acts by the formation of a carbonaceous layer in the condensed phase. The formation of fragments that act by interrupting the radical chain mechanism is also likely.

Ammonium polyphosphate is mainly applied in intumescent coatings. Intumescent systems expand to produce foams. Because of this characteristic they are used to protect materials such as wood and plastics that are combustible and those like steel that lose their strength when exposed to high temperatures. Intumescent agents have been available commercially for many years and are used mainly as fire-protective coatings. They are used as flame retardant systems for plastics by incorporating the intumescent components in the polymer matrix, mainly polyolefins, particularly polypropylene [2].

1.2.1.6 Other inorganic flame retardants

Other inorganic flame retardants, including ammonium sulfamate and ammonium bromide, are used primarily with cellulose-based products and in forest fire-fighting [6].

1.2.2 Halogenated organic flame retardants

Halogenated flame retardants can be divided into three classes: aromatic, aliphatic and cycloaliphatic. Bromine and chlorine compounds are the only halogen compounds having commercial significance as flame-retardant chemicals. Fluorine compounds are expensive and, except in special cases, are ineffective because the C-F bond is too strong. Iodine compounds, although effective, are expensive and too unstable to be useful [11].

With respect to processability, halogenated flame retardants vary in their thermal stability. In general, aromatic brominated flame retardants are more thermally stable than chlorinated aliphatics, which are more thermally stable than brominated aliphatics. Brominated

aromatic compounds can be used in thermoplastics at fairly high temperatures with stabilizers. The thermal stability of the chlorinated and brominated aliphatics is such that, with few exceptions, they must be used with thermal stabilizers, such as a tin compound.

Halogenated flame retardants are either added to or reacted with the base polymer. Additive flame retardants are those that do not react in the application designated. There are a few compounds that can be used as an additive in one application and as a reactive in another; tetrabromobisphenol A is the most stable example. Reactive flame retardants become a part of the polymer either by becoming a part of the backbone or by grafting onto the backbone. The choice of a reactive flame retardants is more complex than the choice of an additive type. The development of systems based on reactive flame retardants is more expensive for the manufacturer, who in effect has to develop novel co-polymers with the desired chemical, physical and mechanical properties, as well as the appropriate degree of flame retardants [5, 11]. Synergists such as antimony oxides are frequently used with halogenated flame retardants.

1.2.2.1 Brominated flame retardant

Bromine-based flame retardants are highly brominated organic compounds with a relative molecular mass ranging from 200 to that of large molecule polymers. They usually contain 50 to 85% (by weight) of bromine [11].

Tetrabromobisphenol A (TBBPA) and decabromodiphenyl ether (DeBDE) are aromatic flame retardants. The primary use of TBBPA is as a reactive intermediate in the production of flame retarded epoxy resins used in printed circuit boards [11]. A secondary use for TBBPA is an additive flame retardant in acrylonitrile-butadiene-styrene (ABS) systems. DeBDE is the second largest volume brominated flame retardant used solely as an additive. The greatest use (by volume) of DeBDE is in high-impact polystyrene, which is primarily used to produce

television cabinets. Secondary uses include ABS, engineering thermoplastics, polyolefins, thermosets, PVC, and elastromers. DeBDE is also widely used in textile application as the flame retardant in latex-based back coatings [5]. Hexabromocyclododecane (HBCD), a major brominated cycloaliphatic flame retardant, is primarily used in polystyrene foam. It is also used to flame-retard textiles.

1.2.2.2 Chlorinated flame retardants

Chlorine-containing flame retardants belong to three chemical groups: aliphatic, cycloaliphatic and aromatic compounds. Chlorinated paraffins are by far the most widely used aliphatic chlorine-containing flame retardants. They have applications in plastics, fabrics, paints and coatings [12].

Bis(hexachlorocyclopentadieno)cyclooctane is a flame retardant having unusually good thermal stability for chlorinated cycloaliphatic. In fact, this compound is comparable in thermal stability to brominated aromatics in some applications. It is used in several polymers, especially polyamides and polyolefins for wire and cable applications. Its principal drawback is the relatively high use levels required, compare to some brominated flame retardants [5]. Aromatic chlorinated flame retardants are not used for flame-retarding polymers.

1.2.3 Organophosphorus flame retardants

One of the principal classes of flame retardants used in plastics and textiles is that of phosphorus, phosphorus-nitrogen and phosphorus-halogen compounds. Phosphate esters, with or without halogen, are the predominant phosphorus-based flame retardant in use. For textiles, phosphorus-containing materials are by far the most important class of compounds used to

impart durable flame resistance to cellulose. These textiles flame retardant finishes usually also contain nitrogen or halogen, or sometimes both [6, 10].

1.2.3.1 Non-halogenated organophosphorus compounds

Although many phosphorus derivatives have flame-retardant properties, the number of those with commercials importance is limited. Some are additive and some reactive. The major groups of additive organophosphorus compounds are phosphate esters include trialkyl derivatives such as triethyl or trioctyl phosphate, triaryl derivatives such as triphenyl phosphate and acyl-alkyl derivatives such as 2-ethylhexyl-diphenyl phosphate. The flame retardancy of cellulosic products can be improved through the application of phosphonium salts. The flame-retardant treatments attained by phosphorylation of cellulose in the presence of a nitrogen compound are also of importance [10].

Plasticizers are mixed into polymers to increase flexibility and workability. The esters formed by reaction of the three functional groups of phosphoric acid with alcohols or phenols are excellent plasticizers. The phosphoric acid esters are also remarkable flame retardants, and for this reason are extensively used in plastics [13].

Aryl phosphate plasticizers are used in PVC-based products. They are also used as lubricants for industrial air-compressors and gas turbines. Miscellaneous uses of aryl phosphates are as pigment dispersants and peroxide carriers, and as additives in adhesives, larger coatings and wood preservatives [14].

1.2.3.2 Halogenated phosphates

In addition to the above type, flame retardants containing both chlorine and phosphorus are used widely. Halogenated phosphorus flame retardants combine the flame-retardant

properties of both the halogen and the phosphorus groups. In addition, the halogens reduce the vapor pressure and water solubility of the flame retardants, thereby contributing to the retention of the flame retardant in the polymer.

One of the largest selling members of this group, tris(1-chloro-2-propyl)phosphate (TCPP) is used in polyurethane foam. Tris(2-chloroethyl)phosphate (TCEP) is used in the manufacture of polyester resins, polyacrylates, polyurethanes and cellulose derivatives. The most widely used bromine-and phosphorus-containing flame retardant used to be tris (2,3-dibromopropyl)phosphate, but it was withdrawn from certain applications in many countries due to carcinogenic properties in animals [7, 13]. However, it was just recently detected in furniture foam and U.S. house dust [15].

1.2.4 Nitrogen-based flame retardants

Nitrogen-based compounds can be employed in flame retardant system or form part of the intumescent flame-retardant formulations. Nitrogen-based flames retardant are used primarily in nitrogen containing polymers such as polyurethane and polyamides. They are also called utilized in PVC and polyolefins and in the formulation of intumescent paint systems [16].

Melamine, melamine cyanurate, other melamine salts quanidine compounds are currently the most used group of nitrogen-containing flame retardants. Melamine is used as flame retardant additive for polypropylene and polyethylene. Melamine cyanurate is employed commercially as a flame retardant for terephthalates and is currently being developed for use in epoxy and polyurethane resins. Melamine phosphate is also used. Melamine salts and melamine formaldehyde are used in thermoset resins [16].

1.3 Usage, toxicity, and environmental fate of three chlorinated organophosphorus flame retardants (TCPP, TDCP, and TCEP)

1.3.1 Usage

Building materials like plastics, wood, carpets, wall coverings and textiles can all act as fuel in the case of fire. Therefore, to provide fire protection and to increase the time available for escape in the case of fire, flame retardants are added to various products to suppress or inhibit the combustion process. Among the phosphorus flame retardants, organophosphate esters are the most commonly used. Different groups of organophosphorus compounds (esters, phosphonates and phosphites) have different properties and thus, are used in different applications mostly as additives (mixed into the material, rather than being chemical bonded. They are frequently utilized as flame retardants in plastics, textiles and building materials. Apart from their use as flame retardants, they are also used as plasticizers, stabilizers, antifoaming and wetting agents and as additives in lubricants and hydraulic fluids. The large volumes consumed and broad application range of those compounds has aroused suspicions that they may escape the technosphere by volatilization, leaching, or abrasion and reach different environmental compartments [17].

In order to trace the sources of individual organophosphorus compounds it is important to consider the areas of application of bulk chemicals. Materials and products in which organophosphorus compounds are used as flame retardants include amongst others, polyvinylchloride (PVC), flexible and rigid polyurethane foams (PUF), thermoset resins, thermoplastic materials, textile finishes, cellulosics, polyesters, phenolics and hydraulic fluids. As plasticizers, organophosphorus are utilized in materials like PVC, cellulose acetate, polyester, acrylo-nitrile-butadiene-styrene (ABS), polystyrene and synthetic rubber. Organophosphorus

flame retardants are also applied as plasticizers in products like paints, lacquers and varnishes [17]. The chlorinated organophosphorus flame retardants are used in both flexible and rigid PUF. They are also used in rubbers and textile coatings. Flexible foams can be found in products such as upholstered furniture and mattresses, while rigid foams are used for thermal insulation [18].

1.3.2 Toxicity

It is known that TDCP (tris(1,3-dichloro-2-propyl)phosphate) is absorbed through human skin [19-25]. Thus, flame retardants added to fabrics may pose a risk of dermal exposure for humans. Young children may also be orally exposed [20]. TCPP can cause skin irritation [18]. TCEP has been found to be teratogenic and has carcinogenic potential in rats and mice, but it is not considered to sensitize skin fate [21]. TDCP, TCPP, TCEP also have documented hemolytic effects [26]. TDCP were detected in human adipose tissues (concentrations ranging from 0.5 to 257 ng/g) [22]. It was found in human seminal fluid at levels between 5 to 50 ppb [22, 23].

1.3.3 Occurrence and fate

1.3.3.1 Water

Organophosphate triesters have been used for decades, so their occurrence in the environment is not a new issue. Since, the 1980s, their detection in surface water [27, 28], groundwaters influenced by wastewater [24, 29, 30] and in drinking water [30] has been reported.

1.3.3.2 Wastewater

The number of studies devoted to the determination of organophosphate triesters in municipal wastewater treatment plants (WWTPs) has been relatively limited. Meyer and Bester [31] conducted a survey on seven triesters in two WWTPs from Germany involving secondary and tertiary treatment. Influent concentrations around or above 0.1 µg/L were found for six of the analytes. Chlorinated aliphatic esters TCEP, TCPP, and TDCP showed no significant removal and, thus, were those triesters with the highest effluent concentrations. In a Swedish study, 12 analytes were monitored in 11 WWTPs [25], representing the average of one week. In agreement with the German study, the chlorinated aliphatic esters were hardly removed in Swedish WWTPs. In this Swedish study, TCPP sorption to activated sludge has been shown to be the major removal process in a WWTPs, resulting in average concentration of 5 µg/g in the sludge [31, 32].

1.4 Reduced sulfur species in the natural environment

The main goal of our research is to investigate the chemical fate of selected flame retardants in the reduced sulfuric environment under anoxic conditions. To understand the fate of these compounds, it is very important to elucidate the reactions of flame retardants in the natural environments containing reduced sulfur species. Flame retardants that are already present in the surface water and wastewater may associate with particles and can eventually become a part of the sediment phase. It is likely that some flame retardants can be transported into salt marshes, sediments, and the bottom layer of estuaries where anoxic conditions are prevalent. Transformation processes under anoxic conditions may represent an important sink for flame retardants. Anoxic conditions can give rise to high concentration of reduced sulfur species, which are versatile environmental “reagent “, that are capable of reacting with a wide array of organic pollutants. Such environmental conditions are simulated in our laboratory.

Inorganic and organic sulfur species have been reported as the most potent nucleophiles present in the environment [33-35]. The potential therefore exists for such highly reduced sulfur nucleophiles to serve as environmental "reagents" affecting the abiotic degradation of flame retardants. H_2S, HS^-, thiolate anions (RS^-), polysulfides (S_n^{2-}, where n >1), are the most common sulfur nucleophiles encountered in natural waters. All of these nucleophiles are thermodynamically unstable in the presence of O_2. Hence, they are most commonly found in hypoxic environment (i.e. natural waters containing very low or undetectable level of dissolved O_2), usually in the presence of high concentration of reduced organic matter. Environments of this type include marine, estuarine, and marsh porewaters, groundwaters containing high concentrations of leachate derived from domestic waste (e.g. beneath landfills), and hydrogeologic zones located far downgradient from their recharge areas. The speciation of sulfur among its various forms in a particular environment arises from a balance among several simultaneous chemical and biochemical processes (usually with microbiological mediation) include oxidation, reduction, hydrolysis, dissolution and precipitation. Bacteria can reduce sulfate to hydrogen sulfide (H_2S) [36], hence HS^- and S^{2-}, as well, which are referred to collectively as $H_2S(T)$. Species, which are partially reduced, have forms in which sulfur exhibits an oxidation state between those of SO_4^{2-} (+VI) and $H_2S(T)$ (-II), such as S_n^{2-}, $S_2O_3^{2-}$, $S_4O_6^{2-}$, and SO_3^{2-} may be produced by the oxidation of $H_2S(T)$ and sulfide minerals, the reductive dissolution of goethite by HS^-, the hydrolysis of polysulfides, and microbial process [36-39]. Thiols may be produced biologically as results of sulfate reduction, biodegradation of organic matter or lithotropic oxidation (the biological oxidation of inorganic compounds to obtain energy)[40]; or abiotically through the reaction of dissolved organic matter with $H_2S(T)$ or elemental sulfur [37, 41].

The simultaneous operation of several of these processes in a particular environment may rise to a complex group of sulfur nucleophiles. For example, Boulegue and coworkers detected $H_2S(T)$, S_8, $S_2O_3^{2-}$, SO_3^{2-}, S_n^{2-}, SO_4^{2-}, and organic polysulfides in the porewater of a salt marsh along the Delaware estuary [37]. They attribute the observed sulfur species speciation to a steady-state interaction between the various reduced sulfur species diffusing upward from hypoxic sediments and O_2 diffusing downward from the sediment-water interface.

Based on equilibrium distribution discussed by Giggenbach [42] and Schwarzenbach and Fischer [43]. We can get a sample distribution of sulfur species over pH values under the condition of 5 mM $[H_2S]_T$ in the presence of excess of elemental sulfur (Figure 1.1). The figure demonstrates that at pH < 6, H_2S is the predominant species in the aqueous solution. As pH increases, the portion of HS^- increases and reaches the maximum at pH = 8. When pH > 9, polysulfide is the predominant species and bisulfide is present in the solution. The much lower proton dissociation constants of S_4^{2-} and S_5^{2-} [43] would result in very low concentrations of HS_4^- and HS_5^- in solution. Therefore, bisulfide and polysulfides are of much more importance than other species in the system containing bisulfide and S(0).

Polysulfides are frequently employed as a 30% aqueous solution in commercial preparation used for agricultural soil conditioning and for fungal, mite, and insect control [44]. Elemental sulfur is also commonly added to soil because of its fungicidal qualities as well as its role as essential nutrients. Under hypoxic conditions, this elemental sulfur could undergo dissimilatory reduction by soil microorganisms to generate polysulfides by the oxidation of bisulfide [45]. Sediment thiosulfate may result from the abiotic reaction of elemental sulfur and sulfite. Thiosulfate is also produced by the oxidation of pyrite (FeS_2) under oxic condition. Many anaerobic sediment bacteria, which lack the ability to reduce sulfate directly, can instead

use sulfur compounds of lower oxidation state, such as SO_3^{2-}, $S_2O_3^{2-}$ and S(0) as electron acceptors to produce HS^-.

The occurrence of organic sulfur compounds in the sediments was also reported [46-48]. Organic sulfur compounds are often abundant in sediment. As stated above, the anoxic environments are favorable for development of microorganisms implied in the formation of inorganic reduced sulfur species, which are liable to react with organic matter to produce low molecular weight organic sulfur compounds and macromolecules reticulated by sulfur. The mechanism involved in the interaction between organic matter and inorganic sulfur species are still subject of many investigators [46, 49-51]. Sediments in the paleoenvironments were anoxic and H_2S has probably exceeded the amount of available iron. These conditions result in free H_2S, which react with organic matter, leading to the formation of organic sulfur species such as thiolanes, thianes, thiophenes and so on [46, 52]. Besides the addition of elemental sulfur and anionic sulfur species on organic matter [53-55] a radical mechanism involving H_2S and elemental sulfur could also be considered as an effective process for the formation of organic sulfur entities. Radicals implied in the incorporation of sulfur into organic matter may be formed by various ways. For instance, the radicals present in humic substance could quench hydrogen from thiols or H_2S leading to formation of sulfur radical. Alternatively, photochemically-induced radical formation could be a potential process in natural environments [47].

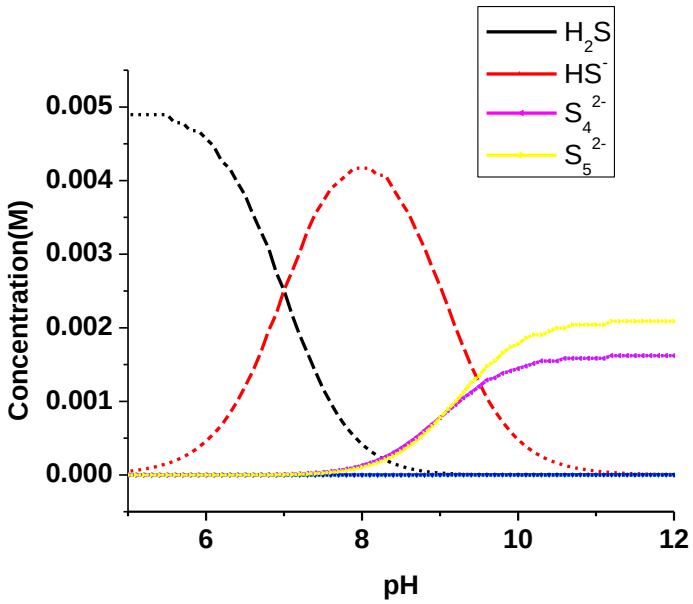


Figure 1.1 Calculated distribution of hydrogen sulfide/polysulfide species over pH 5-10. Assumption: 5 mM $[H_2S]_T$ in equilibrium with excess elemental sulfur based on equilibrium constants reported by [42] and [43]. S^{2-}, HS_4^- and HS_5^- have been included in the calculations, but their concentrations are so small that they have been neglected in the figure.

1.5 General discussion of gas chromatography, high performance liquid chromatography and mass spectroscopy

1.5.1 High-performance liquid chromatography (HPLC)

HPLC is one of the most powerful chromatographic techniques. It is not limited by sample volatility or thermal stability. The result of separation is easily visualized; sample recovery is good. Detection is highly sensitive (down to nanograms in favorable cases). It can separate a wide variety of compounds ranging from macromolecules and ionic species, labile natural products, polymeric materials, to high molecular weight polyfunctional groups. It also

involves specific interactions between sample molecules and both the stationary and mobile phases. It offers a greater variety of stationary phases than gas chromatography (GC), thin layer chromatography (TLC), or column chromatography (CC), allowing more diverse selective interactions and possibilities for separation [56, 57].

In HPLC, solid and liquid samples are dissolved in an appropriate solvent and injected into the instrument. The analyte is forced through a column (stationary phase) by a liquid (mobile phase) at high pressure, which decreases the time they have to diffuse within the column. The components of the mixture are separated by selective retention within the stationary phase. As the analytes flow through, the detector deflects on a chart recorder or computer screen. To collect, store, and analyze the chromatographic data, computer, integrator, and other data processing equipment are frequently used. Available detectors include ultraviolet (UV), refractive index (RI), mass spectrometry (MS), fluorescence, electrochemical, infrared (IR), and evaporative light scattering detector (ELSD).

The mechanism of separation includes adsorption, partition, ion pairing, ion exchange and size exclusion. Two types of HPLC methods are distinguished by the relative polarity of the stationary phase and mobile phase. Normal phase (NP-HPLC) means that the polarity of the stationary phase (e.g. silica) is higher than that of the mobile phase (e.g. hexane or tetrahydrofuran). Reverse phase (RP-HPLC) means that the polarity of the stationary phase (e.g. chemically bonded C_{18} or C_8 alkyl chains) is less than that of the mobile (e.g. mixture of water with either methanol or acetonitrile). For both NP-HPLC and RP-HPLC mixtures of solvent may be used in two elution modes: isocratic (the composition of mobile phase is constant) or gradient (composition of the mobile phase can be made to change in a predetermined way during the elution). Compounds are eluted in order of polarity with both techniques. For RP-HPLC, the

most polar compounds come out first, whereas for NP-HPLC, the least polar come out first. RP-HPLC dominates most current application because it has a broad scope that allows sample types with a wide range of polarities and molecular weights to be separated, features rapid mobile phase column equilibration, and is easier, faster and more reproducible experimentally.

The HPLC instrumentation system consists of an injector unit, degasser, pump, column, and detector. The functions of each part are as follows: the solvent degasser is used to prevent bubbles in the mobile phase; the programmable high-pressure pump mixes the solvents in the prescribed ratios and pumps them through the column and past the detector; the column compartment houses and thermostats the HPLC column; the injection unit (autosampler or injector) draws prescribed volumes from samples volumes from sample vials and injects them onto the column; the in-line detector (UV or other) monitors the absorbance of the column effluent at a selected wavelength at regular intervals.

The very important stationary phase of column used today contains uniform, porous particles with nominal diameters of 10, 5, 3, 1.8 μm. Because of this property, the efficiency of HPLC separation surpasses CC and TLC. That is also the reason that a high-pressure pump is necessary to pass the mobile phase through the column.

1.5.2 Nuclear magnetic resonance (NMR)

NMR is a physical phenomenon based upon the magnetic property of an atom's nucleus. It is one of the principal techniques used to obtain information about molecular structure. NMR is a phenomenon that occurs when the nuclei of certain atoms (such as ^{1}H or ^{13}C) are immersed in a static magnetic field and exposed to a second oscillating magnetic field. Some nuclei experience this phenomenon and others do not, dependent upon whether they possess a property called spin (I). The nuclei with non-zero spin quantum number (e.g. I = 1/2) may be thought to

spin around an axis but with only two possible orientations, up and down. When there is no external magnetic field present there is no energy difference between up and down spin states. If an external magnetic field B_o is applied, then the spin state that gives rise to a nuclear magnetic field aligned with the external magnetic field has the lower energy. The energy difference ($\Delta E = h\nu$) lies in the range of radio frequencies. The populations also differ for the two states, with their ratio given by the Boltzmann distribution [$N_{up}/N_{down} = \exp(-\Delta E/kt)$]. The lower-energy state that has an orientation of nuclear spins parallel to the magnetic field is slightly more populated. The nuclear magnetic resonance phenomenon occurs when nuclei aligned with (or against) an applied field are induced to absorb (or release) energy and change their spin orientation. The energy absorption is a quantized process; the energy absorbed must equal the energy difference between the two states involved

$$\Delta E_{absorbed} = h\nu \quad (1)$$

The energy separation between two different states (Lamar frequency) is given by:

$$\Delta E = h\gamma B_0/2\pi \quad (2)$$

Where γ is the gyro magnetic ratio of spin I, and B_0 is the magnitude of the applied static magnetic field. The observed NMR frequency ν expressed in terms of the gyro magnetic ratio and the applied field

$$\nu = \gamma B_0/2\pi \quad (3)$$

The power of NMR spectroscopy relies on the fact that not all protons in a molecule resonate at the same frequency. This is because the protons in a molecule are surrounded by electrons and exist in slightly different electronic environment from one another. So, different protons in a molecule each resonate at slightly different frequencies. This means that each different proton has a characteristic chemical shift (δ) from a reference proton, typically expressed in

dimensionless units, parts per million (ppm). Because the chemical shift is obtained by measuring Hz (from the reference) divided by ν_0 the operating frequency of the spectrometer, it is independent of spectrometer conditions. Therefore, it is viewed as a characteristic property of the protons. For the spectrum of a given compound, the intensity of ^{1}H NMR absorption is strictly proportional to the number of nuclei giving rise to the absorption. This property is very important for structure determination [56, 58, 59].

Spin-spin coupling refers to scalar coupling between spins, which is characterized by the coupling constant J (a measurement between a pair of protons). The value of J is field independent and is characteristic of each bond type. It depends on chemical environment (e.g. the number and types of bond through which the nuclei are coupled). Usually it is smaller than other magnetic interactions, e.g., for a ^{13}C-^{1}H pair, J can be as much as 150 Hz, for protons separated by three bonds, J is in the range 2-9 Hz, but for geminal protons, J is 12 Hz [59].

1.5.3 Gas chromatography/mass spectroscopy

Mass Spectroscopy is a powerful analytical technique that can provide a myriad of information about analytes in complex mixtures. In principle mass spectroscopy separates ions of a sample based on their mass to charge ratio (m/z). Qualitative as well structural information for both inorganic and organic compounds can be obtained, as well as structural information about a wide variety of molecular species. The use of high resolution instrument allows the determination of isotopic ratios of atoms in samples and with specialized instrumentation the structure and composition of solid surfaces can be investigated. Some significant instrumental advances include the development of double focusing instruments, interfacing mass spectrometers and several new ionization techniques have been developed. Fundamental understanding of the behavior of ions in a magnetic field is the basis for applying mass

spectroscopy to the determination of chemical composition of a sample. Additional developments in the understanding of fragmentation of molecular species in the ion source allow the structural analysis and identification of complex molecules. Further improvements have included the application of Fourier transform, which greatly improves mass resolution and signal-to-noise ratios. Mass spectroscopy has matured into a sophisticated and powerful tool, which is widely used in chemical and biochemical analysis.

All mass spectrometers have five major components, which include an inlet system, ion source, mass analyzer, detector and signal processor. The first four components are typically held at high vacuum (10^{-3}–10^{-8} torr). The inlet system's function is to introduce a small amount of sample (typically 1 μmol or less) into the ionization source with a minimal loss of vacuum. Typical inlets include batch inlets, direct probe inlets and chromatographic inlets. The GC will not only serve as an inlet system but it will also facilitate the separation of our complex mixture before it is introduced to the mass spectrometer.

The ion source is perhaps the most important part of the mass spectrometer. Ion sources are as varied as the types of samples that can be analyzed by mass spectrometry. They can be a simple electron impact ion source, producing ions from the interaction of analyte molecules with energetic electrons in the gas phase, to the more complicated ionization occurring in a MALDI (Matrix Assisted Laser Desorption Ionization) source useful for nonvolatile samples. The ion source used is typically mandated by the physical properties of the sample being analyzed and several types include electron ionization (EI), chemical ionization (CI), field desorption (FD), fast atom bombardment (FAB), secondary ion mass spectrometry (SIMS). Laser desorption (LD), plasma desorption (PD), thermal desorption and matrix MALDI. Understanding the process taking place in the ion source is the key to structure elucidation for qualitative analysis.

If the ion source is the most important part of the mass spectrometer, the ions produced within it would not provide any useful information without the mass analyzer. The main function of the mass analyzer is to separate ions with different mass to charge ratios (m/z) produced in the ion source. Like ion sources, there are a number of mass analyzers, which can be used for this purpose. These are classified as magnetic sector analyzers, double focusing spectrometers, quadrupole mass filters, ion trap analyzers, time of flight analyzers and Fourier transform instruments. Each mass analyzer has its own unique advantages and disadvantages. Typically quadrupole mass filters are coupled to chromatographic instrument because they are typically more rugged, lower priced, and more compact than other mass analyzer. Additionally, quadrupole mass filters are capable of scanning a large range of masses in a short time, which is useful for real-time scanning of chromatography peaks. A quadrupole consists of a focusing lens stack and four cylindrical metal rods, which act as the electrodes of the mass filter. Ions are accelerated and focused into the space between the rods by the lens stack. Opposite rods in a quadrupole are connected electronically, to the positive and negative terminal of a variable DC source. Additionally, there is a variable radio frequency AC potential applied, 180 degrees out of phase to each pair of rods. The quadrupole acts as a mass filter because only ions with a stable trajectory will remain between the rods and pass to the detector.

By simultaneously adjusting the AC and DC potential applied to the rods it is possible to change the m/z of an ion that will have a stable trajectory. Therefore, by rapidly adjusting the AC and DC potentials it is possible to rapidly scan a range of masses and continually collect a complete mass spectrum of the column eluent. The use of a hyphenated GC-MS system is powerful for qualitative as well as quantitative determination of complex mixtures.

Once the ions have been separated by the mass analyzer they must be collected and the beam of ions converted into an electrical signal, which can be recorded, stored and/or displayed for analysis. The most common type of detector is electron multiplier. Other detectors include the Faraday cup, scintillation detectors and photographic plates. Similar to a photomultiplier tube in optical instruments, the electron multiplier ejects a cascade of electrons from a dynode held at a fixed voltage. Though a series of dynodes, each at a successively higher voltage, the current caused by analyte ions is amplified before being recorded and measured. Another electron modifier, and the type used in this instrument, is a continuous dynode type. This detector works on the same principle as a discrete dynode electron multiplier, however it is constructed of a single trumpet shaped device, which acts to collect and amplify the signal from impacting ions. The last component of most mass spectrometers is the signal processor. In most modern instruments analog signals from the detector are converted to digital output that is collected and stored by a computer. The computer can also be used to control instrument parameters of both the GC and MS as well as for data acquisition and manipulation. Through the use of software programs various analysis modes and data work up can be performed depending on the analysis being carried out.

1.5.3.1 Tandem (MS^n) mass spectrometers

Instruments that have more than one analyzer and so can be used for structure determination and sequencing studies by collisionally generating and identifying fragmentation ions inside the mass spectrometer. Two, three, or more analyzers can be incorporated into commercially available tandem instruments, and the analyzers do not necessarily have to be of the same type, in which case the instrument is a hybrid one. More popular tandem mass spectrometer includes those of the quadrupole-quadrupole, magnetic sector-quadrupole, and

more recently, the quadrupole-time-of –flight geometries. In tandem MS, the ion of interest is selected with the first analyzer (MS-1) and then collided with inert gas atoms; the fragments generated by the collision are separated by a second analyzer (MS-2), and so on. In ion trap and Fourier transform experiments, the experiments are carried out in one analyzer, and the various events are separated in time, not in space [60].

Chapter 2

Objectives

The main objectives of the following research are:

1-Determination of second-order rate constants of TCEP, TDCP, and TCPP with reduced sulfur species (polysulfide, bisulfide, and thiophenolate).

2-Identification and quantification of the degradation products of TCEP, TDCP and TCPP with reduced sulfur species.

3-Proposal of possible mechanism of the reaction of TCEP, TDCP, and TCPP with reduced sulfur species.

Chapter 3

Experimental Section

3.1 Preparation

3.1.1 Reaction system

Our laboratory experiments are designed to simulate the natural conditions that are high in reduced sulfur species in order to investigate the role of reduced species in the degradation of TCPP, TDCP, and TCEP. The experimental system was simplified to neglect the influence from other environment factors (e.g., microbial degradation, heterogeneous reactions on metal oxides). Therefore, the experiments were carried out in clean batch system under anoxic conditions.

All reaction solutions were prepared in an anaerobic glovebox (5% H_2, 95% N_2) and aqueous solutions were prepared from argon-purged deionized water (DW) (Mill-Q gradient system, Millipore, Bedford, MA). Glassware used with sulfidic solutions was washed with NaOH/methanol to remove traces of sulfur impurity before acid washing. All glassware was soaked in 1 M HNO_3 overnight, rinsed several times with deionized water, and dried at 200 ^{o}C before use.

3.1.2 Preparation of solution

3.1.2.1 Sodium sulfide stock solution

Sodium sulfide stock solutions were prepared under argon from $Na_2S{\cdot}9H_2O$ crystals (98%; EM Science) using deoxygenated distilled water according to the procedure described by Jans and Miah [61]. Crystals were rinsed with Ar-purged water to remove surface oxidation products, blotted with cellulose wipe, and were then transferred to an Ar-purged three-necked flask. The flask was then connected to an Ar-purged closed glassware system consisting of a

reservoir (containing distilled water), glass tubing, stopcocks and an argon tank. Before rerouting the argon flow and forcing the reservoir content into the flask containing the washed sodium sulfide crystals, the distilled water was purged with argon for one hour. Then, the sodium sulfide stock solution was transferred to the anaerobic chamber.

3.1.2.1 Polysulfide stock solution

The polysulfide stock solution was prepared in 50 mM borate buffer. Na_2S_4 crystals (Na_2S_4, 90% technical grade; Alfa Aesar) were ground into powder in the mortar in the anaerobic glovebox and washed with deoxygenated toluene to remove sulfur. After decanting toluene, the powder was completely dried over argon. The treated powder was dissolved in prepared deoxygenated buffer (50 mM tetraborate buffer containing 100 mM NaCl) and stored in a brown bottle to avoid the exposure to light. The polysulfide stock solutions were kept in the anaerobic chamber.

3.1.2.2 Thiophenol stock solution

The stock solution of 100 mM thiophenol was prepared by dissolving thiophenol (99%, Lancaster Synthesis, Pelham, NH) in deoxygenated methanol and stored in glove box. The commercial thiophenolate was moved into the glove box immediately after reception and kept in the glove box the entire time.

3.1.2.3 TCEP, TDCP, and TCPP stock and standard solutions

Chart 3.1 shows the structures of the three investigated organophosphorus flame retardants (OPFRs). TCEP (98%) and TDCP (95.0%) were obtained from TCI (Portland, OR) TCPP (97%) was purchased from Pflatz and Bauer (Warterbury, CT). The stock solution of

TCEP, TDCP and TCPP were prepared by dissolving exactly weighted TCEP, TDCP, and TCPP in deoxygenated methanol or ethyl acetate in a volumetric flask and stored in a brown bottle in the glovebox. A series of standard solutions of flame retardants were prepared by diluting those flame retardants stock solutions into methanol.

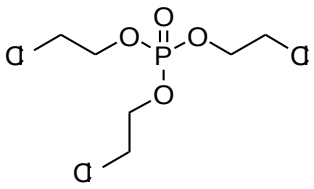
3.1.2.4 Reaction buffer

The reaction solutions containing bisulfide, polysulfide and thiophenol were prepared by dilution of the prepared stock solutions into pH buffer (50 mM sodium tetraborate) in order to maintain a constant pH value in the reaction solution.

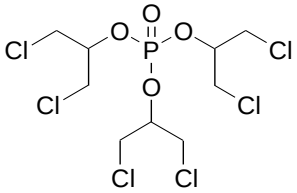
3.1.2.5 Experiment setup and sampling

The reaction solution was prepared and equilibrated overnight in the glovebox and transferred into a 20 mL syringe equipped with polycarbonate stopcock and a PTFE needle tubing. Four glass rings were placed in the syringe to facilitate mixing of the reaction solution. Reactions were initiated by spiking the stock solution of the pesticides into the syringe and vigorously mixing for 30 seconds in the glovebox. The reaction mixtures were maintained anoxic and the syringes were incubated in water bath at selected temperature. During the reactions, samples were taken by transferring aliquots (1 mL) of the reaction mixture into the test tubes containing 1 mL ethyl acetate. The extraction served to quench the reaction. The resulting extracts were stored in the refrigerator and were subjected to later GC analysis. A second sample of the aqueous solution was acidified with one drop of glacial acetic acid and was subjected later to LC-MS analysis.

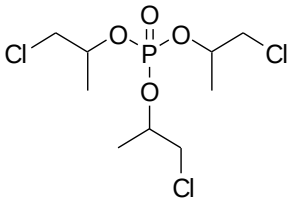
Chart 3.1 Structure of investigated OPFR



TCEP



TDCP



TCPP

The broad application range of TCEP, TCPP, and TDCP and the fact that they are utilized as additives may result in them spreading diffusively into the environment by volatilization, leaching and abrasion [25]. TCEP and TCPP appear to be more recalcitrant and ubiquitous in water [27, 32, 62, 63], air [17, 64-67], sediments [68, 69], soils [70-72] TCPP is a suspected carcinogen while the carcinogenicity of TDCP seems to have been proven more clearly [73]. TCEP, TDCP, and TCPP have been detected in pine needles in the Sierra Nevada Mountains, United States, originated from long range air transportation [74]. TCEP and TDCP have been detected in samples of rainwater from Ireland, in snow in Poland [75] in rainwater collected in Germany [76]. TCPP gives three isomer peaks in the ratio of 9:3:1 that are separated by GC/FID. The quantification has been performed to the first peak only. TCEP, TDCP, and TCPP were selected based on their similar structure to study the influence on the reactivity.

3.2. Methods

3.2.1 Measurement of reduced sulfur concentrations

The thermodynamics and kinetics of the H_2S/ system in natural waters have been investigated in detail [77]. The pK_a of H_2S has been examined as function of temperature and salinity. In our experiments, the total hydrogen sulfide concentration $[H_2S]T$ is the sum of all hydrogen sulfide species ($[H_2S]+[]+[S^{2-}]$). The total thiophenol concentrations $[PhSH]_T$ is the

sum of [PhSH] and $[PhS^-]$. The total reduced sulfur concentration of polysulfide solutions represents the sum $[H_2S] + [HS^-] + [S^{2-}] + [H_2S_n] + [HS_n^-] + [S_n^{2-}]$ for n = 2 - 5.

Concentrations of reduced sulfur species were determined by iodometric titration using a starch endpoint. In order to determine the exact concentration of reduced sulfur species in the reaction solution, iodometric titration was carried out. In the process of iodometric titration I_3^- is the active oxidizing agent. It is formed when potassium iodate (KIO_3) is mixed with an excess of KI in an acidic media. The color of this reaction is richly yellow. In such condition, each mole of (KIO_3) generates three moles of I_3^- as shown below.

$$IO_3^-(aq) + 8I^-(aq) + 6H^+(aq) \rightarrow 3I^-(aq) + 3H_2O \ \ (aq) \qquad (4)$$

Each mole of I_3^- is capable of accepting two moles of electrons hence each mole of KIO_3 has an equivalent of 6 moles of electron accepting capacity

$$I_3^- + 2e^- \rightleftharpoons 3\ I^- \qquad (5)$$

In a normal titration procedure, known volume of the reduced sulfur species is added to an excess of triiodide (I_3^-). The remaining triiodide is back titrated with standardized sodium thiosulfate ($Na_2S_2O_3$). Three titrations were carried out for the standardization of thiosulfate solution. The thiosulfate solution has 1 eq/mol of oxidizing capacity. Thus the concentration of thiosulfate is determined as follows

$$[S_2O_3^{2-}] = \frac{KIO_3 \quad X \quad V_{KIO_3} \quad X \quad 6}{V_{S_2O_3^{2-}}} \qquad (6)$$

Thiosulfate is used as the oxidizing agent because it forms a colorless solution after the completion of titration. Endpoint of titration is detected by the disappearance of the pale yellow

color. Adding a few drops of colloidally dispersed starch aids in this detection since it forms a strongly blue solution in the presence of I_3^- with its concentration as low as 2 x 10^{-7} M. The occurrence of blue color signals the presence of I_3^- at which point the solution is further titrated with thiosulfate. The reduced sulfur concentration is determined by calculating the amount of I_3^- not titrated by thiosulfate and the volume of the sulfur species used. The following equation was used for determining the total reduced sulfur species concentration in the case of thiophenolate, which has the electron donating capacity of 1 eq/mol:

$$[PhSH]_T = \frac{6[KIO_3] \;\; X \;\; V_{KIO_3} - [Na_2S_2O_3] \;\; X \;\; V_{Na_2S_2O_3}}{V_{PhSH}} \tag{7}$$

Since S_n^{2-} has the electron donating capacity of 2 eq/mol, the above equation was divided by 2 in order to obtain the S_n^{2-} concentration. An Accumet pH meter (Fisher Scientific, Pittsburgh, PA) with a Ross combination pH electrode (ThermoOrion, Beverly, MA) was used to measure the pH value in the reduced sulfur reaction solutions. Bisulfide and hydrogen sulfide concentrations were computed from $[H_2S]_T$ and measured pH values via ionization constants for H_2S reported by Millero [77] that were corrected for ionic strength using activity coefficients γ_{HS^-} and $\gamma_{S^{2-}}$ determined from the Davies approximation using MacμQL 2.0. Thiophenolate and thiophenol ion concentrations were determined via the same way as $[HS^-]$ and $[H_2S]$ from $[PhSH]_T$ and the measured pH values. pK_a of thiophenol was reported to be 6.50 at 25 ^oC [78]. The chemistry of aqueous polysulfides solution is complex. The equilibria between polysulfide ions in aqueous solution can generally be described by the equation

$$(n-1)S_{n+1}^{2-} + HS^- + OH^- \rightarrow nS_n^{2-} + H_2O \quad n = 2-5 \tag{8}$$

In our research, the total concentration of polysulfide dianions, $\sum[S_n^{2-}]$ were determined via speciation calculations from the measured $[S(-II)]_T$ and pH values based on the reported equilibrium constants [42, 43]. The resulting values of $[S_n^{2-}]$ were used to compute the second-order rate constant $k''_{S_n^{2-}}$ for the reaction of these OPFRs (organophosphorus flame retardants) with polysulfides. Another method to determine $[S_n^{2-}]$ is reported by Kamyshny et al. However this method requires the difficult synthesis of unstable standards. His method is based on fast, single-phase derivatization with methyl trifluoroethanesulfonate. Under the most aggressive preconcentration treatment involving liquid-liquid extraction, solvent evaporation to dryness, dissolution in *n*-dodecane, and finally HPLC-UV analysis of the dimethylpolysulfane distribution, the minimum detection limits of the individual polysulfides are in the range 15 to 70 nM [79]

3.2.2 GC Analysis

Ethyl acetate extracts from degradation experiments of TCEP with thiophenolate were analyzed using a Series 8000 GC (Fisons Instruments). It is equipped with split/splitless injector as well as on-column injector, a flame ionization detector FID-80 detector (the control unit is EL 980, Fisons Instruments), and an EC^{TM}-5 fused-silica capillary column (30 m x 0.25 mm x 0.25 µm film thickness; Alltech, Deerfield, IL). In gas chromatography, a sample can be injected onto a column in two ways. The sample can be injected into a heated glass liner, which vaporizes the sample. After which the sample vapor is swept onto the column by the carrier gas. This is called split/splitless injection. The sample can also be discharged directly into the column by means of a syringe. This technique is called on-column injection.

Flame ionization detector-FID (EL 980, Fisons Instruments) is mass sensitive rather than concentration sensitive. This gives the advantage that changes in mobile phase flow do not affect the detector's response. The FID is a useful general detector for the analysis of organic compounds; it has high sensitivity, a large linear response range, and low noise. It is also robust and easy to use.

Temperature program: Injector and detector temperature for TCEP, TDCP, and TCPP were set at 250 ^{o}C and 275 ^{o}C, respectively. The column temperature was held at 100 ^{o}C for one minute, then increased at a rate of 20 ^{o}C/min to 275 ^{o}C, and finally held constant at 275 ^{o}C for four minutes for TCEP. The column temperature was set at 150 ^{o}C for one minute, and then increased at a rate of 20 ^{o}C/min for 10 minutes for TDCP. The column temperature was set at 100 ^{o}C for one minute, and then increased to 220 ^{o}C at a rate of 20 ^{o}C/min, to 225 ^{o}C at a rate of 1 ^{o}C/min, to 225 ^{o}C at a rate of 20 ^{o}C/min, and finally held to 275 ^{o}C for 2 minutes for TCPP.

A product peak was observed in the ethyl acetate extracts of the experiment of TCPP with thiophenolate. GC-MS analysis of this peak resulted in the mass spectra summarized in Table 3.1. It was identified as bisphenylthio isopropane (BPTiP), an isomer of bisphenylthio propane (BPTnP). BPTiP is not commercially available. (BPTnP) is commercially available. The mass spectra and relative intensity of BPTnP are summarized in Table 3.1. The molar mass of BPTiP is the same as BPTnP which is 260 g/mol. BPTiP was quantified in the reaction of TCPP with thiophenolate using BPTnP as standard (see Figure 4.11).

Table 3.1: Mass to charge ratio and relative intensities of the mass spectra for BPTnP and BPTiP

BPTiP			BPTnP	
m/z	rel. intensity		m/z	rel. intensity
41.05	52.54		40.00	47.73
65.05	44.02		65.05	44.01
73.05	28.23		78.05	4.70
91.10	6.62		91.10	6.62
109.00	60.21		109.00	60.21
123.05	30.03		117.05	4.68
137.05	20.46		135.05	9.57
151.10	100.00		151.10	100.00
152.10	11.68		260.15	7.66
207.10	4.10			
260.15	7.66			

3.2.3 LC-MS-MS Analysis

The formation of PTEA and BCEP was determined by HPLC and monitored by electrospray ionization tandem mass spectroscopy (ESI-MS-MS) operated in the positive ion mode for PTEA and negative ion mode for BCEP with multiple reaction monitoring (MRM) using a liquid chromatography module UFLC (Shimadzu, Kyoto, Japan) and 4000 Q Trap (Applied Biosystems MDS Sciex, Concord, Canada). BCEP has a low pK_a and is therefore predominantly present as an anion. LC-MS-MS parameters for PTEA are curtain gas: 20 psi, ion spray voltage: +5500 V, ion source gas 1: 65 psi, and ion source gas 2: 65 psi. The ones for

BCEP are curtain gas: 35 psi, ion spray voltage: –4500 V, temperature: 350 ^{o}C, ion source gas 1: 20 psi, ion source gas 2: 15 psi. MRM transitions monitoring for PTEA and BCEP were 155/137 and 221/35, respectively.

The aqueous reaction solution was acidified with one drop of glacial acetic acid. They were used for quantification. Acidified aqueous reaction of TCEP with thiophenolate was analyzed by LC-MS-MS. The chromatograms were examined using Analysis 1.4 software (Applied Biosystems). A Zorbax eclipse XDB-C_{18} column (5 µm, 4.6 mm x 150 mm, Agilent Technologies) was used for detecting PTEA and BCEP. LC conditions for PTEA were: flow rate 1.0 mL/min, mobile phase A water with 0.1% (v/v) formic acid and 4 mM ammonium formate, mobile phase B methanol with 0.1% (v/v) formic acid and 4 mM ammonium formate. The gradient was as follows: 0.01 min, 80% B; 3 min, 95% B; 4 min, 95% B; 5 min, 80% B; 6 min, 80% B. The conditions for BCEP were: flow rate 1.0 mL/min, mobile phase A: methanol /water (20/80), and mobile phase B: methanol/water (95/5), both containing 1 mM tributylamine and 1 mM acetic acid. The gradient was as follows: 0.01 min, 40% B; 3 min, 48% B; 4 min, 100% B; 5 min, 100% B; 6 min, 40% B; 7 min, 40% B. The injection volume was 10 µL.

Another method for the analysis of PTEA was by reverse-phase HPLC–UV/Vis. In this method the samples were analyzed using liquid chromatography module Waters 2690 (Waters Corp., Milford, MA) equipped with Waters 996 photodiode array detector. An Xterra MS C18 column (125 Å, 5 µM, 3.9 mm x 150 mm, Waters Milford, MA) was used for detection 2-phenylthio ethanol. The flow rate was set to 0.7 mL/min. A gradient method was used, which started from 40% of A (methanol) and 60% of C (0.025 M tris(hydroxymethyl)aminomethane as buffer in Milli-Q water); pH was adjusted to 9 with 6 M HCl. The initial condition were held for

3 minutes, then changed to 65% A and 35% C for 14 minutes, then changed to 60% A and 40% for 5 minutes. The injection volume was 10 µL. The chromatogram was examined using Millenium for Windows 98 software. A wavelength of 250 nm was chosen to determine the concentration of 2-phenylthio ethanol.

3.2.4 Synthesis of bis(2-chloroethyl)phosphate, a degradation product of tris(2-chloroethyl)phosphate

Bis(2-chloroethyl)phosphate (BCEP), which is a degradation product of TCEP with thiophenolate, was synthesized and analyzed. The synthesis was performed by hydrolysis of 25 mM TCEP (0.271 g, 1.22 $x10^{-3}$ mol) with 0.100 M sodium hydroxide (0.192 g, 4.81 x 10^{-3} mol) at 50 ^{o}C and pH 12.8 in 50 mL water overnight. 50 mL reaction solution was transferred into a 125 mL separatory funnel that already contained 20 g of sodium chloride. Fifty milliliters of diethylether/acetonitrile (1:1) was added and the solution was acidified with 10 mL hydrochloric acid (6 M). After shaking for 5 min, the separatory funnel was left to settle. The organic phase was transferred into a new separatory funnel, which contained 50 mg potassium carbonate. The aqueous phase was extracted one more time with 50 mL diethylether/acetonitrile (1:1) and the second extract was combined with the first. The organic extracts were concentrated to 1 mL using the Turbovap and evaporated to dryness. 3.75 mL acetonitrile were added to the residue and again dried. The dry residue was then suspended in 7.5 mL acetonitrile. It was transferred and concentrated to 1 mL. The 1 mL extract was transferred to a 2 mL analytical vial and dried in a gentle stream of nitrogen and kept under a vacuum for 4 hours [80]. The actual yield was 0.097 g (4.35 $x10^{-4}$ mol). The percentage yield was 36%. Synthesis of BCEP was carried out two more times with a percentage yield of 41 and 36%, respectively.

3.2.5 Nuclear Magnetic Resonance (NMR) Spectroscopy for Conformation of BCEP

^{1}H NMR spectra were recorded on a Varian Inova 500 NMR spectrometer. *p*-Xylene was used as internal standard to determine the molecular weight of the product. Figure 3.1 and 3.2 show ^{1}H NMR spectrum of *p*-xylene and product, believed to be either BCEP or mono(2-chloroethyl)phosphate (MCEP). The use of *p*-xylene as internal standard allows distinguishing between BCEP and MCEP since the number of methylene protons on BCEP differs from that of MCEP. The molecular weight of the product was calculated by using two ratios: the ratio of the peak area to the number of protons in *p*-xylene, and the respective ratio of peak area to number of protons in BCEP, assuming that the product formed is BCEP. Chemical shift data for the protons was reported in parts per million relative to tetramethylsilane (TMS). ^{1}H NMR data of BCEP was as follows: (500 MHz, $CDCl_3$, δ): 5.398 (s, 1H, OH), 4.101(q, 2H, CH_2), 3.447 (t, 2H, CH_2).

Then a second calculation was executed following the same steps; only this time, assuming the product formed is MCEP. The two calculated molar weights of the product were then compared to the molar weights of the two compounds.

When the product was presumed to be MCEP, the calculated molar weight was 159% of the actual molar weight. Whereas when BCEP was assumed as the product, the resulting calculated molar weight was 104%. Therefore, the product of hydrolysis of TCEP at high pH is BCEP. The calculations of the respective molar weight are shown in the appendix for both assumptions.

3.2.6 Synthesis of bis(1,3-dichloropropyl)phosphate and bis(monochloropropyl)phosphate

Bis(1,3-chloroethyl)phosphate (BDCP) and bis(monochloropropyl)phosphate (BDCP) were synthesized and analyzed. The synthesis was performed by hydrolysis of (0.22 g, 0.5 10^{-3} mol) TDCP (0.16 g, 0.5 10^{-3} mol) TCPP in 2 mL of 2 M NaOH in water/methanol (20/80). The tube was closed, heated to 100 ^{o}C for 2 h, and allowed to cool. Sodium chloride (2.01 g) was added to the test tube. Two milliliters of diethylether/acetonitrile (1:1) was added and the sample was acidified with 1 mL hydrochloric acid (6 M). After shaking for 5 min, the separatory funnel was left to settle. The organic phase was transferred into a new tube, which contained 10 mg potassium carbonate. Afterwards, the extraction was repeated with 2 mL diethylether/ acetonitrile (1:1) and the second extract was combined with the first. The extracts were concentrated to 1 mL using the Turbovap. Afterwards, it was evaporated using the Turbovap. 750 microliter acetonitrile were added to the residue and again dried. The dry residue was then suspended in 1.5 mL acetonitrile. It was transferred and concentrated to 1 mL using the Turbovap. The 1 mL extraction was added to a 2 ml analytical vial and dried in a gentle stream of nitrogen and kept under a vacuum pump for 4 hours[80]. The actual yield was 0.0404 g (9.38 x10^{-5} mol) for BDCP and 0.041 g (1.25 x10^{-4} mol) for BCPP, respectively. The percentage yield was 25% for BDCP and 32% for BCPP. (Figure A10 and A11 show the NMR spectra of these isolated products).

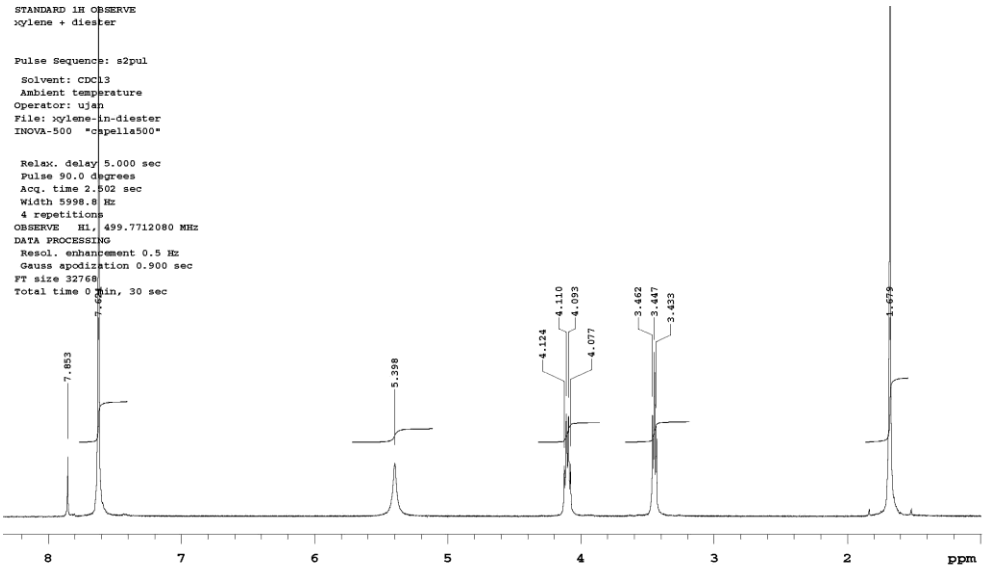


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Figure 3.1 ^{1}H NMR of *p*-xylene and bis(chloroethyl)phosphate

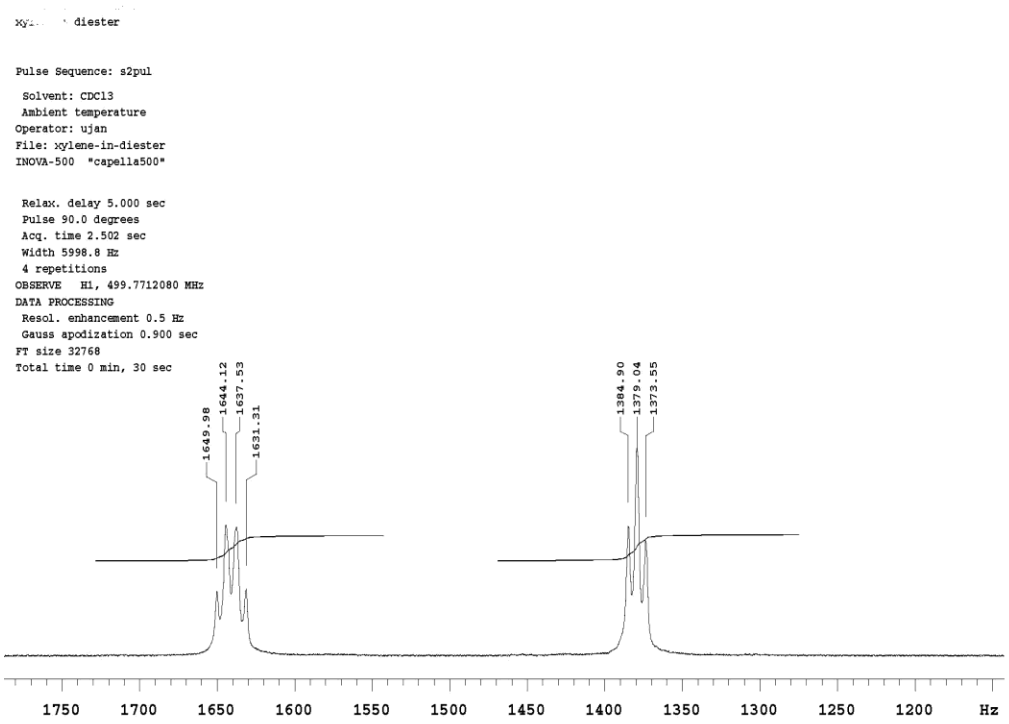


Figure 3.2 ^{1}H NMR of *p*-xylene and bis(chloroethyl)phosphate

Chapter 4

Results and Discussion

4.1 Kinetics of investigated OPFR

4.1.1 Hydrolysis

A time-course of the hydrolysis of TCEP at 50 ^{o}C and pH 9.20 is shown in Figure 4.1. The pH dependence for TCEP at 50 ^{o}C was determined (Figure 4.2). It has been reported that hydrolysis of organophosphorus triesters in general is dominated by a base-catalyzed mechanism. Therefore, as expected the hydrolysis rate of TCEP is increasing with increasing pH. The pseudo-first-order hydrolysis rate will depend on the base-catalyzed reaction assuming the contribution of the neutral reaction can always be neglected. From the hydrolysis experiment of TCEP at 50 ^{o}C and pH 8, a half-life at 25 ^{o}C of approximately 2 years was estimated using base-catalyzed reactions rate and assuming an activation energy of 50 kJ/mol. That is equivalent to an increase in reaction rate by a factor of 2 for an increase in temperature of 10 ^{o}C. Therefore, it can be concluded that hydrolysis is a very slow pathway of degradation of TCEP in the environment.

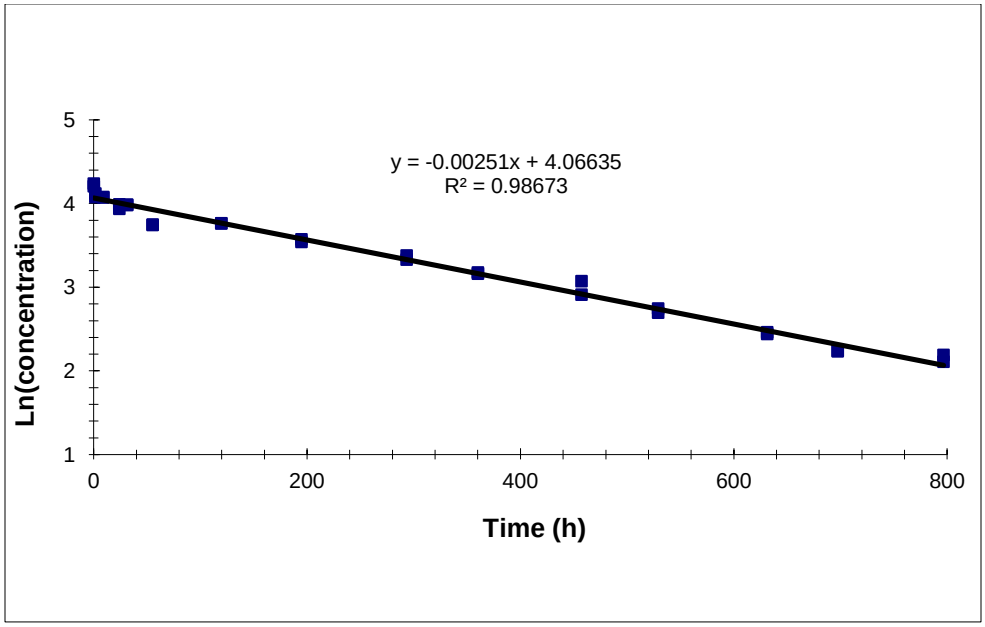


Figure 4.1 Hydrolysis of TCEP at pH 9.20 and 50 ^{o}C

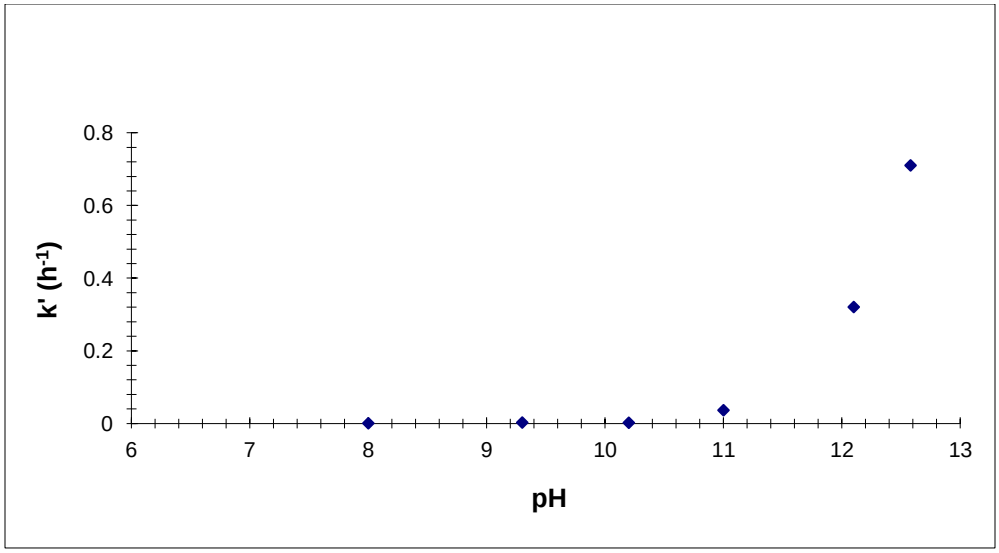


Figure 4.2 Hydrolysis of TCEP at different pH and 50 ^{o}C

Table 4.4 Hydrolysis rate constant of TCEP at different pH and 50 °C

pH	k
8.0	8×10^{-8} s^{-1}
9.3	6.4×10^{-7} s^{-1}
11.0	1.0×10^{-5} s^{-1}
12.1	8.9×10^{-5} s^{-1}
12.6	2.0×10^{-4} s^{-1}

4.1.2. Reaction of THAP with reduced sulfur species (bisulfide, polysulfides, and thiophenolate)

Since the hydrolysis is very slow, reactions of these OPFRs with reduced sulfur species (bisulfide, polysulfides, and thiophenolate) might be relevant in the environment. The semilogarithmic plots for time course of these investigated OPFRs in the presence of excess sulfide species over 2-3 half-lives is indicative of first-order dependence in these OPFRs (Figure 4.3). The slope in such semilogarithmic plot yields a pseudo-first-order reaction rate constant (k_{obs}). The observed first-order rate constant can be expressed in the following manner for the reaction with bisulfide (neglecting the very slow hydrolysis):

$$k_{obs} \approx k''_{HS^-} \quad HS^- \quad + \quad k''_{H_2S} \quad H_2S \qquad (9)$$

The term k"$_{H_2S}$ [H_2S] is much smaller than k" [ ] at pH greater than 7, since most of the species are in form and since is a better nucleophile than H_2S. Therefore, at pH > 7 the expression can be simplified to

$$k_{obs} \approx k''_{HS^-} \quad HS^- \qquad (10)$$

For the reaction with polysulfides expression for the observed rate constant is

$$k_{obs} \approx k''_{HS^-}\ [HS^-] + k''_{H_2S}\ [H_2S] + k''_{HS_n^-}\ [HS_n^-] + k_{S_n^{2-}}\ [S_n^{2-}] \quad (11)$$

By choosing a pH greater than 8.5 and assuming that the solutions are saturated with elemental sulfur. $[S_n^{2-}]$ will be present at much higher concentration than (HS^-). The expression for the observed rate constant can be simplified

$$k_{obs} \approx k''_{S_n^{2-}}\ [S_n^{2-}] \quad (12)$$

The expression for the observed rate constant for the reaction of OPFRs in the presence thiophenolate is given by:

$$k_{obs} \approx k''_{PhS^-}\ [PhS^-] + k''_{PhSH}\ [PhSH] \quad (13)$$

For experiment at pH >7 most of the thiophenol will be in forms. Therefore, for experiment at pH >7, the observed rate constant can be expressed as:

$$k \approx k''_{PhS^-}\ [PhS^-] \quad (14)$$

4.1.3 Reaction of THAP with polysulfides

The reactions of polysulfides with TCEP, TDCP, and TCPP under anoxic conditions were investigated. A representative time-course profile for the reaction TCEP with 3.81 mM polysulfide at 25 ^{o}C and pH 9.29 is shown (Figure 4.3). The second order rate constant for the reaction of TCEP with polysulfides was determined by plotting k_{obs} vs. $[S_n^{2-}]$ (Figure 4.4). The

determined second-order rate constant for the reaction of TCEP with polysulfides at 25 ^{o}C is 5.0 (± 1.4) 10^{-4} $M^{-1}s^{-1}$. Second-order and relative rate constants of TCEP, TDCP, and TCPP with polysulfides are reported in Table 4.1. The table shows that TCEP reacts the fastest with polysulfides while TCPP reacts the slowest with polysulfides.

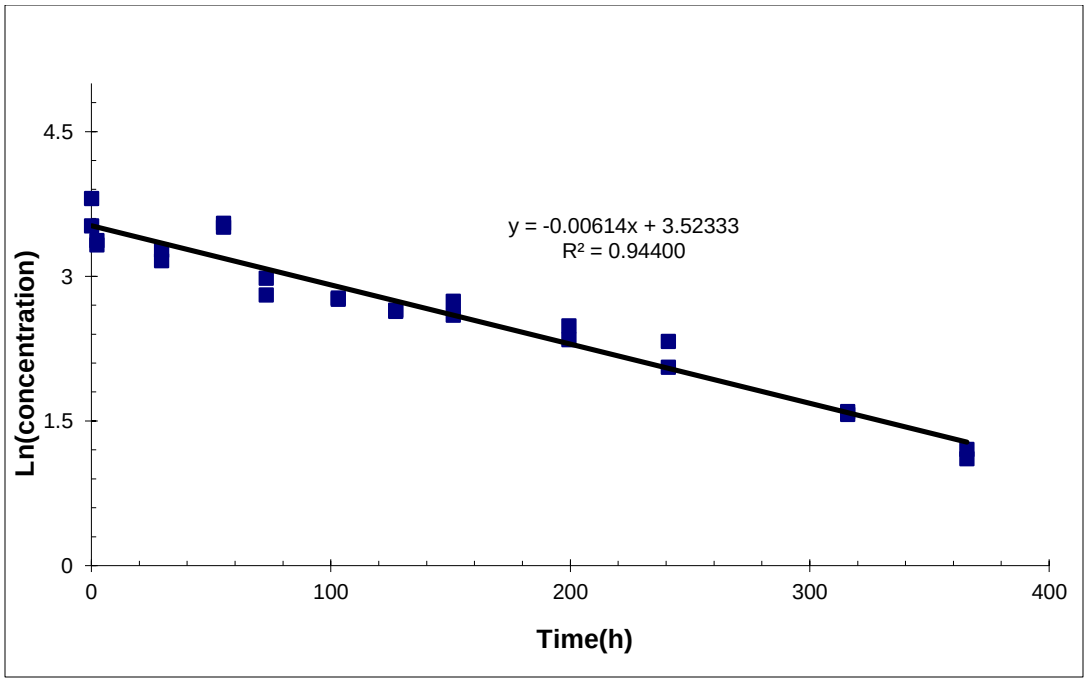


Figure 4.3 Degradation of 50 µM TCEP with 3.81 mM polysulfides at 25 ^{o}C and pH 9.29

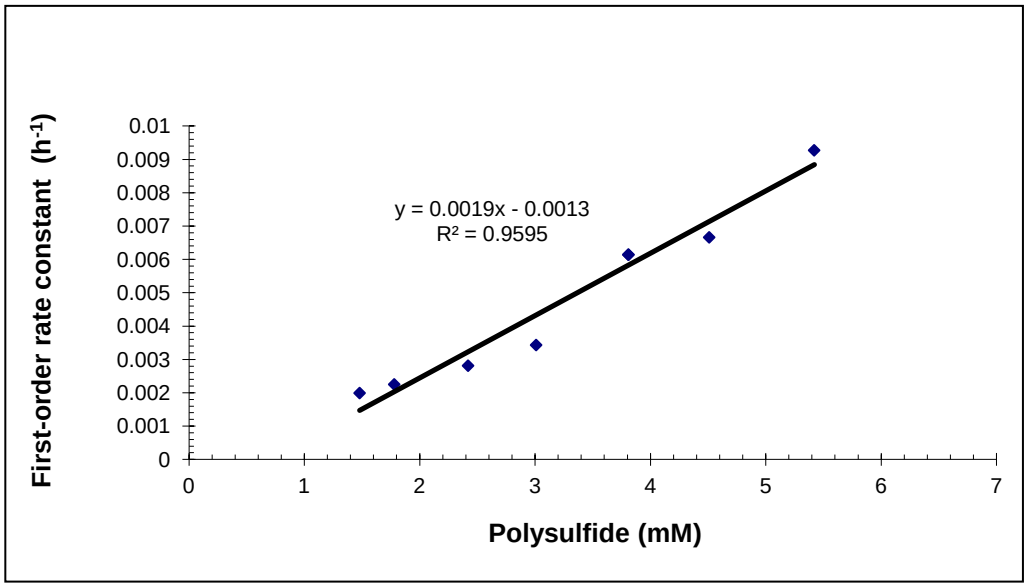


Figure 4.4 First-order rate constant of TCEP vs polysulfides concentration at 25 ^{o}C

Table 4.1 Second-order and relative rate constants of TCEP, TDCP, and TCPP with polysulfides at 25 ^{o}C, thiophenolate, and bisulfide at 50 ^{o}C.

Flame retardant	TCEP	TDCP	TCPP
$k''_{S_n^{2-}}$ (10^{-4} $M^{-1}s^{-1}$)[a] (25 ^{o}C)	5.0 (± 1.4)	3.3 (± 8.3)	0.61 (± 0.08)[a]
k''_{PhS^-} (10^{-4} $M^{-1}s^{-1}$)[a] (50 ^{o}C)	34 (± 1.8)	6.4 (± 1.4)	1.8 (± 0.2)[a]
k''_{HS^-} (10^{-4} $M^{-1}s^{-1}$) (50 ^{o}C)	0.89	0.45	0.045[b]
Relative rate constant for	8	5	1
Relative rate constant for	19	4	1
Relative rate constant for	20	10	1

[a] Stated uncertainties represent 95% confidence limits.

[b] No confidence interval is reported since the experiment was only performed at two different concentrations for TCEP, and once for TDPP and TCPP

4.1.4 Reaction of THAP with thiophenolate

Thiophenolate is used as a model for reduced sulfur species because it is expected to react similar as bisulfide and polysulfides. However, thiophenolate has the advantage that the products of substitution reactions will be neutral and therefore they can be more easily isolated and analyzed. A representative time-course profile for the reaction of TCEP with 7.09 mM thiophenolate at 50 ^{o}C and pH 9.29 are shown (Figure 4.5). Five experiments with varying thiophenolate concentrations were performed and the determined second-order rate constant is 34 (± 1.8) $\times$ 10^{-2} $M^{-1}s^{-1}$ (Figure 4.6). Second-order and relative rate constants of TCEP, TDCP, and TCPP with thiophenolate at 50 ^{o}C are summarized in table 4.1. It can be seen that TCEP is the fastest reacting compound with thiophenolate, while TCPP is the slowest reacting compound with thiophenolate at 50 ^{o}C. The relative rate constants of those organophosphorus flame retardants with thiophenolate are similar to those with polysulfides.

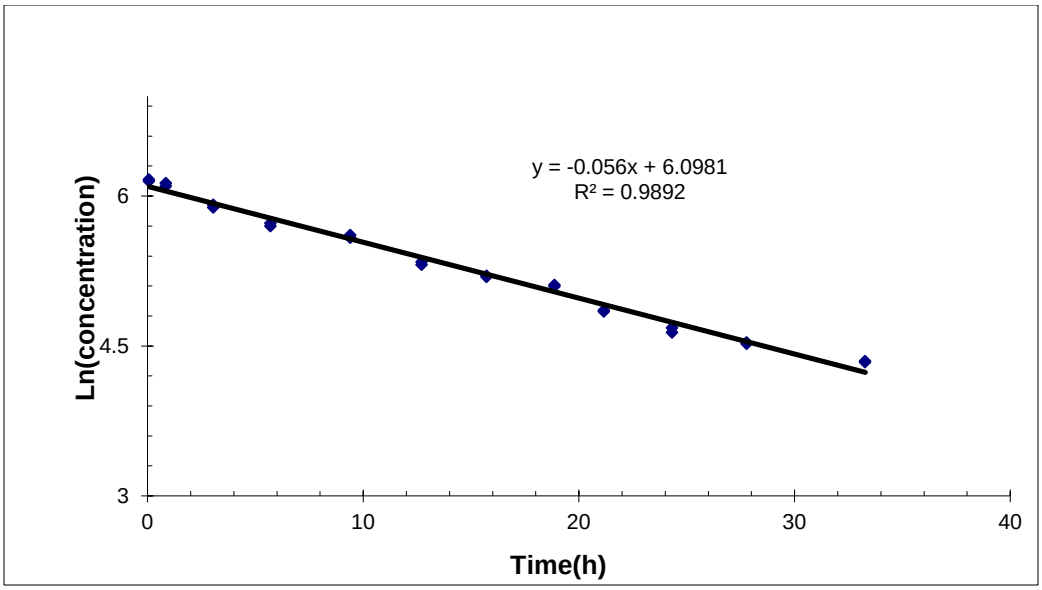


Figure 4.5 Degradation of 50 µM TCEP with 7.09 mM thiophenolate at pH 9.04 and 50 °C

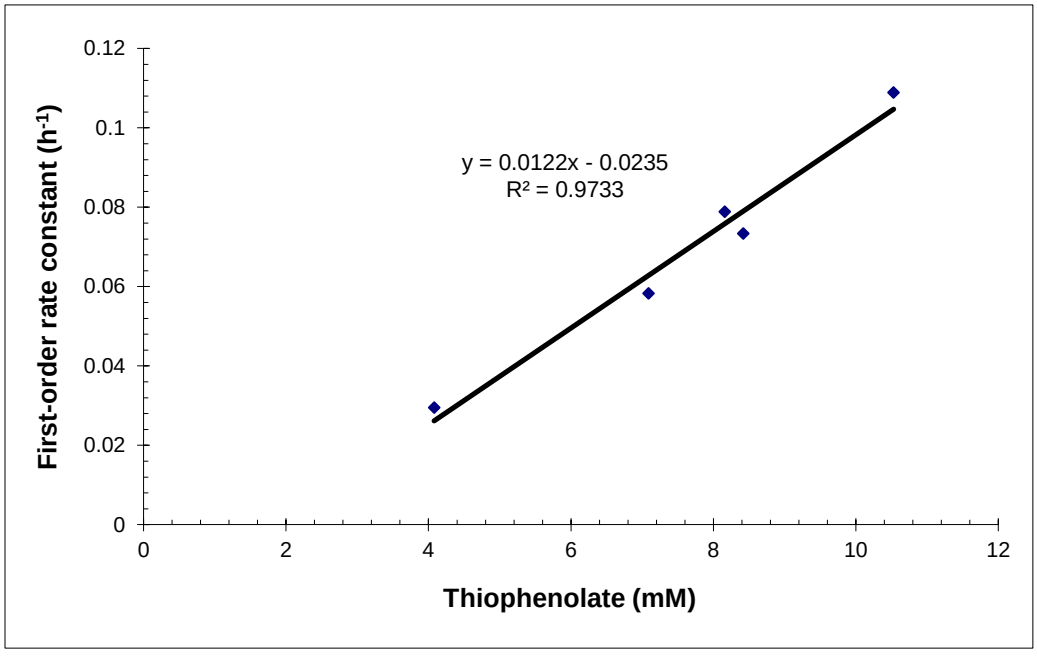


Figure 4.6 First-order rate constant TCEP vs thiophenolate concentration at 50 °C

4.1.5 Reaction of THAP with bisulfide

Table 4.1 shows the second-order and relative rate constants of those three OPFRs with bisulfide at 50 ^{o}C. Those experiments were performed only once because of the extended time they took to reach 2 to 3 half-lives. The same relative reaction pattern for the reaction of bisulfide with those three OPFRs as for polysulfides and thiophenolate was observed. TCEP is the fastest reacting compound with bisulfide, while TCPP is the slowest reacting compound with bisulfide at 50 ^{o}C. This is in good agreement with the difference in reactivity that has been reported for nucleophilic substitution reactions at a carbon center [81-83].

4.1.6 Discussion of the relative rate constants in light of reactivity

Steric, electronic, and "statistical" effects are contributing factors in the reaction of TCEP, TDCP and TCPP with thiophenolate, polysulfides, and bisulfide. All three factors have to be considered when attempting to explain the relative degradation rate constant of the structurally related OPFR with the three reduced sulfur species.

From previous studies investigating the reaction of organophosphorus insecticides with reduced sulfur species, it is known that reduced sulfur species attack the insecticides at the α-carbon of ethoxy and methoxy groups and not at the phosphorus atom [83, 84]. Such an attack at the α-carbon seems also likely in TCEP, TDCP, and TCPP. However, TCEP, TDCP, TCPP also possess chlorine atoms and it seems reasonable to also consider a nucleophilic substitution of a chloride by a reduced sulfur species. When considering steric effects for nucleophilic attack at the α-carbon (carbon that is adjacent to the oxygen atom) (Scheme 4.1, pathway II) and we compare TCEP and TCPP, the additional methyl group in TCPP will enhance steric hindrance therefore leading to a slower rate constant for the nucleophilic substitution at the α-carbon (Table

4.2). On the one hand, when comparing TCEP and TDCP, the additional chloromethyl group increases steric hindrance; the expected rate constant of TDCP would be slower than of TCEP.

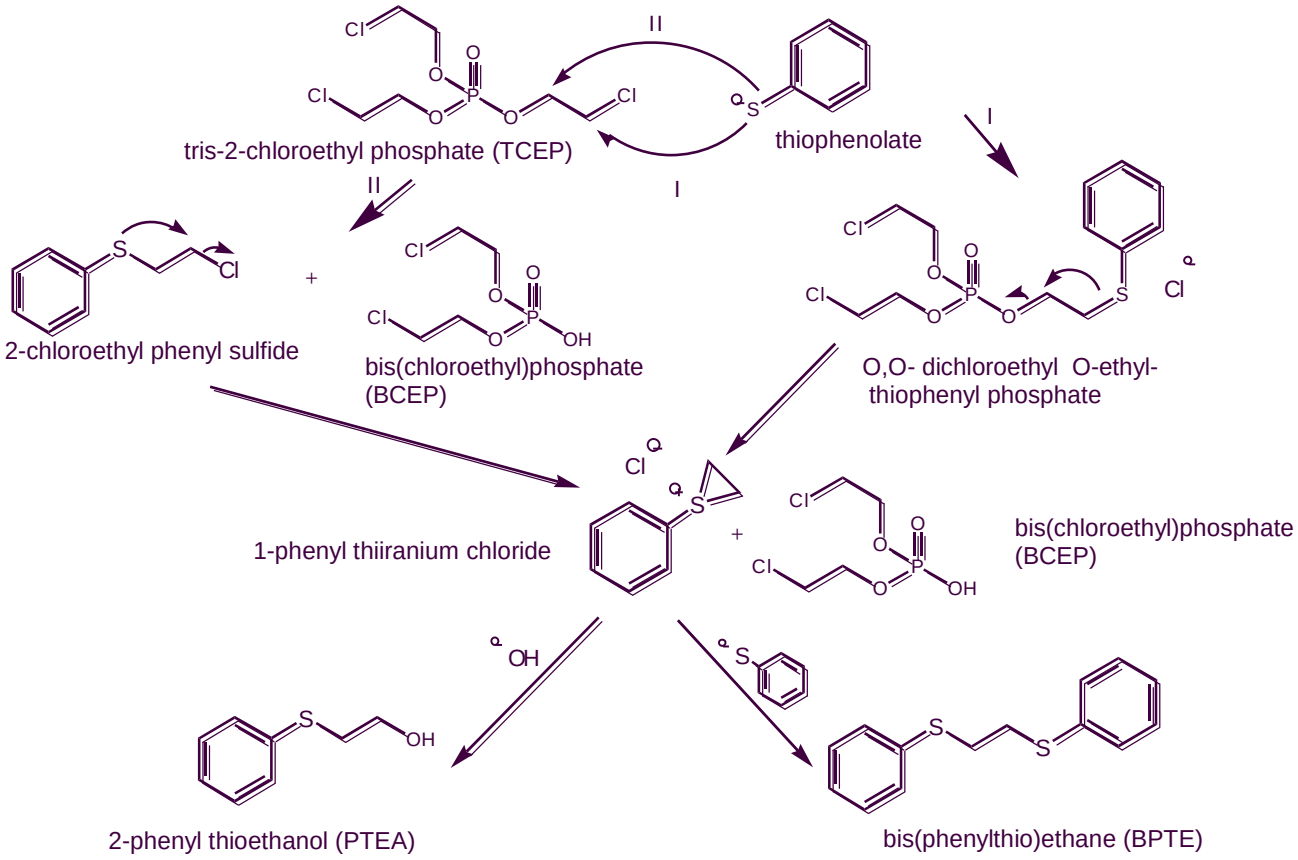
When considering electronic effects for nucleophilic attack at the α–carbon (carbon that is adjacent to the oxygen atom) and we compare TCEP and TCPP, the additional methyl group in TCPP is an electron donating group increasing the negative charge of the reaction center and therefore leading to a slower rate constant for nucleophilic substitution. However, when comparing TCEP and TDCP, the additional chloromethyl group is an electron-withdrawing group; the expected rate constant of TCPP would be faster than TCEP. Therefore, it can be concluded that a combination of the two effects can lead to the observed relative rate constant with TCEP being the fastest reacting compound, followed by TDCP and TCPP the slowest reacting compound in a nucleophilic substitution.

When considering steric effects for nucleophilic attack at the β-carbon (carbon atom that is adjacent to the chlorine atom (Scheme 4.1, pathway I) and we compare TCEP and TCPP, the additional methyl group in TCPP is at the adjacent carbon to the nucleophilic center and will not enhance steric hindrance significantly therefore leading to a slghtly slower rate constant for nucleophilic substitution at the β-carbon (Table 4.3). However, when comparing TCEP and TDCP, the additional chloromethyl group at carbon to the adjacent nucleophilic center will not increase steric hindrance in TDCP that much, the expected rate of relative rate constant of TCPP would only be a little bit slower than TCEP.

When considering electronic effects for nucleophilic attack at the β-carbon and we compare TCEP and TCPP, the additional methyl group in TCPP is an electron donating group that is adjacent to the nucleophilic center and therefore will increase the negative charge of the reaction center less than in the attack of the α-carbon attack therefore leading to only slightly

slower rate constant for nucleophilic substitution of TCPP versus TCEP. However, when comparing TCEP and TDCP, the additional chloromethyl group is electron-withdrawing group at the adjacent nucleophilic center will make the relative rate constant slighly faster. We should also note that a "statistical effect" has to be taken in consideration when comparing TDCP to TCPP. Both chlorines of the dichloropropyl side chains in TDCP can be replaced leading to the same product. Assuming that steric effects are more important than electronic effects, when combining steric and electronic effects for nucleophilic attack at the reaction center could also explain why TCEP reacts the fastest. From this discussion it can be concluded that both pathways (Scheme 4.1, pathway I and II) potentially explain the relative reaction of the three investigated OPFR with reduced sulfur species that is reported here 2-chlorophenylthio ethane is the intermediate of TCEP reacting with thiophenolate. It undergoes the same type of reaction as mustard gas. A systematic investigation into the mechanism of nucleophilic substitution of mustard gas analogs was completed by a research group at the University of Alabama in [54] Huntsville [85]. Using deuterated mustard gas analogs such as $C_6H_5SCH_2CD_2Cl$, these researchers detected complete deuterium scrambling in the presence of a series of nucleophiles for almost all types of organic and aqueous solvent mixtures. Therefore, it can be concluded that the nucleophilic substitution of mustard or its analogs proceeds exclusively via S_n1 mechanism. The sulfur in these molecules is located at a position to participate internally in the cleavage of the C-Cl bond by forming a transient cyclic ethylenesulfonium ion intermediate. Any external nucleophile Y (including water or another molecule) cannot compete with the internal sulfur. Only one exception is reported in pure dimethyl sulfoxide in the presence of thiophenolate anion, there was no scrambling of the isotopes and the substitution was, therefore S_n2 [85].

It is important to note that the observed rate of S_n1 substitution reaction can increase in the presence of Y. This is not because of a mechanistic change but because of the competition between Y and the chloride ion as reflected in the relative magnitude of ($k_{-1}[Cl^-]$) and ($k_Y[Y]$) , k_{-1} is the reverse rate of the reaction. Since the magnitude of $k_Y[Y]$ increases with both the strength of the nucleophile and the concentration of Y, the observed rate enhancement by a nucleophile can be significant and this enhancement may even be proportional.



Scheme 4.1 Possible mechanism of the reaction of TCEP with thiophenolate.

Table 4.2 Discussion of the relative rate constant in light of relative reactivity at the α-carbon

Flame retardant	Steric effect	Electronic effect
TCEP	reference	reference
TCPP	slow	slower
TDCP	slower	faster

Table 4.3 Discussion of the relative rate constant in light of relative reactivity at the β-carbon

Flame retardant	Steric effect	Electronic effect
TCEP	reference	reference
TCPP	slightly slower	slightly slower
TDCP	slightly slower	slightly faster

4.2 Products of the reaction of THAP with reduced sulfur species

4.2.1 Products of hydrolysis

Figure 4.7 shows the time-course for hydrolysis of TCEP at pH 10.79 and 50 ^{o}C. From the figure, it can be seen that the TCEP concentration decreases with time while the concentration of BCEP increases with time. The figure also shows that TCEP is converted in a 1:1 ratio to BCEP. The mass balance is stable over the duration of the experiment indicating that BCEP is the sole and stable product of the hydrolysis of TCEP.

Figure 4.8 shows the time-course for the hydrolysis of TCPP at pH 13.00 and 50 ^{o}C. From the figure, it can be seen that TCPP concentration decreases with time while the concentration of BCPP (bis(chloropropyl)phosphate) increases with time. The mass balance is increasing over the duration of the experiment. Such a behavior could be explained with the fact that the BCPP used to prepare the standards was not pure. The BCPP was synthesis from hydrolysis of TCPP with NaOH followed by extraction with acetonitrile and diethyl ether. The presence of impurities is supported by HRMS of BCPP (see Appendix A3). The HRMS of synthesized BCPP resulted in additonal molecular masses beside the one expected for BCPP. The impurities in BCPP may be organic (e.g., TCPP that was not hydrolyzed). They may also be inorganic (e.g., salts extracted into acetonitrile/diethyl ether phase) during liquid-liquid extraction).

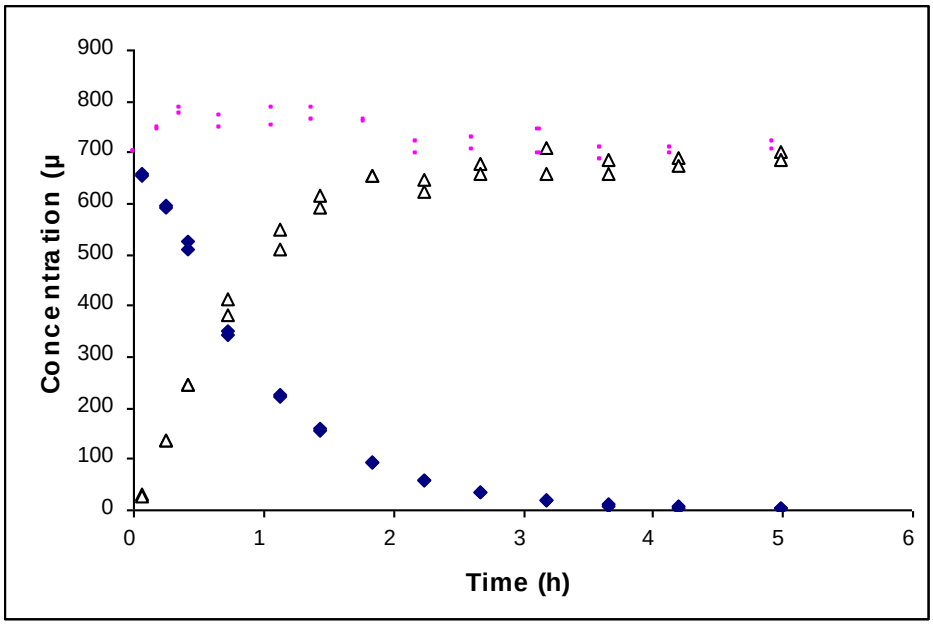


Figure 4.7 Hydrolysis of TCEP (♦) at pH 10.00 and 50 °C including degradation product BCEP (Δ) and mass balance (■)

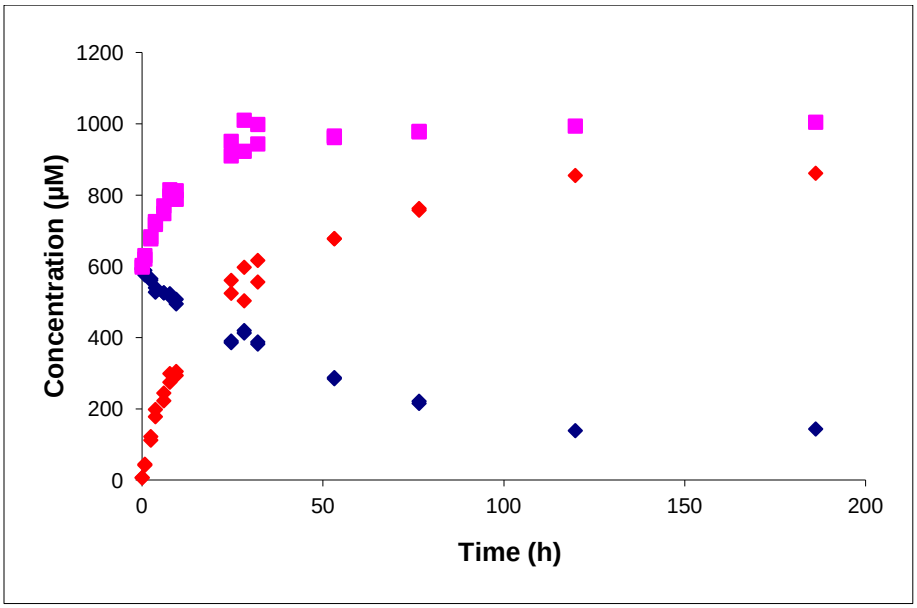


Figure 4.8 Hydrolysis of 500 µM TCPP (♦) at pH 13.00 and 50 °C including degradation product BCPP (■) and mass balance (■)

4.2.2 Products of the reaction of THAP with thiophenolate Figure 4.9 illustrates the reaction of TCEP with 1.15 mM thiophenolate at pH 9.18 and 50 ^{o}C. The figure shows that three products are formed. BCEP is formed at the highest concentration. BPTE and PTEA are also formed and their concentrations are approximately half of the concentration of BCEP. 2-chloroethyl phenyl sulfide a potential intermediate in this reaction was not observed. The mass balance remains high throughout the time-course indicating that most of the degradation products for this reaction were analyzed and that the products are stable over the course of the experiment. In order to elucidate the stability of BCEP in thiophenolate solution an experiment of BCEP with thiophenolate was performed. The degradation of BCEP with thiophenolate at 50 ^{o}C and pH 9.25 is illustrated in Figure 4.10. The concentration of BCEP in Figure 4.10 is higher than the one in Figure 4.9. The reaction time in Figure 4.10 is longer than the one in Figure 4.9.

Figure 4.11 illustrates the reaction of TCPP with 18.2 mM thiophenolate at pH 9.35 and 50 ^{o}C. The figure shows that two products are formed. BCPP is formed at the highest concentration. BPTiP (bisphen is also formed at the lowest concentration. 2-chloropropyl phenyl sulfide a potential intermediate in this reaction was not observed. The mass balance slightly increases initially and then slightly decreases. Figure 4.12 illustrates the reaction of BCPP with 18.2 mM thiophenolate at pH 9.35 and 50 ^{o}C. The figure shows the reaction is increasing at the beginning and remains constant at the end.

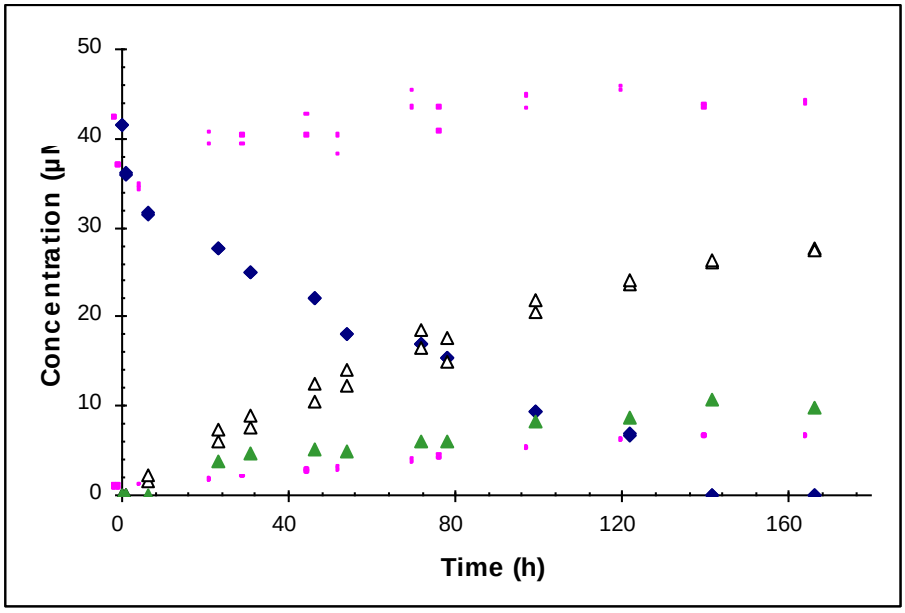


Figure 4.9 Degradation of TCEP (♦) 1.15 mM thiophenolate at pH 9.18 and 50 °C including BCEP (Δ), BPTE (▲), PTEA (●) as products and the mass balance (■).

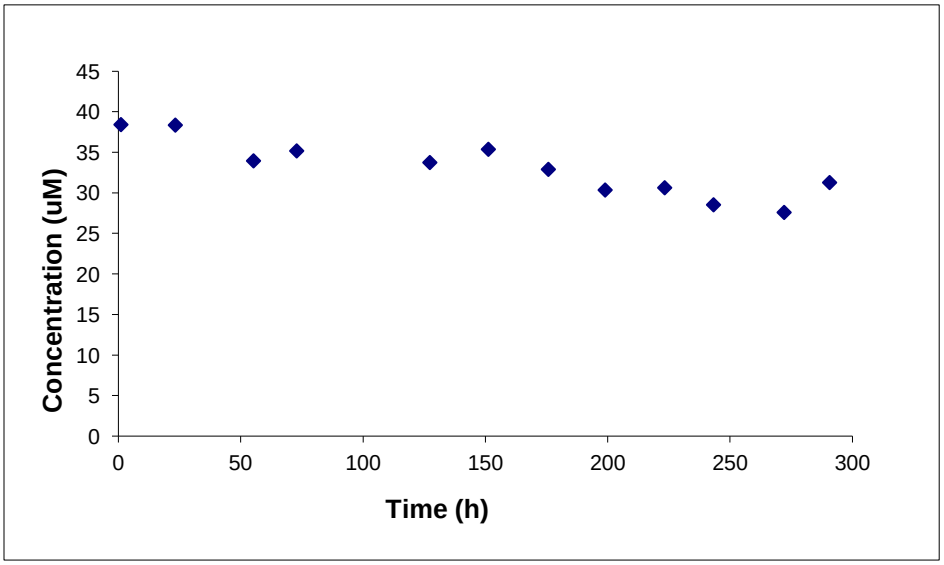


Figure 4.10 Degradation of 30 µM BCEP with 2.37 mM thiophenolate at 50 °C and pH 9.25

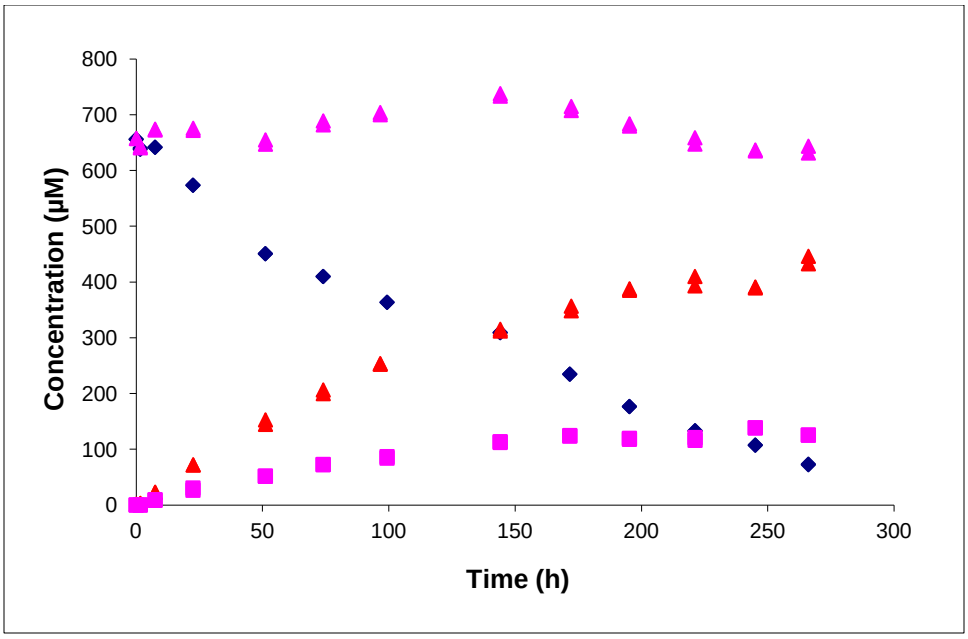


Figure 4.11 Degradation of 500 µM TCPP (♦) with 18.20 mM thiophenolate at pH 9.35 and 50 °C including BCPP (♦), BPTiP (■) as products and mass balance (▲)

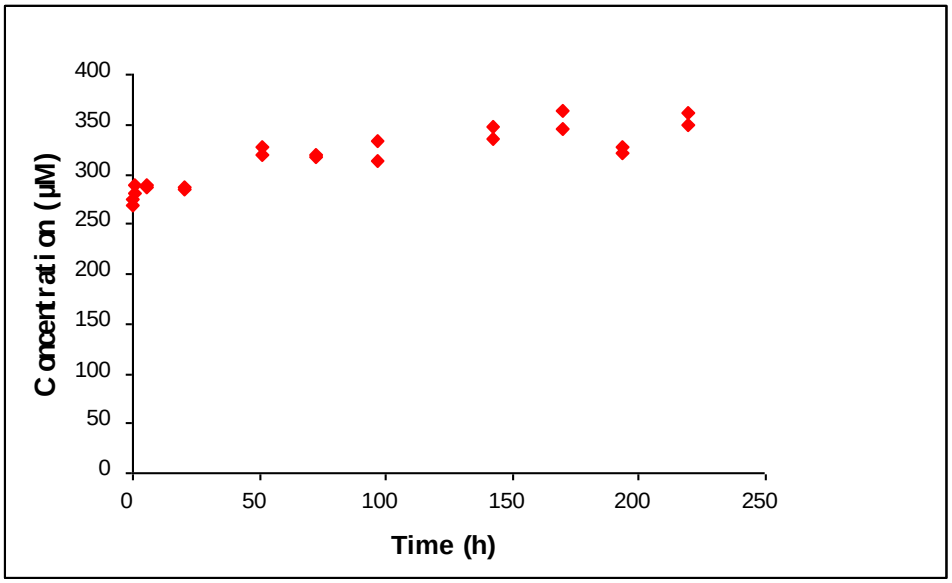


Figure 4.12 Degradation of 500 µM BCPP with 18.20 mM thiophenolate at pH 9.35

4.2.3 Products of the reaction of THAP with bisulfide

Figure 4.13 shows the time-course of the reaction of TCEP with 43.7 mM bisulfide at 50 ^{o}C and pH 9.70. BCEP is again detected to be the main product of this reaction. However, the mass balance is declining with time indicating that either not all products are detected or that the product formed is not stable under the reaction conditions. One possible reason that no additional products were observed is the possibility that they might be charged or they might be thiols. Charged products and thiols are difficult to extract and analyze. Figure 4.13 also includes fitted curves obtained by simultaneously fitting the data for TCEP and BCEP using Scientist. The following model was used to fit the data (Scheme 4.2). Experiments starting with BCEP were also conducted in order to determine the stability of BCEP in bisulfide solutions. Two experiments of BCEP with 63.1 mM [HS^-] and 71.7 mM [HS^-] were performed, respectively. The first-order rate constants for the reaction of BCEP with 63.1 and 71.7 mM were determined to be 0.0046 and 0.0051 h^{-1}, respectively. This corresponds to a second-order reaction rate constant of the reaction of BCEP with [HS^-] at 50 ^{o}C of 0.073 and 0.071 $M^{-1}h^{-1}$, respectively. In comparison, the second-order rate constant for the reaction of BCEP with bisulfide that was derived by fitting the data in Figure 4.13 is 0.12 $M^{-1}h^{-1}$. One can see that the second-order rate constant for the reaction of BCEP with bisulfide obtained by fitting the TCEP and BCEP concentrations in Figure 4.13 is almost 2 times larger than the rate constant directly determined in the experiments starting with BCEP. Such a discrepancy might be explained with the existence of a competing reaction between TCEP and [HS^-] that does not lead to the formation of BCEP. This competing reaction would have to contribute about 25% to the overall degradation of TCEP under the chosen conditions to explain the observed discrepancy in the rate

constants. Nevertheless, we can conclude that the reaction of BCEP with [HS^-] is one reason why the mass balance in Figure 4.13 is decreasing with time, in addition there might be an additional reaction product formed that was not identified.

Scheme 4.2

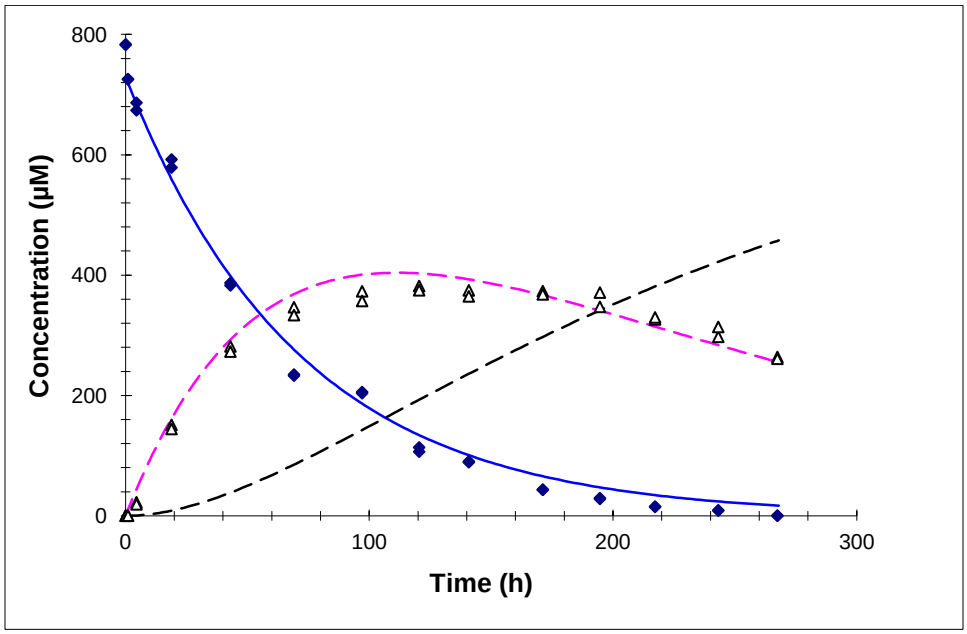


Figure 4.13 Degradation of TCEP(♦) with 43.7 mM bisulfide at pH 9.18 and 50 °C including degradation product BCEP (Δ). **The lines represent model fits; the solid line represents the formation of an unknown compound (scheme 4.2)**

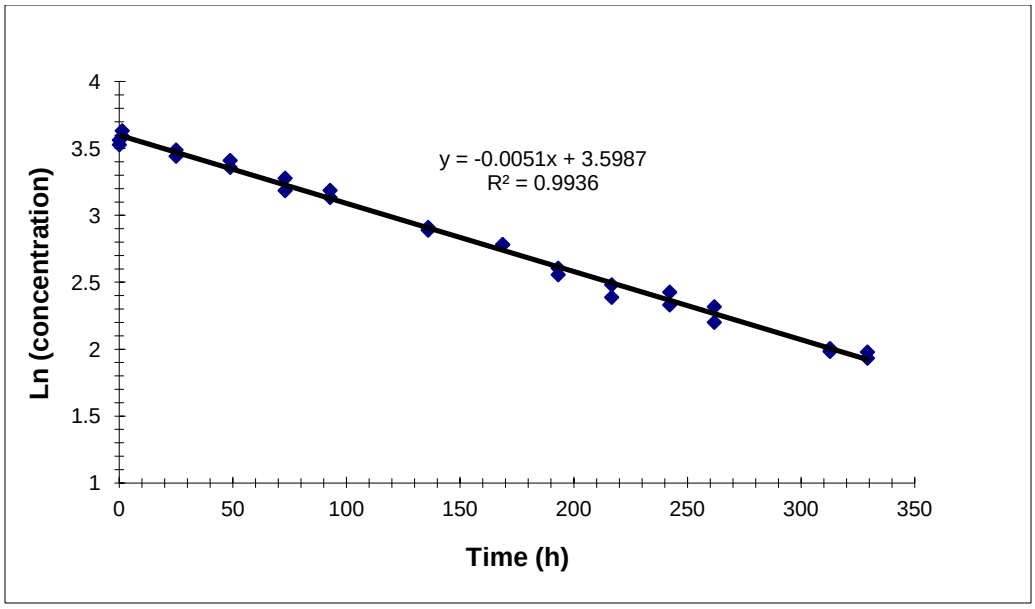


Figure 4.14 Degradation of 30 µM BCEP with 71.7 mM bisulfide at 50 °C and pH 9.84

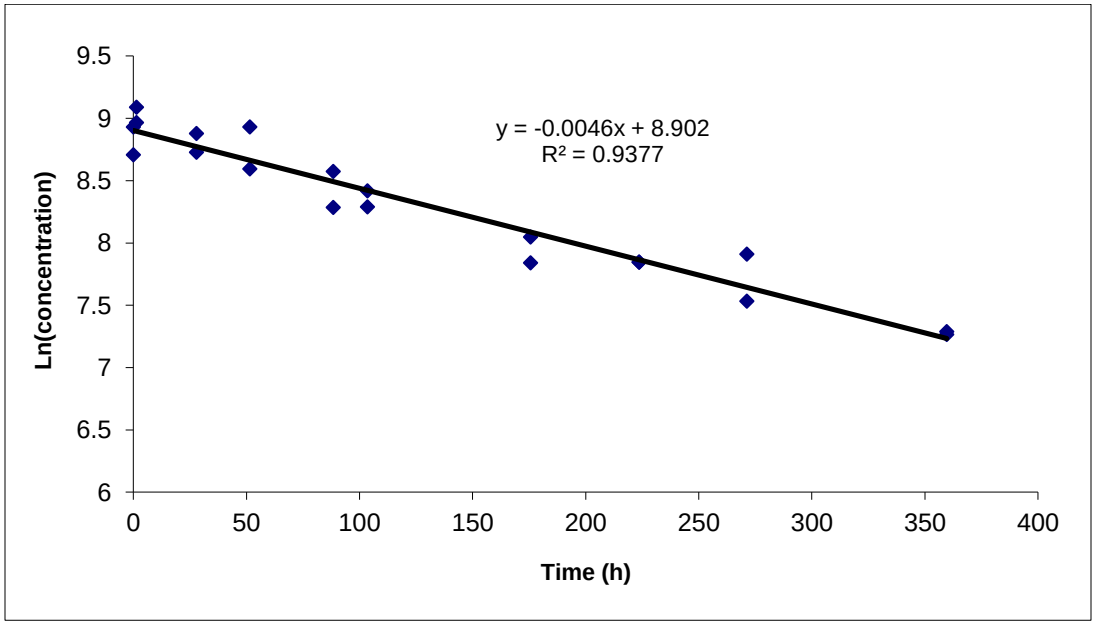


Figure 4.15 Degradation of 30 µM BCEP with 63.1 mM bisulfide at 50 °C and pH 9.75

4.2.4 Products formed in the reaction of THAP with polysulfides

Similar findings in regards to product formation were made with polysulfide as the nucleophile than with bisulfide. Figure 4.16 shows a time-course for the reaction of TCEP with 5.4 mM polysulfides at pH 9.18 and 50 ^{o}C. It can be seen that the TCEP concentration is decreasing while the BCEP concentration increases. It can also be seen that TCEP is almost converted in a 1:1 ratio to BCEP and therefore the mass balance is nearly constant. In order to elucidate the stability of BCEP in polysulfide solution an experiment of BCEP with S_n^{2-} was performed. A time-course for the reaction of BCEP with 8.05 mM polysulfides at pH 9.27 and 50 ^{o}C was measured. The first-order rate constant for the reaction of BCEP with 8.05 mM S_n^{2-} is 0.0023 h^{-1} (see Figure 4.17). This corresponds to a second order reaction rate constant of the reaction of BCEP with S_n^{2-} of 0.28 $M^{-1}h^{-1}$ at 25 ^{o}C. The first-order rate constant obtained by fitting the data in Figure 4.16 is 0.11 ± 0.09 $M^{-1}h^{-1}$. Thus, the second-order rate constant for the reaction of BCEP with polysulfide that was derived by fitting the data in Figure 4.16 is in the same order of magnitude than the rate constant determined by the direct experiment of BCEP with polysulfide. This is suggesting that all the major reactions in the reaction of TCEP with polysulfide are accounted for.

Figure 4.18 shows the time-course of TCPP with 35.95 mM polysulfides at pH 9.46 and 50 ^{o}C. From the figure, it can be seen that the TCPP concentration decreases with time while the concentration of BCPP increases with time. The mass balance climbs up to 120%. This might be potentially due to impurities in the synthesized BCPP. Figure 4.19 illustrate that BCPP is not stable in polysulfide solution. Its concentration goes up and down due the impurity in the synthesized BCPP.

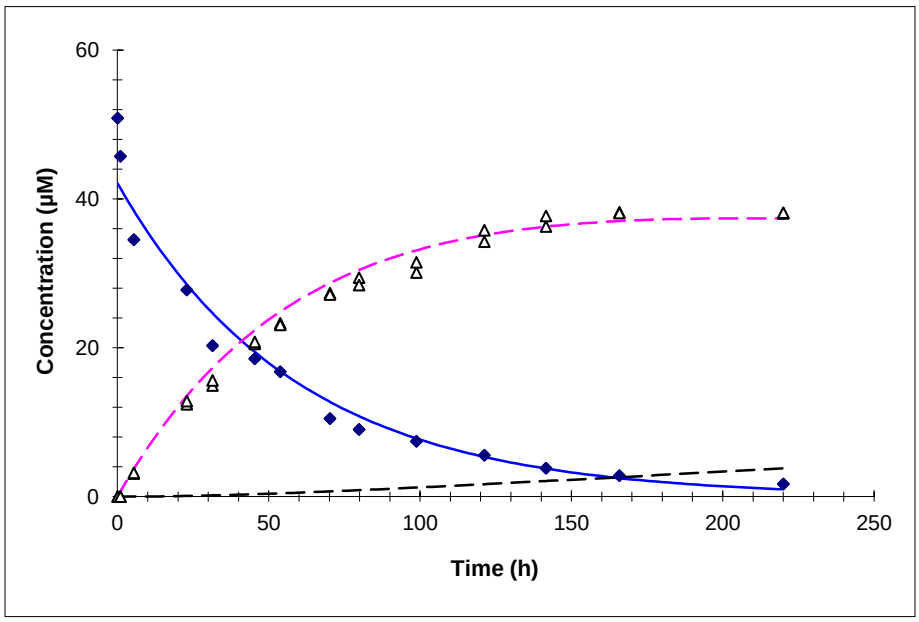


Figure 4.16 Degradation of TCEP (♦) with 5.44 mM polysulfides at pH 9.15 and 25 °C including degradation product BCEP (Δ). The lines represent model fits; the dashed line represents the formation of an unknown compound (scheme 4.2)

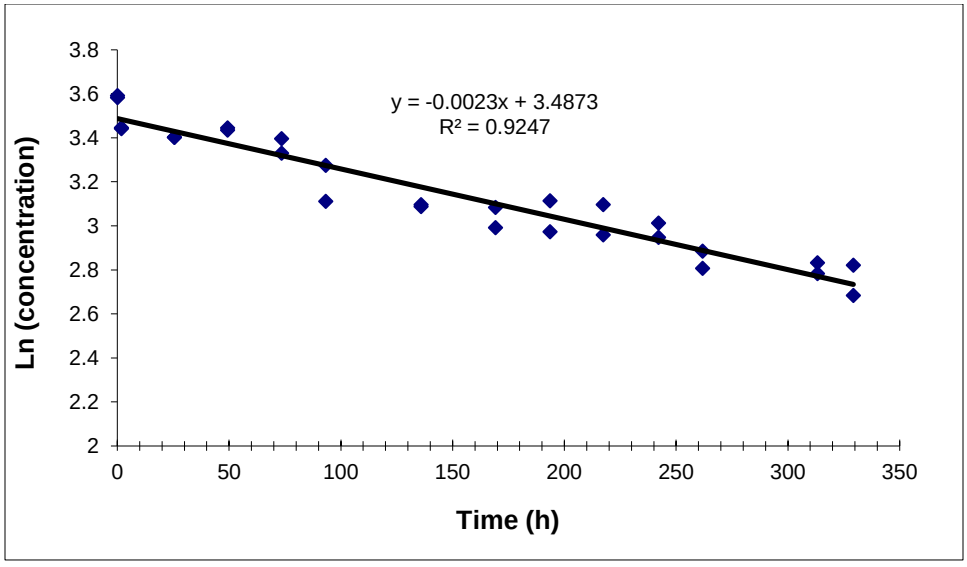


Figure 4.17 Degradation of 30 μM BCEP with 8.05 mM polysulfides at 25 °C and pH 9.27

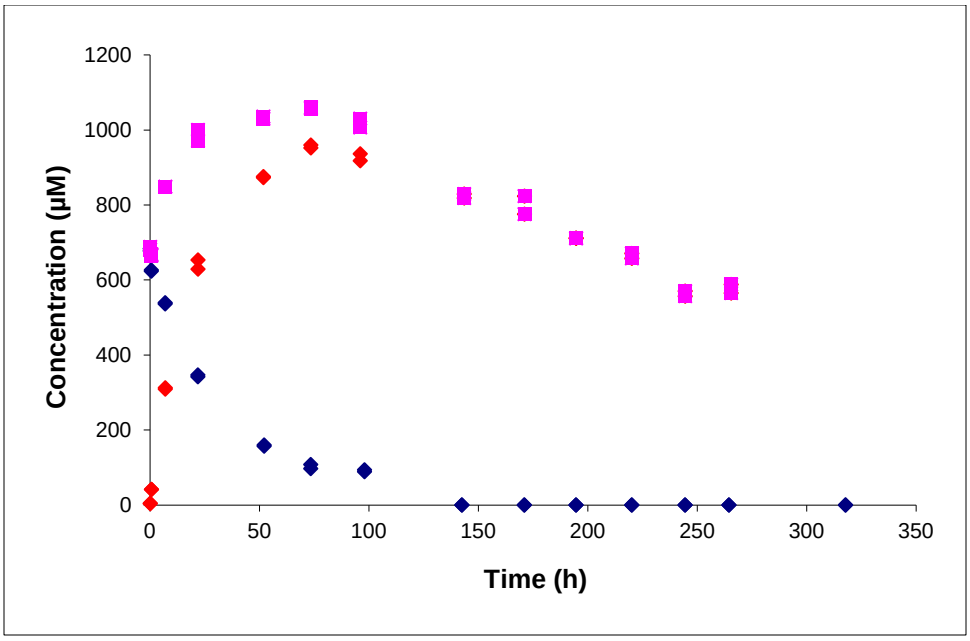


Figure 4.18 Degradation of 500 µM TCPP (♦) with 36.0 mM polysulfides at pH 9.46 and 50°C including BCPP (♦) as product and mass balance (■)

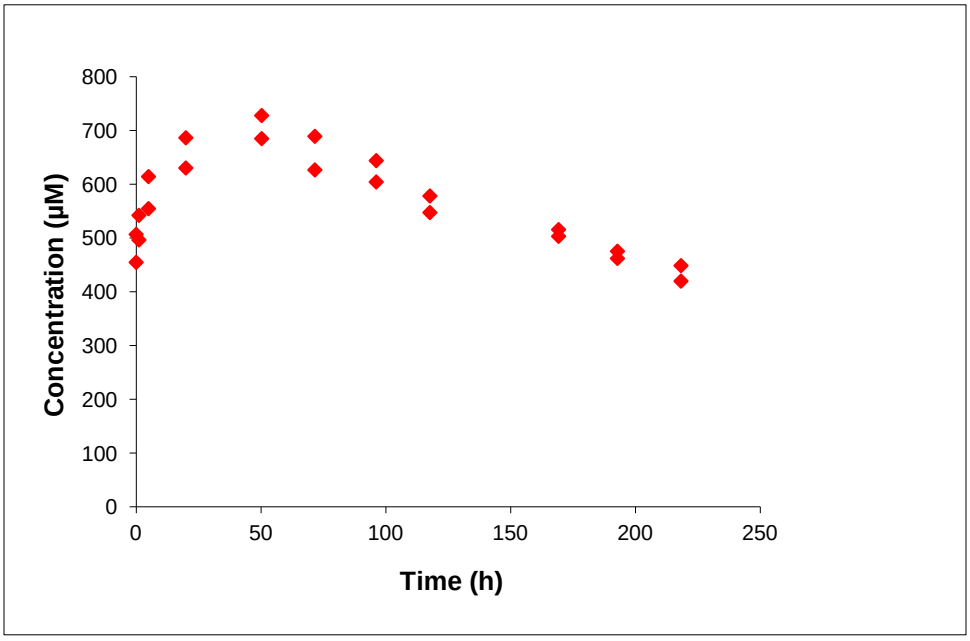
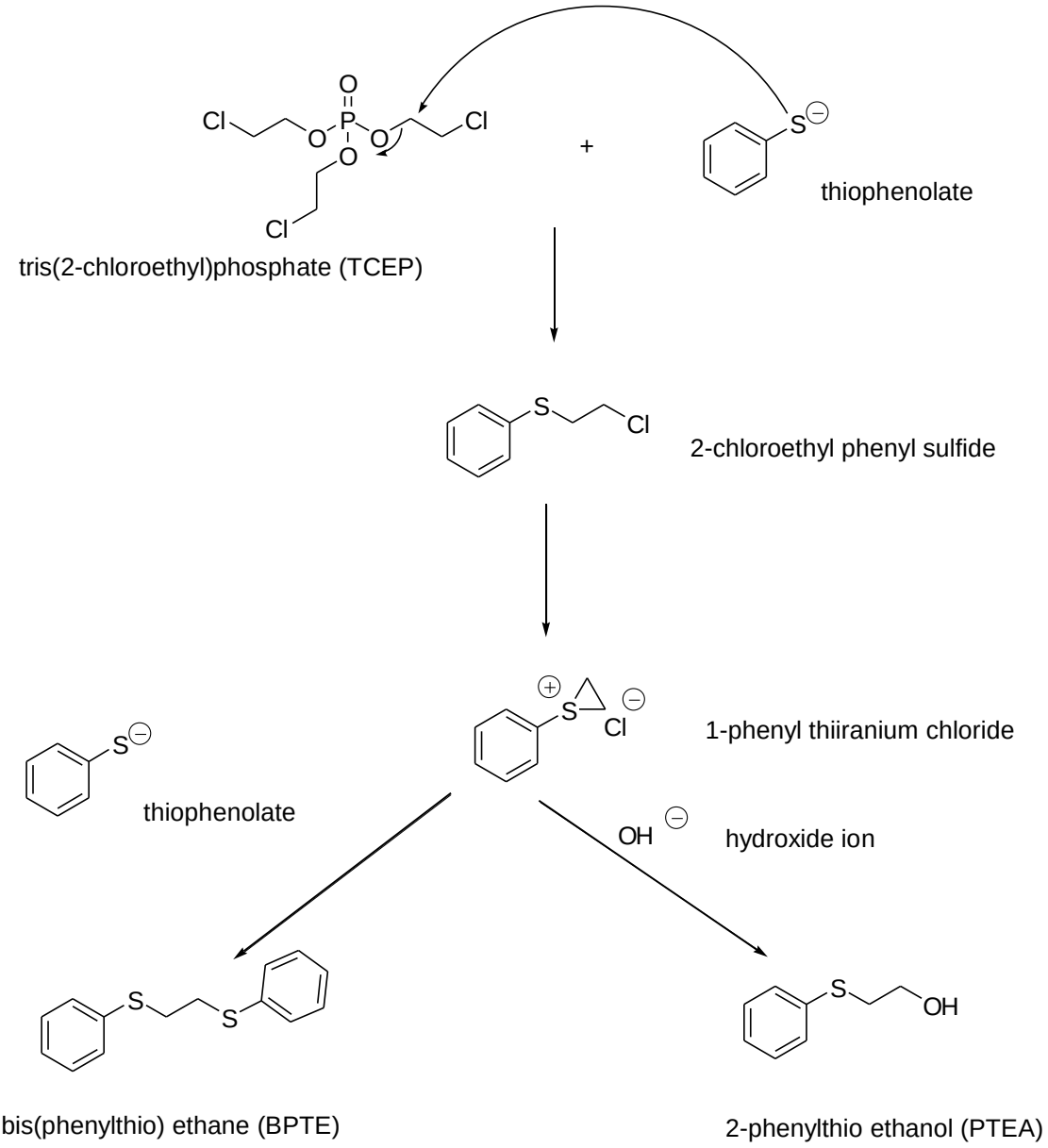


Figure 4.19 Degradation of BCPP with 36.0 mM polysulfides at pH 9.46 50 °C

4.2.5 Reaction of 2-chloroethyl phenyl sulfide, a potential intermediate for reaction of TCEP with thiophenolate, with hydrolysis and thiophenolate

In order to elucidate 2-chloroethyl phenyl sulfide as a potential intermediate for the reaction of TCEP with thiophenolate. The hydrolysis and reaction with thiophenolate 2-chloroethyl phenyl sulfide were investigated. At first, the hydrolysis of 2-chloroethyl phenyl sulfide was studied. A first-order rate constant of 1.4×10^{-4} s^{-1} was obtained at 25 ^{o}C and pH 9.14 (Figure 4.20). A similar experiment was repeated, the only difference was the addition of 5.16 mM thiophenolate (Figure 4.21). The first-order rate constant of this reacton of 2-chloroethyl phenyl sulfide at 50^{o}C, pH 9.14 and 5.16 mM thiophenolate was 1.7×10^{-4} s^{-1}. It cab be argued that the first-order rate constant of 2-chloroethyl phenyl sulfide is not significantly increased by the addition of 5 mM thiophenolate compared to the experiment without thiophenolate. This finding might indicate that the reaction of 2-chloroethyl phenyl sulfide is independent of nucleophiles such as thiophenolate. The degradation of 2-chloroethyl phenyl chloride at 50^{o}C, pH 9.14 and 5.16 mM thiophenolate and degradation product BPTE are shown (Figure 4.22). It can be seen BPTE is formed as a product. (PTEA was not quantified for this experiment Figure 4.22.

We can also explain why we are not actually able to detect 2-chloroethyl phenyl sulfide as intermediate in the reaction of TCEP with thiophenolate assuming the mechanism in Scheme 4.2. 2-Chloroethyl phenyl sulfide would be formed from a slow reaction of TCEP with thiophenolate (the reaction rate constant in the presence of 5 mM thiophenolate is 0.005 h^{-1}). It will be however reacting much faster with hydroxide and thiophenolate ion in the following step (0.5-0.6 h^{-1}), which is by a factor 100 faster than the first step.



Scheme 4.2 Mechanism of 2-chloroethyl phenyl sulfide with thiophenolate when nucleophilic attack occurs at α-carbon of TCEP

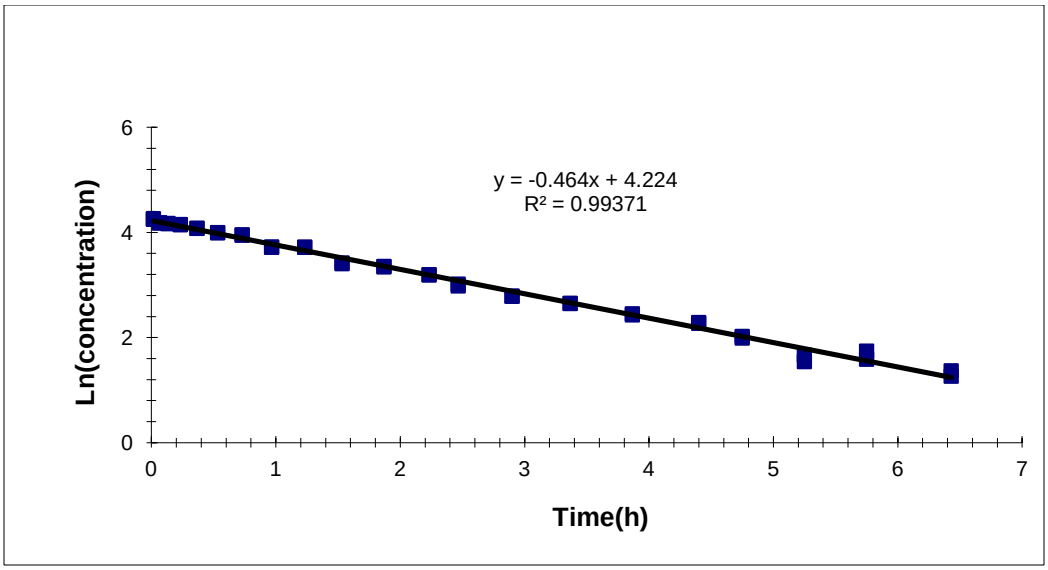


Figure 4.20 Hydrolysis of 2-chloroethyl phenyl sulfide at pH 9.14 and 25 ^{o}C

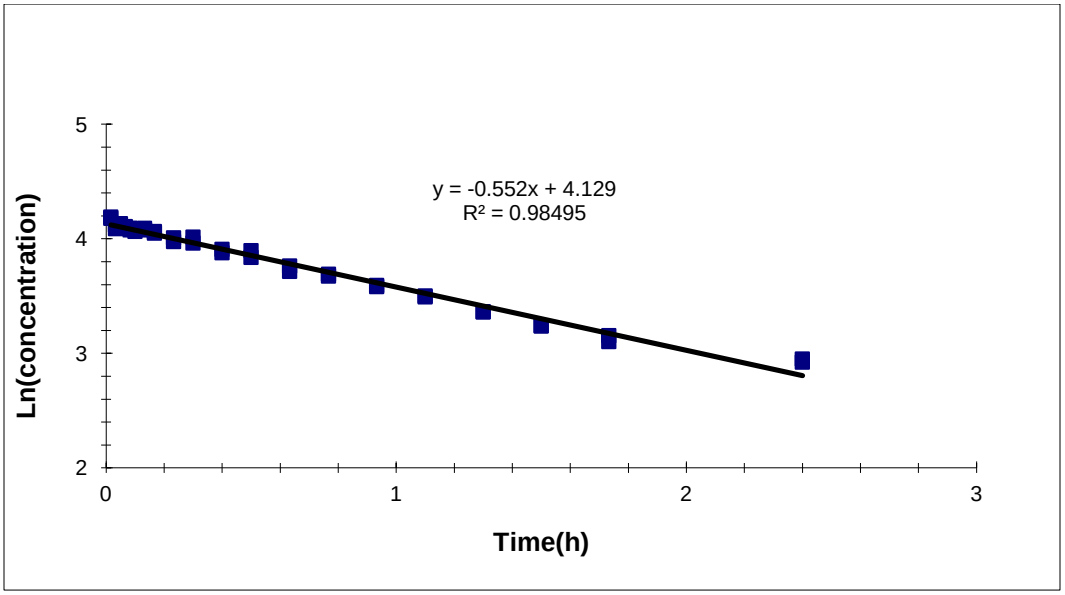


Figure 4.21 Degradation of 2-chloroethyl phenyl sulfide with 5.2 mM thiophenolate at pH 9.19 and 25 ^{o}C

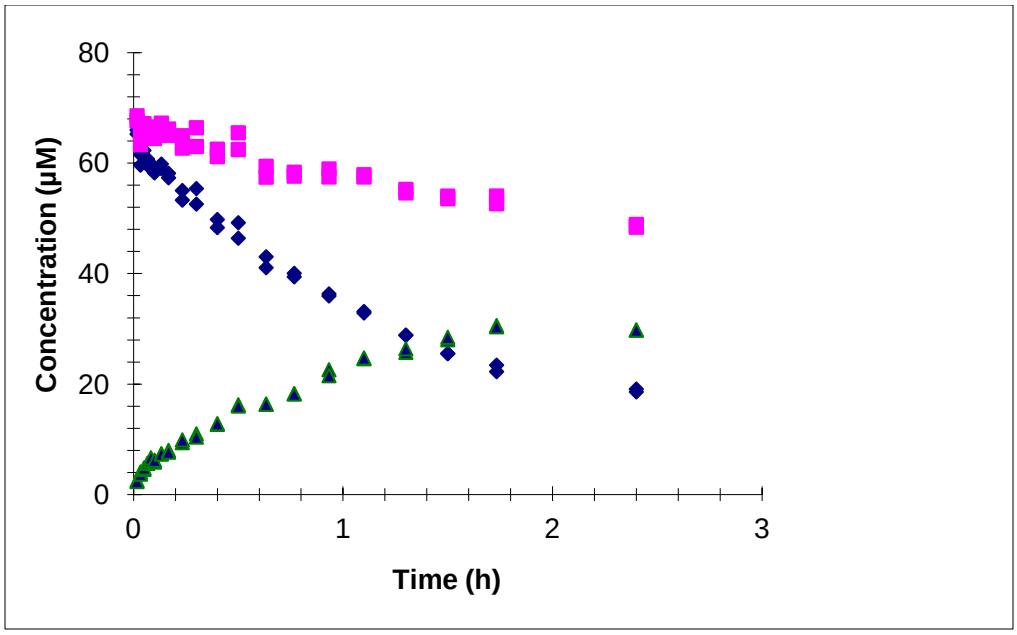


Figure 4.22 Degradation of 2-chloroethyl phenyl sulfide (♦) with 5.16 mM thiophenolate at pH 9.19 and 25 °C and BPTE (▲) as product and massbalance (■)

Chapter 5

Impact and Environmental Significance

The series of experiments indicate that the three reduced sulfur species (HS^-, S_n^{2-}, PhS^-) react with TCEP, TDCP, and TCPP. TCEP reacts with these reduced sulfur species in a nucleophilic substitution reaction with BCEP being the major product. The product formation can be explained by the proposed reaction mechanism. However, our experiments are not conclusive in determining whether the nucleophilic attack occurs at the α-carbon or at the β-carbon of TCEP.

The environmental fate of TCEP is controlled by a number of abiotic and biotic processes. Our results suggest that HS^-, S_n^{2-}, PhS^- are sufficiently reactive as to control the fate of TCEP in anoxic and suboxic environments where reduced sulfur species are abundant. Half-lives for TCEP, TDCP, and TCPP in marine porewater containing reduced sulfur species were calculated by multiplying the second-order rate constants by the maximum concentrations of [HS^-] and $\sum$ [S_n^{2-}] reported by MacCrehan [87]. The results indicate that the calculated half-lives of TCEP at pH 7.0, 5.6 mM HS^-, and 0.33 mM S_n^{2-} at 25 ^oC are 90 and 30 days, respectively compared to a half-life of 2 years for hydrolysis of TCEP. Hence, our results demonstrate that significant degradation of TCEP can occur with polysulfides and bisulfide under anoxic condition relative to hydrolysis. Therefore, reduced sulfur species present at environmentally relevant concentrations can represent an important sink for TCEP, TDCP, and TCPP in anoxic coastal marine environment. The calculated half-life of TDCP at pH 7.0, 5.6 mM HS^-, and 0.33 mM S_n^{2-} at 25

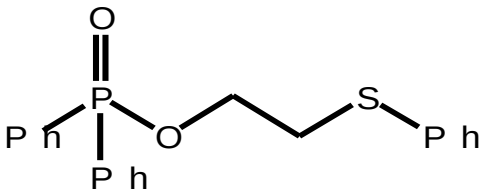
^{o}C are 180 and 74 days, respectively. The calculated half-life of TCPP at pH 7.0, 5.6 mM HS^-, and 0.33 mM S_n^{2-} at 25 ^{o}C are 1800 and 402 days, respectively. Analysis of lake samples from three volcanic lakes located in the Lazio area (Central Italy) showed that organophosphorus flame retardants and plasticizers were found at low concentration with tributyl phosphate and tripropyl phosphate as the most abundant [88].

Appendix

A1. Synthetic Part

The goal of the synthesis was to understand the position of attack (at α or β position) by reduced sulfur species of our investigated organophosphorus flame retardants (especially TCEP). The identified products from our kinetic experiments suggests that reduced sulfur species such as thiophenolate could potentially attack TCEP at the α or β carbon to form the degradation products bis(phenylthio)ethane and 2-phenylthio ethanol. The intention was to synthesize a compound that is structurally related to TCEP which was 2-chloroethyldiphenyl phosphinate (Fig.A1). 2-chloroethyldiphenyl phosphinate would actually help to elucidate the mechanism of TCEP with thiophenolate. It has one active side chain with two reactive carbons compare to TCEP that has three active side chains with two reactive carbons. The first part of the synthesis was to synthesize 2-chloroethyldiphenyl phosphinate from diphenyl phosphinic chloride, and diisopropylethylamine at ambient temperature (Scheme A1). The second step of the synthesis was to react 2-chloroethyldiphenyl phosphinate with thiophenol at room temperature.

Figure A1- Structure of (2-phenylthio)ethyl diphenylphosphinate



A1.1 Experimental Section

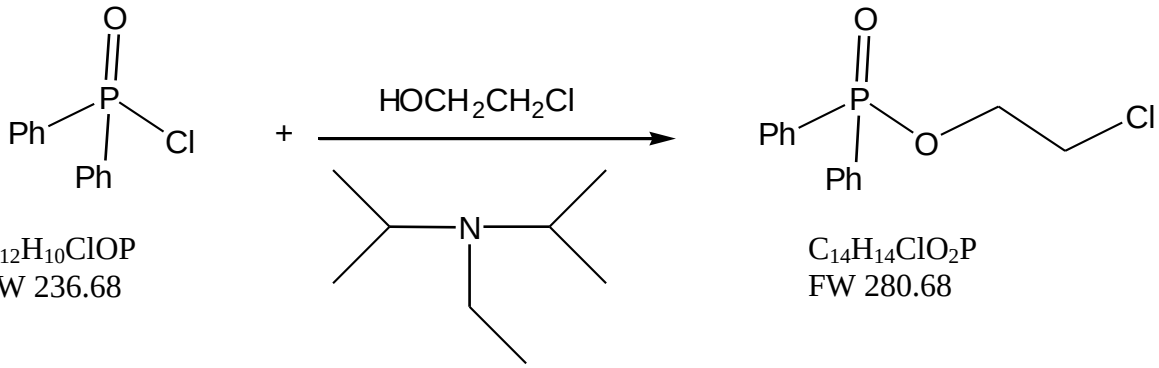
All commercial reagents and solvents were used without further purification unless otherwise specified. ^{1}H NMR spectra were recorded on a Varian Mercury 300 NMR spectrometer using $CDCl_3$ 7.26 ppm (parts per million) as standard. ^{31}P NMR spectra were recorded on a Varian Mercury Chemical 300 NMR spectrometer using 85% phosphoric acid as standard. Chemical shift data for the proton and phosphorous NMR were reported in ppm. Chromatographic solvent proportions were expressed on volume basis. Thin layer chromatography was run using Analtech silica gel. Plates were visualized under UV light. A pair of NaCl salt plates was used for the infrared measurement. The infrared spectra were recorded on a Nicolet 4700 FT IR. A drop of sample was placed in the center of one plate, and the second plate was placed gently on top.

A1.2 Synthesis of 2-chloroethyldiphenyl phosphinate

Diphenyl phosphinic chloride was obtained from Alfa Aesar (98%). Scheme A1 shows the chemical equation for formation of 2-chloroethyldiphenyl phosphinate. To a 250 mL one-necked flask, 1.00 g diphenyl phosphinic chloride (0.0042 mol), 0.37 g 2-chloroethanol (0.0046 mol) and 0.59 g diisopropylethylamine (0.0046 mol) were added together at room temperature. The reaction was kept at room temperature for about 2 hours. 50 mL of water was poured into the reaction mixture and the crude product was extracted with methylene chloride (25 mL, three times). The organic layer was dried with anhydrous magnesium sulfate. Methylene chloride

was removed by rotary evaporator and vacuum pump overnight. The residue was analyzed with ^{1}H NMR and ^{31}P NMR. The yield percentage was 56%. The actual yield was 0.67 grams (0.0024 mol). ^{1}H NMR data of 2-chloroethyldiphenyl phosphinate are as follows: (300 MHz, $CDCl_3$, δ): 8.00 (m, 4H, ArH), 7.5 (m, 6H, ArH), 4.3 (m, 2H, CH_2), 3.8 (t, 2H, CH_2) for 2-chloroethyldiphenyl phosphinate (see Figures A2 and A3).

Scheme A1- Formation of 2-chloroethyldiphenyl phosphinate



$C_{12}H_{10}ClOP$
FW 236.68

$C_{14}H_{14}ClO_2P$
FW 280.68

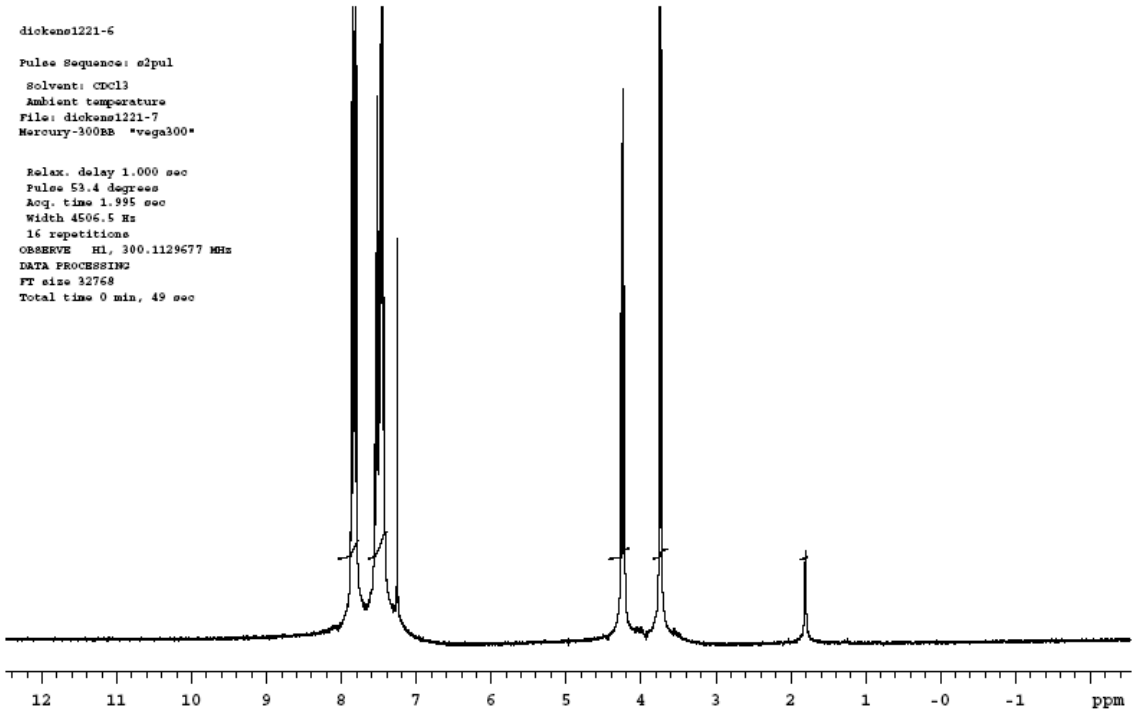


Figure A2- ^{1}H NMR spectrum of 2-chloroethyldiphenyl phosphinate in $CDCl_3$

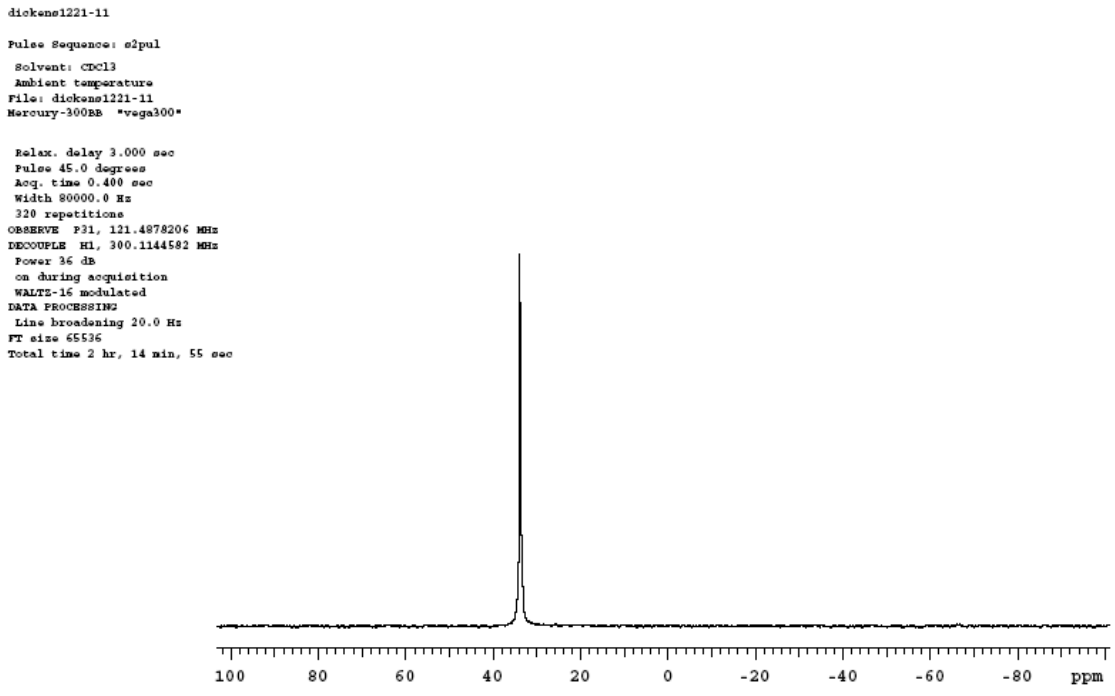


Figure A3- ^{31}P NMR spectrum of 2-chloroethyldiphenyl phosphinate in $CDCl_3$

A1.3 Synthesis of (2-phenylthio)diphenyl phosphinate

a) The reaction of 2-chloroethyldiphenyl phosphinate with thiophenol and potassium hydroxide in a 10:1 ratio was carried out. 10 mL of 5% potassium hydroxide (0.0089 mol), 0.0872 g thiophenol (0.00080 mol), and 0.20 g 2-chloroethyl diphenyl phosphinate (0.00071 mol) were added to 7.0 mL ethanol at room temperature in a 50 mL two-neck flask equipped with a nitrogen balloon. The reaction time was 2 hours at room temperature. 4% Hydrochloric acid was added drop wise in order to neutralize the pH of the solution to pH 7. 50 mL of water was added into the reaction mixture. The crude product was extracted with methylene chloride (25 mL, three times). Column chromatography was performed on the reaction mixture to separate the two spots observed in thin layer chromatography. Solvent for the column chromatography was 90% methylene chloride and 10% ethyl acetate. Rotatory evaporator and vacuum pump were used to remove the solvent from individual material after the column chromatography. The higher spot on the thin layer chromatography plate corresponded to 2-(phenylthio)ethanol as indicated by ^{1}H NMR. The chemical shifts of 2-(phenylthio)ethanol are as follows: 7.40 (m, 5H, ArH), 3.8 (t, 2H, CH_2), 3.2 (t, 2H, CH_2), 2.0 (s, 1H, OH). They are shown in Figure A4. Deuterated water exchange experiment was performed in order to verify the presence of alcohol. Figure A5 showed a shift of the alcohol peak in the ^{1}H NMR compared to Figure A4. IR spectrum of 2-(phenylthio)ethanol had a broad peak around 3360.87 cm^{-1}, which corresponds to an alcohol group in Figure A6 (on a Nicolet FT-IR Instrument). The chemical shifts of ethyl diphenyl phosphinate are as follows: 8.00 (m, 4H, ArH), 7.6 (m, 6H, ArH), 4.2 (m, 2H, CH_2), 1.4 (t, 3H, CH_3). They are shown in Figure A7. The lower spot on the thin layer chromatography plate corresponded to ethyl diphenyl phosphinate as shown in Figure

A7. Synthesis of (2-thiophenol)-1-ethyldiphenylphosphinate was not successful since the expected product was not obtained. Other products such as 2-(phenylthio)ethanol (0.0011 mol, 0.17 g) and ethyl diphenyl phosphinate (0.00012 mol, 0.030 g) were obtained. Ethyl diphenyl phosphinate and 2-(phenylthio)ethanol were obtained from reaction of 2-chloroethyl diphenyl phosphinate with potassium hydroxide to thiophenol at 10:1 ratio. The formation of ethyl diphenyl phosphinate can be explained by solvolysis of 2-chloroethyl diphenyl phosphinate in alkaline ethanol. 2-(phenylthio)ethanol is likely generated by two reactions. The first reaction is thiophenolate attack at the α-carbon of 2-chloroethyl diphenyl phosphinate to form 2-(phenylthio)ethyl chloride. The second reaction is the attack of the hydroxide ions from potassium hydroxide at the carbon bond to chloride atom of 2-(phenylthio)ethyl chloride. The fact that almost 1.5 equivalent of 2-(phenylthio)ethanol is formed might be explained by the solvent being still present in the compound.

b) The reaction of 2-chloroethyldiphenyl phosphinate with thiophenol and potassium hydroxide in a 1:1 ratio was performed. 1 mL of 5% potassium hydroxide (0.00089 mol), 0.088 g thiophenol (0.00084 mol) and 0.20 g of 2-chloroethyldiphenyl phosphinate (0.00071 mol) were added to 3.0 mL ethanol at room temperature in 50 mL two neck flask equipped with a nitrogen balloon. The reaction time was 1 day at room temperature. 4% Hydrochloric acid was added drop wise in order to neutralize the pH of the solution to pH 7. 50 mL of water was added into the reaction mixture. The crude product was extracted with methylene chloride (25 mL, three times). Rotatory evaporator and vacuum pump (overnight) were used to remove solvent from individual material after the column chromatography. No products were isolated.

c) The same reaction was carried out as above but with sodium hydride instead of potassium hydroxide. 0.0209 g sodium hydride (0.00080 mol), 0.0872 g thiophenol (0.00080

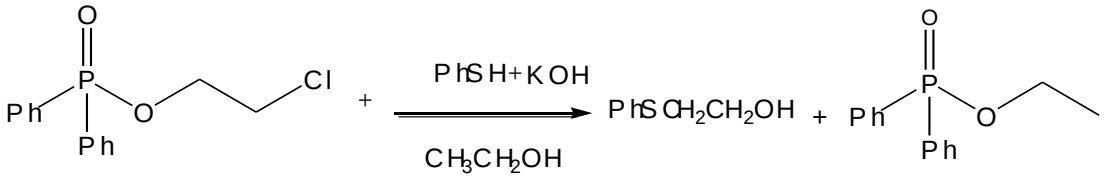
mol), and 0.20 g of 2-chloroethyldiphenyl phosphinate (0.00071 mol) were added to 6.0 mL of ether at room temperature in 50 mL two-neck flask equipped with nitrogen balloon. The reaction time was 5 days at room temperature. 4% Hydrochloric acid was added drop wise in order to neutralize the pH of the solution to pH 7. 50 mL of water was added into the reaction mixture. The crude product was extracted with methylene chloride (25 mL, three times). Rotatory evaporator and vacuum pump (overnight) were used to remove solvent from individual material after column chromatography. No products were isolated.

d) A related reaction was performed with diisopropyl ethylamine as a base instead of sodium hydride. Diisopropyl ethylamine (0.00080 mol, 0.10 g), 0.0872 g of thiophenol (0.00083 mol), and 0.1983 g of 2-chloroethyldiphenyl phosphinate (0.00071 mol) were added to 7.0 mL ether at room temperature

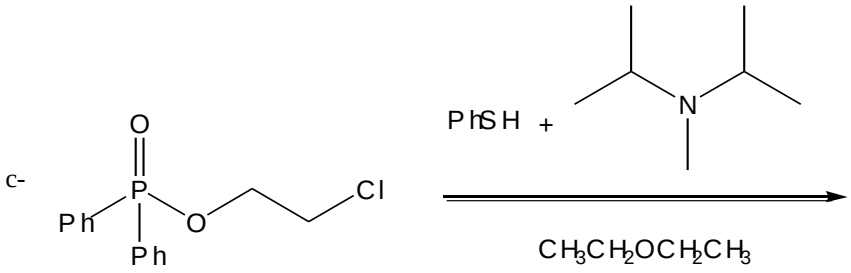
in 50 mL two-neck flask equipped with nitrogen balloon. The reaction time was 5 days at room temperature. 4% hydrochloric acid was added drop wise in order to neutralize the pH of solution to pH 7. 50 mL of water was added into the reaction mixture. The crude product was extracted with methylene chloride (25 mL, three times). Rotatory evaporator and vacuum pump (overnight) were used to remove solvent from individual material after column chromatography. No products were isolated.

Scheme A2- Reaction of 2-chloroethyldiphenyl phosphinate with thiophenolate

a-b



c-



d-

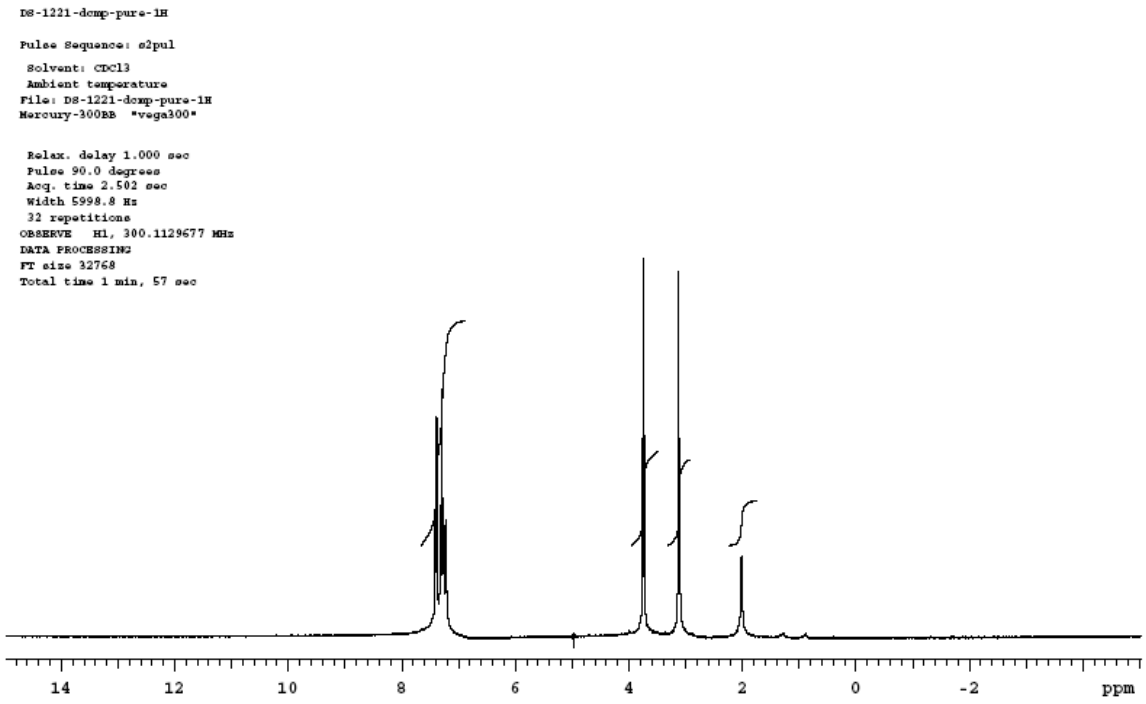


Figure A4- ^{1}H NMR spectrum of 2-(phenylthio)ethanol in $CDCl_3$

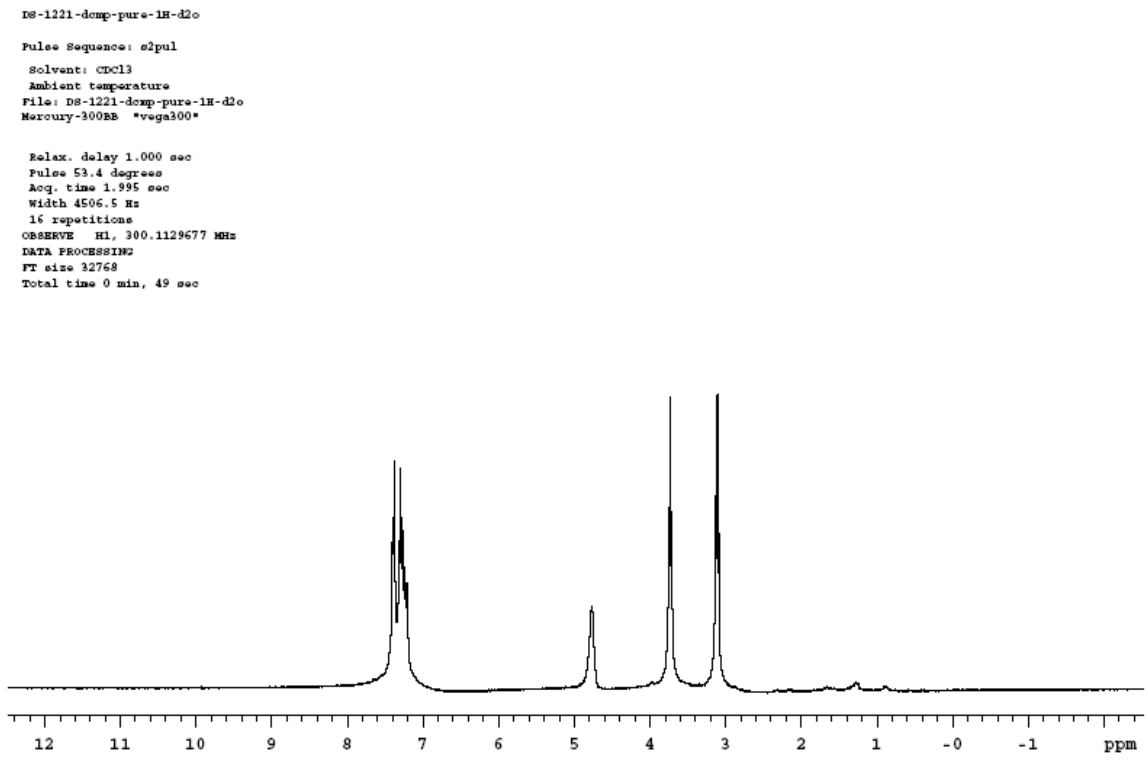


Figure A5- ^{1}H NMR spectrum of 2-(phenylthio)ethanol in D_2O

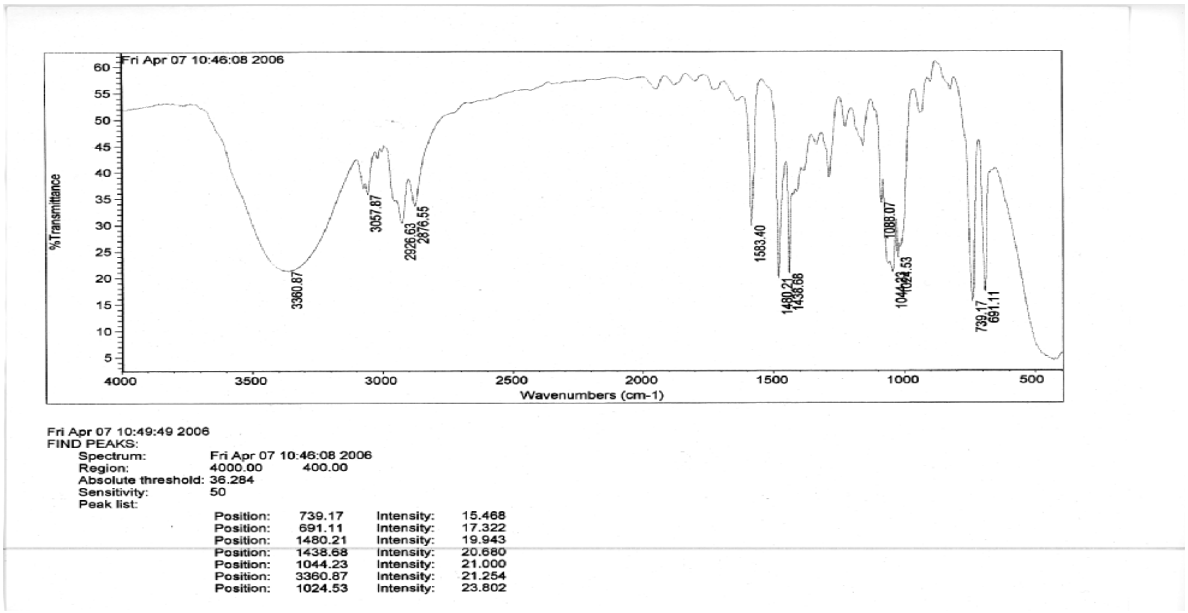


Figure A6- IR spectrum of 2-(phenylthio) ethanol in NaCl salt plates

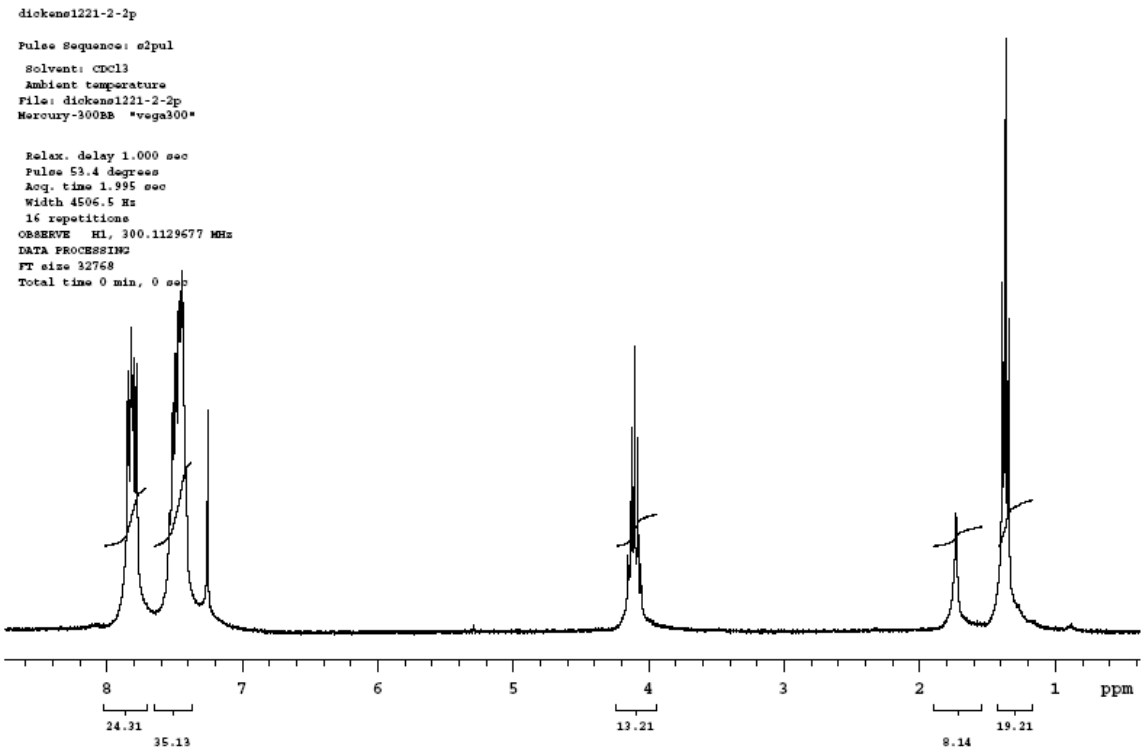


Figure A7- ^{1}H NMR spectrum of ethyl diphenyl phosphinate in $CDCl_3$

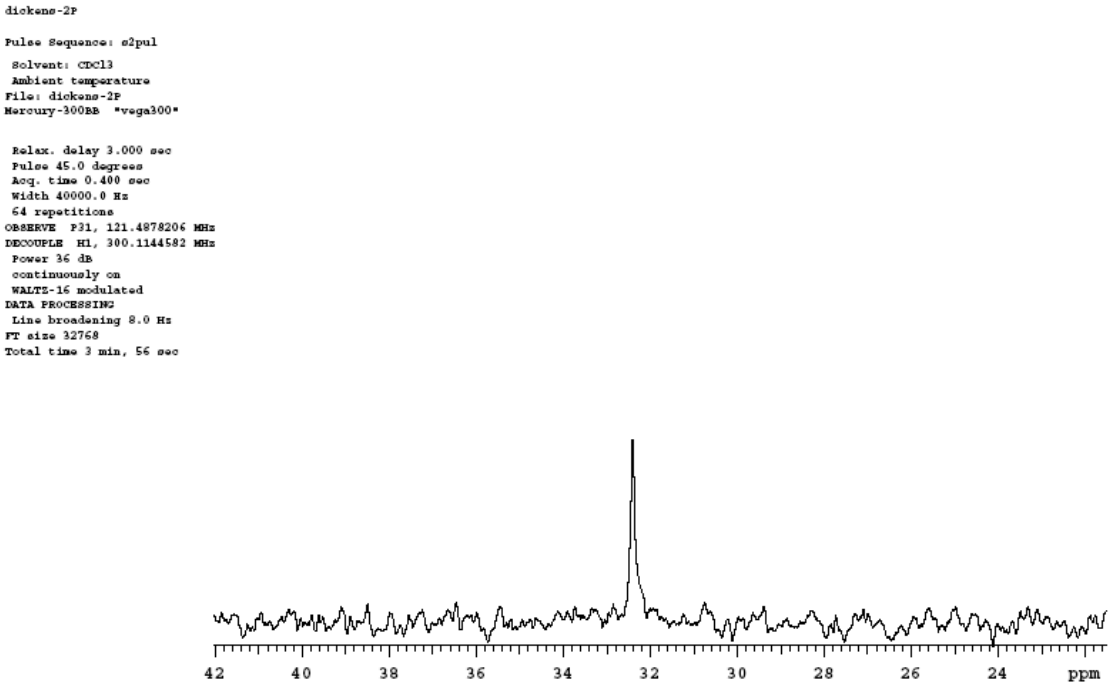
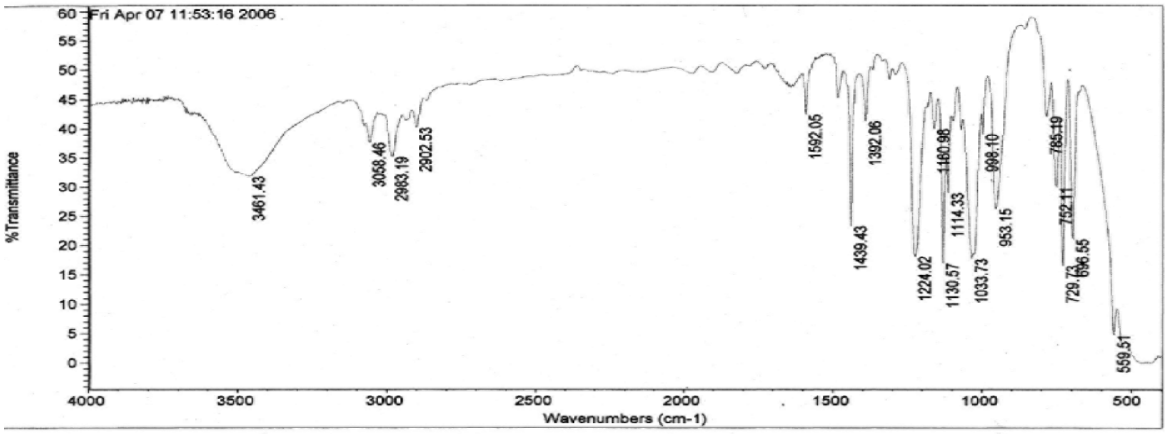


Figure A8- ^{31}P NMR spectrum of ethyl diphenyl phosphinate in $CDCl_3$



Fri Apr 07 11:57:41 2006
FIND PEAKS:
Spectrum: Fri Apr 07 11:53:16 2006
Region: 4000.00 400.00
Absolute threshold: 42.654
Sensitivity: 50
Peak list:

Position:	559.51	Intensity:	4.791
Position:	729.73	Intensity:	16.626
Position:	1130.57	Intensity:	16.965
Position:	1033.73	Intensity:	17.751
Position:	1224.02	Intensity:	18.162
Position:	696.55	Intensity:	21.108
Position:	1439.43	Intensity:	23.325

Figure A9- IR spectrum of ethyl diphenyl phosphinate in NaCl salt plates

The failure to synthesize (2-phenylthio)ethyl diphenyl phosphinate could be explained with the attack of thiophenolate at the α–carbon of the 2-chloroethoxy group and not at the β-carbon. The attack of thiophenolate at the α–carbon of 2-chloroethyl diphenyl phosphinate would lead to the formation of diphenyl phosphinic acid and 2-chloro-(phenylthio)ethane. Diphenyl phosphinic acid does not extract into organic solvents and would be lost in water that was used to clean the reaction. In the case of thiophenol and potassium hydroxide in 1:10 ratio, 2-chloro-(phenylthio)-ethane the other product of an attack at the α-carbon might hydrolyze and form the 2-(phenylthio)ethanol which was isolated. If the corresponding reaction (attack at the α-carbon) is dominating in the reaction of TCEP with polysulfide, then the attack of polysulfide might occur at the α-carbon of the chloroethoxy group. The expected product would be a phosphate diester. Therefore, an attack of reduced sulfur species at a chloroethoxy group of a phosphate ester might preferentially occur at the α-carbon.

However, I could also be possible that thiophenolate attacked at the β carbon of 2-chloroethyldiphenyl phosphinate to form (2-phenylthio)ethyl diphenylphosphinate which undergoes solvolysis attack by ethanol to form ethyl diphenyl phosphinate. The predicted rate constant of 2-phenylthio tosylates in 100% ethanol and 25 ^{o}C is 2 x 10^{3} s^{-1} based on McManus' data [86]. The desired product that was not able to isolate is very similar in structure to 2-phenylthio tosylate. The half-life for the predicted rate constant of 2-phenylthio tosylates in 100% ethanol and 25 ^{o}C is 3 x 10^{-4} s^{-1}. The solvent is the same between 2-chloroethyldiphenyl phosphinate and 2-phenylthio tosylates.

A2 Calculation of the respective molar weight of BCEP

The calculations of the respective molar weight are shown below for both assumptions. The formation and isolation of BCEP from the hydrolysis of TCEP was also supported by HRMS.

Molar weight percent of BCEP was determined by using the following calculations:

Known concentration of p-xylene: 0.0874 M

Number of protons from methyl group in p-xylene is 6.

Number of protons from methylene group in BCEP is 4.

Area of protons from methyl group in p-xylene is 6.00.

Area of protons from methylene group in BCEP is 1.89.

$\frac{(6.00)}{(6)}$ = 1.00 ratio of area to protons for p-xylene

$\frac{(1.89)}{(4)}$ = 0.4725 ratio of area to protons for BCEP

$$\frac{[p-xylene]_{known}}{[Bis(2-chloroethyl)\ phosphate]_{unknown}} = \frac{ratio\ area\ to\ protons\ p\text{-}xylene}{ratio\ area\ to\ protons\ bis(2-chloroethyl)\ phosphate}$$

$$\frac{(0.0874\ M)}{X} = \frac{(1.000)}{(0.473)}$$

X = 0.0874 M x 0.473 = 0.0413 M of BCEP

Since 4.44 mg were added to 0.50 mL of $CDCl_3$ and has a concentration of 0.0413 M, the molar weight can be calculated

$$MW = \frac{mass}{concentration\ \text{x volume}} = \frac{4.44\ \text{x}10^{-3}\ \text{g}}{0.0413\ \text{M x}\ 0.5\ \text{x}\ 10^{-3}\ \text{L}} = 215.0\ \frac{\text{g}}{\text{mol}}$$

Molar weight of MCEP was determined by using the following calculations.

Known concentration of p-xylene: 0.0874 M

Number of methyl protons in p-xylene is 6

Number of protons from methylene group in MCEP is 2.

Area of protons from methyl group in p-xylene is 6.00

Area of protons from methylene group in MCEP is 1.89

$\frac{(6.00)}{(6)}$ = 1.00 ratio of area to protons for p-xylene

$\frac{(1.89)}{(2)}$ = 0.945 ratio of area to protons for MCEP

$$\frac{[p-xylene]_{known}}{[Mono(2-chloroethyl)\ phosphate]_{unknown}} = \frac{ratio\ area\ to\ protons\ p\text{-}xylene}{ratio\ area\ to\ protons\ mono(2-chloroethyl)\ phosphate}$$

$$\frac{(0.0874\ M)}{X} = \frac{(1.000)}{(0.945)}$$

X = 0.0874 M x 0.945 = 0.0826 M

Since 4.44 mg were added to 0.50 mL of $CDCl_3$ and has a concentration of 0.0826 M, the molar weight can be calculated

$$MW = \frac{mass}{concentration \text{ x volume}} = \frac{4.44 \text{ x} 10^{-3}\ g}{0.0826 \text{ M x } 0.50 \text{ x} 10^{-3} \text{ L}} = 107.5 \frac{\text{g}}{\text{mol}}$$

Table A1 Summary calculations of assumption 1: MCEP and assumption 2: BCEP

	Assumption 1 Mono(2-chloroethyl) phosphate	Assumption 2 Bis(2-chloroethyl) phosphate
Number of protons from methylene groups	2	4
Number of protons from methyl groups in p-xylene	6	6
Area of protons from methyl group in p-xylene	6.00	6.00
Area of protons from methylene group	1.89	1.89
Ratio of area to protons for p-xylene	6.00/6 = 1.00	6.00/6 = 1.00
Ratio of area to protons for	1.89/2 = 0.945	1.89/4 = 0.4725
Known concentration of p-xylene (M)	0.0874	0.0874
Calculate concentration (M)	0.0826	0.0413
Mass of sample added to 0.50 mL $CDCl_3$ (mg)	4.44	4.44
Calculate molar weight (g/mol)	107.5	215.0

A3. HRMS results for BCEP, BCPP and BDCP

HRMS was performed using a Q-TOF. The Q-TOF is a hybrid quadrupole time of flight mass spectrometer with MS/MS capability. The quadrupole is operated as an ion guide in MS mode and as mass selection device in MS/MS mode. A reflection time of flight (TOF) analyzer is placed orthogonally to the quadrupole and serves as a mass-resolving device for both MS and MS/MS modes. A collision cell is located between the quadrupole and the TOF analyzer to induce fragmentation in MS/MS experiments. The final detector is a microchannel plate with high sensitivity. The ESI interface consists of an electrospray probe and a Z-spray source. Sample introduction is through an infusion pump, loop injection, or from an HPLC column. A nanoflow electrospray interface is also available for analysis of very small amounts of sample. The Q-TOF has very sensitivity, resolution and mass accuracy. The high mass measurement accuracy allows exact mass measurements. The parameters that are used in TOF/Q-TOF mass spectrometer in the determination of BCEP are gas temperature 300 ^{o}C, nebulizer 40 (psi), 222.96883 as mass, and retention time 5.5 min.

The mass balance found in Figure 4.8 is not 100%. This might be due to impurites in the synthesized BCPP. BCPP was synthesized from TCPP and NaOH in aqueous solution followed by extraction with acetonitrile and diethyl ether. Possible impurities could be organic or inorganic. They might side products from the synthesis or possibly salts extracted from the reaction mixture. The HRMS of the synthesis of BCEP shows only one mass at 222.99. The HRMS of the synthesis of BCPP is 248.9847 and 500.8764. The HRMS of the synthesis of BDCP is 318.9234, 320.9206, 322.9176, 327.0087, 329.00401, 331.0009, 332.9082, 334.9356, 335.9401, 336.9334, and 338.9331.

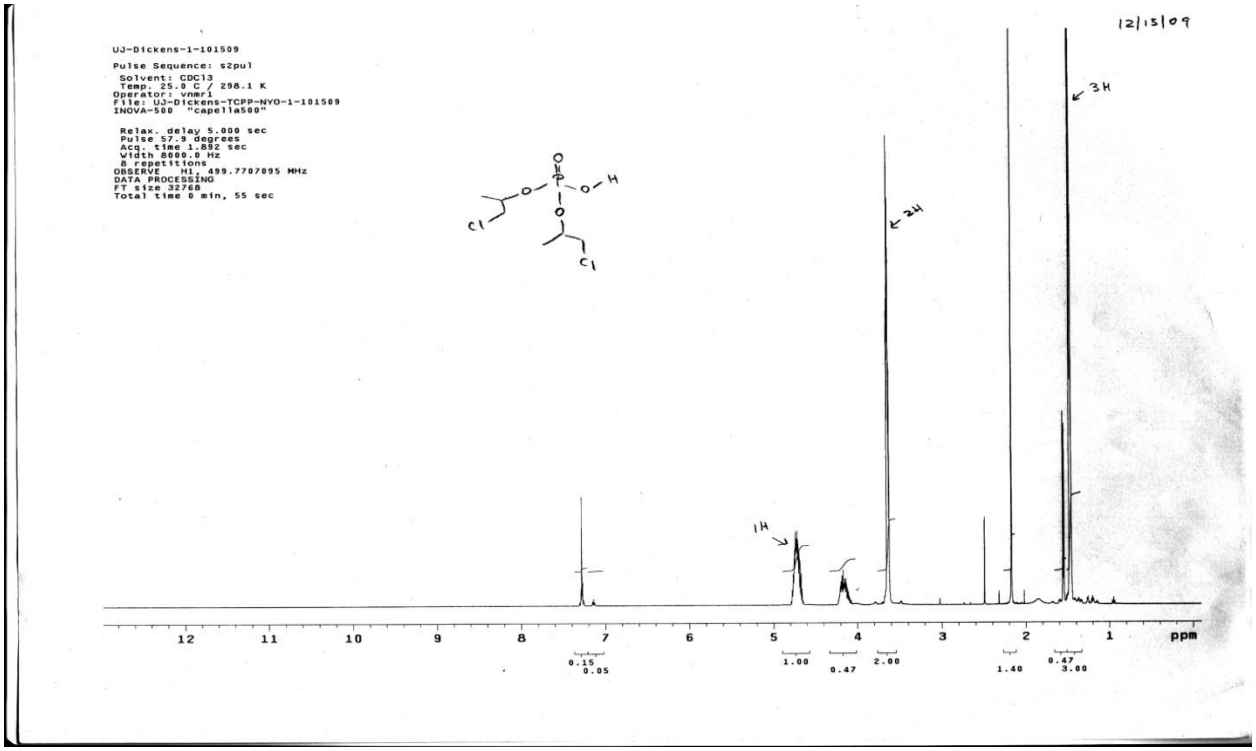


Figure A10. ^{1}H NMR of TCPP hydrolysis

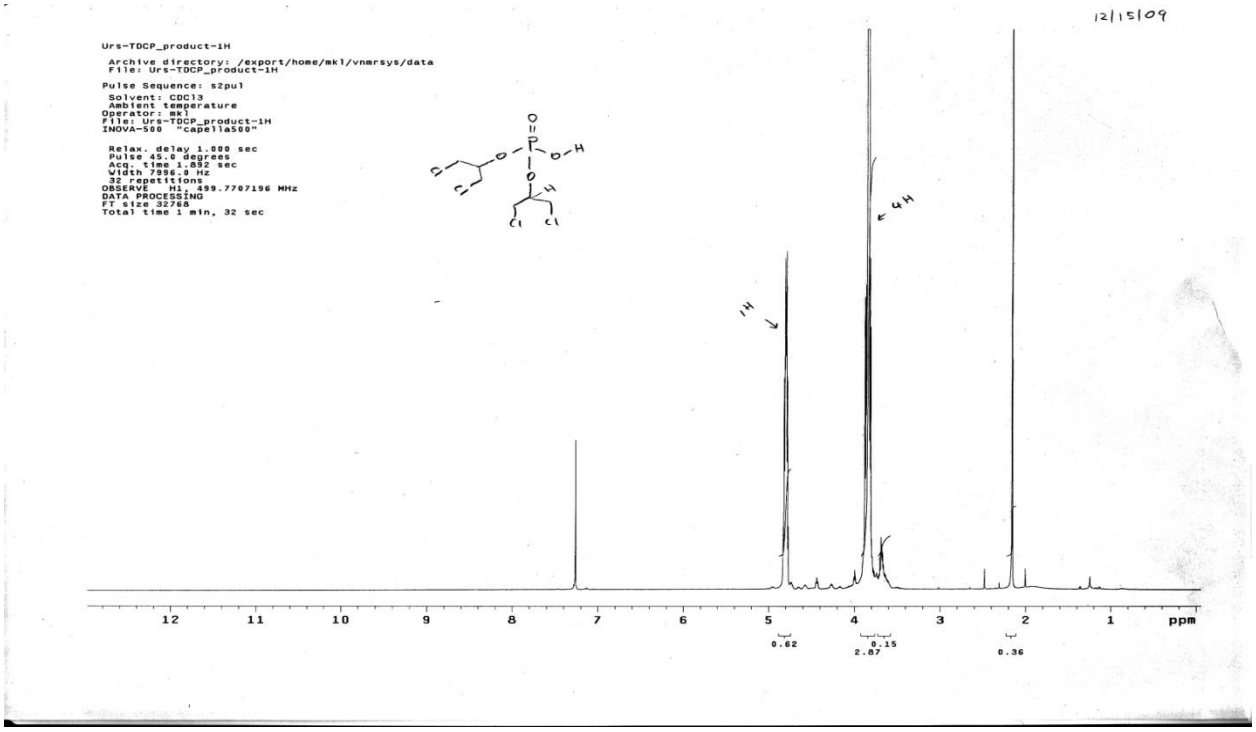


Figure A11. ^{1}H NMR of the product of TDCP hydrolysis

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