Isotopic Fractionation of Chlorinated Hydrocarbon Compounds During Sorption to Activated Carbon

by

Andrew Gates Gibson

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Approved by

Ann S. Ojeda, Assistant Professor of Geology
Ming-Kuo Lee, Professor of Geology
Natalia Malina, Assistant Research Professor of Geology

Abstract

Activated carbon (AC) amendments are leading remediation efforts of groundwater systems containing organic contaminants. More recently, *in situ* AC treatment is being explored to sequester contaminants in place. However, evaluating the effectiveness of *in situ* AC amendments is difficult because the reduction in concentration of parent compounds alone cannot differentiate between degradative and adsorption processes. Compound-specific isotope analysis (CSIA) has emerged as an important tool in identifying subsurface (bio)transformation of compounds like chlorinated hydrocarbons (CHCs) during remediation processes that can reveal subsurface processes. In principle, (bio)chemical transformations induce isotopic fractionation in organic compounds, whereas processes like adsorption, diffusion, and advection do not affect isotope ratios. This study seeks to understand the degree to which multi-step sorption contributes to the overall change in the isotope ratios of compounds treated with AC. Frist, working standards for a set of chlorinated organic compounds were analyzed using GC-qMS to determine the analytical uncertainty in δ^{37}Cl. When international or interlaboratory standards were available, working standards were calibrated to Standard Mean Ocean Chlorine, the international calibration standard for chlorine isotope measurements. Second, single and multi-step sorption batch experiments were conducted to understand kinetics and the extent of isotope fractionation during sorption to AC. All single-step batch experiments showed no trends in isotope enrichment nor depletion during sorption. However, in the multi-step experiments, perchloroethene (PCE), 1,2-dichlorobenzene (1,2-DCB) and 1,2,4-trichlorobenzene (1,2,4-TCB) produced cumulative shifts in δ^{37}Cl exceeding their calculated analytical uncertainties. These observations are in agreement with the findings of Wanner et al. (2017), which found similar trends for another CHC, 1-2 dichloroethane in low permeable soils. This study provides a foundation for future research to

analyze the total shift in isotope ratios due to small physical processes by remediation technology and low permeable material present in field scenarios. Lastly, this study continues to stress the importance of interlaboratory comparisons when reporting isotope data.

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List of Abbreviations

AC	Activated Carbon
ATSDR	Agency for Toxic Substances and Disease Registry
AU-WS	Auburn University Working Standard
CB	Chlorinated Benzenes
cDCE	1,2-cisDichloroethylene
CE	Chlorinated Ethenes
CHC	Chlorinated Hydrocarbons
CSIA	Compound Specific Isotope Analysis
DIF	Diffusion-Induced Fractionation
DNAPL	Dense Non-Aqueous Phase Liquid
EPA	Environmental Protection Agency
GAC	Granular Activated Carbon
GC-IRMS	Gas Chromatograph – Isotope Ratio Mass Spectrometer
GC-qMS	Gas Chromatograph – Quadrupole Mass Spectrometer
HA	Humic Acid
LD50	Lethal Dose 50%
MCB	Monochlorobenzene
MSD	Mass Selective Detector
MTBE	Methyl-*tert*-butyl-ether
NOM	Natural Organic Matter
PCE	Tetrachloroethylene

PFO	Pseudo-First-Order
PSO	Pseudo-Second-Order
P&T	Pump & Treat
PTC	Purge and Trap Concentrator
SIF	Sorption-Induced Fractionation
SIM	Selected Ion Monitoring
SMOC	Standard Mean Ocean Chloride
TCE	Trichloroethylene
UPW	Ultra-Pure Water
VC	Vinyl Chloride
VOA	Volatile Organic Analysis
1,2-DCB	1,2-Dichlorobenzene
1,2,4-TCB	1,2,4-Trichlorobenzene

Chapter 1

Motivation

1.1. Introduction

Activated carbon (AC) amendments are leading remediation efforts of groundwater systems containing organic contaminants[1–3]. More recently, *in situ* AC treatment is being explored to sequester contaminants in place[2]. However, evaluating the effectiveness of *in situ* AC amendments is difficult because the reduction in concentration of parent compounds alone cannot differentiate between degradative and adsorption processes. Compound-specific isotope analysis (CSIA) has emerged as an important tool in identifying subsurface (bio)transformation of compounds like chlorinated hydrocarbons (CHCs) during remediation processes that can reveal subsurface processes[1–7]. In principle, (bio)chemical transformations induce isotopic fractionation in organic compounds, whereas processes like adsorption, diffusion, and advection do not affect isotope ratios[7–11]. Typically, lab-based experiments are conducted to constrain the isotopic fractionation and are then applied to interpret processes in the field[4,11]. However, many sorption experiments that are relevant to AC amendments are static batch experiments and therefore neglect subsurface dynamic like flowing conditions, that promote sorption/desorption cycling[11]. Particularly for CSIA, it is unclear if small isotope fractionation observed during static equilibrium experiments is magnified during sorption/desorption cycling or multiple sorption steps which are prevalent under flowing conditions[1,2,5,7].

Among CHC compounds classified as priority pollutants, chlorinated ethenes (CEs) (trichloroethylene (TCE) and tetrachloroethylene (PCE)) and chlorinated benzenes (CBs) (monochlorobenzene (MCB), 1,2-dichlorobenzene (DCB), and 1,2,4-trichlorobenzene (TCB)) are common and persistent in the environment[12–14]. Inadvertent releases of these CHCs into the environment often occur during the production and improper disposal methods used during prior decades[14].

1.2. Chlorinated Ethenes

CEs like TCE and PCE are widely used in commercial industry as solvents for cleaning and degreasing as well as lubricants and adhesives[14,15]. CEs can originate from a variety of sources including manufacturing facilities, military installations, hazardous waste sites (Figure 1)[14,16,17]. CEs like TCE and PCE are listed among the national priority pollutants list and are classified as a hazardous substance and a dense nonaqueous phase liquids (DNAPLs), identified by the Environmental Protection Agency (EPA)[14]. Other physical and chemical properties such as high specific gravity, and tendency to remain sorbed to organic matter, make treating CEs difficult. These CE properties are summarized in Table 1.

The mismanagement of these substances leads to unintentional or illicit spills which can result in contaminated surface water and groundwater. Once spilled, CEs will migrate down into the water table through soil by percolation of water[18]. Typically, the more precipitation, the greater the chance for CHCs to leach from soil into groundwater systems[18]. Once in the subsurface, TCE & PCE may migrate through vapor intrusion or advective and/or dispersive flow in groundwater[19].

The Capital City Plume in downtown Montgomery, Alabama, is an example of a designated site under the Comprehensive Environmental Response, Compensation, and Liability Act where the soil and groundwater are contaminated by CEs (1.0 – 240 ppb) in a 30-50 block area[20]. A city ordinance restricts the use of groundwater and soil in the area to protect people from exposure[9].

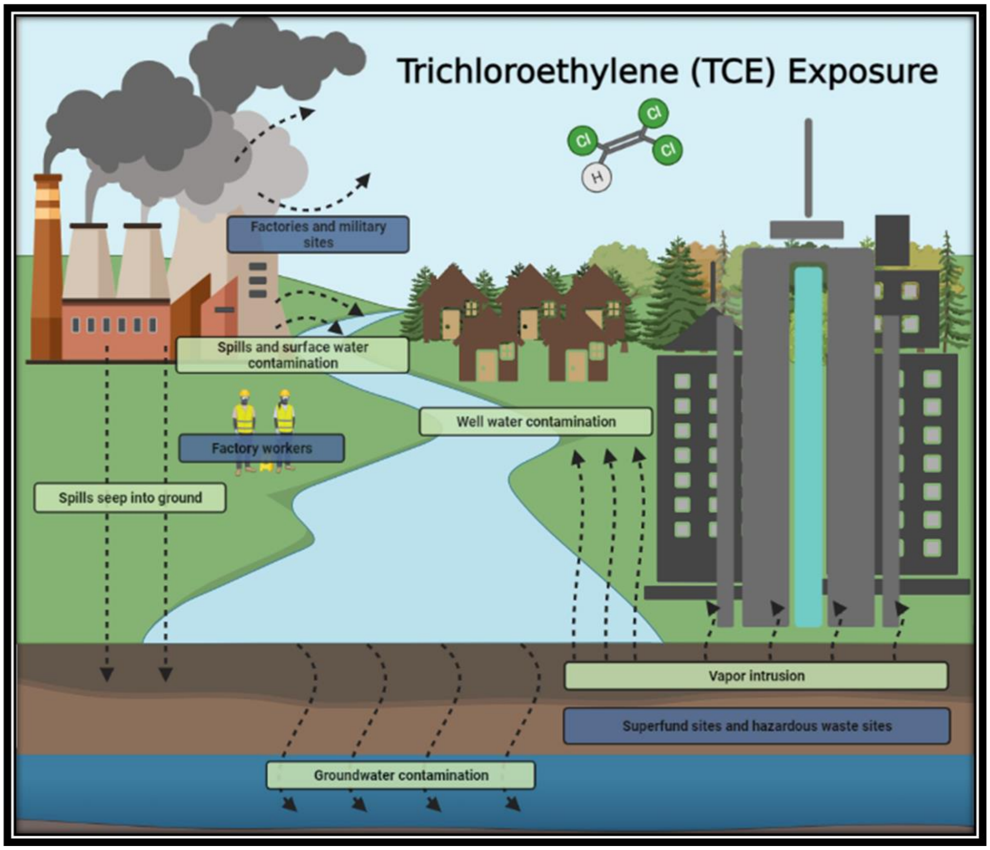
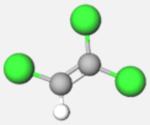
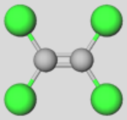
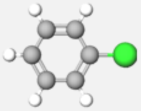
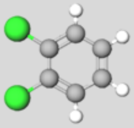
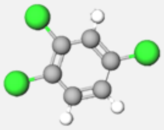


Figure 1. Illustrates the various sources of chloroethanes (specifically TCE) contamination and the pathways they may enter the environment. Potential exposure routes post spill can take happen through vapor intrusion or the ingestion of contaminated well water.

1.3. Chlorinated Benzenes

Chlorobenzenes (CBs) are a group of substituted benzene compounds possessing only carbon, hydrogen and chlorine atoms. CBs may contain 1 to 6 chlorine atoms in their structure and many of these compounds are listed on the national priority pollutants list[13,21]. Table 1 summarizes properties of some CBs. CBs, such as MCB, and DCB are commonly used as solvents and intermediates in odorizers, herbicides, insecticides, fungicides, fire retardants, degreasers, and chemical intermediates for dyes and pharmaceuticals[22–24]. Of the twelve common CB species, mono- and dichlorobenzenes are produced in large volumes, with MCB constituting ~70% of the total world production of all CBs[25]. Much like CEs, CBs are released into the environment from industrial as effluents and wastes, or by incineration of wastes containing organochlorine compounds[26].

Table 1. Summary of compound structure, priority according to the EPA, LD50 (oral), and Industry applications of CE and CB compounds

Compound	Structure	Priority Pollutant Rank (EPA)	LD50 (Oral) (mg/kg) *	Industry Applications *
Trichloroethylene (C_2HCl_3)		14	2,402 mg/kg	Degreaser, pesticides, flame retardants, dry cleaning
Tetrachloroethylene (C_2Cl_4)		15	1,780 mg/kg	Degreasing, dry cleaning, chemical synthesis
Monochlorobenzene (C_6H_5Cl)		7	2,000 mg/kg	Chemical intermediates, solvents
1,2-Dichlorobenzene ($C_6H_4Cl_2$)		25	1,516 mg/kg	Chemical intermediates, solvents, insecticides
1,2,4-Trichlorobenzene ($C_6H_3Cl_3$)		8	766 mg/kg	Dye carrier, herbicides, Heat-transfer medium

* = Information obtained using ATSDR publications [16,17,22,23,24], structures created using Molview.

1.4. Remediation of CEs & CBs

Human exposure routes to CHC compounds may occur through the inhalation of ambient air, dermal exposure while swimming or bathing, or in the context of groundwater, through the ingestion of contaminated drinking water[14]. Sources and exposure routes of TCE are illustrated in Fig 1. By all routes of exposure, both CE and CB are carcinogenic and present noncancer toxicity to the central nervous system, kidney, liver, immune system, and reproductive health[14,27]. The lethal dose 50 (LD50), is a measure of the amount (mg/kg) of toxic substance required to kill half of a dosed population of animals[16,17,22–24]. According to the Agency for Toxic Substances and

Disease Registry (ATSDR), LD50s can be used to measure the acute toxicity of substances[16,17,22–24]. The ATSDR has determined that the LD50 in rat populations through oral ingestion for each of the following compounds to be; TCE: 2,402 mg/kg; PCE: 1,780 mg/kg; MCB: 2,000 mg/kg; 1,2-DCB: 1,516 mg/kg; 1,2,4-TCB: 766 mg/kg (Table1)[16,17,22–24]. The LD50 (oral) for many of the compounds listed (Table 1) lower, or become more toxic, with increasing chlorination.

Due to the toxicity of many chlorinated solvents to humans and the environment, remediation efforts are focused on stabilizing contaminant plumes through adsorption to AC and then bio-stimulating or bio-augmenting the system to transform chlorinated compounds into benign CO_2[2,14]. The persistence of chlorinated compounds like CEs and CBs in the environment is partly due to the (C-Cl) bond, and the limited capability of microbial populations found in nature to dechlorinate organic compounds[28]. Reductive dechlorination by specialized communities like *dehalococcoides* has been identified as the targeted route to remediation of CBs and CEs to transform these compounds into lower- or non-chlorinated substances[29–31].

A common treatment technology for groundwater remediation is *ex situ* pump & treat (P&T), the process of physically removing contaminated soils and water from aquifer systems through pumping and then treating the extracted medium in storage tanks containing AC amendments. Newer innovative treatment technologies such as *in situ* bioremediation forego the removal process of contaminated mediums and instead inject AC into the subsurface to promote sorption of the contaminant. During this time, the goal of this process is to bio-stimulate complete dichlorination directly in the contaminated soils or aquifers[2,7,14,32].

1.5. CSIA

The effectiveness of any *in situ* treatment is usually assessed by monitoring the concentration of contaminants over time[2,31]. However, both biodegradation and sorption would cause contaminant concentrations to decrease[7,8,11,31,41]. Compound-specific isotope analysis (CSIA) has emerged as a diagnostic tool that aids in remediation efforts by identifying changes in the isotopic signature of the contaminant within the plume[42]. Furthermore, it has become a popular tool to unravel competing mechanisms in contaminant assessment, including differences between degradative (biotransformation) and non-degradative (sorption) processes[3,5,42–45]. CSIA involves measuring the ratio of a heavy isotope of an element (^{H}E) to the light isotope (^{L}E) in a compound. The small differences in the physio-chemical properties of the isotopes of the same element leads to the partial separation or discrimination of isotopes known as isotopic fractionation. During reactions, chemical bonds containing exclusively the light isotopes (e.g., ^{12}C, ^{35}Cl) are preferentially broken over those containing the heavier isotopes (e.g., ^{13}C, ^{37}Cl). For example, bond-cleaving transformations like those that occur during biodegradation of TCE induce isotope fractionation and lead to an isotopic enrichment of daughter products containing primarily light isotopes[40]. This process results in an isotopic enrichment in the residual contaminant pool seen as an increase in the proportion of $\delta^{H}E$. Physical processes like diffusion show little isotopic fractionation, typically leading to negligible changes in the ratio of $^{H}E/^{L}E$ [42,46].

Research studies focusing on the processes that govern contaminant transport in low-permeability zones are garnering increased attention. While diffusive isotopic fractionation (DIF) has been investigated in recent literature[7,11,33], very little research has been conducted on sorptive isotopic fractionation (SIF)[7]. To date, the studies of this process remain predominantly restricted to a small number of pure elements (boron)[34], minerals (goethite)[35] and non-CHC (benzene and toluene)[36] compounds[7]. Previous studies of SIF in single-step batch experiments using AC amendments have reported no significant isotope fractionation (within analytical uncertainty of ±0.5‰) for carbon[8]. Sorption studies conducted on carbon and hydrogen isotopes using different carbonaceous and microporous materials concluded similar results in that no significant fractionation occurred during equilibrium sorption (within ±0.5‰ for $\delta^{13}C$ and ±8‰ for $\delta^{2}H$)[9]. It was also concluded that the adsorption/pore-filling in the case of activated carbon had no distinguishable effect on the isotopic signatures of hydrogen and carbon within the error range of their measurements[9].

More recently, studies have analyzed SIF of carbon and chlorine under field conditions with key findings providing evidence that sorption has a measurable effect on carbon and chlorine isotope patterns[11]. This study was the first time that SIF was recognized for both carbon and chlorine in saturated low permeability units and can cause fractionation of isotope ratios of around 2‰ for $\delta\, ^{13}C$ in 1,2-dichloroethane (1,2-DCA). A shift to this extent is considered a threshold value for identifying processes affecting organic compounds[7,10,11,33].

1.6. Gap in Knowledge

Stable isotope ratios of carbon in the past have shown sorption has little-to-no effect on isotope fractionation[3,5,7,37]. However, it is unclear if small isotope fractionation observed during equilibrium experiments is magnified during sorption/desorption cycling or multiple sorption steps which is likely to be encountered in field scenarios. This project will address how field conditions affect rates of adsorption/desorption in AC amendments, the ability to detect the potential isotopic fractionation occurring under these conditions, and factors that contribute to its uncertainty. Additionally, the potential for δ^{37}Cl SIF has not yet been measured on chlorinated benzene compounds like monochlorobenzene, 1,2-dichlorobenzene, and 1,2,4-trichlorobenzene, something this project seeks to establish. Likewise, this research seeks to connect potential isotope fractionation to intrinsic sorption parameters within isotherm models. Lastly, little research has quantified the effects that increasing chlorination may have on adsorption/desorption characteristics.

1.7. Research Questions and Hypothesis:

This research was organized around two research questions and hypotheses:

RQ1: How does the process of multiple adsorption steps affect the isotopic composition of chlorinated ethenes and chlorinated benzenes?

Hypothesis: Multiple adsorption steps will enhance the isotope fractionation measured through CSIA. The extent of fractionation is proportional to the number of steps and physio-chemical characteristics of the sorbate.

RQ2: How do intrinsic sorption parameters within the isotherm model affect the extent of isotopic fractionation during single and multi-step sorption experiments for chlorinated ethenes and chlorinated benzenes?

Hypothesis: Differences in molecular characteristics between chlorinated ethenes and chlorinated benzenes, as well as differences within each compound class (extent of chlorination), will affect sorption to activated carbon amendments and the resultant extent of isotopic fractionation.

1.8. Summary and Outlook

A main criterion to establish the presence of isotopic fractionation for a given process is to first ensure that the degree of fractionation is greater than the analytical uncertainty of the stable isotope measurement[5,38]. To this extent, it was necessary to first establish a baseline for stable isotope measurement uncertainty within the Auburn University working standards (AU-WS) as described in Chapter 2 prior to experimental work described in Chapter 3.

Chapter 2

Uncertainty in Chlorine Stable Isotope Ratios of Chlorinated Solvents using GC-qMS

2.1. Abstract

Differences in techniques and instrumentation used for the CSIA of chlorine has brought about a greater need for the comparison of accuracy and total uncertainty in measurements made across laboratories[39]. This, in-part, stems from the differences in data acquisition between the two most popular CSIA techniques for chlorinated hydrocarbons: gas chromatography/isotope ratio mass spectrometry (GC-IRMS) and gas chromatograph-quadrupole mass spectrometry (GC-qMS). This chapter seeks to establish a baseline uncertainty for each of the AU working standards (CE and CB compounds) analyzed and calibrate the applicable compounds to the SMOC scale. For the purpose of this study, offline methods are not considered where samples are converted to methyl chloride or chloride salts prior to analysis[40]. Likewise, as no international standard exists for the CB compounds, their analytical uncertainty is set to an arbitrary zero. Lastly, areas in-which additional uncertainty may be introduced during experimentation and data derivations are also explored.

2.2. Introduction

GC-IRMS is the traditional analytical method for online CSIA of carbon, hydrogen and nitrogen. Typically, a mixture is separated into components on the GC, then combusted (in the case of carbon) or pyrolyzed (in the case of hydrogen) online to produce a gas (e.g., CO_2, H_2, N_2). Then, stable isotope ratios are measured for each resultant gas peak. For chlorine-CSIA intact non-combusted chlorinated hydrocarbon compounds are directly transferred into the IRMS ion source where fragments of the original molecule are created during ion bombardment in the mass spectrometer[41,42]. This prevents the need for off-line separation techniques that may potentially introduce isotopic fractionation[41,42]. The ion fragments for each isotopologue are recorded simultaneously. Isotope ratios obtained using a GC-IRMS are later used to determine the extent of fractionation relative to the international standard mean ocean chloride (SMOC) by external calibrations. This approach demonstrates high precision, however, the specific cup configurations required limit the range of compounds that can be analyzed.[4,39,41–43]. Other drawbacks include artifacts of previous measurements within the GC-IRMS system that may influence the isotopic composition of subsequently analyzed samples[41,42]. Artifacts like these tend to skew isotopic fractionation results over a long period of time.

Alternatively, a method to quantify chlorine isotope ratios using GC-qMS was developed by Sakaguchi-Söder et al[44], and later modified by Aeppli et al[45], to obtain chlorine stable isotope ratios on the SMOC scale[45]. This is made possible because the two stable isotopes of chlorine occur at relatively similar abundances (^{35}Cl ~ 75.78% and ^{37}Cl ~ 24.22%) and are two mass units apart[46]. Both characteristics allow a scanning qMS to record mass spectral data of sufficient precision to calculate isotope ratios using ion abundances[45,47]. However, instrumental parameters in a qMS, such as dwell time and number of selected masses, are crucial factors for the reproducibility and precision of chlorine CSIA using GC-qMS [47].

The basis for evaluating changes in isotopic ratios within a sample is illustrated in Eq (1). The traditional stable isotope equation values are derived and reported in delta notation[3,48]

$$\delta^{37}\mathrm{Cl}(‰) = \left(\frac{R_{sample}}{R_{SMOC}} - 1\right) \times 1000 \tag{1}$$

where δ^{37}Cl is the isotopic value in per mil (‰) and R is the ratio of heavy to light isotopes in for the sample and international isotopic standard like that of SMOC. However, the limitations of GC-qMS mentioned above require the use of additional evaluation schemes to determine ^{37}Cl/^{35}Cl ratios[47]. Previous studies by Sakaguchi-Söder et al[44] derived a multiple ion method, by which the two most abundant ions in each ion group were used for chlorine isotope determination by GC-qMS[44]. Aeppli et al[45] later modified this evaluation scheme to only use molecular ions[45]. Comparisons of evaluation schemes and the impacts of instrumental settings in terms of precisions and accuracy of chlorine CSIA using qMS systems was evaluated in a study by Jin et al[47]. The authors revealed evaluation schemes that included more ions in their calculations were most precise for compounds containing less than three chlorine atoms. For this reason, this study uses the Aeppli et al[45] molecular ion method evaluation scheme illustrated in Eq (2). Additionally, Jin et al[47] found that for compounds containing increasing numbers of ^{13}C in the target analyte, the chlorine isotope ratios tended to be overestimated[47]. This is normally corrected for using a modified version of the Aeppli et al[45] molecular ion formula, however, this requires the carbon isotopic composition of the sample and isotopic standard to be known[45]. Elsner and Hunkeler[48] later recognized the importance of these evaluation schemes to calculate chlorine isotope ratios and quantify isotope fractionation for a GC-qMS using molecular/fragment ions. However, this concept only proves viable if the mass spectrometer is free from bias or drift[48].

The purpose of this study was to 1) calibrate the AU-WS to other inter-laboratory standards and 2) establish uncertainty constraints for the AU-WS using GC-qMS, which are then compared to interlaboratory standards. For many of the CB compounds, these standards do not exist and thus uncertainty had to be constrained using only AU-WS.

2.3. Materials & Methods

Methanol was used to prepare stock solutions (6,000 ppm in 10 mL methanol) for the following CE and CB compounds used for chlorine isotope analysis: TCE, PCE, chlorobenzene, 1,2-dichlorobenzene, and 1,2,4-trichlorobenzene. Samples for the AU-WS at different aqueous concentrations (10-80ppm) were prepared by dosing 20mL of ultra-pure water (UPW, 18 Ω) into borosilicate glass volatile organic analysis (VOA) vials (22mL capacity), from the previously mentioned stock solutions, immediately capped with mininert-valve screw caps.

A purge and trap concentrator (PTC) connected to a (8890) gas chromatograph (GC) system paired to a (5977B) quadrupole mass selective detector (MSD) (from Tekmar Lumin and Agilent, respectively) was used to measure ion abundances. Sample injection was facilitated by manual syringe injection of 5mL UPW, 18 Ω dosed with various concentrations from the selected chlorinated compound stock solutions (10 – 100 ppm). The PTC was operated under specific parameters depending on the compound being analyzed. PTC methods for CEs were operated using (Table 6), while PTC methods for CBs were operated using (insert method). The GC was equipped with a capillary column (DB-624 30m x 250μm, 1.4μm film thickness) and used helium as a carrier gas. Oven temperatures were programmed according to the compound being injected (chlorinated benzene compounds required higher transfer line temperatures (200 – 250 C°) to be sent from the PTC to the GC-qMS). A split ratio of (1:70) was used for all compounds.

The qMS was operated in selected ion monitoring (SIM) mode, considering the following two most abundant ions of the target analytes: TCE: 130, 132 *m/z*; PCE: 164, 166 *m/z*; monochlorobenzene: 112, 114 *m/z*; 1,2-dichlorobenzene: 146, 148 *m/z*; 1,2,4-trichlorobenzene: 180, 182 *m/z*. Specific GC-qMS parameters used for each compound were recorded into Table X., located in the appendix.

2.4. Data Analysis

Chromatograms obtained after sample injection were manually integrated to obtain areas for light and heavy isotopes for each of the targeted CE and CB molecular ions. Uncertainty calculations were conducted in Excel using a correction developed by Aeppli et al[45], and then substituted in for the traditional stable isotope equation Eq. (1). This study used the molecular ion method Eq (2) derived by Aeppli et al[45] for each compound analyzed, whereby each $^{H}E/^{L}E$ ratio is multiplied by the ratio of chlorine atoms on a single molecule. The areas for each molecular ion were substituted in for the appropriate ion (*I*) in Eq (2). The values obtained from Eq (2) were then substituted into Eq (3) to calculate the δ^{37}Cl offset within the AU-WS in permil (‰). Lastly the analytical uncertainty was calculated using Eq (4), taking the average of Eq (3) values for each CE and CB compound.

$$R_{TCE} = \frac{1}{3} \cdot \frac{I_{132}}{I_{130}} \qquad R_{PCE} = \frac{1}{4} \cdot \frac{I_{166}}{I_{164}}$$

$$R_{MCB} = \frac{1}{1} \cdot \frac{I_{114}}{I_{112}} \quad R_{DCB} = \frac{1}{2} \cdot \frac{I_{148}}{I_{146}} \quad R_{TCB} = \frac{1}{3} \cdot \frac{I_{182}}{I_{180}} \tag{2}$$

$$\delta^{37}\text{Cl Offset (‰)} = \left(R_{avg} - R_{\text{i}}\right) \times 1000 \tag{3}$$

$$\text{Analytical Uncertainty} = \text{AVG}(|\delta^{37}\text{Cl(‰)}|) \tag{4}$$

To better define the uncertainty within AU-WS, standards for TCE and PCE, obtained from other stable isotope laboratories, were analyzed similarly to the AU-WS. Standards from the University of Oklahoma Stable Isotope Lab (Kuder 2023, personal communication) were used to calibrate the AU-WS using 1- & 2-point calibration curves according to Ebert et al[49]. Calibrations for both CE and CB compounds were used to define uncertainty within the AU-WS.

2.5. Results

2.5.1. Uncertainty within the AU-WS

The concentration for each compound in nanograms/microliter was plotted against the changes in δ^{37}Cl (‰) for the AU-WS (Figure 2, A-E). No trends were seen as a result of increasing concentration on column (10-200 ng) for both CE and CB compounds (Figure 2, A-E). The analytical uncertainty calculated by Eq (4) for each compound is reported in the end-of-chapter (Table 2) and is reported on each graph from which they were derived (Figure 2, A-E). Uncertainty in δ^{37}Cl measurements obtained in this chapter were used to constrain error from data collected and analyzed in Chapter 3 for single-step and multi-step experiments.

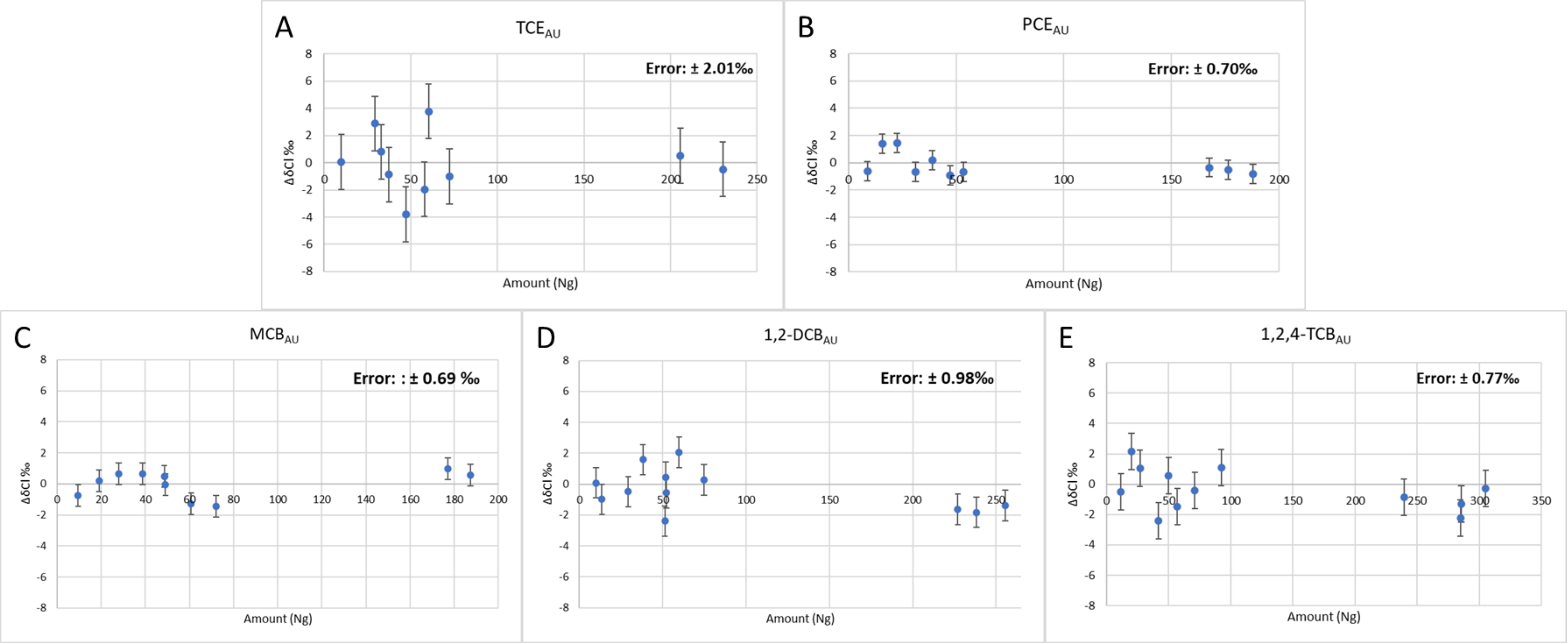


Figure 2. Analytical uncertainty in δCl‰ for each CE and CB standards run at concentrations of 10-200 ng/ul (n=11-14). Error bars were calculated using Eq (4). Plots generated compared the amount of each CE and CB compound in nanograms on column (x-axis) to the resultant δCl‰ (y-axis). Analytical uncertainty as a result of increasing concentration in ng on column for A). TCE - ± 2.01‰; B). PCE - ± 0.70‰; C). MCB - ± 0.69‰; D). 1,2-DCB - ± 0.98‰; E).1,2,4-TCB - ± 0.77‰.

2.5.2. Comparison to International Standards

TCE standards were designated as TCE_{OU1} and TCE_{OU2} to represent different sample lots with known δCl‰ of +3.3 ± 0.5 ‰ SMOC and +0.2 ± 0.1 ‰ SMOC, respectively. A PCE standard obtained through Dr. Kuder was designated PCE_{OU} and have a known δCl‰ of +0.3 ± 0.5 ‰ SMOC. To compare uncertainty in the isotopic signatures of each standard to the AU-WS, $TCE_{OU1,}$ TCE_{OU2} and PCE_{OU} samples were prepared and analyzed at various concentrations (10-100ppm) using GC-qMS following the same stock solution procedures. The concentrations for each standard in nanograms was plotted against $\delta^{37}Cl$ (‰) (Figure 3, A-B). Much like the AU-WS, no trends were seen in the standards as a result of increasing concentrations (figure 3, A-B). The analytical uncertainty for each standard was calculated using Eq (4) and is reported on each graph from which they were derived. The uncertainty in $\delta^{37}Cl$ obtained using GC-qMS methods and computational equation schemes for TCE_{OU1} & TCE_{OU2}, and PCE_{OU} were ±1.29‰ and ±1.53‰ respectively (figure 3, A-B)

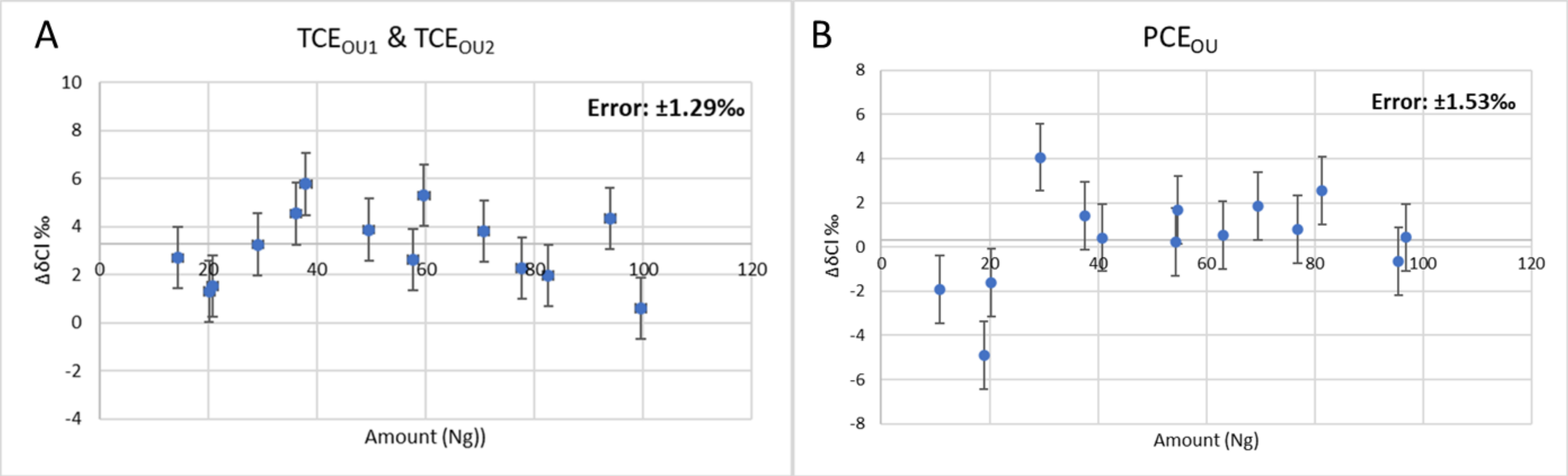


Figure 3. demonstrates the analytical uncertainty for the CE standards obtained from Kuder 2023, personal communication. A). Illustrates the resultant δ^{37}Cl of 1.29‰ obtained from TCE$_{OU}$ (Kuder 2023, personal communication). B). Illustrates the resultant δ^{37}Cl of 1.53‰ obtained from PCE$_{OU}$ (Kuder 2023, personal communication). Standards were run at concentrations of 10-100ppm (n=14).

2.5.3. Calibration to the SMOC Scale

To obtain δ^{37}Cl values on the international SMOC scale, 2-point calibration curve was performed on the AU-WS for TCE & PCE. (Figure 4, A-B). The AU-WS were calibrated using the SMOC standards obtained from Dr. Kuder. With no available secondary calibration point for PCE, TCE_{OU1} was substituted as a secondary anchor. The δ^{37}Cl value for the AU-WS was calculated using the slope of the anchor points and the average of the experimentally determined R in Eq 2 for both TCE and PCE. The results for both TCE and PCE calibrations of the AU-WS to SMOC were (+1.79‰ & -1.59‰) respectively (Table 2). The value obtained for the AU-WS for PCE would be better characterized with a 2-point calibration using solely PCE standards. No standards exist to calibrate δ^{37}Cl for MCB, 1,2-DCB, and 1,2,4-TCB, therefore we assigned these compounds an arbitrary zero value.

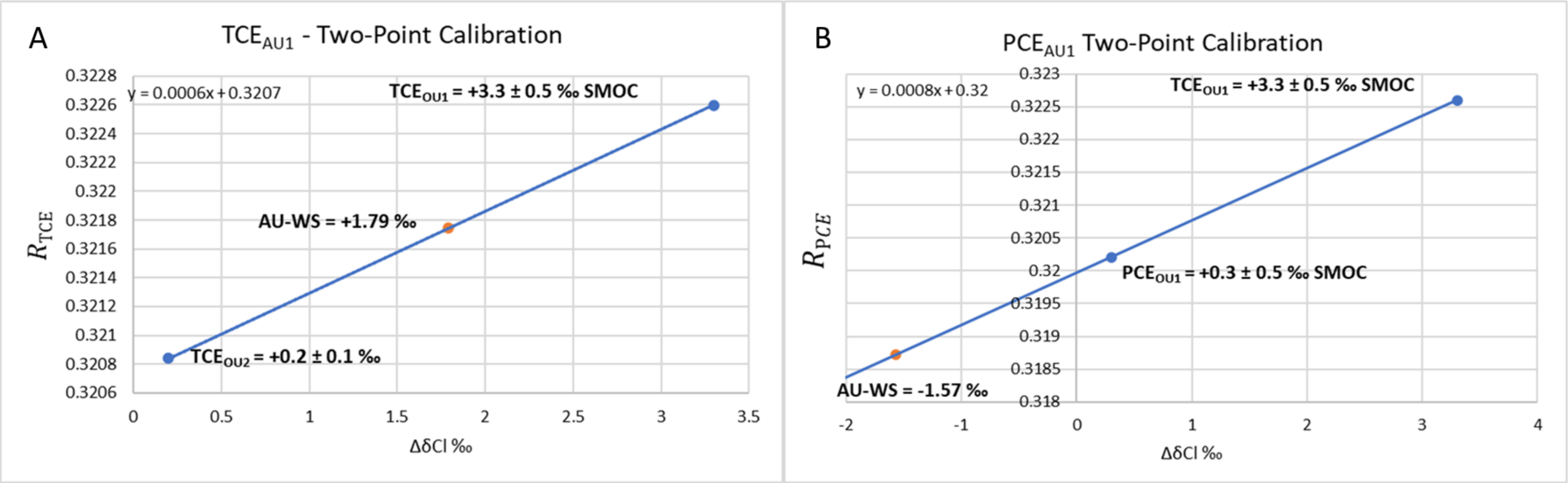


Figure 4. Illustrates the anchoring of the AU-WS for TCE and PCE to other international standards to generate relative values on the SMOC scale. A). R_{TCE} for the AU-WS was used calculated a value of + 1.79‰ SMOC. B). No secondary PCE standard was available and thus a TCE standard was used as a secondary anchor. R_{PCE} for the AU-WS was used to calculate a value of – 1.57‰ SMOC. This calibration would be better if a secondary PCE anchor was used.

Table 2. Summary of δ^{37}Cl and uncertainty (‰) for AU-WS and standards as determined by GC-qMS at Auburn University

Compound	δ^{37}Cl (‰) SMOC	Samples (n)
Standards for Inter-Laboratory Comparisons		
TCE_{OU1}	3.3 ± 0.5	n = 14
TCE_{OU2}	0.2 ± 0.1	n = 5
PCE_{OU}	0.3 ± 0.5	n = 14
AU Working Standards		
TCE_{AU1}	1.8 ± 2.0	n = 12
PCE_{AU1}	-1.6 ± 0.7	n = 13
MCB_{AU1*}	0 ± 0.7	n = 11
1,2-DCB_{AU1*}	0 ± 0.9	n = 11
1,2,4-TCB_{AU1*}	0 ± 0.8	n = 13

* - Chlorinated benzenes have not been calibrated and therefore are exceptions to SMOC (set to 0)

2.6. Discussion

It is important to recognize sources of uncertainty when making interpretations of isotope data. Here, these sources of uncertainty are likely to be during the measurement of samples on the GC-qMS (measurement uncertainty) and during data manipulation (mathematical uncertainty). These factors are discussed as it related to chlorine stable isotope measurements of the studied compounds.

2.6.1. Measurement Uncertainty

While a GC-qMS can produce a δ^{37}Cl for very small signals, signal sizes below a certain threshold can affect both the accuracy and reproducibility of δ^{37}Cl measurements[33]. According to Sherwood Lollar et al[50], variations introduced by operating parameters such as signal size and split-ratio settings within the GC inlet, can contribute to total instrumental uncertainty associated with CSIA using IRMS. These factors also likely influence GC-qMS measurements. Likewise, instrument linear range varies for each compound and between instruments[33]. This further stresses the importance of interlaboratory comparisons of analytical uncertainties related to instrumentation and evaluation schemes.

In a broad analysis, asserted by Berstein et al[39], the average uncertainty of the calibrated SMOC values were within the range of 0.08‰ to 0.11‰ for the Agilent GC-qMS systems tested. The Agilent GC-qMS analysis using purge & trap sample injection method produced an average uncertainty calibrated to SMOC of 0.08‰ ± 0.01‰. Comparatively, the average uncertainty measured here for TCE using similar purge & trap methodology was 1.8‰ ± 2.0‰ (Table 2). The uncertainty values obtained for OU and AU for TCE and PCE could be driven by differences in techniques regarding GC-qMS. The chlorine isotopic standards TCE_{OU1} - +3.3 ± 0.5 ‰ SMOC and TCE_{OU2} - +0.2 ± 0.1 ‰ SMOC and PCE_{OU} - +0.3 ± 0.5 ‰ SMOC obtained through personal communications with Dr. Kuder, were acquired using a GC-qMS using a cryofocusing trap[51,52]. Cryogenic cold trapping is frequently used for narrowing the chromatographic band and improving the detection limit by way of focusing the analytes at the head of the column by cooling before being sent to the MSD. These slight differences in instrumentation may in-part be responsible for the differences in uncertainty reported for the isotopic standards in this study and data obtained by Dr. Kuder.

2.6.2. Mathematical Uncertainty

The uncertainty associated with mathematical calculations, manipulations or evaluation schemes are often overlooked, but can have significant impacts on the interpretations of isotopic data as well[6]. In particular, due to instrumental parameter limitations in GC-qMS systems, the use of evaluation schemes is important to aid in the determination of $^{37}Cl/^{35}Cl$ ratios (R). As previously mentioned, Jin et al[47] compared the primary evaluation schemes used for chlorine isotopes and found that the chlorine isotope ratio values (R) varied from $0.319 \pm 4.8 \times 10^{-4}$ to $0.320 \pm 2.4 \times 10^{-4}$ for PCE and from $0.322 \pm 6.3 \times 10^{-}4$ to $0.321 \pm 2.2 \times 10^{-4}$ for TCE using the molecular ion method. In comparison, this study obtained values of $0.319 \pm 1.4 \times 10^{-4}$ to $0.317 \pm 8.8 \times 10^{-5}$ for PCE and 0.323 ($\pm 1.5 \times 10^{-3}$) to 0.319 ($\pm 6.2 \times 10^{-4}$) for TCE using the molecular ion method. Differences between values reported in Jin et al and here are potentially due to measurement uncertainty detailed above or due to the pitfalls of using the molecular ion method for compounds containing three or more chlorine atoms[47]. In contrast, the multiple ion method (method using more than just the most abundant ion fragments during GC-qMS analysis), was considered to be the most precise[47]. This has major implications for the calculated $\delta^{37}Cl$ (‰) for compounds containing 3 or more chlorines such as TCE, PCE, and 1,2,4-TCB.

Other sources of uncertainty to be accounted for when using evaluation schemes include the number of ^{13}C atoms in the target analyte. The chlorine isotope ratios mentioned above tend to be overestimated in the presence of increasing ^{13}C atoms[47]. While corrections have been made by Aeppli et al to evaluation schemes to account for the increasing number of carbons in chlorinated compounds such as CBs, these corrections could not be applied in this study due to lack of carbon stable isotope data for the AU-WS. This could account for some uncertainty in the AU-WS for all three CB compounds, but it is not likely to affect CEs.

Lastly, each chromatogram obtained after GC-qMS analysis is manually integrated to obtain the area used in Eq (2) rather than use an auto-integrate function within the Agilent software. This is to account for the loss of data (area) within the shoulders of the peak/signal on the chromatogram. Slight discrepancies between each manual integration may introduce uncertainty in the resultant data; therefore, it is not uncommon for automated scripts to be developed by research labs to account for this. This often leads to better reproducibility of the data; however, no such script was used during this experiment.

2.6.3. Uncertainty in 2-point calibrations

In a review paper published by M. Elsner[53], it is noted that the use of a single “in-house” standard, otherwise known as a one-point calibration, for the purpose of correcting offset, can only show that no significant fractionation occurs during gas chromatography and chemical conversion. Furthermore, the use of a one-point calibration cannot make claims about samples with isotope values differing from that of the standard used and can only correct samples for which values are similar to those of the standard[53]. While this can be corrected by introducing a secondary standard to create a two-point calibration, the use of two “in-house” standards can only ensure the reproducibility of measurements over time in a given laboratory. Coplen et al[54], reported that uncertainty values obtained across laboratories often differ by ten times the reported total uncertainty of their measurements. To account for this discrepancy in reported data, two or more isotopically different reference materials should be sufficient to isotopically bracket sample measurements.

To test this assertion, Bernstein et al[39] compared 2-point calibration curves using identical standards across different laboratories and found that calibration slopes not only changed across laboratories, but also changed across time in GC-qMS systems. To this extent, Bernstein et al,[39] found it mandatory to include a minimum of two compound-specific calibration standards with defined $\delta^{37}Cl_{SMOC}$ values. This is necessary in order to obtain absolute values on the international SMOC scale, however, in practice, obtaining a universal standard for chlorine isotopes is not easy[39]. In this study, the $\delta^{37}Cl_{SMOC}$ value obtained for PCE_{AU} (-1.57‰) would be better characterized if a secondary international standard for PCE was used (Figure 4). Additionally, $\delta^{37}Cl_{SMOC}$ values for each CB compound would be better characterized if standards existed, further stressing the importance of interlaboratory comparisons for these compounds.

2.7. Conclusion

The results of this chapter summarized in (Table 2) seek to further stress the importance of interlaboratory comparisons of accuracy and total analytical uncertainty in GC-qMS systems and evaluation schemes if alternatives to traditional GC-IRMS systems are to continue to develop. As stated before, it is important to include a minimum of two well defined compound-specific calibration standards with known $\delta^{37}Cl_{SMOC}$ to obtain true differences and to convert values to the SMOC scale for accuracy. This however, is not an easy feat to accomplish as many standards for chlorine isotope analysis of various CHCs do not exist or have no universal standard. Therefore, it is important to compare results to those obtained by other laboratories. The results of this section will be further applied to the following chapter to constrain the analytical uncertainty and to aid in the determination of the presence of sorption induced fractionation of chlorine isotopes among CE and CB compounds using AC amendments.

Chapter 3

Sorption of Chlorinated Hydrocarbons onto Activated Carbon

CSIA is an important tool to decipher the transformations of CHCs in the subsurface through biotic and/or abiotic means. To date, research has heavily focused on the effects that biodegradation and diffusive processes have on the isotopic signatures of the contaminant pool in aquifer systems[7,10,38,55,56]. This chapter will analyze factors that may influence sorption characteristics that in-turn may affect the extent of isotopic fractionation. These factors include the types of AC amendments used in industry for contaminant sequestration and remediation, as well as how surficial chemistry and AC heterogeneity may affect the extent of sorption that occurs. Additionally, this chapter will explore the kinetics of sorption onto AC amendments and its effects on varying CHC compounds. This chapter also seeks to define the extent of the research that has focused on the isotopic fractionation that may occur through sorption equilibrium processes that are expected to occur in field scenarios. Lastly, areas in which additional uncertainty may be introduced into the data will be discussed.

Introduction

3.2.1. Activated Carbon Amendments

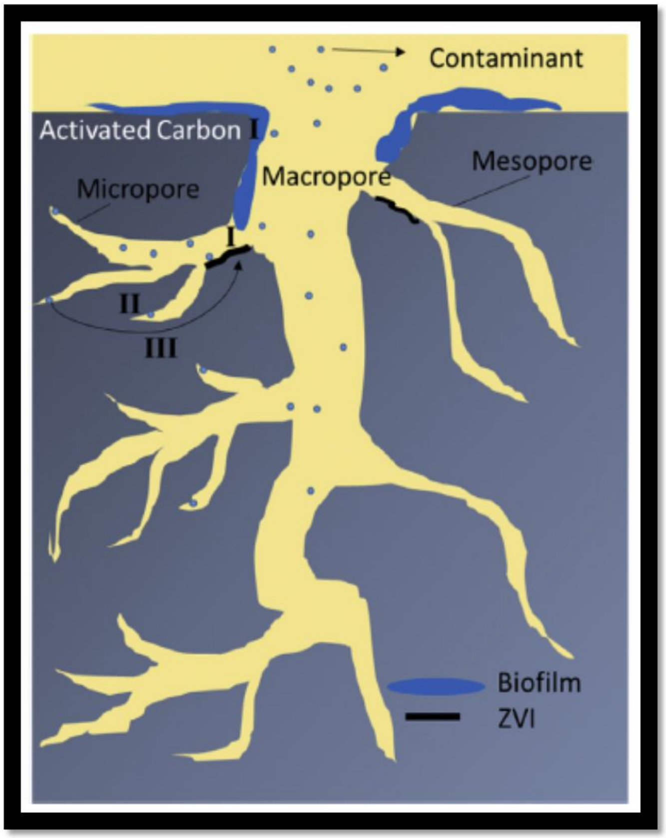


Figure 5. Schematic that illustrates the size and distribution of pore spaces in AC amendments. Pore sizes range from micropores (≤2 nm) and mesopores (2-50 nm) to macropores (>50nm). (Fan et al,. 2017).

AC amendments are a popular remediation technology for inorganic, organic and toxic metal ions. This is in-part due to its high surface area in the form of micro, meso and macro pores throughout its carbonaceous material illustrated in (Figure 5)[57]. Previous studies have shown that the microporosity of AC controls the rate of adsorption for CHCs, and that the micropore volume determines the amount of sorption that may occur[58–60]. AC amendments also play a key role in promoting the biodegradation of organic pollutants by enhancing the interspecies electron transfer between AC and microbial communities responsible for breaking the pollutants down into non-toxic daughter products[2,32,61–63]. Adsorption of CEs and CBs onto AC occurs through weak van der Waals forces primarily in micropores (≤2 nm) and mesopores (2-50 nm) of the AC matrix[59]. The adsorption process onto AC amendments is naturally reversible. Additionally, competitive adsorption may affect the long-term effectiveness of *in situ* activated carbon amendments[59,64]. This has unforeseen consequences for strategies currently leading remediation efforts today. For example, strongly adsorbed compounds that have high binding affinities may displace weakly adsorbed compounds with lower binding affinities, resulting in the release of the latter[32]. A common example is benzene being displaced by xylene in hydrocarbon plumes[4]. Likewise, in CE plumes, daughter products generated such as cis-1-2-dichloroethene (cDCE) or vinyl chloride (VC) may be displaced by PCE and TCE.

3.2.2. Effects of Activated Carbon during Remediation

Granular activated carbon (GAC) is traditionally used in amendment remediation efforts for groundwater systems containing organic contaminants through *ex situ* P&T technology[2,4,26]. Remediation occurs by sorption of contaminants in extracted groundwater onto AC, leaving the treatment tank effluent cleaner than before[32]. One drawback of P&T technology is that contaminants in low permeability zones are poorly targeted. Slow back-diffusion of contaminants out of low permeability zones can extend the time it takes to reach site cleanup goals[32].

An emerging remedial technique is *in situ* AC treatment where a suspension of AC is directly injected into the subsurface. Similar to *ex situ* treatment, sorption stabilizes the contaminant plume. But in contrast, *in situ* treatment provides long term remediation for low permeability zones as long as the sorption capacity is not reached[2,4,32]. Additionally, *in situ* treatment can enhance bioremediation of organic contaminants through the formation of biofilms on the AC surface [2,4,32]. AC's ability to sequester contaminants and bring them into close contact with microbial biofilms (Figure 6), allows for potential biodegradation of the organic contaminants as well as restore AC's adsorption capacity[2,32,63–65].

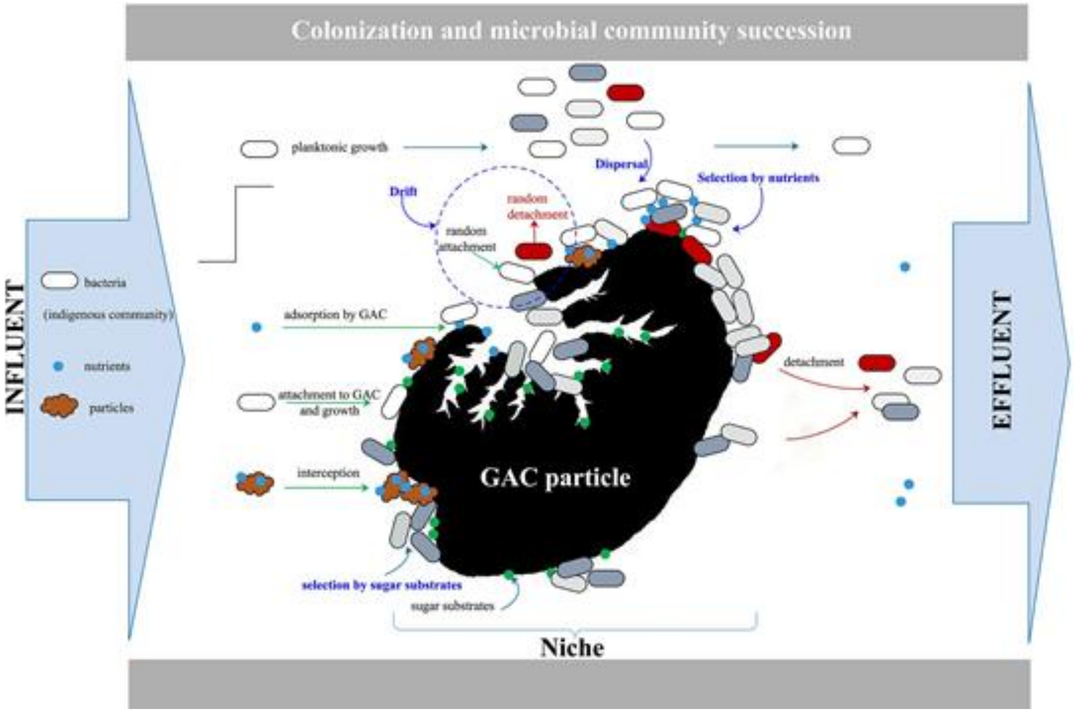


Figure 6. Illustrates the formation and interactions between microbial biofilms and adsorbed contaminants on a GAC particle. This highlights the strategy used in in situ contaminant remediation[65]**.**

3.2.3. Factors Influencing AC Sorption Characteristics

Both powdered and granular AC amendments are used in the contaminant remediation process and are typically manufactured from relatively heterogeneous base materials. These materials include bituminous coal, coconut shells, lignite, or wood. The activation of the base material through carbonization is predominantly responsible for the internal pore structure, however, the base material also affects this property[59,66]. Likewise, the surficial chemistry of AC is dependent on the base material and treatment process during AC formation[66].

The size and number of pores in the AC amendment affects the adsorption of organic contaminants in two ways. First, adsorption strength will increase as pore size decreases due to the heightened number of contact points between the adsorbent and adsorbate[66]. Secondly, if pore sizes are too small, size exclusion will ultimately affect the extent of sorption[66]. According to Li et al[66], size exclusion is observed when pore width is smaller than ~1.7 times the second largest dimension of the adsorbate. An example of this effect was demonstrated by Guo et al[59], who concluded that the adsorption of MCB onto AC was greatly affected by the physical structure of the AC. More specifically, the microporosity controls adsorption, and the micropore volume determines the total amount adsorbed. Additionally, AC treated to modify surficial functional groups to be more basic, showed greater adsorption behaviors of chlorobenzene[59].

3.2.4. Sorption Studies using Single-Step Batch Experiments

The earliest investigations into potential shifts in isotopic signatures (primarily carbon and hydrogen) during sorption onto carbonaceous materials such as lignite, graphite, and pore-filling AC, began with studies by Slater et al[8], and Schuth et al[9]. To better elucidate changes in isotopic fractionation contributed by equilibrium sorption, Slater et al, performed a series of single-step experiments containing varying concentrations of PCE, TCE, benzene, and toluene, using an AC sorbent. Vials were allowed to reach equilibrium over a period of 24 hours and samples were analyzed using a GC-C-IRMS. The results reported in $\delta^{13}C$ (‰) to percent sorbed showed that for both aromatic and chlorinated solvents, there was no significant isotopic fractionation within accuracy and reproducibility (±0.5‰) over the range of 0-90% equilibrium sorption[8].

Similarly, Schuth et al, performed triplicate benchtop single-step experiments using TCE, *cis*-dichloroethylene (*c*-DCE), vinyl chloride (VC), benzene, toluene, and *p*-xylene. All vials were allowed to reach equilibrium over a seven-day period. Stable carbon and hydrogen isotope measurements were performed using a GC-IRMS. The results indicated that the isotope effects were in range of the reproducibility limit (±0.5‰ for $\delta^{13}C$ and ±8‰ for $\delta^{2}H$) for the instrument used[9]. No significant trends were seen in $\delta^{13}C$ (‰) values as a result of increasing sorption, however, much like Slater et al, larger data scatter was observed for $\delta^{2}H$ isotopes with higher degrees of sorption. Schuth et al surmised that as concentrations approach the detection limits of the analytical instruments, the isotope measurements in general become associated with larger error[9]. This data provides evidence that single-step equilibrium experiments do not show changes in isotopic signatures. However, under environmental conditions, there is the potential for several sorption/desorption cycles, and it is unclear if small isotope fractionation observed during equilibrium experiments are magnified during sorption/desorption cycling.

3.2.5. Multi-Step Batch Experiments

During the migration of CHCs in aquifers and low permeable units, CHCs are likely to undergo successive sorption-desorption steps. Research by Kopinke et al[67], Imfeld et al[68], and Wanner et al[11], have modified the standard single-step batch experiments to include multiple equilibrium sorption steps to understand isotope fractionation more deeply under field-relevant conditions. This is accomplished by allowing single-step batches to reach sorption equilibrium and then transferring a portion of the remaining liquid onto fresh sorptive materials such as AC or low permeable clay. This experiment is repeated until concentrations reach the detection limit of the analytical machinery or when concentrations exceed 90% sorption.

Kopinke et al, [67] studied the sorptive effects of suspended humic acid (HA) on $\delta^{13}C$ of methyl-*tert*-butyl-ether (MTBE), benzene, and toluene diluted in deionized water to concentrations of 100, 200, and 1000 ppm[67]. Batch experiments were allowed to equilibrate over an hour and suspended solids were centrifuged and the remaining clear liquid was transferred to a new vial for use in the next batch experiment. Decreasing concentrations were attributed to sorption onto the suspended HA[67]. Isotopic compositions of headspace samples were analyzed using GC-C-IRMS. MTBE did not significantly bind to HA; therefore, no fractionation was observed. Sorption onto HA by benzene and toluene resulted in an observed $\Delta\delta^{13}C$ ‰ of (1.09 ± 0.24 and 1.20 ± 0.19) respectively[67]. The results of this study, for the first time, stressed the importance of sorption induced fractionation when considering real contaminated aquifer systems. More specifically, sorptive affects need to be taken into account when isotope fractionation is being used to assess *in situ* biodegradation. It is noted that these isotope-based effects are small compared to other mixing effects and biodegradation within a migrating plume, however, significant isotope shifts are observed along real contaminant plumes and need to be better understood[67].

Imfeld et al,[68] further conducted multi-step batch experiments, analyzing carbon and hydrogen isotope effects during the partitioning of benzene and toluene between water and organic sorbents that represent the typical structural components of natural organic matter (NOM). Similar to Kopinke et al, batch experiments for benzene and toluene were dosed to 300 ppm and allowed to equilibrate before being analyzed on GC-IRMS[68]. Among the NOM sorbents, only benzene-octanol showed a significant carbon and hydrogen isotope fractionation during the partitioning between water and 1-octanol in the multi-step batch experiment[68]. The resultant $\Delta\delta^{13}C$ and $\Delta\delta^{2}H$ for benzene as a result of sorption onto 1-octanol were 1.6 ± 0.3‰ and 88 ± 3‰, respectively[68]. The results of the study suggests that functional groups of sedimentary organic matter (SOM), may interact with contaminants migrating through an aquifer system, resulting in potentially relevant isotope fractionation.

More recently, Wanner et al, [11] sought to investigate whether sorption had a significant influence on the isotope ratios of CHCs in a field scenario through the use of multi-step batch experiments and a mock field scenario using 1,2-DCA and DCM. Bench-top batch experiments were conducted in duplicate to quantify the isotope fractionation due to sorption, using a well characterized, saturated low permeable sediment with strong sorptive characteristics. Batch experiments were dosed to concentrations of 7,226 mg/L and 5,983 mg/L for 1,2-DCA and 572 mg/L and 745 mg/L for DCM. All batch experiments were allowed to reach equilibrium before transferring 10 mL of solution to fresh sorbent. The experiment continued until concentrations were too low to determine the isotope ratios. During these multi-step sorption experiments, 1,2-DCA and DCM became increasingly enriched in ^{13}C and ^{37}Cl after each sorption step. This infers that light isotopes were preferentially sorbed compared to heavy isotopes. A larger enrichment of ^{37}Cl was seen compared to ^{13}C for 1,2-DCA, resulting in an isotope enrichment factor of ($\varepsilon_{Cl,sorption}$ = -0.55‰) and ($\varepsilon_{C,sorption}$ = -0.40‰) respectively. For DCM, the enrichment of ^{13}C was larger compared to 1,2-DCA, with an isotope enrichment factor of ($\varepsilon_{C,sorption}$ = -0.54‰). This experiment showed for the first time that isotope fractionation due to sorption occurs for chlorine isotopes in organic compounds. Furthermore, this study showed that sorption induced isotope fractionation can cause shifts of carbon isotope ratios of around 2‰ in saturated low permeable units. This data set provides a starting point to continue to evaluate the relevance of sorption-induced isotope fractionation especially when considering the application of AC remediation amendments which were not used in this study.

The purpose of this study was to 1) determine the extent of chlorine isotope fractionation that may occur through sorption of CE and CB compounds onto GAC during multiple sorption steps; 2) elucidate factors influencing the sorption of CE and CB compounds onto GAC to better determine AC amendment usage and its impact on potential isotope fractionations in the future.

3.3. Materials & Methods

Activated carbon manufactured from Thermo Fisher Scientific (Alfa Aesar) was used in this study. Particle size, as described by the manufacturer, varied in the range of 0.250 to 2 mm (Mean particle diameter 1.55 mm). Activated carbon was washed and dried by manufacturer with a moisture content of 0% and an apparent density, dry of 0.48 g/mL. Activated carbon was kept dry and at constant temperature when not in use.

Methanol was used to prepare stock solutions (6,000 ppm in 10 mL methanol) for the following CE and CB compounds used for chlorine isotope analysis: TCE, PCE, chlorobenzene, 1,2-dichlorobenzene, and 1,2,4-trichlorobenzene.

3.3.1. Instrument Parameters

A purge and trap concentrator (PTC) connected to a (8890) gas chromatograph (GC) system paired to a (5977B) quadrupole mass selective detector (MSD) (from Tekmar Lumin and Agilent, respectively) was used to measure ion abundances. Sample injection was facilitated by manual syringe injection of 5mL UPW, 18 Ω dosed from the benchtop mesocosms. The PTC was operated under specific parameters depending on the compound being analyzed. PTC methods for CEs and CBs were operated according to Table 7 (Appendix I). The GC was equipped with a capillary column (DB-624 30m x 250µm, 1.4µm film thickness) and used helium as a carrier gas. Oven temperatures were programmed according to the compound being injected (chlorinated benzene compounds required higher transfer line temperatures (200 – 250 C°) to be sent from the PTC to the GC-qMS).

The qMS was operated in selected ion monitoring (SIM) mode, considering the following two most abundant ions of the target analytes: TCE: 130, 132 *m/z*; PCE: 164, 166 *m/z*; monochlorobenzene: 112, 114 *m/z*; 1,2-dichlorobenzene: 146, 148 *m/z*; 1,2,4-trichlorobenzene: 180, 182 *m/z*. Specific GC-qMS parameters used for each compound were recorded into Table 6, located in the appendix.

3.3.2. Sorption Experiments

Sorption experiments, both single-step and multi-step, were conducted using 20 mL amber borosilicate glass VOA vials capped with miniert-valve screw caps. Activated carbon (dry) was pre-weighted and placed into trays, to be added as necessary (0.2g ±0.01g). Vials were half-filled with ultrapure water (UPW 18 Ω) and all contaminants were dosed at an initial concentration of 50 mg/L and filled to 20 mL with UPW. Experiments were performed in triplicate. Batches were analyzed prior to the addition of activated carbon for an initial $\delta^{37}Cl/\ \delta^{35}Cl$ isotopic ratio.

3.3.3. Single-Step Sorption Experiments

Pre-weighted activated carbon was added to vail containing TCE, PCE, MCB, 1,2-DCB, or 1,2,4-TCB and allowed to sorb for ~20 minutes with frequent agitation. Sample concentration and changes in isotope ratios were analyzed every ~20 minutes via syringe injection to identify any potential fractionation during active sorption. Single-step sorption experiments were stopped after ~180-200 minutes once the concentrations was below 90% of the initial starting concentration.

Chromatograms obtained after sample injection were manually integrated to obtain areas for light and heavy isotopes for each of the targeted CE and CB molecular ions. Changes in isotopic composition were calculated accordingly as described in Chapter 2 Section 2.3 Rate constants for each compound (k) was derived using Eq (5), where Ct is concentration at time (t), and Ci is the initial concentration. Rate constants were then compared to other literature values and to evaluate factors that influence isotopic fractionation.

$$\ln[C_t] = -kt + \ln[C_i] \tag{5}$$

3.3.4. Langmuir & Freundlich Isotherms

Model isotherms were conducted for each compound using 20 mL amber borosilicate glass VOA vials capped with screw septa caps. Activated carbon (dry) was pre-weighted and placed into each vial (0.2g ± 0.01g). Concentration intervals for each compound were treated at 10, 20, 30, 40, 50, 60, 70 mg/L respectively. This concentration range was selected as not to exceed the water-solubility limit of the chlorinated benzenes. All vials were then placed on a rocking tray upside-down to prevent loss to volatilization, and left to reach equilibrium over 48 hours (previous experimentation had shown 48 hours was more than sufficient to reach equilibrium). Langmuir and Freundlich constants were calculated using Eq (6 &7) respectively, where q_e is the amount sorbed per unit sorbate, K_{Fr} is the Freundlich constant, Ce is the concentration at equilibrium, n is the linearity constant, and q_{max} is the calculated sorption capacity. All values are compared to those reported in literature for accuracy and precision.

$$\log(q_e) = \log(K_{Fr}) + \frac{1}{N}\log(C_e) \tag{6}$$

$$\frac{1}{q_e} = \frac{1}{K_L q_{max}} \cdot \frac{1}{C_e} + \frac{1}{q_{max}} \tag{7}$$

3.3.5. Multi-Step Sorption Experiment

Pre-weighted activated carbon was added to each vial containing TCE, PCE, MCB, 1,2-DCB, or 1,2,4-TCB and allowed to sorb for ~20 minutes with frequent manual agitation, this constituted one step of sorption. Analyte concentrations and stable isotope ratios were measured at the end of each step. After each sorption step, 10 mL of the previous sample was transferred to a new vial containing fresh activated carbon (0.2g ± 0.01g) and topped to 20 mL using UPW and allowed to sorb for an additional 20 minutes. In total, this experiment included 5-6 steps or until concentrations in the vials were below the detection limit of the method (10ng on column), however, the purge volume in the PTC could be adjusted to obtain signals on the GC-qMS within the detection limit to account for loss due to half serial dilutions between each step.. To avoid loss through volatilization, the transfer syringe was submerged into 10ml of fresh UPW in the new vial and capped immediately. Chromatograms obtained after sample injection were manually integrated to obtain areas for light and heavy isotopes for each of the targeted CE and CB molecular ions. Changes in isotopic composition were calculated according to the chapter 2, section 2.3 data analysis section. To quantify the isotopic fractionation factor for sorption, Freundlich constants for the heavy and light isotopes for each compound were substituted into Eq. (8), following Wanner et al, where the light isotope constant ${}^{L}K_F$ and the heavy isotope constant ${}^{H}K_F$ were used for each compound to calculate the fractionation factor for sorption ($\alpha_{sorption}$). Additionally, substituting $\alpha_{sorption}$ into Eq. (9), the enrichment factor for each compound can be derived where applicable.

$$\alpha_{sorption} = \frac{{}^{H}K_d}{{}^{L}K_d} \approx 1 + \Delta\delta_i / \left[1000m\left(1 - X_{dissovled}\right)\right] \qquad \textbf{(8)}$$

$$\epsilon = \left((\alpha - 1) \times 1000\right) \qquad \textbf{(9)}$$

3.4.Results

3.4.1. Single-Step Sorption Experiments

Single-step sorption experiments showed a time-dependent decrease in CHC concentrations, summarized in Figure 7. Control experiments containing no AC were run parallel to sorption experiments to identify any loss of CE and CB compounds due to volatilization, however, minimal changes were seen over the course of the experiment (Figure 7, orange points), suggesting sorption is the primary process affecting CHC concentrations in each batch experiment. The speed and extent of sorption are likely not the result of the pore size, volume, and abundance within the GAC amendment used. However, the size difference between CEs and CBs may in-part be responsible for the sorption characteristics seen in Figure 7 [58–60].

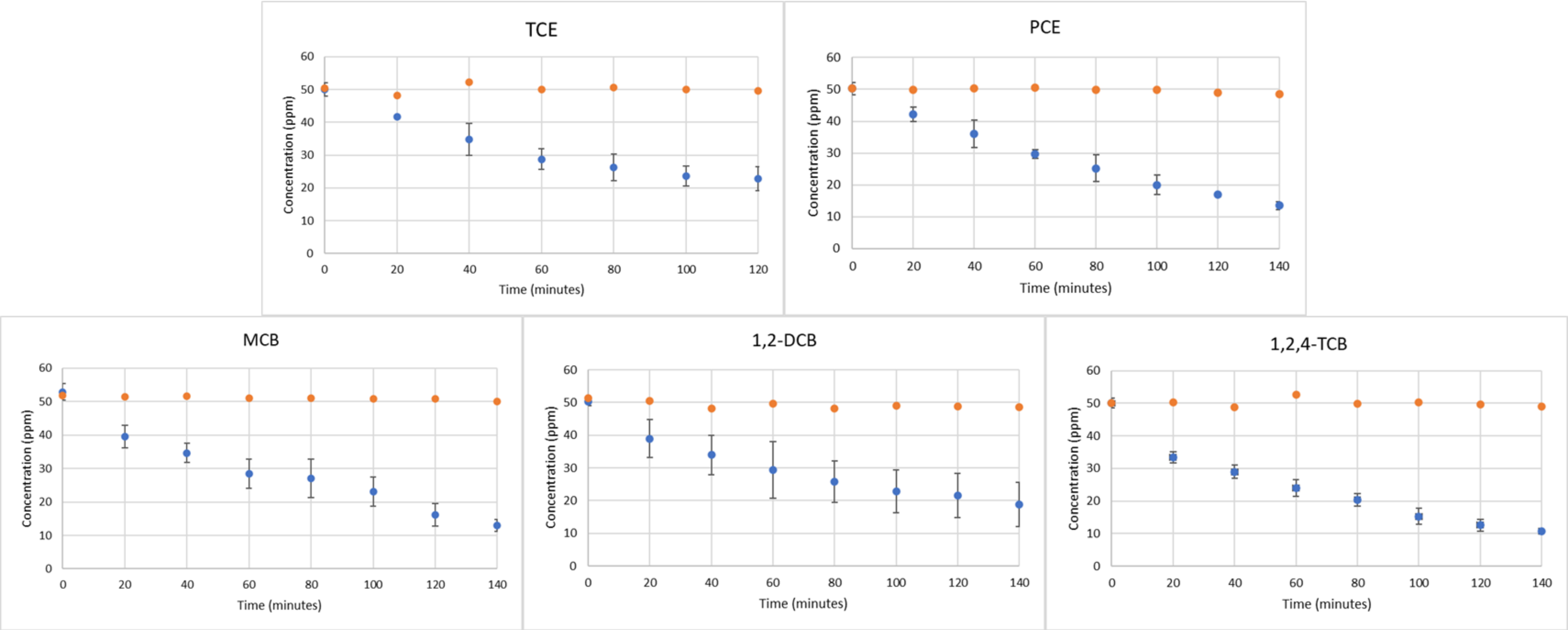


Figure 7. Shows the results of the single step sorption batch experiments for each of the CE and CB compounds onto GAC. The average time for each compound to reach a near equilibrium state was ~120 minutes. Blue points represent the sorption batch experimental data, while orange points represent static control experiments without GAC. Error bars were calculated using the averaged standard deviations obtained for each triplicate experiment.

3.4.2. Kinetics

To better characterize and define potential factors that may influence the extent of sorption within batch experiments rate constants were derived from each of the single-step, single adsorbate batch experiments for each compound (Figure 8). The two most commonly used kinetic models, pseudo-first-order (PFO) and pseudo-second-order (PSO), are employed during experimentation to elucidate trends in resultant data. Typically, single adsorbate systems fall under PFO reaction rates, where the solvent (water) component can be considered a secondary reactant in greater excess relative to the adsorbate. In this scenario, during sorption, the concentration of water will remain unchanged and therefore the rate of adsorption is expressed solely on the concentration of the adsorbate. Rate-limiting factors for systems like these include diffusion and the concentration of the adsorbate[69].

The averaged reaction rate constants (k_1) calculated for TCE, PCE, MCB, 1,2-DCB, and 1,2,4-TCB were 0.00723 ± 0.00119 m^{-1}, 0.0933 ± 0.00833 m^{-1}, 0.0933 ± 0.00143 m^{-1}, 0.0069 ± 0.00236 m^{-1}, and 0.0107 ± 0.000651 m^{-1} respectively (Table 3). Each compound fits the pseudo-first-order kinetics model (Figure 8). In this kinetic model, physisorption through Van der Waals forces is considered to be the controlling factor. Similar experiments conducted using AC materials derived from various sources achieved rate constants that bracket the results reported in this study (Table 3). For CEs, TCE's rate constant falls within the range of reported values by Tseng et al[70], who's experiment used AC at a pH of 7 and produced rate constants ranging between 0.072 - .0.0091 (Table 3). For PCE, the rate constant obtained in this study is comparable to those reported by Yang et al[71] using biochar materials (Table 3). The rate constants obtained in this study do not vary by a large degree given standard deviation (Table 3), therefore it is believed that processes such as steric hinderance have little-to-no effect.

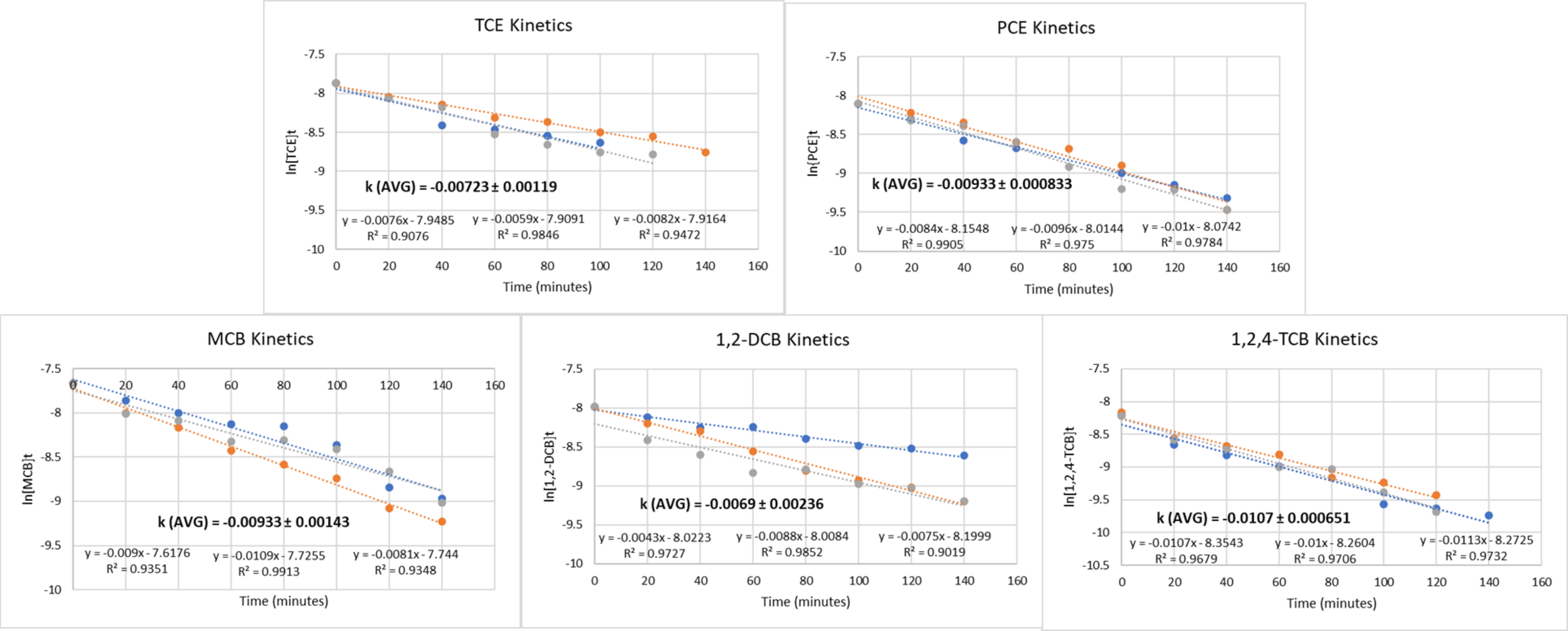


Figure 8. Shows the graphical derivation of the kinetics for each CE and CB compound using data obtained through single-step sorption experiments. Each compound, when the natural log of their concentrations at time t, were plotted against time (t), indicated a PFO model.

Table 3. Summary of rate constants (k) obtained in this study and comparisons to literatue values using similar adsorbents.

Compound	k (m^{-1})
TCE	**0.0072 ± 0.0012 - AC (n = 21)** 0.0072 – 0.0091* - AC @ pH 7 - 11 (Tseng et al., 2011)
PCE	**0.00933 ± 0.000833 - AC (n = 21)** 0.00120 - 0.0158* - Biochar (Yang et al., 2022)
MCB	**0.00933 ± 0.00143 - AC (n = 21)** 0.00994 - 0.00999* - AC (Jia et al., 2023) 0.070 - 0.105* Coconut derived AC (Zhao et al., 2018) 0.19 - PAC (Lin et al., 2015)
1,2-DCB	**0.0069 ± 0.00236 - AC (n = 21)** 0.001 - 0.0085* - Biochar (Lu Han et al., 2016) 0.19 - PAC (Lin et al., 2015)
1,2,4-TCB	**0.0107 ± 0.000651 - AC (n = 21)** 0.001 - 0.0085* - Biochar (Lu Han et al., 2016) 0.42 - PAC (Lin et al., 2015)

Footnote: **Bold** = data obtained in this study, * = converted to m^{-1}

3.4.3. Isotherm Model Fitting

Equilibrium isotherm models were employed to better understand the sorption behavior between CHC compounds and GAC. No single model is universally applicable[72], but instead, model fits are tested with empirical data to deduce sorption behavior. Langmuir isotherms (Lineweaver-Burk)[72] were initially employed to explore these relationships under the assumption of adsorption onto a homogenous surface. For the CE compounds, Langmuir linear-fit tests (Figure 9), produced a Langmuir constant (K_L) of 0.064 L mol^{-1} for TCE and 0.97 L mol^{-1} for PCE indicating interaction between the adsorbent and adsorbate (Table 4). The Langmuir constant related to the energy of adsorption (R_L) for TCE and PCE were 0.24 and 0.021 respectively, indicating the interaction between the adsorbent and GAC to be favorable (Table 4). However, the CB compounds failed the linear-fit test indicating a failure of the Langmuir isotherm model to accurately describe equilibrium interactions for CBs (Figure 9).

Further assessments were made using both linear and non-linear Freundlich isotherm models, which models adsorption onto heterogenous surfaces. Freundlich linear-fit tests (Figure 10) produced Freundlich constants (K_f) of 1.39 and 1.03 for TCE and PCE respectively (Table 4). Non-linear Freundlich models produced in Excel (Figure 10), gave Freundlich constants (K_f) of 0.000143, 0.000164, and 0.327 (mg/g).$(L/mg)^{1/n}$ for MCB, 1,2-DCB, and 1,2,4-TCB respectively. The isotherm data for both models is summarized in Table 4.

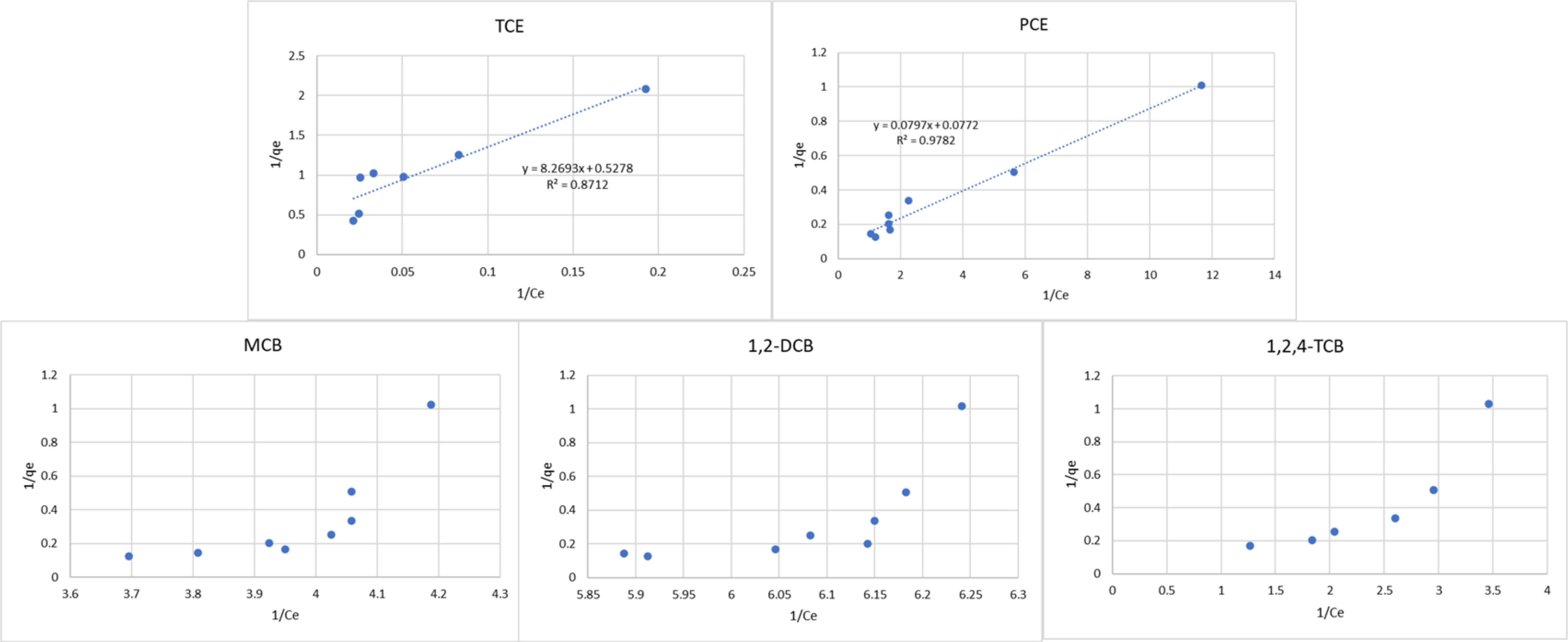


Figure 9. Illustrates the application of Langmuir linear-fit tests. For the CE compounds, the linear-fit test produced Langmuir constants (K_L) of 0.064 for TCE and 0.97 for PCE after using Eq (6). The CB compounds failed the Langmuir linear-fit test, indicating additional surficial interactions between the CB compounds and GAC.

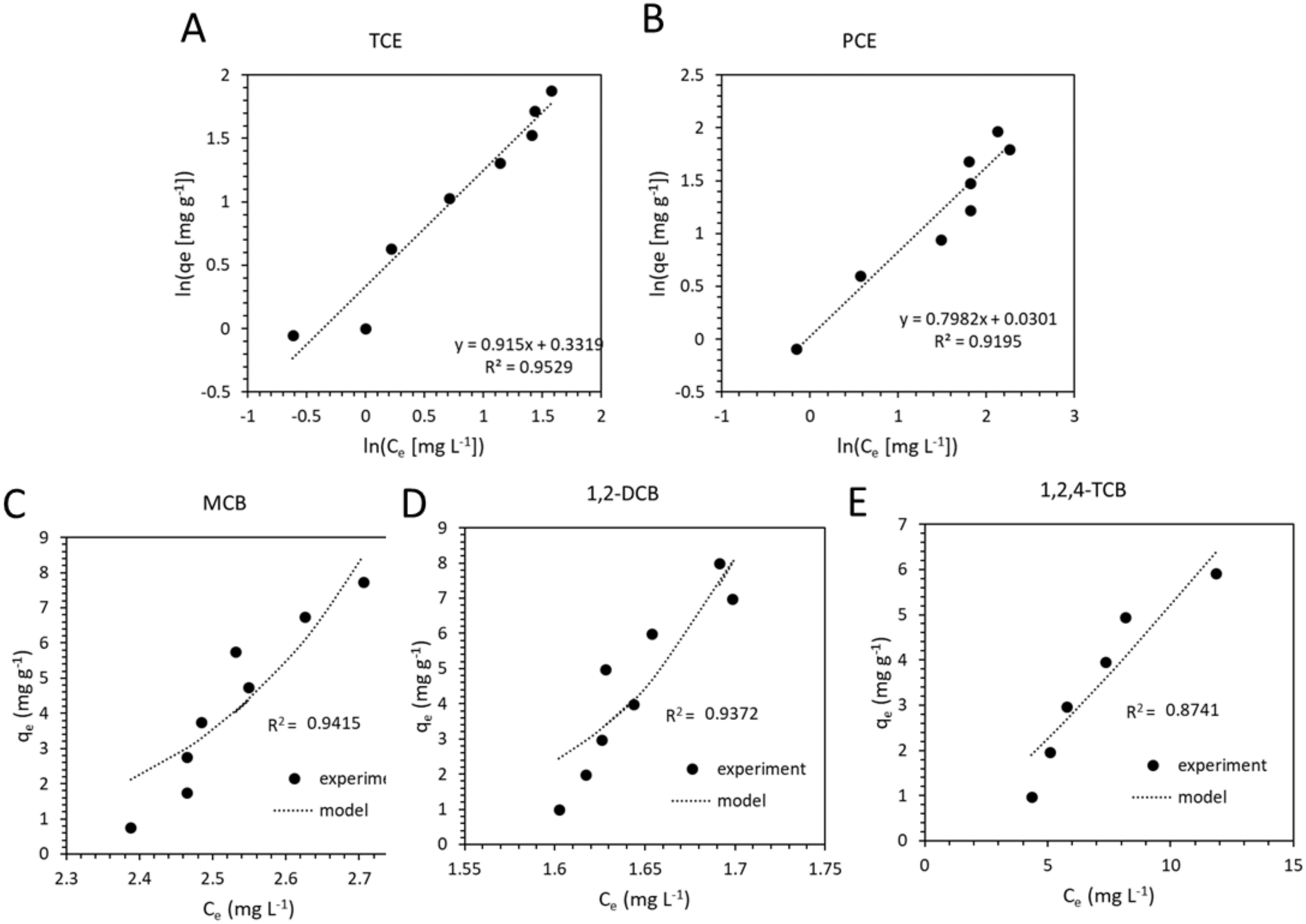


Figure 10. Illustrates the application of both linear and non-linear Freundlich model isotherms to better describe sorption interactions between CE and CB compounds and GAC. A-B; the Freundlich linear-fit tests produced Freundlich constants (K_f) of (1.39) and (1.03) for TCE and PCE respectively. C-E; the non-linear Freundlich models suggested that the CB compound concentrations had yet to plateau. More data points are necessary to find a concentration in which sorption interactions to GAC and multi-layered sorption interactions reach sorption capacity.

Table 4. Summary of Langmuir and Freundlich Model Isotherm constants where K_L is a measure of the level of interaction between the GAC surface and adsorbates, and K_F is a measure of adsorption capacity. Results obtained in other studies are converted to the units used in this study for the purpose of comparison.

Langmuir			**Freundlich**	
Compound	**K_L (L mol-1)**	**q_{max}**	**K_f (mg/g).(L/mg)$^{1/n}$**	**1/n**
TCE	**6.91 (R^2 = 0.99)**	**1.86 (mg/g)**	**1.39 (R^2 = 0.98)** 1.73 - 2.30 (Speth & Miltner et al) 0.337 - 2.08 (Erto et al)	**0.92** 0.45 - 0.52 (Speth & Miltner et al)
PCE	**0.891 (R^2 = 0.96)**	**11.7 (mg/g)**	**1.03 (R^2 = 0.92)** 3.52 - 4.67 (Speth & Miltner et al) 0.337 - 2.08 (Erto et al)	**0.8** 0.48 - 0.56 (Speth & Miltner et al)
MCB	**No Fit**		***0.000143 (R^2 = 0.94)** 7.17 - 11.7 (Speth & Miltner et al)	**11** 0.298 - 0.398 (Speth & Miltner et al)
1,2-DCB			***0.000164 (R^2 = 0.94)** 3.32 - 7.45 (Speth & Miltner et al)	**20.4** 0.57 - 0.81 (Speth & Miltner et al)
1,2,4-TCB			***0.327 (R^2 = 0.87)**	**1.2**

Footnote: **Bold** = data obtained in this study, * = Non-linear model equation

3.4.4. Multi-Step Sorption Experiments

Multi-step sorption experiments reached 90% sorption in approximately 4 steps (Figure 11). Similar to single-step sorption experiments, minimal loss due to volatilization was observed during control experiments that ran parallel to multi-step sorption experiments (Figure 11, orange points). Additionally, greater variance in the data was observed in the CB compounds (MCB and 1,2,4-TCB) compared to the CEs (Figure 11).

3.4.5. Stable Isotope Fractionation During Multi-Step Sorption Experiments

During sorption, shifts in δ^{37}Cl (‰) for CE and CB compounds were measured across individual sorption steps. TCE and PCE showed predicable kinetic behavior and Langmuir behavior, and for these compounds, the cumulative shift in δ^{37}Cl was 1.40 ‰ and 2.22 ‰ for TCE and PCE, respectively (Figure 12). The kinetic behavior of CBs agreed with literature (Table 4), but the isotherm models did not produce suitable fits for MCB or 1,2-DCB. Therefore, the isotope fractionation will only be evaluated for 1,2,4-TCB where sorption behavior is well described. The average cumulative isotope shift observed in the multi-step experiments for 1,2,4-TCB compounds was 4.42 ‰ (Figure 12). Throughout the multistep sorption experiment, CEs and 1,2,4-TCB became increasingly enriched in ^{37}Cl after each individual step (Figure 12). This data is summarized in Table 5.

Following similar experimental procedures conducted by Wanner et al[11], the isotope fractionation factor for sorption was quantified for TCE, PCE, and 1,2,4-TCB using the Freundlich constants for the heavy and light isotopes ($^{H}K_{Fr}$ & $^{L}K_{Fr}$) according to Eq (8). Enrichment factors were further derived from the isotope fractionation factors for sorption and reported in Table 5.

To figure 11

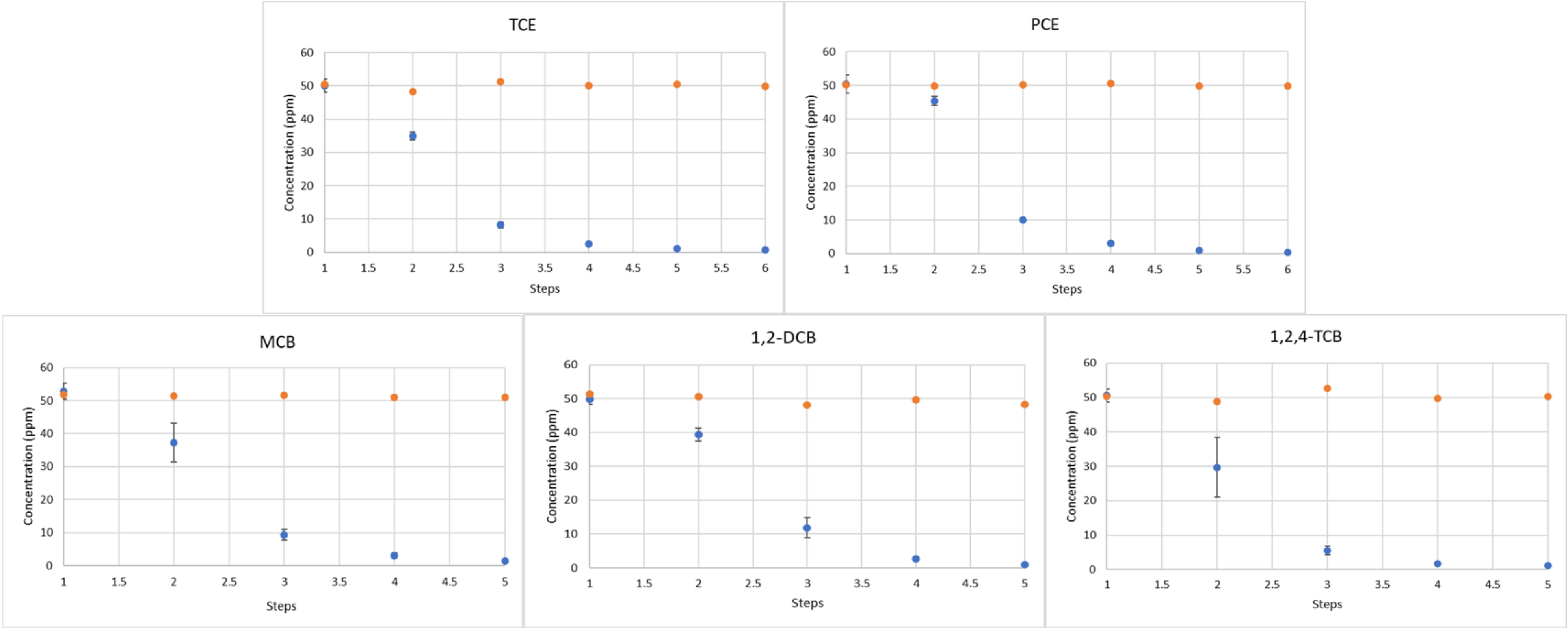


Figure 11. Illustrates the sorption of CEs and CBs onto GAC over the course of multiple sorption steps (blue points), wherein 10ml aliquots of the previous batch experiment was drawn out and placed over fresh GAC. Experiments were cutoff after a ~90% decrease in concentration was seen. Orange points represent control experiments run alongside batch experiments to test for loss due to volatilization.

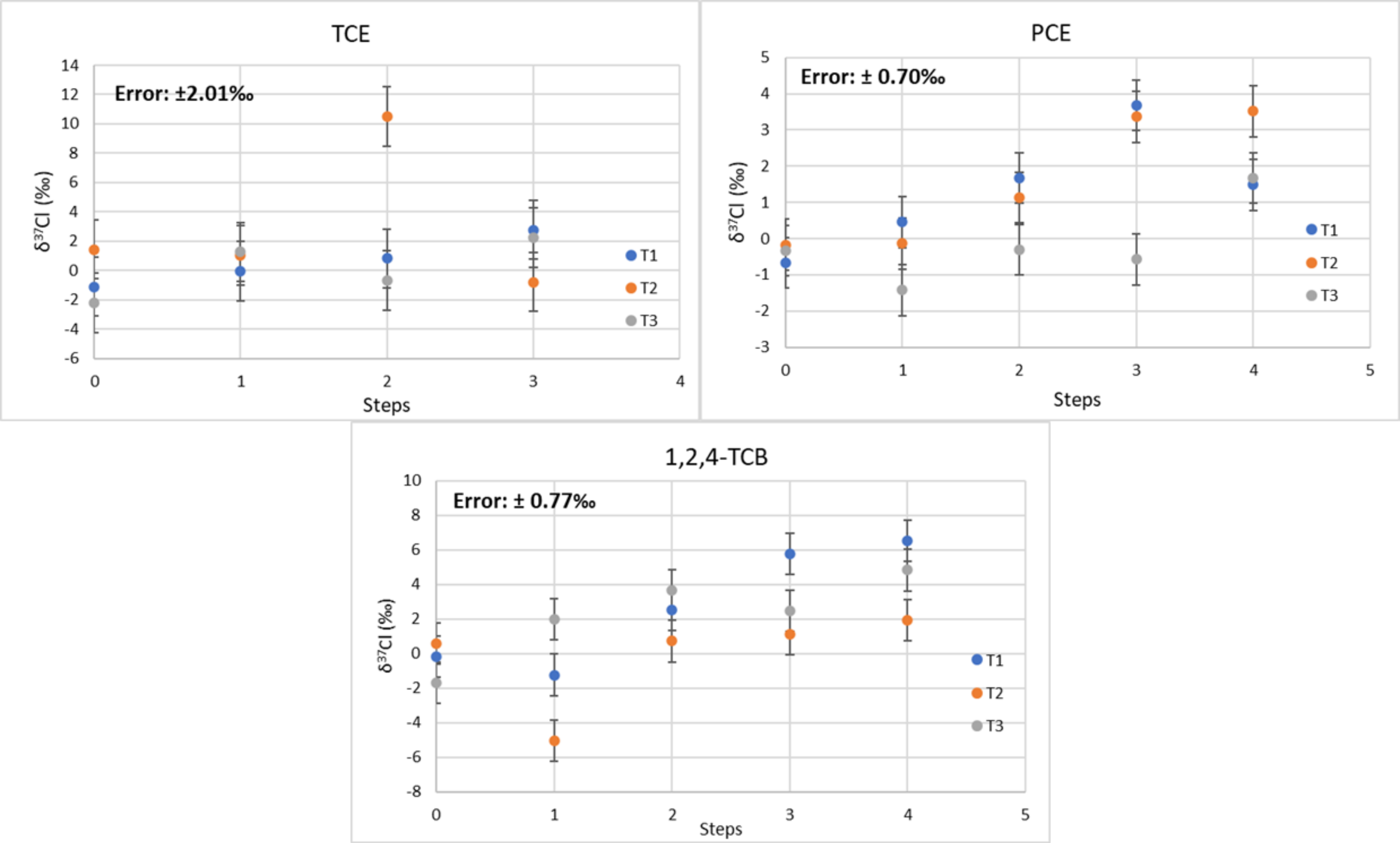


Figure 12. Illustrates the resultant changes in δ^{37}Cl (‰) for each AU-WS CE and CB compounds (trials 1, 2 & 3) after sorption during each step and the associated analytical uncertainty (error bars) calculated in chapter 2.

Table 5. Summary of δ^{37}Cl and uncertainty (‰) for AU multi-step sorption experiments as determined by GC-qMS at Auburn University

AU Multi-Step Sorption Experimental Data

Compound	**δ^{37}Cl (‰) SMOC**	**α**	**ε**
TCE	1.4 ± 2.01	-	-
PCE	2.2 ± 0.70	0.998 ± 0.2	-1.2
1,2,4-TCB	4.4 ± 0.77	0.994 ± 0.06	-5.4

3.5.Discussion

It is important to better understand the factors that may influence sorption characteristics that in-turn may affect the interpretations of isotopic fractionation. The importance of these factors is further stressed when applying industry remediation amendments such as AC to field scenarios.

3.5.1. Kinetics and influence on Sorption Characteristics

While single-step sorption experiments showed little-to-no change in the isotopic composition of $\delta^{37}Cl$ (‰) for both CE and CB compounds, the kinetic data derived from these experiments along with the model isotherms provided great insight into the underlying processes that may affect isotopic fractionation through sorption in the future. The kinetic data calculated through Eq (5) produced results comparable to literature values using similar AC amendments (Table 3). These amendments include GAC and biochar, however coconut-based AC materials and PAC amendments produced greater rate constants (k_1) suggesting faster rates of CE and CB sorption to these materials (Table 3).

As an example, 1,2,4-TCB yielded a rate constant (m^{-1} of 0.0107 ± 0.000651 m^{-1}. Similar to Lu Han et al[73], the rate constant obtained in this study matched the rate constant obtained by Lu Han using biochar 0.001 ± 0.0085 m^{-1}, however, Lin et al[74] determined PAC amendments produced a rate constant of 0.42 m^{-1} suggesting 1,2.4-TBC sorbs much quicker to PAC materials. This same demonstration was made for five other chlorinated benzene compounds including MCB & 1,2-DCB[74].

While the rate constants obtained in this study are relatively close between the CE and CB compounds, previous studies by Lu Han et al[73] revealed faster rate constants for CEs like TCE compared to CB compounds (k_1 ranged from 0.06 to 1.14 with TCE towards the faster end of this spectrum). Typically, there is a positive correlation between the k_1 of CHC compounds and the

$\log K_{OW}$ of the compounds, however, TCE demonstrates the opposite trend, possibly explaining why CBs having strong hydrophobicity are readily attracted to AC surfaces[73]. The relatively high aqueous solubility and small molar volume of TCE is likely to contribute to faster rate constants[75]. Additionally, TCE, with a molecular size of 4.9 Å, is not greatly affected by steric hindrance during micropore-filling compared to larger CB compounds[76,77]. However, with the GAC used in this study, steric hindrance may not play an important role as all the rate constants are all relatively similar.

To further elucidate factors influencing sorption onto GAC, Langmuir and Freundlich model isotherms were created (Figures 9-10). Of the Langmuir model isotherms, only the CEs conformed to the Langmuir model, evident by the linear fit tests producing a straight line (Figure 9). The Langmuir constants (K_L) obtained from this model for TCE and PCE were 6.91 & 0.891 L mol^{-1} respectively, suggest a stronger interaction between TCE and GAC compared to PCE, which may be the result of its aqueous solubility and small molar volume.

The CBs did not conform to the Langmuir model and instead produced a curved graph (Figure 9). The failure of the Langmuir model for the CB compounds suggests surficial interactions between the CBs and GAC not accounted for by the Langmuir model. Such interactions include multi-layered sorption occurring between CB molecules[79], represented by the curved plot (Figure 9). For this reason, Freundlich model isotherms were employed to better elucidate the surficial interactions taking place.

The CE linear-Freundlich model isotherms produced Freundlich constants K_F of 1.39 and 1.03 $(mg/g)/(L/mg)^{1/n}$ for TCE and PCE respectively (Table 4). This constant is a measure of the adsorption capacity, with a larger K_F suggesting greater adsorption capacities. Erto et al[78], conducted a study analyzing various intrinsic properties of AC materials and the affects they had on the adsorption capacities of TCE and PCE Freundlich model isotherms[78]. Among the 12 AC materials used, the Freundlich constants obtained ranged from 0.337 – 2.08 (mol/kg)/(mol/L)^n for TCE and PCE[78], which generally agree with the results observed here.

By dividing 1 by the model isotherm constant n, the degree of linearity ($1/n$) for each compound across their concentration ranges can be measured. For the CEs, the values of $1/n$ were 0.92 and 0.80 for TCE and PCE respectively (Table 4). The typical values of $1/n$ will range from 0.7 to 1.0 in L-type isotherms and show that as concentrations increase, the relative adsorption decreases due to the saturation of adsorption sites and no additional processes impeded adsorption[80]. Additionally, when paired with the R^2 values for TCE and PCE which were 0.98 and 0.92 respectively, which suggests that the behavior of TCE and PCE is confidently described (Table 4)[80].

The CB compounds failed the Freundlich linear-fit test and thus the non-linear Freundlich model isotherm was employed to better describe the surficial interactions taking place between the CBs and GAC. Among the CB compounds, 1,2,4-TCB produced a 1/n of 1.20 while MCB and DCB failed this test (Table 4). Therefore 1,2,4-TCB was the only compound that produced a K_F equaling 0.327 (mg/g)/(L/mg)$^{1/n}$. No literature comparison exists for 1,2,4-TCB sorption to AC materials; additionally, the value of K_F lies outside the typical range of 0.5 to 50 (mg/g)/(L/mg)$^{1/n}$. Lastly, the R^2 for 1,2,4-TCB was 0.87, lower than that of the CEs.

The nonlinear-Freundlich models for MCB and 1,2-DCB seem to suggest sorption capacity was never reached, even at the highest concentration (100 ppm). The range of concentrations in this experiment are orders of magnitude smaller compared to other studies[7,11,67,68], in an attempt to reflect field-relevant concentrations. Likewise, multi-layered sorptive interactions would have a considerable effect on the resultant K_F, evident by the Freundlich constant $1/n$ being extremely large, indicating an S-type isotherm. Additionally, S-type isotherms, while relatively uncommon, are often observed at low concentration ranges[80].

Comparisons of the Freundlich constants obtained in this study to those obtained by Speth and Miltner et al[81], show varying levels of interactions (Table 4). The range of Freundlich constants (95% confidence intervals) reported by Speth & Miltner for TCE and PCE were 1.73 -2.30 $(mg/g)/(L/mg)^{1/n}$, and 3.52 – 4.67 $(mg/g)/(L/mg)^{1/n}$ respectively[81]. The Freundlich constants obtained in this study 1.39 and 1.03 $(mg/g)/(L/mg)^{1/n}$ respectively, while lower, may be the result of differences in concentration ranges and the amount of GAC used in the isotherms. The degree of linearity (1/n) obtained in this study for TCE and PCE were 0.92 and 0.8 respectively, indicative of the saturation of adsorption sites on GAC.

Of the CB compounds in Speth and Miltner et al's[81] study, only MCB and 1,2-DCB were analyzed and had Freundlich constants reported. The constants reported for MCB and 1,2-DCB were 7.17 – 11.7 and 3.32 – 7.45 $(mg/g)/(L/mg)^{1/n}$ respectively, and are orders of magnitude higher than the Freundlich constants obtained in this study (Table 4). One potential reason for the difference in obtained Freundlich constants could be the concentration range used in this study (10-100 ppm), whereas Speth and Miltner et al[81] ran adsorption isotherm concentrations in ppb. Additionally, the degree of linearity suggests adsorption equilibrium was never reached, potentially as a result of continued multi-layered sorption interactions. For these reasons, MCB and 1,2-DCB are not considered when determining the extent of isotopic fractionation as a result of multiple sorption steps.

3.5.2. Multi-Step Isotope Fractionation

In the first step of each batch experiment, the extent of fractionation was within the analytical uncertainty for all compounds. This, as previously discussed in Chapter 1: Introduction, agrees with the findings of Slater et al[8], and Schuth et al[9], who observed no significant sorption-induced isotope effects in single-step sorption experiments.

Beyond this initial first step, the isotope ratio of TCE remained within the analytical uncertainty calculated in Chapter 2, with a total averaged isotope shift of ($\delta^{37}Cl = 1.4 \pm 2.01$‰) for this reason, the fractionation factor (α) & the enrichment factor (ε) cannot be quantified. For PCE and 1,2,4-TCB the isotopic fractionation exceeded the analytical uncertainty after the second sorption step and resulted in cumulative averaged isotope shifts of $\delta^{37}Cl = 2.2 \pm 0.70$‰ and $\delta^{37}Cl = 4.4 \pm 0.77$‰, respectively.

It is noted that among the compounds tested, the failure to observe considerable shifts in isotope ratios was for compounds containing less than 3 chlorine atoms in their molecular structure. As detailed in chapter 2, section 2.6.2, the molecular ion method used to calculate the total shift in the isotopic signatures of each compound is not as reliable on compounds containing fewer than 3 chlorine atoms[45]. These results are likely an indication that the multiple ion method, encompassing ion fragments beyond the parent peaks, is needed to accurately detect changes in these compounds.

The enrichment factors, ε, measured in these experiments are more negative than those reported by Wanner et al[11]. First, this is likely due to the higher affinity for AC in the compounds studied here. This may, in part, be explained by a potential relationship between the organic carbon-water partitioning coefficient (K_{OC}) also known as the adsorption coefficient and the extent of fractionation observed. Compounds with a higher $Log(K_{OC})$ are less mobile organic chemicals and tend to sorb onto soil and AC surfaces more readily, while lower Log(K_{OC}) values indicate chemicals with higher solubility and thus higher mobility. 1,2,4-TCB with a Log(K_{OC}) of 2.64 demonstrated the greatest enrichment factor at $\varepsilon = -5.4$ compared to PCE, who's Log(K_{OC}) = 2.37, had an enrichment factor of $\varepsilon = -1.2$. More research is needed to suggest a potential relationship and its effect on the isotope ratios and enrichment factors in future studies. Second, the magnitude of the enrichment factor is likely affected by the sorptive material being used. Wanner et al.[11] used saturated, low permeable sediments, while this study focuses on AC amendments, which as discussed in the chapter 3 introduction, has a greater microporosity which controls the rate of adsorption for CHCs onto its surface and pores compared to low permeable soils. More research is needed to better quantify the extent to which AC amendment types effects the resultant isotope fractionations observed. These types of research include the modification of AC to better suit the types of contaminant remediation projects through surficial chemistry modification and better techniques to track changes in isotope ratios that match the speed in-which AC amendments tend to reach equilibrium compared to low permeable soils.

3.6.Conclusion

This study provides additional insight into the evidence for sorption-induced isotope fractionation initially brought forth by Wanner et al[11]. Through the use of multiple sorption steps, a shift in the isotopic signatures for the CE and CB compounds (excluding MCB due to the failing of the Freundlich model isotherms to accurately predict sorption interactions) was detected. However, very few of the compounds analyzed (PCE and 1,2,4-TCB) managed to produce isotopic shifts beyond the analytical uncertainties derived in chapter 2. To this extent, this study continues to stress the importance and greater need for inter-laboratory comparisons to more accurately constrain the analytical uncertainty (both in measurements and mathematical analysis) in benchtop experiments reflecting field scenarios. This is especially necessary for experiments in which these inter-laboratory and international standards do not exist, where-by the precision and accuracy of the results are in question.

Chapter 4: Summary and Outlook

The effectiveness of any *in situ* treatment during contaminant remediation is usually assessed by monitoring the concentrations of contaminants over time, however, as address in this study, the monitoring of concentration alone is not enough to prove the complete remediation of contaminants in field scenarios. This is in-part due to physical processes that are likely to sequester contaminants and through processes such as back-diffusion, continue to contaminate groundwater systems for prolonged periods of time. CSIA has proven that it is a great diagnostic tool that aids in remediation efforts by identifying changes in the isotopic signature of the contaminants and can aid in unraveling competing mechanisms in contaminant assessment, including differences between degradative (biotransformation) and non-degradative (sorption) processes. However, physical processes that may have a cumulative effect on the isotopic signatures of CHC compounds in field scenarios are important to decipher if *in situ* remediation techniques are to be deployed successfully for contaminant removal. As previously discussed, *in situ* contaminant remediation techniques are leading the industry, however, little is known about how remediation amendments such as AC alter the isotopic signatures of target contaminants compared to known isotopic shifts induced through biotransformation.

This study sought to study any potential effects that AC may have during future uses in *in situ* applications and quantify changes in isotopic signatures. Throughout this study, a greater need for interlaboratory comparisons of accuracy and total analytical uncertainty in GC-qMS systems and evaluation schemes were and still are needed if alternatives to traditional GC-IRMS systems are to continue to be developed and aid in future studies. A larger body of shared knowledge and the further development of laboratory and international standards are necessary if processes present in field scenarios are to be evaluated accurately. As previously stated in Chapter 2, it is important to include a minimum of two well defined compound-specific calibration standards with known $\delta^{37}Cl_{SMOC}$ to obtain true differences and to convert values to the SMOC scale for accuracy. However, many standards for chlorine isotope analysis of various CHCs do not exist or have no IAEA standard[54], as seen in the CB compounds used in this experiment. Additionally, the differences in techniques and instrumentation have a significant effect on the analytical uncertainty. For example, differences obtained by measuring the interlaboratory standards provided by Kuder et al, personal communications, may be attributed to his use of cryogenic traps[51,52]. Likewise, the best mathematical schemes used to calculate the isotope ratios are dependent on the number of chlorine atoms present in each compound, and are potentially masked by the increasing presence of carbon atoms.

To illustrate areas in which continued research is necessary, previous batch experiments conducted during the preliminary period of this study found that PAC reduced the overall CE and CB compound concentrations too quickly to track changes in isotope ratios between steps. This may pose greater difficulty in analyzing/quantifying shifts in isotope ratios in future studies where PAC (commonly used in *in situ* studies) or low permeable soils with similar characteristics are present. New developments in analytical methodology are necessary to track changes in isotope ratios during sorption to these materials. Likewise, research focusing on the duality of AC amendments and soil sorption behaviors in tandem is likely necessary to better quantify the overall change in isotope ratios due to physical processes from both, as both are likely to be present in field scenarios.

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Appendix I

Table 6. Summary of Purge & Trap parameters according to CHCs.

Compound	Purge Temp.	Purge Time	Flow	Desorb Temp.	Desorb Time	Bake Temp	Bake Time	Transfer Line Temp.
TCE	20 °C	11.00 min	40 mL/min	200 °C	2.00 min	280 °C	2.00 min	200 °C
PCE								
MCB	20 °C	11.00 min	40 mL/min	250 °C	2.00 min	280 °C	2.00 min	220 °C
1,2-DCB								
1,2,4-TCB								