2014

The Study of the Degradation Rates of the Hexabromocyclododecane Isomers with Polysulfides at 25 oC and pH 9 in 80% Water / 20% Methanol

John H. Wilson
CUNY City College of New York

How does access to this work benefit you? Let us know!
Follow this and additional works at: http://academicworks.cuny.edu/cc_etds_theses
Part of the Chemistry Commons

Recommended Citation
http://academicworks.cuny.edu/cc_etds_theses/490

This Thesis is brought to you for free and open access by the City College of New York at CUNY Academic Works. It has been accepted for inclusion in Master's Theses by an authorized administrator of CUNY Academic Works. For more information, please contact AcademicWorks@cuny.edu.
The Study of the Degradation Rates of the
Hexabromocyclododecane Isomers with Polysulfides at 25 °C
and pH 9 in 80% Water / 20% Methanol

A Thesis Presented to
The Faculty of the Chemistry Program
The City College of New York

In (Partial) Fulfillment
of the Requirements for the Degree
Master of Science

By
John H Wilson III
December 2013
APPROVED FOR THE CHEMISTRY PROGRAM

Prof. Urs Jans 12/12/2013

Prof. Pengfei Zhang 12/12/2013

Prof. Barbara Zajc 12/12/2013
1. Abstract

The three dominant isomers (\(\alpha\), \(\beta\), \(\gamma\)) of hexabromocyclododecane (HBCD) were observed for their individual rates of degradation with polysulfide under anoxic conditions at pH 9 and 25 °C. Research performed by others indicate that in non-biotic media such as soil and sludge the average distribution of the three main isomers are 15% \(\alpha\), 10% \(\beta\), and 75% \(\gamma\)-HBCD, whereas in marine mammals the distributions are 95% \(\alpha\), 2% \(\beta\), and 3% \(\gamma\)-HBCD, respectively. The three predominant isomers exist in the industrial grade HBCD product with the following distribution: 13% \(\alpha\), 2% \(\beta\), and 85% \(\gamma\)-HBCD. This indirectly indicates that \(\alpha\)-HBCD is the most persistent of the three main isomers of HBCD in the environment. This study is geared towards understanding why \(\alpha\)-HBCD bio-accumulates, by studying the rates of reaction of \(\alpha\),\(\beta\),\(\gamma\)-HBCD with polysulfide and bisulfide. Polysulfide and bisulfides are found in anoxic pore water and sediment systems, and may prove to be an important pathway for \(\alpha\),\(\beta\),\(\gamma\)-HBCD degradation in the environment. The second order rate constants of \(\alpha\),\(\beta\),\(\gamma\)-HBCD degradation with polysulfide, hydrogen sulfide, and the first order rate constant for hydrolysis were determined at pH 9 and 25 °C in 80% water/20% methanol solutions. Triple injections were made on a LC-MS/MS system using electron spray ionization in negative mode with MRM. The order of the reactions with respect to the polysulfide concentration at pH 9 and 25 °C in 80% water/20% methanol were found to be: \(\alpha\) = 1.12 ± 0.93, \(\beta\) = 1.37 ± 0.95, and \(\gamma\) = 1.01 ± 0.89. The order of the reaction with respect to polysulfide was confirmed as pseudo first order for all three isomers. The second order rate constants of \(\alpha\),\(\beta\),\(\gamma\)-HBCD with polysulfide at pH 9 and 25 °C in 80% water/20% methanol were found to be 2.37 ± 2.08 M\(^{-1}\)hr\(^{-1}\), 22.6 ± 18.4 M\(^{-1}\)hr\(^{-1}\) and 24.2 ± 36.0 M\(^{-1}\)hr\(^{-1}\). The second order rate constants of \(\alpha\),\(\beta\),\(\gamma\)-HBCD with bisulfide at pH 9.5 and 25 °C in 80% water/20% methanol were found to be 0.414 ± 0.059 M\(^{-1}\)hr\(^{-1}\).
1.54 ± 0.118 M⁻¹ hr⁻¹ and 4.62 ± 0.71 M⁻¹ hr⁻¹. The first order rate constants of α,β,γ-HBCD with hydrolysis at pH 9 and 25 °C in 80% water/20% methanol were found to be 3.0 (± 0.7) x 10⁻⁴ hr⁻¹, 8.0 (± 0.8) x 10⁻⁴ hr⁻¹ and 4.3 (± 1.6) x 10⁻⁴ hr⁻¹. The result of these experiments, indicate that the α-HBCD isomer is more persistent than the β and γ-HBCD isomers under these conditions, and that polysulfides and bisulfide in the natural environment may provide a faster degradation process than hydrolysis alone.

2. Introduction:

HBCD (1,2,5,6,9,10-hexabromocyclododecane) is the third most widely used brominated flame retardant in the world. The U.S. import/production of HBCD was 10 – 50 million pounds in 2002 and 2006, respectively. HBCD is used as a flame retardant in expanded polystyrene foam (thermal insulation boards), extruded polystyrene foam (thermal insulation boards), textile back coating (upholstery), and high impact polystyrenes (used in electrical equipment). These materials are common construction materials and household items that end up in city landfill or are shipped out for incineration. Disposal in landfills will result in leachate migration of HBCD into surrounding waterways. Incineration of HBCD containing materials will result in ash and small particulates that may disperse a far distance through atmospheric transport. The methods currently used for disposal and the relative persistence of HBCD to environmental degradation allows for the migration and detection of HBCD in remote areas, and bioaccumulation in animals.

In 2010 the US EPA wrote an HBCD action plan. The EPA is currently reviewing HBCD presence in humans, its persistence in the environment, bio-accumulative ability as well as
bio-magnification potential, and toxic characteristics to determine if they will list HBCD under TSCA (Toxic Substance Control Act) sections 4 and 5, Emergency Planning and Community Right-to-Know Act (EPCRA) 313. HBCD is of international concern and has been added to ECHA’s (European Chemicals Agency) list of Substances of Very High Concern on October 28, 2008 [14].

HBCD has been detected in air with concentrations ranging from 2 – 610 pg/m$^3$[15]. Suspended solids and soils have been found to have HBCD levels of 1,700 ng/g (suspended particulate material), and 111 – 23,200 ng/g dry weight in soils near HBCD processing factories [15]. HBCD concentrations in fish have been found to be as high as 10,275 ng/g lw (lipid weight) [16]. Porpoises stranded on the Irish and Scottish coasts were found to have an average HBCD concentration of 2,900 ng/g lw and a maximum of 9,600 ng/g lw, while dolphins off the west coast of Ireland were found to have HBCD lipid weight concentrations of 900 ng/g [17]. Norwegian human breast milk was found to have HBCD concentrations as high as 20 ng/g lw [18]. The distribution of the three main HBCD stereoisomers varies between biotic and non-biotic media. In non biotic media such as sediment soil and sludge the average distribution is 15% $\alpha$, 10% $\beta$, and 75% $\gamma$-HBCD. In air and dust the distribution is approximately 45% $\alpha$, 10% $\beta$, and 45% $\gamma$-HBCD, respectively [15]. In comparison, the average stereoisomer distribution in biotic organisms such as aquatic invertebrates, marine fish, freshwater fish, birds, and marine mammals is approximately 80% $\alpha$, 5% $\beta$, and 15% $\gamma$-HBCD. Food chain trends have been observed with the trend increasing in $\alpha$-HBCD %. Aquatic invertebrates have been observed with an HBCD stereoisomer ratio of 70% $\alpha$, 5% $\beta$, and 25% $\gamma$. Marine mammals have been observed with an HBCD stereoisomer ratio of 95% $\alpha$, 2% $\beta$, and 3% $\gamma$ HBCD [15]. The accumulation of $\alpha$-HBCD in larger marine mammals is indirectly due to several factors. One factor is the persistence of $\alpha$-HBCD to abiotic
environmental degradation pathways such as reduction, oxidation, hydrolysis, nucleophilic substitution, elimination reactions, and photolysis. Once it persists in the environment it then enters into the food chain where it is persistent in most organisms and then accumulates at higher levels in predatory species. This leads to the second factor for $\alpha$-HBCD bio-accumulation, which is the persistence of $\alpha$-HBCD to biotic environmental degradation pathways such as lower organism chemical transformation of $\alpha$-HBCD via metabolic transformation/digestion, and mammalian metabolic transformation/digestion. This study focuses on the abiotic elimination and or nucleophilic substitution reactions that $\alpha,\beta,\gamma$-HBCD undergo with reduced sulfur species. In particular, this study looks at the chemical kinetics and degradation pathways of $\alpha,\beta,\gamma$-HBCD with polysulfides, hydrogen sulfide, and hydrolysis.

HBCD is synthesized by bromination of the four possible isomers of cyclododecane-1,5,9-triene (CDT). Bromination of CDT results in six stereo centers at positions 1,2,5,6,9 and 10. As a result of the bromination of the four possible CDT isomers there are sixteen stereoisomers of HBCD that can be formed. There are six diastereomeric pairs of enantiomers and four meso forms [2]. The three predominant diastereoisomers $\alpha, \beta, \gamma$ exist in the industrial grade HBCD product with the following percentages: $\gamma = 85\%$, $\alpha = 13\%$, and $\beta = 2\%$, respectively [15]. The structure of the enantiomer pairs of $\alpha,\beta,\gamma$-HBCD are shown below in schematic 1.
Schematic 1. Structure of the three main diastereomers $\alpha,\beta,\gamma$-HBCD, and their enantiomer pairs.

The water solubility of the three main isomers are as follow: $\alpha$-HBCD = 48.8 ± 1.9 $\mu$g/L, $\beta$-HBCD = 14.7 ± 0.5 $\mu$g/L, and $\gamma$-HBCD = 2.1 ± 0.2 $\mu$g/L [13], and the formula weight of HBCD is 641.7 g/mol. The solubility of the three main isomers in a saturated saltwater media are: $\alpha$-HBCD = 34.3 $\mu$g/L, $\beta$-HBCD = 10.2 $\mu$g/L, and $\gamma$-HBCD = 1.76 $\mu$g/L [18]. In abiotic samples (sediment and sludge) it has been shown that the predominant isomer present is $\gamma$-HBCD, which agrees with the known concentration of $\gamma$-HBCD in technical HBCD. Bioaccumulation in seal blubber, fish and bird eggs have been observed. The elevated levels of $\alpha$-HBCD in seal blubber and fish is most likely due to the greater persistence of $\alpha$-HBCD in the environment. Another possible method of HBCD intake into mammalian species is via inhalation. HBCD crystals are 100 micron’s in size which would allow for deep lung penetration followed by diffusion into the blood stream and accumulation in fatty tissue. It has also been shown that HBCD is present in air levels of 1.2 – 1.8 pg/m$^3$ [3].
It is believed that due to HBCD’s hydrophobic nature, HBCD will partition onto available solid phases such as surface soils and will eventually make their way into anaerobic sediment layers where reduced sulfur species are present. Concentrations of hydrogen sulfide and polysulfide in coastal marine sediment pore waters have been reported to be as high as 5.6 mM and 0.33 mM, respectively [4]. The most probable mechanisms for the degradation of HBCD is the E2 type oxidation/reduction between bromine and a reduced sulfur species resulting in the formation of a double bond to yield tetrabromocyclododecene and HSBr [schematic 2]. The polysulfide or bisulfide attacks the vicinal bromides and act as reducing agents. The arrangement of the bromines must be in an anti-coplanar transition state in order for this mechanism to proceed.

A)

B)

Schematic 2. A) General mechanism for E2 reduction of a hydrocarbon with vicinal bromines. B) Proposed mechanism for the stepwise degradation of HBCD by polysulfides. Structure 1: 1,2,5,6,9,10-Hexabromocyclododecane, Structure 2: 5,6,9,10-Tetrabromo-cis-1-cyclododecene, Structure 3: 9,10-Dibromo-cis-trans-1,5-cyclododecadiene, Structure 4: Cis-trans-trans-1,5,9-cyclododecatriene
The instrument of choice for HBCD analysis is LC-MS/MS. GC-MS or GC-FID/GC-ECD techniques are often not reproducible due to the chemical nature of HBCD. Separation of the three predominant individual isomers may be hindered by the ability of HBCD isomers to undergo thermally induced isomerization at 160 °C after 15 minutes [20]. The thermal decomposition temperature for HBCD has been found to be 220 °C and it is autocatalytic. The thermal decomposition of HBCD results in HBr production and therefore multiple HBCD products are observed [21]. Both of these issues may further complicate the experiments and present a false sense of degradation product identification when using gas chromatography. The use of gas chromatography techniques are also unable to measure the rates of individual HBCD isomer degradation, because separation of the three main isomers of HBCD is not possible when using gas chromatography. In addition when using GC-MS, the ion counts of daughter ions would be in abundance in comparison to the parent HBCD m/z ion of 640.8. This would decrease the signal to noise ratio. It is also known that halogenated aliphatic hydrocarbons are often more difficult to ionize in positive mode MS due to their electronegativity in comparison to their non-halogenated counter parts. This would also lead to a lower amount of parent ions produced, which would result in a lower signal to noise ratio of the parent compound and would require higher concentrations of HBCD for detection and quantification.

Experiments at 25 °C with a solvent ratio of 80% water and 20% methanol will require experiments to be conducted at very low concentration due to the low solubility of HBCD in water. LC-MS/MS methods separate the HBCD isomers at room temperature were thermal degradation of HBCD is not possible and thermally induced isomerization is eliminated as a potential reproducibility issue for separation of the three dominant isomeric forms. External
HBCD standards were analyzed each time samples were analyzed to assure the instrument produced reproducible results for each isomer. If thermal isomerization or degradation were to occur in this experimental setup, it would only take place after separation during the ionization process. LC-MS/MS ESI (electron spray ionization) MRM (multiple reaction monitoring) in negative mode with a 0.2% or 0.1% formic acid in methanol and water are used in this work. The use of LC-MS/MS MRM techniques allowed for the detection of the three HBCD isomers at concentrations between 100 nM and 10 nM total HBCD. The isomeric composition of the technical grade HBCD was not considered in these experiments. The concentration of each isomer is substituted with the total values for both calibration curves and experiments.

The focus of this work was to determine the second order rate constant of $\alpha,\beta,\gamma$–HBCD by varying the concentration of polysulfide (reducing agent) at a fixed pH of 9, at a temperature of 25 ºC in a solvent of 80% water/ 20% methanol. The second order reaction rate constant of total HBCD with polysulfide in 80% methanol/20% water at 40 ºC was reported as $2.2 \times 10^{-2} \text{ M}^{-1}\text{s}^{-1}$ ($79 \text{ M}^{-1}\text{hr}^{-1}$) by Lo and Jans in previous work [1]. The temperature was changed from 40 ºC to 25 ºC and the solvent was changed from 20% water/ 80% methanol to 80% water/ 20% methanol in this study to make the conditions more environmentally relevant and to study any solvent effect that may occur. Prior to this series of experiments several additional experiments were conducted to establish a means of approximation for the maximum concentration of HBCD under the chosen experimental conditions. The limit of detection and quantification was determined using the IUPAC method for each isomer. The actual solubilities of the three main stereoisomers of HBCD were not determined in a 80% water/ 20% methanol solution at 25 ºC because the appropriate equipment was not available.

The issue of solubility and sorption was experimentally evaluated by performing a hydrolysis experiment. The hydrolysis experiment at 25 ºC shows only a slight change in
concentration of 20% for β, and γ, and only 5% for α-HBCD over 75 days. This in no way rules out sorption, but if this disappearance of HBCD is from either hydrolysis or sorption (due to lack of solubility) it may be neglected. The rates of reaction of α,β,γ-HBCD degradation with respect to polysulfides occurred at a factor of 5 times faster or more for β and γ-HBCD, but remained the same for α-HBCD in comparison to the rate of reaction of α,β,γ-HBCD hydrolysis/sorption. In order to assure reproducible LC-MS/MS results, the limit of detection and limit of quantification were determined to work at the lowest concentrations possible to observe three half-lives. The limiting factor for the limit of quantification was α-HBCD with a method LOQ of 4.67 nM. This LOQ value was taken into consideration when determining the starting concentration of the experiments. The starting concentration of 100 nM total HBCD was used because it would result in an ending concentration of 12.5 nM HBCD after three half-lives. This allowed for some error in preparation measurements, instrument error, and timing errors for extractions. The ending concentration is also 2.5x larger than the limiting LOQ. The concentration of 100 nM was chosen also because it was the lowest reasonable starting concentration to work with respect to the limit of quantification and solubility. Without measuring the solubility of each isomer it was determined that working at the lowest reasonable concentration with respect to the limiting LOQ would be the best way to assure solubility of all three isomers.

Several problems plagued initial experiments at low concentration. Erratic points of large concentrations were often observed. These led to poor semilogarithmic plots at the beginning of this research project. Therefore, glassware cleaning procedures, instrument method development, quality control techniques and procedures were carefully evaluated in order to decrease the detection limit for α-HBCD and assure that the glassware was free of α,β,γ-HBCD. Ethyl acetate blanks and triple injections of all standards and samples were made to assure there
was no HBCD carry over. Statistical analysis was performed on the linear regression plots to eliminate outlier points in an appropriate manner.

In work conducted by Lo and Jans [1], on-column GC/MS was used to attempt to identify two possible degradation products formed by the reaction of 100 μM HBCD with polysulfides at 40 °C in an 80% methanol/20% water solvent. The formula weight of HBCD is 641.7 g/mol, which should correspond to the M+ peak in positive mode GC/MS analysis for injections of HBCD. The retention times in the GC of these degradation products were less than the retention time of HBCD which is the usual trend for molecules of less mass. Both degradation products contained the m/z 79.1, 81.1 single bromine isotope ion fragments as well as the dibromide m/z 158, 160, 162. It is important to note that the m/z = 79 and m/z = 81 peaks for each of these fragments are approximately the same size which is an indication of bromine due to its naturally occurring isotopic ratio. This m/z = 79 and m/z = 81 peak ratio is found in HBCD as well as all of its fragments. It should also be noted that the single bromine fragment and the dibromide fragment should be present in all degradation product spectra, which was observed in Lo and Jans’s research. The expected HBCD (RT=18.48 min) degradation products in their work are tetrabromocyclododecene MW=481.9 g/mol (RT=17.18 min) and dibromocyclododecadiene MW=322.1 g/mol (RT=15.98 min). The MS of the peak at 17.18 minutes showed an M+ peak of around 641.7 which was explained as the tetrabromocyclododecene with the addition of a Br2- during ionization. The second peak at 15.98 minutes showed an M+ at around 320 which agrees with the mass of dibromocyclododecadiene. Their discussion of the degradation products that they observed, stated that the peaks observed were in fact degradation products of HBCD, but the assignment of their number of bromine and hydrogen atoms based on the GC/MS data they obtained was not definitive. They showed that the peaks observed using GC/FID were degradation products, by plotting the formation of the products as a function of time on the same
plot of HBCD degradation as a function of time. This plot clearly shows a trend of HBCD degradation and the formation of products that are in good agreement.

In LC-MS/MS, degradation product identification can be performed by using transitions specific to individual products formed. The main difference in negative ionization mode is that a (m/z or mass equivalent) m/z = +1.0 is ejected in the ionization process in large molecules such as HBCD, instead of the electron in the positive MS mode (GC/MS). In negative mode MRM MS/MS a negative charge is therefore induced during ionization in the MS. For this reason the parent HBCD M- ion will have an m/z of 640.8. The tandem MS/MS takes a specific mass after ionization in the first MS then collides that mass with an inert collision gas and looks for another specific mass fragment in the next MS. By performing this procedure of only looking for this specific mass transition, the background noise is dramatically reduced, resulting in a larger signal to noise ratio. The end result is a much lower limit of quantification and detection. The technique that creates the analytical excellence of this instrument hinders its ability to identify degradation products. Multiple transitions can be entered into the method to search for degradation products, but this ultimately results in a decrease in the sensitivity of the parent transitions (counts) for HBCD and poorer isomer peak resolution. It is believed that increasing the number of transitions will result in a decrease in the dwell time per transition. The dwell time could be decreased to improve the resolution but not the level of detection. The dwell time was not changed in anyway during these experiments. For this reason only six transitions were entered into the method for HBCD and product identification analysis. HBCD and degradation products containing bromine can be assumed to be real, if both the parent/78.9 and parent/80.8 transitions are both present, and the two transitions peaks overlap with the same retention time. The two peak areas of the parent product/78.9 and parent product/80.8 transitions should show the same ratio as the natural isotope distribution of Br-79 versus Br-81. In this research
degradation product peaks were observed with the 321/78.9 and 321/80.9 transitions (refer to figure 14 and 14a-c). The parent transition mass matches the mass of dibromocyclododecadiene. The only problem in the observed peaks is the fact that the corresponding isotope peaks are not of equivalent size. The corresponding 321/78.9 and 321/80.9 transition peaks are in a 1:2 ratio.

3. Experimental:

3.1 Chemicals:

1,2,5,6,9,10-Hexabromocyclododecane (HBCD) was purchased from TCI Japan (99%, TCI, Tokyo, Japan). Tetrahydrofuran (HPLC grade, Fisher Scientific, Fair Lawn, NJ), ethyl acetate (EMD Chemicals, Gibbstown, NJ), methanol (HPLC grade, EM Chemicals, Gibbstown, NJ), methanol (LC-MS grade), water (LC-MS grade), formic acid (for MS, Sigma-Aldrich, St. Louis, Mo), sodium tetraborate decahydrate (Fisher Scientific, Fair Lawn, NJ), sodium thiosulfate pentahydrate (Fisher Scientific, Fair Lawn, NJ), potassium iodate (J.T.Baker, Philipsburg, NJ) and potassium iodide (J.T. Baker, Philipsburg, NJ) were used without further purification.

3.2 Reduced sulfur solutions:

Bisulfide stock solutions (130 mM) and polysulfide stock solutions (86.9 mM) were prepared according to Wu and Jans and stored in an anaerobic glove box [5]. All reaction mixtures were prepared by diluting the polysulfide and hydrogen sulfide stock solutions with deoxygenated methanol and deoxygenated water in the anaerobic glove box. The total hydrogen sulfide and polysulfide concentration were determined by iodometric titrations with a starch end
point. The pH was measured with an Accument pH meter (Fisher Scientific, Pittsburgh, PA) with a Ross combination pH electrode (ThermoOrion, Beverly, MA). The speciation of hydrogen sulfide was assumed to be 100% bisulfide in these experiments (pH 9). The speciation of polysulfide were calculated based on the total reduced sulfur species concentration \([S(-II)]_T = \left[H_2S\right]_T + \left[S_{n^2}^-\right]_T\) which was determined by iodometric titration. The data was then processed using published equilibrium constants [12]. The resulting \([S_{n^2}^-]_T\) was used to compute the second order rate constant \(k''_{Sn2}\) for the reaction of the flame retardant HBCD with polysulfide by dividing the first order rate constant by the concentration of polysulfide.

### 3.3 Hydrolysis experiments:

All hydrolysis experiments were prepared in the anaerobic glove box. The solutions were prepared using sodium tetraborate and the appropriate ratio of deoxygenated water and methanol. The pH was measured with an Accument pH meter (Fisher Scientific, Pittsburgh, PA) with a Ross combination pH electrode (ThermoOrion, Beverly, MA) in a 100 mL Erlenmeyer flask. Hydrolysis experiments were conducted for at least one month but no more than three months. The concentration of the hydroxide ion is calculated using the formulas below.

\[
pH = - \log [H^+] \\
14 = pH + pOH \\
pOH = 14 - pH \\
pOH = - \log [OH^-] \\
[OH^-] = 10^{-pOH} \\
[OH^-] = 10^{-(14-pH)}
\]

The mechanism by which hydrolysis degrades \(\alpha,\beta,\gamma\)-HBCD has not yet been studied and can not be explained with the few hydrolysis experiments presented in this work. In order to
elucidate the mechanism of hydrolysis it is necessary to determine the pH dependence of the hydrolysis of \( \alpha,\beta,\gamma \)-HBCD. The hydrolysis experiments presented in this work were performed to confirm that sorption to the reaction vessel walls is not an issue and that hydrolysis under the chosen conditions can be neglected. This can be achieved by comparing the rates of the hydrolysis experiment with those for the reactions with polysulfide and hydrogen sulfide with the same solvent make up, temperature, and pH. The equations above are only presented as an example of how to calculate the hydroxide ion concentration. The same set of equations can be used to calculate the hydronium ion. This information can be used in later studies to calculate the second order rate constants at low and high pH levels accordingly.

3.4 Glassware cleaning method # 1:

The solutions were collected into the appropriate waste collection bottles. Glassware was then rinsed once with acetone, and the effluent was collected into an organic waste bottle. The glassware was then rinsed once with water and that effluent was placed into a waste collection bottle. The glassware was rinsed again with water, then rinsed with a 1 M NaOH in methanol, and then rinsed thoroughly with water. Glassware was then filled with aqua regia for 24 hours to remove any contaminants. That aqua regia was carefully placed back into its (Aqua regia) labeled bottle for reuse. The glassware was then extensively rinsed three times with tap water, rinsed again thoroughly with deionized water and then placed into an oven set to 200 °C overnight to dry.
3.5 Glassware cleaning method # 2:

The solutions were collected into the appropriate waste collection bottles. Glassware was then rinsed once with acetone, and the effluent was collected into an organic waste bottle. The glassware was then rinsed once with water and that effluent was placed into a waste collection bottle. The glassware was rinsed again with tap water, then rinsed with a 1 M NaOH in methanol (this was recollected for reuse in the Erlenmeyer flask labeled sodium hydroxide/methanol mixture), and then rinsed thoroughly with tap water. Glassware was then cleaned with soap and a brush (the brush was rinsed off after each use to prevent contamination of soap solution and other pieces of glassware), rinsed with tap water five times, washed with soap again, rinsed with tap water 10 times, rinsed with acetone twice (acetone rinses were collected into organic waste bottles with a proper label), rinsed with tap water once and then rinsed twice with deionized water. The glassware was then allowed to air dry with a loose piece of aluminum foil covering them.

3.6 Glassware quality control method:

The glassware was allowed to dry and then every piece of glassware (e.g., test tube, 20 mL syringe, volumetric flasks) was labeled individually. Complimentary 2 mL brown sample vials were labeled as well. Ethyl acetate was then added to each piece of glassware (approximately 2 mL in smaller vessels, 4 mL in larger vessels). The ethyl acetate was gently swirled around in each piece of glassware for ten seconds. The ethyl acetate was then removed by a disposable pasteur pipette and placed into its complimentary labeled brown vial for analysis. The glassware extractions were then run on the LC-MS/MS with the appropriate method. A fresh set of standards were run each time. Double injections of the standards, one prior to glassware extractions and one after were made. The glassware extractions were only injected once. Ethyl
acetate blanks were also injected to monitor the method baseline and purity of the ethyl acetate. The glassware was then determined clean only if the ethyl acetate extracts were below the method limit of detection for \(\alpha\)-HBCD (1.4 nM), and 1 nM for \(\beta\)-HBCD and \(\gamma\)-HBCD, respectively.

### 3.7 Method limit of detection and quantification:

The baseline was determined by finding the level at which the integrated area over the entire ethyl acetate blank was as close to zero as possible. This level (cps-counts per second) was recorded as the baseline. Then the ten largest peaks in the blank ethyl acetate were integrated from the determined baseline and recorded. The standard deviation of these peaks was calculated, and the inverse of the calibration curves of each isomer was determined. The following equation was used to determine the method limit of detection (LOD):

\[
C_L = C_{ib} + k s_{ib} S
\]

where:

- \(C_L\) = smallest measure that can be detected with reasonable certainty.
- \(C_{ib}\) = the mean of the blank measures = 0 for the blank.
- \(k = 3\) the IUPAC assigned value of 3, to yield a 90% confidence level.
- \(s_{ib}\) = standard deviation of the blank measure.
- \(S = \text{sensitivity} = \text{inverse slope from calibration curve} = \Delta \text{concentration}/\Delta \text{intensity}\)

The method limit of quantification (LOQ) was then calculated as 3.3 x LOD. This procedure was repeated three times with three separate ethyl acetate blank injections during an actual analysis, once at the beginning, once in the middle, and once at the end of the run. The
values were then averaged, and the standard deviation and 90% confidence level were calculated using an Excel spreadsheet.

3.8 General experimental setup:

Each experiment was prepared in the anaerobic glove box. The solutions were prepared in a 100 mL volumetric flask. The volumetric flasks were pre-filled with sodium tetraborate salt to yield a 10 mM concentration in a 100 mL volumetric flask; 50 mL of deoxygenated water was then added, the solution was swirled for 10 to 15 minutes and the salt was allowed to completely dissolve, then 20 mL of deoxygenated methanol was added, then the appropriate amount of polysulfide or hydrogen sulfide stock solution was added, then deoxygenated water was again added to the line on the 100 mL volumetric flask to yield a solvent ratio of 80% water/20% methanol. This solution was mixed for 5 minutes. This solution was then transferred to a 250 mL Erlenmeyer flask with a stopper. The pH was adjusted by adding 6 M deoxygenated HCl when needed (usually 2 – 4 drops to adjust to pH 9, measured with a pre-calibrated Accument pH meter), the appropriate amount of HBCD in deoxygenated methanol was then injected (this time was recorded as the start time) into the solution with a microliter syringe, the top of the Erlenmeyer flask was covered with the rubber stopper and the solution was mixed gently by swirling the Erlenmeyer flask for 30 seconds, a rubber stopper with a stainless steel needle through it (with a Teflon needle through that) was then placed on the Erlenmeyer flask and the solution was taken up through the Teflon needle into each of the pre-labeled 20 mL glass syringes. This way the reaction solution was the same for α,β,γ-HBCD. The first syringe was used for α-HBCD sampling which could be taken over a total reaction time of two months. The second syringe was used for β- and γ-HBCD sampling which could proceed at much shorter
time intervals of several days or a couple of weeks depending on the experiment. The third syringe was used for iodometric titration to determine the total concentration of reduced sulfur species, \([S(-II)]_T\), as mentioned in method section 3.2 above, and a forth syringe could be filled and used if glassware is available, in the event of a solution spill this would be a backup sample syringe for any of the isomers.

Extractions were then taken as 1 mL aliquots of the reaction solution. The 1 mL aliquots of the reaction solution were added to pre-weighed test tubes containing 1 mL ethyl acetate. The weight of the test tube after the aliquot was added was also measure to be able to determine the exact amount added. Thereafter, 2 mL of a 5 M sodium chloride solution was added to the mixture to assist in phase separation and to aid in the extraction of HBCD into the ethyl acetate phase. The test tube was moderately shaken side to side only, for 10 seconds. The solution was allowed to separate for 1 minute, and then the ethyl acetate phase was transferred into a brown 2 mL vial.

External standards were prepared under a hood in an oxygen rich environment. A series of three stock solutions were prepared: 12.08 mM in tetrahydrofuran, 500 \(\mu\)M HBCD in ethyl acetate, and 10 \(\mu\)M HBCD in ethyl acetate. Dilutions were made accordingly to yield standards in ethyl acetate with concentrations of 200 nM HBCD, 150 nM HBCD, 100 nM HBCD, 80 nM HBCD, 60 nM HBCD, 40 nM HBCD, 20 nM HBCD, and 10 nM HBCD.

Experimental extracts and standards were then analyzed using LC-MS/MS. The HBCD concentration was then determined using an external calibration curve (refer to figures 2a, 2b and 2c). A correction was performed considering the exact volume of ethyl acetate and the exact volume of reaction solution taken for the extraction. The order of the reaction was determined to be pseudo first order with respect to the polysulfide concentration in this study for each of the three main HBCD isomers. First order kinetics with respect to HBCD concentration was
observed in previous work conducted by Lo and Jans [1]. The kinetics were therefore assumed to be second order overall. The data was plotted accordingly. Standard calibration curves were evaluated for the individual alpha, beta, gamma isomer at the total measured HBCD concentration, but it was assumed that the purchased HBCD has the following isomeric α, β, γ composition: 13% α, 2% β, 85% γ [1]. The results below do not take this isomeric composition into account. The results represent the total HBCD concentration. The reason for not making the correction is because it would be just an approximation. Standards of the individual isomers would be needed. The pure individual isomer could then be used to calculate the exact ratio of the α,β,γ-HBCD in the technical grade HBCD used in all work.

3.9 LC-MS/MS analysis #1:

The MS conditions are listed in table 1 below. Ethyl acetate extracts were analyzed with a LC-MS/MS system (LC20AD, Shimadzu USA, Canby, OR/QTrap 4000, Applied Biosystems, Foster City, CA). The column used was a Zorbax Eclipse XDB-C18 column (4.6 mm x 150 mm, 5 µm particle size, 80 Å pore size, double end capped; Agilent Technology, Santa Clara, CA).

Experiments #14-18 were analyzed by LC-MS/MS using a gradient mobile phase with a flow rate of 1 mL/min and a 0.2% formic acid concentration in both water and methanol components independently as described in table 2 below.
Table 1:

<table>
<thead>
<tr>
<th>MS Conditions # 1</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionization type:</td>
<td>Electrospray ionization (ESI)</td>
</tr>
<tr>
<td>Ion source mode:</td>
<td>Negative</td>
</tr>
<tr>
<td>MS monitoring method:</td>
<td>Multiple reaction transition (MRM)</td>
</tr>
<tr>
<td>Transitions for quantification:</td>
<td>640.8 - 78.90 and 640.80 - 80.80</td>
</tr>
<tr>
<td>Transitions for Product ID:</td>
<td>481.0 – 78.90 and 481.0 – 80.80</td>
</tr>
<tr>
<td>Transitions for Product ID:</td>
<td>321.0 - 78.90 and 321.0 – 80.80</td>
</tr>
<tr>
<td>CAD Collision gas:</td>
<td>Nitrogen at 8 arbitrary units</td>
</tr>
<tr>
<td>Curtain gas:</td>
<td>Nitrogen at 25 arbitrary units</td>
</tr>
<tr>
<td>DP declustering potential:</td>
<td>negative 75 V</td>
</tr>
<tr>
<td>EP Entrance potential:</td>
<td>negative 10 V</td>
</tr>
<tr>
<td>GS1 Nebulizer gas</td>
<td>nitrogen 60 arbitrary units</td>
</tr>
<tr>
<td>GS2 heater gas:</td>
<td>nitrogen 40 arbitrary units</td>
</tr>
<tr>
<td>IS ion spray voltage:</td>
<td>negative 1500 V</td>
</tr>
<tr>
<td>TEM source heater temperature:</td>
<td>400 °C</td>
</tr>
<tr>
<td>Interface Heater:</td>
<td>ON</td>
</tr>
<tr>
<td>Collision Energy:</td>
<td>-34</td>
</tr>
<tr>
<td>Collision Cell Exit Potential:</td>
<td>-3</td>
</tr>
</tbody>
</table>

Table 2:

<table>
<thead>
<tr>
<th>Gradient method # 1 : mobile phase composition vs time: 0.2% Formic acid composition in methanol and water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (minutes)</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>6.1</td>
</tr>
<tr>
<td>7</td>
</tr>
</tbody>
</table>

3.10 LC-MS/MS method #2:

The MS conditions are listed in table 3 below. Ethyl acetate extracts were analyzed with a LC-MS/MS system (LC20AD, Shimadzu USA, Canby, OR/QTrap 4000, Applied Biosystems, Foster City, CA). The column used was a Phenomenex Kinetex C18 (150 mm x 4.60 mm, 5 μm
particle size, 100 A pore size, double end capped, Phenomenex, www.phenomenex.com). A Phenomenex SecurityGuard Ultra, guard column was also used.

Experiments 20 - 27 were analyzed by LC-MS/MS using a gradient mobile phase with a flow rate of 1 mL/min and a 0.2% formic acid concentration in both water and methanol components independently as described in table 4 below.

Table 3:

<table>
<thead>
<tr>
<th><strong>MS Conditions # 8</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionization type:</td>
<td>Electrospray ionization (ESI)</td>
</tr>
<tr>
<td>Ion source mode:</td>
<td>Negative</td>
</tr>
<tr>
<td>MS monitoring method:</td>
<td>Multiple reaction transition (MRM)</td>
</tr>
<tr>
<td>Transitions for quantification:</td>
<td>640.8 - 78.90 and 640.8 - 80.80</td>
</tr>
<tr>
<td>Transitions for Product ID:</td>
<td>481.0 – 78.90 and 481.0 – 80.80</td>
</tr>
<tr>
<td>Transitions for Product ID:</td>
<td>321.0 -78.90 and 321.0 – 80.80</td>
</tr>
<tr>
<td>CAD Collision gas:</td>
<td>Nitrogen at 8 arbitrary units</td>
</tr>
<tr>
<td>Curtain gas:</td>
<td>Nitrogen at 30 arbitrary units</td>
</tr>
<tr>
<td>DP declustering potential:</td>
<td>negative 40 V</td>
</tr>
<tr>
<td>EP Entrance potential:</td>
<td>negative 10 V</td>
</tr>
<tr>
<td>GS1 Nebulizer gas:</td>
<td>nitrogen 60 arbitrary units</td>
</tr>
<tr>
<td>GS2 heater gas:</td>
<td>nitrogen 40 arbitrary units</td>
</tr>
<tr>
<td>IS ion spray voltage:</td>
<td>negative 2500 V</td>
</tr>
<tr>
<td>TEM source heater temperature:</td>
<td>250 °C</td>
</tr>
<tr>
<td>Interface Heater:</td>
<td>ON</td>
</tr>
<tr>
<td>Collision Energy:</td>
<td>-42</td>
</tr>
<tr>
<td>Collision Cell Exit Potential:</td>
<td>-5</td>
</tr>
</tbody>
</table>

Table 4:

<table>
<thead>
<tr>
<th>Gradient method # 2 : mobile phase composition vs time:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2% Formic acid composition in methanol and water</td>
<td></td>
</tr>
<tr>
<td>Time (minutes)</td>
<td>% A (LC-MS water)</td>
</tr>
<tr>
<td>----------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>0.5</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>8.1</td>
<td>15</td>
</tr>
<tr>
<td>9</td>
<td>15</td>
</tr>
</tbody>
</table>
3.11 Graphical analysis to determine first and second order rate constants:

The first order rate constants were determined by plotting the natural logarithm of the corrected HBCD concentration (corr. conc) as a function of time. In the plot of ln (corr. conc.) vs. time (hr⁻¹) a linear regression was assumed, and the equation of the linear regression was calculated using Microsoft excel, and the first order rate constant was taken as the negative slope of the regression equation. The sample concentrations were plotted until 3 half-lives were observed; samples with a concentration of less than the concentration expected after the 3rd half-life were omitted for the plots of ln (corr.conc.) vs. time (hr⁻¹). The sample corrected concentration values used had to be above the estimated LOQ values for each isomer. In addition, final samples of a time-course with corrected concentration values that were more than double that of the previous sample were omitted.

The order of the reaction with respect to polysulfide was determined by plotting log k'(sec⁻¹) as a function of log [polysulfide concentration (M)]. The linear regression equation of such a plot was added to each of the plots. The slope of the linear regression was taken as the order of the reaction with respect to polysulfide.

The overall second order rate constant for each of the individual α, β, γ-HBCD isomers were found by plotting k'(hr⁻¹) as a function of the polysulfide concentration (M). The linear regression line was added to this plot. The slope of the linear regression was then taken as k'' (the second order rate constant).
3.12 Statistical analysis # 1: 90% Confidence intervals for k’ and for triple injections:

The 90% confidence interval was calculated for all regression lines of the semilogarithmic plots. The LINEST function in Microsoft excel was utilized for most of the calculations. The LINEST function was performed by selecting a 5 row and 2 column block of cells and highlighting them. Then the following was typed: =LINEST (Y series, X series, True, True) then shift/Control/and enter were pressed at the same time. Note: Y series = the highlighted Y data, and X series= the highlighted X data.

The information obtained is in the following format:

<table>
<thead>
<tr>
<th>Least squares predicted slope</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope standard error</td>
<td>Intercept standard error</td>
</tr>
<tr>
<td>$R^2$ (square of correlation coefficient)</td>
<td>Y Tolerance</td>
</tr>
<tr>
<td>$F$ (square of slope/standard error of slope)</td>
<td>Degrees of freedom (DF)</td>
</tr>
<tr>
<td>Regression sum of squares</td>
<td>Residual sum of squares</td>
</tr>
</tbody>
</table>

The 90% confidence interval for the slope of the regression equations were then calculated by multiplying the standard error of the slope by the t-value for the corresponding degrees of freedom. The 90% confidence interval for triple injections were calculated by dividing the standard deviation by the square root of three (N) and multiplying by the corresponding t-value.

3.13 Statistical analysis #2: Outlier identification:

Statistical plots were developed on Microsoft excel to provide a means of eliminating outlier points. This was done on all linear regression plots of ln [corrected concentration (M)] versus time. The upper and lower tolerance boundary, upper and lower 90% confidence and 95% confidence level lines were calculated and plotted along with the evaluated linear regression plot (trend line added by Microsoft excel). Error bars for k’ where added to the graph. The error bars
were the 90% confidence intervals calculated in statistical method #1. All points located outside the tolerance boundary lines were omitted. This was done until all the points were between the upper and lower tolerance boundary lines. The data had to have at least three points, with one at the beginning, middle and end to assure a linear regression. If one injection point was outside the upper or lower tolerance boundary lines then all injection of that sample were omitted. In addition, sample sets with error bars that extended outside the tolerance boundary levels were omitted in plots of k’ vs. [polysulfide (M)]. The series of equations used for these calculations are listed below:

1) \( \bar{x} = \frac{\sum x_i}{n} \)  
2) \( \bar{y} = \frac{\sum y_i}{n} \)  
3) \( S_{xx} = \sum (x_i - \bar{x})^2 \)  
4) \( S_{yy} = \sum (y_i - \bar{y})^2 \)

5) \( S_{xy} = \sum (x_i - \bar{x})(y_i - \bar{y}) \)  
6) \( b_1 = \frac{S_{xy}}{S_{xx}} \)  
7) \( \hat{y} = \bar{y} + b_1(x_i - \bar{x}) \)

8) \( s = \sqrt{\frac{\Sigma(y_i - \hat{y}_i)^2}{n - 2}} \)  
9) \( TB_{\hat{y}} = \hat{y} \pm (t_{n-2})s \sqrt{1 + \frac{1}{n} + \frac{(x_i - \bar{x})^2}{S_{xx}}} \)

10) \( CL_{\hat{y}} = \hat{y} \pm (t_{n-2})s \sqrt{\frac{1 + \frac{1}{n} + \frac{(x_i - \bar{x})^2}{S_{xx}}}{k}} \)

Where:
\( \bar{x} = \text{the average } x \text{ value} \)  
\( x_i = \text{an individual point } x \)  
\( \bar{y} = \text{the average } y \text{ value} \)  
\( y_i = \text{an individual point } y \)  
\( n = \text{number of data points} \)  
\( S_{xx}, S_{yy}, S_{xy} = \text{Intermediate Formulas} \)  
\( b_1 = \text{Slope} \)  
\( \hat{y} = \text{estimated } y \text{ value} \)  
\( s = \text{standard deviation of the error} \)  
\( t_{n-2} = t \text{ value for the desired confidence level} \)  
\( TB_{\hat{y}} = \text{Tolerance boundary} \)  
\( CL_{\hat{y}} = \text{Confidence Interval Boundary} \)  
\( k = \text{the number of times a measurement was repeated for the same } x \)
4. Results and Discussion:

The LOD and LOQ were approximated by using the IUPAC method for the final calculations. LC-MS/MS method # 1 (0.1% Formic acid in both the methanol and water mobile phase) resulted with the following LOD values: \( \alpha \)-HBCD = 3.05 nM, \( \beta \)-HBCD = 0.95 nM, and \( \gamma \)-HBCD = 0.65 nM. LC-MS/MS method # 1 (0.1% Formic acid in both the methanol and water mobile phase) also resulted in the following values for the LOQ for each isomer: \( \alpha \)-HBCD = 10.05 nM, \( \beta \)-HBCD = 3.15 nM, and \( \gamma \)-HBCD = 2.13 nM, refer to table 5. The idea was to use the lowest possible starting concentration with the ability to still observe all three half-lives without going below the LOQ. This is why 100 nM total HBCD was chosen for the starting concentration in 80% water/ 20% methanol experiments at 25 °C. The concentration after three half-lives would be 12.5 nM total HBCD which is slightly above the LOQ for \( \alpha \)-HBCD.

For LC-MS/MS method # 2 the following limits of detection were determined: \( \alpha \)-HBCD 1.41 ± 0.27 nM, \( \beta \)-HBCD 0.27 ± 0.05 nM, and \( \gamma \)-HBCD = 0.08 ± 0.01 nM. LC-MS/MS method # 2 also resulted in the following values for the limit of quantification for each isomer: \( \alpha \)-HBCD = 4.67 ± 0.89 nM, \( \beta \)-HBCD 0.90 ± 0.17 nM, and \( \gamma \)-HBCD = 0.26 ± 0.05 nM. Refer to tables 6a-d.

LC-MS/MS method development played a key role in the progress of this research. Increasing the formic acid content in both mobile phases resulted in larger peak areas for all three \( \alpha, \beta, \gamma \)-HBCD isomers. There was an observed increase by a factor of 3.6 for \( \alpha \)-HBCD, 3.1 for \( \beta \)-HBCD, and 3.8 for \( \gamma \)-HBCD, when the formic acid content was changed from 0.1% to 0.2% by volume in both mobile phases.

\( \alpha \)-HBCD is ionized at a much lower percentage resulting in a much smaller peak area for
α-HBCD and therefore a larger LOD and LOQ. MS conditions # 8 of LC-MS/MS analysis # 2 was developed to yield a lower LOD and LOQ for α-HBCD while still maintaining low enough LOD and LOQ’s for β-HBCD and γ-HBCD to proceed with the experiments in a similar fashion. The development of MS conditions # 8 resulted in larger peak areas again for α, β, γ-HBCD. There were observed area increases of a factor of 3.9 for α-HBCD, 2.3 for β-HBCD, and 1.4 for γ-HBCD.

The observed area/concentration ratios using the isomeric ratio assumed above are: 290 for α-HBCD, 7193 for β-HBCD, and 2009 for γ-HBCD. This clearly shows that ionization of the three main isomers in negative mode MRM MS/MS is in the order β>γ>α, with α-HBCD a factor of 6.9 times less ionized than γ-HBCD and 24.8 times less ionized than β-HBCD. An attempt to add an additional 18 transitions was made to observe HBCD degradation products. However, this resulted in a loss of resolution and sensitivity. This was explained by the decrease in the number of scans in a given time-period documented in the mass spectrometer method properties for the acquisition method. For this reason, only six transitions were used throughout this study.

Throughout this study there were issues of random points of high concentration in some of the time courses. Several hypotheses were created, but the cleanliness of the glassware was the most logical. Glassware cleaning method # 1 used aqua regia to remove the HBCD, where as method # 2 used soap and water followed with an acetone and then a water rinse. The percentage of clean glassware was 26% for glassware cleaned using method # 1, refer to figure 1a. The percentage of clean glassware was 76% for glassware cleaned using method # 2, refer to figure 1b. There are also a few advantages to method # 2. Glassware cleaning method # 2 is safer, no neutralization is needed for disposal, and it is less expensive. The glassware quality control method was then developed. It was believed that this would eliminate these random
points of high concentration, and therefore better degradation plots would be observed. The
glassware cleaning method #2 and the glassware quality control method were used for
experiments # 20 – 27. However, some of the degradation plots still had outliers. It should be
noted that there were four types of observations in the degradation plots that were of concern.
The four observations were: 1) sample points that had concentrations larger than twice the
starting concentration that would appear randomly throughout some of the experiments, 2)
sample points that were larger than 120% of the previous sample point, 3) samples that were
much lower than the expected sample concentration at a given time, and 4) individual injections
of the same sample differed by more than 20%.

Outlier identification was then performed using statistical analysis # 2. In some cases the
first order rate constants were changed by a factor of 1.6 after the outliers were eliminated (refer
to figure 4a-e). There was also another issue that arose. When the plot of k’ vs. [polysulfide]
was created, one experiment had a much faster rate than the others and was most likely an outlier
(refer to figure 8, 9, 10). The statistical analysis procedure was carried out until all of the points
were inside the tolerance boundaries (refer to figure 8, 9, 10). The results for the k” for HBCD
reactions with polysulfide from the experiments performed were 2.37 ± 2.08 M⁻¹ hr⁻¹ for
α-HBCD, 22.6 ± 18.4 M⁻¹ hr⁻¹ for β-HBCD, and 24.2 ± 36.0 M⁻¹ hr⁻¹ for γ-HBCD, refer to
figure 8, 9, 10. These results indicate that α-HBCD reacts ten times slower than its β and
γ-HBCD counterparts. Previous research indicated the second order rate constant of 100 μM
HBCD with polysulfide in 80% methanol/ 20% water at 40 °C is 79.2 ± 10.8 M⁻¹ hr⁻¹[1]. This
rate constant is most likely the combined second order rate constant for β and γ-HBCD. These
reactions were performed at a temperature of 40 °C. They differ only by a factor of 3.3 from the
γ-HBCD and 3.5 from the β-HBCD second order rate constant found in this study.
The order of the reaction with respect to polysulfide was confirmed as first order. The logarithm of the first order rate constants for each HBCD isomer were plotted vs. the logarithm of the polysulfide concentration. The points were not in good agreement but the slopes were as follows: \( \alpha = 1.12 \pm 0.93 \), \( \beta = 1.37 \pm 0.95 \) and \( \gamma = 1.01 \pm 0.89 \) (refer to figure 5-7).

Sorption to the glassware was a concern due to the estimation of the solubility of the three main isomers of HBCD. To evaluate the relevance of sorption to materials used in the experiments, a hydrolysis experiment was performed at pH 9 and 25 ºC in 80% water/ 20% methanol. This experiment was sampled over a two month period (refer to figure 11-13). It should be noted that for \( \alpha \)-HBCD there was very little change in the concentration. The first order rate constants were: \( \alpha \)-HBCD \( 0.0003 \pm 0.0001 \) hr\(^{-1} \), \( \beta \)-HBCD \( 0.0008 \pm 0.0001 \) hr\(^{-1} \) and \( \gamma \)-HBCD \( 0.0004 \pm 0.0002 \) hr\(^{-1} \). This data reveals that the first order rate constants were small, and by evaluating the size of the 90% confidence interval they are not zero because the 90% confidence interval is smaller than the first order rate constant. The samples taken within the first 600 hours of the experiment have error bars that all overlap. These results indicate that either a very slow hydrolysis reaction with a first order rate constant of \( 0.0003 \pm 0.0001 \) hr\(^{-1} \) was observed, or there may be a small amount of sorption, but both are possible. It should also be noted that the rate of reaction for 3.38 mM bisulfide with \( \alpha \)-HBCD had a faster first order reaction rate constant of \( 0.0014 \pm 0.0002 \) hr\(^{-1} \) (refer to figure 11 and table 7), than hydrolysis and polysulfide. The reaction with bisulfide was observed for 647 hours and one half life was observed, the second order rate constant was determined to be \( 0.414 \pm 0.059 \) M\(^{-1}\)hr\(^{-1} \) for the reaction of \( \alpha \)-HBCD with 3.38 mM bisulfide at pH 9.5 and 25 ºC in 80% water/ 20% methanol. The reaction with 0.226 mM polysulfide was observed for 1705 hours, had a first order reaction rate constant of \( 0.0004 \pm 0.0001 \) hr\(^{-1} \) and one half-life was observed. The second order rate constant was determined to be \( 2.37 \pm 2.08 \) M\(^{-1}\)hr\(^{-1} \) for the reaction of \( \alpha \)-HBCD with 0.226 mM.
polysulfide at pH 9 and 25 °C in 80% water/ 20% methanol. It is important to observe that there is no difference in the first order rate constant for the α-HBCD reaction with 0.226 mM polysulfide compared to the hydrolysis reaction. This can be concluded since the two 90% confidence intervals of the first order rate constants of the two reactions (refer to table 7) overlap.

The results of β-HBCD in the same hydrolysis experiment revealed that over 388 hours 14% of the β-HBCD was consumed (refer to figure 12a). The polysulfide experiment conducted under the same conditions with a 0.226 mM polysulfide concentration had a decrease in concentration of 78% during the same amount of time. These results indicate that either a very slow hydrolysis reaction with a first order rate constant of 0.0008 ± 0.0001 hr⁻¹ was observed, or there may be a small amount of sorption, but both are possible. It should also be noted that the reactions with 3.38 mM bisulfide and 0.226 mM polysulfide had significantly faster first order reaction rate constants (refer to figure 12 and table 7) than hydrolysis. The reaction with bisulfide was observed for 647 hours and one half-life was observed. The first order rate constant was determined to be 0.0052 ± 0.0004 hr⁻¹ and the second order rate constant was determined to be 1.53 ± 0.06 M⁻¹hr⁻¹ for the reaction of β-HBCD with 3.38 mM bisulfide at pH 9.5 and 25 °C in 80% water / 20% methanol. The reaction with 0.226 mM polysulfide was observed for 388 hours and the first order reaction rate constant was determined to be 0.0031 ± 0.0012 hr⁻¹, and one half-life was observed. The second order rate constant was determined to be 13.7 ± 5.3 M⁻¹hr⁻¹ for the reaction of β-HBCD with 0.226 mM polysulfide at pH 9 and 25 °C in 80% water/ 20% methanol. It is important to observe that the first order rate constants for β-HBCD reactions with 0.226 mM polysulfide and hydrolysis differ by a factor of three and that the reactions with bisulfide is faster than hydrolysis by a factor of six and a half.
The results for γ-HBCD in the same hydrolysis experiment revealed that over 150 hours (total time polysulfide experiment was observed), a 12% concentration change was observed, (refer to figure 13). The polysulfide experiment conducted under the same conditions with a 0.226 mM polysulfide concentration had a decrease in γ-HBCD concentration of 76% during the same amount of time. These results indicate that either a very slow hydrolysis reaction with a first order rate constant of $0.00043 \pm 0.00016 \text{ hr}^{-1}$ was observed, or there may be a small amount of sorption, but both are possible. It should also be noted that the reactions with 3.38 mM bisulfide and 0.226 mM polysulfide had faster first order reaction rate constant (refer to figure 13 and table 7) than hydrolysis. The reaction with bisulfide was observed for 142 hours and one half-life was observed. The first order rate constant was determined to be $0.0156 \pm 0.0024 \text{ hr}^{-1}$ and the second order rate constant was determined to be $4.62 \pm 0.71 \text{ M}^{-1}\text{hr}^{-1}$ for the reaction of γ-HBCD with 3.38 mM bisulfide at pH 9.5 and 25°C in 80% water/ 20% methanol. The reaction of γ-HBCD with 0.226 mM polysulfide was observed for 120 hours, had a first order reaction rate constant of $0.0031 \pm 0.0012 \text{ hr}^{-1}$, and two half-lives were observed. The second order rate constant was determined to be $55.8 \pm 4.4 \text{ M}^{-1}\text{hr}^{-1}$ for the reaction of γ-HBCD with 0.226 mM polysulfide at pH 9 and 25 °C in 80% water/ 20% methanol.

Previous work conducted by Jans and Lo (1) conducted in 20% water/ 80% methanol solutions at 40 °C, pH 9.2 and 100 μM total HBCD resulted in a second order rate constant of $79.2 \pm 10.8 \text{ M}^{-1}\text{hr}^{-1}$ for reactions with polysulfides and $3.2 \pm 1.0 \text{ M}^{-1}\text{hr}^{-1}$ for reactions with bisulfide. The second order rate constants in this study for γ-HBCD were $24.2 \pm 36.0 \text{ M}^{-1}\text{hr}^{-1}$ for reactions with polysulfides and $4.62 \pm 0.71 \text{ M}^{-1}\text{hr}^{-1}$ for reactions with bisulfide. The second order rate constants for bisulfide in the two independent studies are in the same order of magnitude. The second order rate constants for polysulfide in the two independent studies are also in the same order of magnitude. The second order rate constant of γ-HBCD in this study is
three times slower than that obtained in experiments conducted by Jans and Lo (1). This is due to the difference in temperature between the experiments and the solvent composition. The rate observed by Jans and Lo is comparable to the γ-HBCD data collected in this experiment because Jans and Lo only studied the total HBCD degradation using GC/MS which does not observe individual rates of degradation for each isomer of HBCD. The observed total degradation of HBCD was due to the 85% composition of γ-HBCD in the total HBCD observed in their experiments. This is also supported by the fact that γ-HBCD is the fastest of the three isomers of HBCD to react under these conditions at 25 °C.

Previous work conducted by Wilson, Lo and Jans (19) revealed second order rate constants of 8.28 M⁻¹hr⁻¹ for α-HBCD, 227 M⁻¹hr⁻¹ for β-HBCD and 147 M⁻¹hr⁻¹ for γ-HBCD in a 50% water/50% methanol solution at 40 °C with polysulfide and 28.8 M⁻¹hr⁻¹ for α-HBCD, 79.2 M⁻¹hr⁻¹ for β-HBCD and 79.2 M⁻¹hr⁻¹ for γ-HBCD in a 20% water/80% methanol solution at 40 °C with polysulfide. The results in this study, conducted at 80% water/20% methanol at pH 9 and 25 °C were 2.37 ± 2.08 M⁻¹hr⁻¹ for α-HBCD, 22.6 ± 18.4 M⁻¹hr⁻¹ for β-HBCD, and 24.2 ± 36.0 M⁻¹hr⁻¹ for γ-HBCD. The results at 40 °C show that when the amount of water is increased from 20% to 50% by volume the rate constant with polysulfide decreases by a factor of 3 for α-HBCD, but an increase of a factor of 2.9 for β-HBCD and 1.9 for γ-HBCD. This trend agrees with the proposed mechanism presented in schematic 2 above for β-HBCD and γ-HBCD. The transition state of the E2 debromination has a partial charge, this partial charge will be stabilized by the addition of the more polar solvent. The more water that is present the lower the activation energy that is needed for the reaction to proceed. The α-HBCD isomer does not follow this trend and is very slow in comparison to the other degradation rates. This may indicate that the α-HBCD isomer undergoes a different mechanism from its β and
γ-HBCD counterparts. The second order rate constants for α, β, and γ-HBCD are a factor of 3, 10, and 6 times larger in a 50% water/ 50% methanol when compared to the rates obtained at 25 °C in a 80% water/ 20% methanol solution in this study. The increase in temperature will result in a faster rate of degradation, but the decrease in the amount of water (polarity of the solution) and increase in methanol should result in slower reaction rate according to the proposed mechanism presented in schematic 2. Therefore the overall solvent effect can not be predicted based on these results. It can be stated that the temperature has a greater effect on the reaction rate than the solvent effect in this system under these conditions.

**Degradation product identification:**

Degradation product investigation revealed the observation of what is believed as one product peak containing bromine and two possible product peaks that do not contain bromine, refer to figures 14 and 14 a-c. The product peak containing bromine (refer to figure 14 and 14a) appears at a later retention time (5.62 minutes) than the three α,β,γ-HBCD isomers (4.23, 4.51, and 4.65 minutes respectfully), and contains both of the necessary transitions (321/80.8 and 321/78.9) expected for dibromocyclododecadiene. These peaks are observed in several experiments and appear to be getting smaller overtime. The first requirement for the identification of a degradation product containing bromine is that the two transitions, mass of parent ion/bromine isotope mass are both present at the same retention time (they must overlay each other) and theoretically be present close to 1:1 ratio (bromine isotope ratio), and the second requirement is that there is a gradual peak area increase overtime (if it is not still reacting, dibromocyclododecadiene would still be reacting further). The transitions found for the degradation product at 5.62 min are 321/78.9 and 321/81.8 which corresponds to the
dibromocyclododecadiene product (refer to figure 14a). The integrated area of the 321/78.9 transition was 1174 counts, while the area for the 321/80.8 transition was 2230 counts. These two transitions appear in an integrated area ratio of 1:2. The ideal ratio of these two transitions would 1:1. It should be noted as a reference, that there is some variation in the ratio of related transitions. For example, hexabromocyclododecane has some variation in its observed transition ratios. At high concentrations 640/78.9 and 640/80.8 have the integrated area ratios of 0.8:1, were as at lower concentrations the ratio becomes closer to a 1:2 ratio. The reason for the theoretical 1:1 ratio originally stated is due to the ratio of the naturally occurring bromine isotopes 78.9 and 81.9 which is 1:0.973. The most probable reason that this ratio varies from the theoretical 1:0.973 at lower concentrations is that the instrumental error at low concentrations is greater. As the concentration of the analyte decreases to concentrations close to the LOQ and LOD the signal to noise ratio increases, this results in an increase in the instrument error.

Two additional peaks were observed. However, these peaks may be degradation products without bromine (refer to figure 14). The first peak appears at 4.19 minutes with the 321/78.9 transition in a large amount with the other transition of 321/80.8 at the baseline level. The integrated area of the 321/78.9 peak was 3136 counts. A second peak was observed at 3.75 minutes with the 321/80.8 transition peak and an area of 1801 counts. The other transition peak of 321/78.9 was observed at baseline level. Ethyl acetate blanks were analyzed between all sample and standard sets that were analyzed, and none of these peaks were observed.

The reason why these peaks were rejected as bromine containing products is the fact that the parent ion mass peak and daughter bromine isotopes (the transition) must be present at the same time and with a reasonable ratio for both transitions. The formula weight of cyclododecatriene is 162.28 g/mol, add one bromine isotope 78.9 and one 80.8, and the expected molecular mass is 322, subtraction 1.0 in negative mode MRM MS/MS and the result is a mass to charge ratio of
approximately 321 (the mass of the parent ion of dibromocyclododecadiene). This is why the two transitions of 321/80.8 and 321/78.9 were selected for the observation of a possible dibromocyclododecadiene product.

**Future work – To further improve time-courses**

The two hypotheses for the reason why the semilogarithmic plots still show some occasional outliers are: 1) The points with greater than 120% of the corrected concentration values of the previous sample in a time course were most likely due to evaporation of the samples while they were stored in the refrigerator in the brown vial waiting to be analyzed, and 2) Samples that were much lower in corrected concentration than the expected sample corrected concentration at a specific time might be the result of large water droplets in the bottom of the brown sample vials. The first hypothesis can be easily tested by making measurements of the brown vials; empty, with initial extraction, immediately prior to LC-MS/MS analysis and then making a second correction for the concentration according to the volume of extract prior to LC-MS/MS analysis. The second hypothesis can be easily tested by performing experiments and making sure there are no large water droplets on the bottom of the extraction brown vials. Another way to test hypothesis # 2 is by using an internal standard. Hypothesis # 2 is based on the assumption that while LC-MS/MS injections were made, the needle may have taken up some water in the bottom brown vials, therefore resulting in these lower than usual points. Two simple modifications to the existing procedure can be made in future experiments to possibly obtain degradation plots with less outliers, which will result in more accurate measurements of the rates of reaction.

Concerns about sorption to the reaction vial, Teflon stopcock, and Teflon needle can be tested by extracting the stopcock, needle and reaction vial with a known volume of ethyl acetate.
after the reactions have gone to completion and all the reaction solution was removed from the syringe. This should of course be done prior to glassware cleaning. This must be done with a measured amount of ethyl acetate. The brown vial should be measured empty, directly after the extraction and then immediately prior to LC-MS/MS analysis.

**Future work – additional suggestions**

The synthesis of the degradation products would be the first goal to reach. Once the tetrabromocyclododecene and dibromocyclododecatriene products are formed they must be separated, and negative mode mass spectroscopy must be used to observe all of the fragments. Then the limits of detection and quantification must again be calculated with the existing LC-MS/MS method # 2 listed in section 3 above. The negative mode ionization of these products may result in a much higher limit of detection and quantification, which would explain the reason why they are not observed in the work conducted so far. Without the determination of each degradation product LOD and LOQ, two things can be done to advance this study; 1) increase the volume fraction of methanol in the solutions to work at increased starting HBCD concentrations so that degradation products will be at higher concentrations as well, and 2) write additional methods similar to method #2 that decrease the LOD and LOQ for the products found in step one above.

The structure of the $\alpha,\beta,\gamma$-HBCD should be further studied. Two attempts were made to use the CUNY College of Staten Island High Performance Computing Center to perform computational calculations to determine the most stable conformation of each of the three main HBCD isomers. Unfortunately, the alpha HBCD isomer ran for about a week and then the server crashed at the end of the semester. After the most stable conformation of each isomer is
computationally calculated, the following important physical properties for each individual isomer may be obtained; 1) the bond angles for each pair of vicinal bromines, 2) bond lengths between bromine-carbons at each of the six sites as well as the carbon-carbon and carbon-hydrogen bonds, 3) partial charges on all atoms, 4) distance between bromines on adjacent carbons, 5) bond rotational energies for all bonds present, 6) torsional energy for each bond, and 7) the bond strength of each bond. This data may provide an insight into the observed reactivity difference between $\alpha$-HBCD as compared to $\beta$, and $\gamma$-HBCD.

5. Conclusion:

The degradation of HBCD with respect to polysulfide is first order. Reactions in 80% water/20% methanol and an initial concentration of 100 nM total HBCD seemed to be reasonable. These experiments obeyed second order kinetics overall. We suspect that the reaction mechanism is the E2 mechanism via removal of the bromine by the reduced sulfur species. This study revealed that the $\alpha$-HBCD isomer reacts at a much slower rate than its $\beta$ and $\gamma$-HBCD counterparts. These results imply that the reason for the bioaccumulation of $\alpha$-HBCD over $\beta$– or $\gamma$-HBCD could also be due to the $\alpha$-HBCD isomer being more persistent toward abiotic second order elimination. This research also indicates that the rates of reaction for HBCD degradation in pore water systems are faster with reduced sulfur species such as polysulfide and bisulfide in comparison to hydrolysis alone (refer to Table 7 and Figures 11 a,b,c – 13a,b,c). The research conducted in this study is also in good agreement with previous studies.

The cleaning method and the glassware quality control method seemed to resolve the problem with random samples with corrected concentration values of twice that of the original amount of HBCD. Future experiments should be conducted with the measurement of the brown
vial weight before extraction, after extraction with removal of any water droplets, and then prior to LC-MS/MS analysis. These method modifications can then be tested with statistical method # 2 for outlier identification and compared to prior results to see if there is a decrease in the amount of outliers per experiment.
6. Literature

(1) Lo, Ka; Saha-Roy, Sumon; Jans, Urs. 2012. Investigation of the reaction of hexabromocyclododecane with polysulfide and bisulfide in methanol/water solutions. Chemosphere 87, 158-162.


(29) Heeb, N., Schweizer, W., 2007. Solid-state conformations and absolute configurations of (+) and (-) $\alpha$-, $\beta$- and $\gamma$-hexabromocyclododecanes (HBCDs). Chemosphere 68, 940-950.


7. List of Tables:

Table 1. MS conditions # 1 of LCMSMS method # 1.

Table 2. LC gradient method #1, LCMSMS method # 1.

Table 3. MS condition # 8, LCMSMS method #2.

Table 4. LC gradient method #2, LCMSMS method #2.

Table 5. Approximated LOD and LOQ for α,β,γ-HBCD using LCMSMS method #1.

Table 6a-d. Approximated LOD and LOQ for α,β,γ-HBCD using LCMSMS method #2.

Table 7. Measured first order rate constants for individual HBCD isomers with hydrolysis (HO-) at a pH 9.09, 3.38Mm bisulfide at a pH 9.5, and 0.226 mM polysulfide at a pH 9.08 in 80% water / 20% methanol at 25°C

Table 8. Comparison of k” for α,β,γ-HBCD obtained in this work with values obtained in previous work.
8. List of Figures

Figure 1a-b. Percentage of clean glassware recovered using glassware cleaning method # 1 and #2.

Figure 2a. External calibration curve for α-HBCD.

Figure 2b. External calibration curve for β-HBCD.

Figure 2c. External calibration curve for γ-HBCD.

Figure 3a. Time course of α-HBCD (exp17)

Figure 3a1. Plot of ln[Corrected Concentration] versus time for α-HBCD. Prior to statistical omission of any points. (exp17)

Figure 3a2. Plot of ln[Corrected Concentration] versus time for α-HBCD. After statistical omission of all necessary points. (exp17)

Figure 3b. Time course of β-HBCD. (exp17)

Figure 3b1. Statistical Plot of ln[Corrected Concentration] versus time for β-HBCD to confirm no outliers present. (exp17)

Figure 3c. Time course of γ-HBCD. (exp17)

Figure 3c1. Plot of ln[Corrected Concentration] versus time for γ-HBCD and statistical plot to identify outliers. (exp17)

Figure 3c2. Plot of ln[Corrected Concentration] versus time for γ-HBCD. After statistical omission of all necessary points. (exp17)

Figure 4a-f. Step by step approach to eliminating outlier points from a data set. (exp25)

Figure 5. α-HBCD order of the reaction with respect to polysulfide.

Figure 6. β-HBCD order of the reaction with respect to polysulfide.

Figure 7. γ-HBCD order of the reaction with respect to polysulfide.

Figure 8. Determination of the second order rate constant of α-HBCD reacting with polysulfide at pH=9 and 25°C in 80% water/20% methanol solutions.

Figure 9. Determination of the second order rate constant of β-HBCD reacting with polysulfide at pH=9 and 25°C in 80% water/20% methanol solutions.
Figure 10. Determination of the second order rate constant of $\gamma$-HBCD reacting with polysulfide at pH=9 and 25°C in 80% water/20% methanol solutions.

Figure 11. Comparison of $\alpha$-HBCD degradation at 25°C in 80% water/20% methanol with hydrolysis at pH 9, 3.38 mM bisulfide at a pH 9.5, and 0.226 mM polysulfide at pH 9.0.

Figure 12. Comparison of $\beta$-HBCD degradation at 25°C in 80% water/20% methanol with hydrolysis at pH 9, 3.38 mM bisulfide at a pH 9.5, and 0.226 mM polysulfide at pH 9.0.

Figure 13. Comparison of $\gamma$-HBCD degradation at 25°C in 80% water/20% methanol with hydrolysis at pH 9, 3.38 mM bisulfide at a pH 9.5, and 0.226 mM polysulfide at pH 9.0.

Figure 14. XIC MRM chromatogram showing the bromine containing product dibromocyclododecene, the non-bromine containing products, and $\alpha,\beta,\gamma$-HBCD peaks.

Figure 14a. MS/MS Spectrum of the degradation product dibromocyclododecene with both 321/78.9 and 321/80.8 transitions.

Figure 14b. MS/MS Spectrum of a possible degradation product not containing bromine with only a 321/78.9 transition.

Figure 15b. MS/MS Spectrum of a possible degradation product not containing bromine with only a 321/80.8 transition.
Determination of Limit of Detection and Quantification for HBCD on the LC MSMS

Ethyl Acetate Blank: Sample 80 of 81
Baseline: 5.1 cps
Integrated entire area: 24,332 cps
Blank after Exp # 3 (HBCD:Polysulfide) 3rd Inj.

<table>
<thead>
<tr>
<th>Peak #</th>
<th>R.T (min)</th>
<th>Area (cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.54</td>
<td>233.78</td>
</tr>
<tr>
<td>2</td>
<td>5.02</td>
<td>30,972</td>
</tr>
<tr>
<td>3</td>
<td>5.66</td>
<td>24,818</td>
</tr>
<tr>
<td>4</td>
<td>5.31</td>
<td>28,488</td>
</tr>
<tr>
<td>5</td>
<td>3.32</td>
<td>74,916</td>
</tr>
<tr>
<td>6</td>
<td>0.759</td>
<td>48.47</td>
</tr>
<tr>
<td>7</td>
<td>4.02</td>
<td>26,427</td>
</tr>
<tr>
<td>8</td>
<td>5.22</td>
<td>18.35</td>
</tr>
<tr>
<td>9</td>
<td>1.93</td>
<td>34,838</td>
</tr>
<tr>
<td>10</td>
<td>2.04</td>
<td>27,186</td>
</tr>
</tbody>
</table>

Noise
Average: 54.8245 cps
Calculation Method #1: ehow

<table>
<thead>
<tr>
<th>Calculation Method # : IUPAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>N - 1 = 10</td>
</tr>
<tr>
<td>( X_{L} ) smallest measure that can be detected with reasonable certainty</td>
</tr>
<tr>
<td>( X_{bi} ) the mean of the blank measures.</td>
</tr>
<tr>
<td>( N - 1 = 9 )</td>
</tr>
<tr>
<td>( T ) 99% CL 4.03 ( k ) is the ( t ) value for the 99% confidence interval</td>
</tr>
<tr>
<td>( s_{bi} ) standard deviation of the blank measure</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Conversion to concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{L} ) smallest measure that can be detected with reasonable certainty</td>
</tr>
<tr>
<td>( C_{bi} = 0 ) the mean of the blank measures = 0 For Blank.</td>
</tr>
<tr>
<td>( k ) is the ( t ) value for the 99% confidence interval</td>
</tr>
<tr>
<td>( s_{bi} ) standard deviation of the blank measure</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>S sensitivity = ( \frac{\text{concentration}}{\text{intensity}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>INVERSE SLOPE: 1/ slope</td>
</tr>
</tbody>
</table>

Using 99% T value = \( k \) Using IUPAC \( k \) value = 3, to yield a 90% confidence interval

<table>
<thead>
<tr>
<th>LOD’s</th>
<th>LOQ’s</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{L} ) = 12.06 n M</td>
<td></td>
</tr>
<tr>
<td>( C_{L} ) = 3.15 n M</td>
<td></td>
</tr>
<tr>
<td>( C_{L} ) = 2.13 n M</td>
<td></td>
</tr>
</tbody>
</table>

Assuming the Following isomer ratios in the starting material:

| \( \alpha \) HBCD     | 0.13 |
| \( \beta \) HBCD    | 0.02 |
| \( \gamma \) HBCD  | 0.85 |

The End resulting Limit of QUANTIFICATION with the % composition correction

| \( \alpha \) HBCD = 1.31 n M |
| \( \beta \) HBCD = 0.06 n M |
| \( \gamma \) HBCD = 1.81 n M |

HBCD FW:\( = 641.7 \) Converted to units of g/L the LOD’s are:

<table>
<thead>
<tr>
<th>LOD’s</th>
<th>LOQ’s</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{L} ) = 838.52587 ng/L</td>
<td></td>
</tr>
<tr>
<td>( C_{L} ) = 40.38638 ng/L</td>
<td></td>
</tr>
<tr>
<td>( C_{L} ) = 1162.64880 ng/L</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Must get injection volume for final

<table>
<thead>
<tr>
<th>Converted to grams of HBCD the LOD’s are:</th>
</tr>
</thead>
<tbody>
<tr>
<td>With a 10μL injection</td>
</tr>
<tr>
<td>( C_{L} ) = 8.39E-12 g</td>
</tr>
<tr>
<td>( C_{L} ) = 2.54E-12 g</td>
</tr>
<tr>
<td>( C_{L} ) = 4.04E-13 g</td>
</tr>
<tr>
<td>( C_{L} ) = 1.22E-13 g</td>
</tr>
<tr>
<td>( C_{L} ) = 1.16E-11 g</td>
</tr>
<tr>
<td>( C_{L} ) = 3.52E-12 g</td>
</tr>
</tbody>
</table>

Table 5: Approximated Limit of Detection and Limit of Quantification for \( \alpha,\beta,\gamma \)-HBCD using LC-MS-MS method #1.
### Determination of Limit of Detection and Quantification for HBCD on the LC MSMS

**Method:** HBCD-ESI-NEG-MRM-JWGS1GS2.dam  
**File name:** DATA062613JWB

**Baseline:** 5 cps  
**Integrated entire area:** 170.9 cps  
**Blank in the very beginning of the run**

<table>
<thead>
<tr>
<th>Peak #</th>
<th>R.T (min)</th>
<th>Area (cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.66</td>
<td>110.22</td>
</tr>
<tr>
<td>2</td>
<td>5.58</td>
<td>37.74</td>
</tr>
<tr>
<td>3</td>
<td>5.51</td>
<td>24.425</td>
</tr>
<tr>
<td>4</td>
<td>3.58</td>
<td>28.449</td>
</tr>
<tr>
<td>5</td>
<td>4.41</td>
<td>26.692</td>
</tr>
<tr>
<td>6</td>
<td>5.24</td>
<td>20.402</td>
</tr>
<tr>
<td>7</td>
<td>8.1</td>
<td>16.973</td>
</tr>
<tr>
<td>8</td>
<td>5.8</td>
<td>17.001</td>
</tr>
<tr>
<td>9</td>
<td>5.91</td>
<td>18.276</td>
</tr>
<tr>
<td>10</td>
<td>6.61</td>
<td>26.466</td>
</tr>
</tbody>
</table>

**Noise**  
**Average:** 24.05 cps  
**std dev:** 6.77

- **Calculation Method # 1: ehow**
  - **LOD = 3 x Average Noise Peak Area**
  - **LOD = 72 cps**

- **Calculation Method # 2: IUPAC**
  - **LOD = 10 x Average Noise Peak Area**
  - **LOQ = 240 cps**

**Noise Average:** 24.05 cps

**Calculation:**  
\[ X \text{bias} = \frac{X}{N - 1} \]  
\[ s_{bi} = \text{standard deviation of the blank measure} \]

**Inverse Slope:**  
\[ \text{INVERSE SLOPE} = \frac{1}{\text{slope}} \]

Using 99% T value = k  
Using IUPAC k value = 3, to yield a 90% confidence interval

**Conversion to concentration**

\[ C_L = C_{bi} + k_{bi}s_{bi} \]

**Assuming the Following isomer ratios in the starting material:**

- **HBCD FW= 641.7**

### Table 6a: Approximated Limit of Detection and Limit of Quantification for α,β,γ−HBCD using LC-MS-MS method # 2 (Beginning of analysis results).

| α HBCD Slope exp # 20: | 18.344 | \( \alpha_{CL} = 1.33 \text{ nM} \) | \( \alpha_{CL} = 1.11 \text{ nM} \) |
| β HBCD Slope exp # 20: | 95.228 | \( \beta_{CL} = 0.26 \text{ nM} \) | \( \beta_{CL} = 0.21 \text{ nM} \) |
| γ HBCD Slope exp # 20: | 323.94 | \( \gamma_{CL} = 0.08 \text{ nM} \) | \( \gamma_{CL} = 0.06 \text{ nM} \) |

Using 99% T value = k  
Using IUPAC k value = 3, to yield a 90% confidence interval

| α HBCD Inverse Slope exp # 20: | 0.054514 | \( \alpha_{CL} = 4.40 \text{ nM} \) | \( \alpha_{CL} = 3.67 \text{ nM} \) |
| β HBCD Inverse slope exp # 20: | 0.010501 | \( \beta_{CL} = 0.85 \text{ nM} \) | \( \beta_{CL} = 0.71 \text{ nM} \) |
| γ HBCD Inverse Slope exp # 20: | 0.003087 | \( \gamma_{CL} = 0.25 \text{ nM} \) | \( \gamma_{CL} = 0.21 \text{ nM} \) |

Assuming the Following isomer ratios in the starting material:

- α HBCD = 0.13
- β HBCD = 0.02
- γ HBCD = 0.85

**The End resulting Limit of QUANTIFICATION WITH % composition correction**

| α HBCD | 0.48 nM |
| β HBCD | 0.01 nM |
| γ HBCD | 0.18 nM |

**HBCD FW= 641.7**

**Converted to units of g/L the LOQ's are:**

| α HBCD | 0.48E-12 g |
| β HBCD | 9.07E-14 g |
| γ HBCD | 113 ng/L |

**Converted to grams of HBCD the LOQ's are:**

| α HBCD | 3.06E-12 g |
| β HBCD | 9.27E-13 g |
| γ HBCD | 3.42E-13 g |

**Note:** Must get injection volume for final calculation.
### Determination of Limit of Detection and Quantification for HBCD on the LC MSMS

**Method:** HBCD-ESI-NEG-MRM-JWGS1GS2.dam

**Baseline:** 5 cps

**File name:** DATA062613JWB

**Ethyl Acetate Blank 1 - 52nd Injection**

**Middle of Analysis run**

**Baseline:** 5 cps

**Integrated entire area:** 204.36 cps

**Blank in the very beginning of the run**

<table>
<thead>
<tr>
<th>Peak #</th>
<th>R.T. (min)</th>
<th>Area (cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.89</td>
<td>60.88</td>
</tr>
<tr>
<td>2</td>
<td>4.7</td>
<td>34.17</td>
</tr>
<tr>
<td>3</td>
<td>4.33</td>
<td>25.54</td>
</tr>
<tr>
<td>4</td>
<td>4.62</td>
<td>42.04</td>
</tr>
<tr>
<td>5</td>
<td>4.97</td>
<td>35.61</td>
</tr>
<tr>
<td>6</td>
<td>5.81</td>
<td>30.85</td>
</tr>
<tr>
<td>7</td>
<td>6.07</td>
<td>53.03</td>
</tr>
<tr>
<td>8</td>
<td>8.31</td>
<td>50.78</td>
</tr>
<tr>
<td>9</td>
<td>8.64</td>
<td>24.04</td>
</tr>
<tr>
<td>10</td>
<td>0.51</td>
<td>35.17</td>
</tr>
</tbody>
</table>

#### Calculation Method # 1: ehow

- LOD = 3 x Average Noise Peak Area
- LOQ = 10 x Average Noise Peak Area

#### Calculation Method # : IUPAC

\[
X_{\text{L}} = X_{\text{bi}} + k_s
\]

- \(X_{\text{bi}}\): the mean of the blank measures.
- \(k_s\): standard deviation of the blank measure

#### Conversion to concentration

\[
C_{\text{L}} = C_{\text{bi}} + k_s X_{\text{bi}}
\]

- \(C_{\text{L}}\): smallest measure that can be detected with reasonable certainty
- \(C_{\text{bi}}\): the mean of the blank measures
- \(k_s\): standard deviation of the blank measure
- \(S\): sensitivity = \(\Delta\) concentration/\(\Delta\) intensity

#### Conversion to grams of HBCD

- Multiplying by the molecular weight of HBCD (641.7)

<table>
<thead>
<tr>
<th>Isomer</th>
<th>LOD's</th>
<th>LOQ's</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha) HBCD</td>
<td>1.84E-12 g</td>
<td>5.53E-12 g</td>
</tr>
<tr>
<td>(\beta) HBCD</td>
<td>1.28E-12 g</td>
<td>3.02E-12 g</td>
</tr>
<tr>
<td>(\gamma) HBCD</td>
<td>6.33E-13 g</td>
<td>1.06E-13 g</td>
</tr>
</tbody>
</table>

#### Conversion to grams of HBCD

- Multiplying by the molecular weight of HBCD (641.7)

<table>
<thead>
<tr>
<th>Isomer</th>
<th>LOD's</th>
<th>LOQ's</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha) HBCD</td>
<td>2.61E-12 g</td>
<td>7.48E-12 g</td>
</tr>
<tr>
<td>(\beta) HBCD</td>
<td>1.37E-12 g</td>
<td>3.45E-12 g</td>
</tr>
<tr>
<td>(\gamma) HBCD</td>
<td>7.16E-13 g</td>
<td>1.71E-13 g</td>
</tr>
</tbody>
</table>

**Note:**

- Must get injection volume for final injection calculation.
- Using 99% T value = \(k\) and IUPAC \(k\) value = 3, to yield a 90% confidence interval.

**HBCD FW = 641.7**

**Converted to units of g/L the LOQ's are:**

- \(\alpha\) HBCD = 0.72 n M
- \(\beta\) HBCD = 0.02 n M
- \(\gamma\) HBCD = 0.05 n M

**The End resulting Limit of QUANTIFICATION WITH the % composition correction**

- \(\alpha\) HBCD = 0.13
- \(\beta\) HBCD = 0.02
- \(\gamma\) HBCD = 0.05

**Table 6b:** Approximated Limit of Detection and Limit of Quantification for \(\alpha\),\(\beta\),\(\gamma\)-HBCD using LC-MS-MS method # 2 (Middle of analysis results).
Determination of Limit of Detection and Quantification for HBCD on the LC MSMS

Method: HBCD-ESI NEG-MRM JWGS1GS2.dam
File name: DATA06513JWB

Baseline: 5 cps
Integrated entire area: 204.36 cps
Blank in the very beginning of the run

Peak # R T (min) Area (cps) Calculation Method # 1: ehow
1 5.06 43.476
2 4.42 28.125
3 5.83 22.3
4 7.07 17.794
5 7.37 18.658
6 6.12 14.143
7 6.38 32.726
8 2.06 10.033
9 8.56 8.348
10 8.79 6.8067

LOD = 3 x Average Noise Peak Area
LOQ = 10 x Average Noise Peak Area
LOD = 53 cps
LOQ = 177 cps

Converted to grams of HBCD the LOD's are:
g ng/L
0.003087

LOQ = 10 x Average Noise Peak Area
323.94

LOQ = 3 x Average Noise Peak Area
3.3 x LOD

Xb = X0 + ksb

N = 10
Xb the mean of the blank measure.
N = 9
Xb the mean of the blank measures.

LOD's

Using IUPAC k value = 3, to yield a 90% confidence interval

Using 99% T value = k

Using IUPAC k value = 3, to yield a 90% confidence interval

Assuming the Following isomer ratios in the starting material:

α HBCC = 0.13
β HBCC = 0.02
γ HBCC = 0.85

The End resulting Limit of QUANTIFICATION WITH the % composition correction

Assuming the ratios provided are by weight

Note: Must get injection volume for final Calculation. 10µl

Converted to grams of HBCD the LOD's are:

Converted to grams of HBCD the LOQ's are:

Table 6c: Approximated Limit of Detection and Limit of Quantification for α,β,γ–HBCD using LC-MS-MS method # 2 (End of analysis results).
Summary of LOD and LOQ Calculated using Method: HBCD-ESI-NEG-MRM-JWGS1GS2.dam

### Limits of Detection

<table>
<thead>
<tr>
<th>Isomer</th>
<th>Beginning</th>
<th>Middle</th>
<th>End</th>
<th>Average (nM)</th>
<th>Standard Deviation (nM)</th>
<th>90% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>α HBCD (nM)</td>
<td>1.11</td>
<td>1.67</td>
<td>1.45</td>
<td>1.41</td>
<td>0.28</td>
<td>0.27</td>
</tr>
<tr>
<td>β HBCD (nM)</td>
<td>0.21</td>
<td>0.32</td>
<td>0.28</td>
<td>0.27</td>
<td>0.06</td>
<td>0.05</td>
</tr>
<tr>
<td>γ HBCD (nM)</td>
<td>0.06</td>
<td>0.09</td>
<td>0.08</td>
<td>0.08</td>
<td>0.02</td>
<td>0.01</td>
</tr>
</tbody>
</table>

### Limits of Quantification

<table>
<thead>
<tr>
<th>Isomer</th>
<th>Beginning</th>
<th>Middle</th>
<th>End</th>
<th>Average (g)</th>
<th>Standard Deviation (g)</th>
<th>90% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>α HBCD (g)</td>
<td>9.27E-13</td>
<td>1.4E-12</td>
<td>1.21E-12</td>
<td>1.18E-12</td>
<td>2.38E-13</td>
<td>2.26E-13</td>
</tr>
<tr>
<td>β HBCD (g)</td>
<td>2.75E-14</td>
<td>4.15E-14</td>
<td>3.16E-14</td>
<td>3.35E-14</td>
<td>7.20E-15</td>
<td>6.84E-15</td>
</tr>
<tr>
<td>γ HBCD (g)</td>
<td>3.42E-13</td>
<td>5.18E-13</td>
<td>4.48E-13</td>
<td>4.36E-13</td>
<td>8.86E-14</td>
<td>8.42E-14</td>
</tr>
</tbody>
</table>

**Table 6d:** Summary of approximated Limit of Detection and Limit of Quantification for α,β,γ-HBCD using LC-MS-MS method # 2 (note: excel functions were used to calculate the standard deviations and confidence at 90%). LODs and LOQs in units of nM are not corrected for the % composition of each isomer in the industrial grade HBCD. LOD and LOQ values in units of grams are corrected for the % composition in the technical grade HBCD.
### Table 7

Measured first order rate constants for individual HBCD isomers with hydrolysis (HO-) at a pH 9.09, 3.38 mM bisulfide at a pH 9.5, and 0.226 mM polysulfide at a pH 9.08 in 80% water/20% methanol at 25 ºC. The results listed are for actual individual experiments performed under the listed conditions.

<table>
<thead>
<tr>
<th>Reduced sulfur species</th>
<th>Hydrolysis [HO-]</th>
<th>Bisulfide [HS-]</th>
<th>Polysulfide [Sₙ²⁻]</th>
</tr>
</thead>
<tbody>
<tr>
<td>kₐ' [hr⁻¹]</td>
<td>0.0003 ± 0.00007</td>
<td>0.00139 ± 0.0002</td>
<td>0.00041 ± 0.00009</td>
</tr>
<tr>
<td>kₐβ' [hr⁻¹]</td>
<td>0.0008 ± 0.00008</td>
<td>0.00518 ± 0.0004</td>
<td>0.0031 ± 0.0012</td>
</tr>
<tr>
<td>kₐγ' [hr⁻¹]</td>
<td>0.00043 ± 0.00016</td>
<td>0.0156 ± 0.0024</td>
<td>0.0126 ± 0.0010</td>
</tr>
</tbody>
</table>

### Table 8

Measured second order reaction rate constants for the individual α,β,γ-HBCD isomers with polysulfide in water/methanol solutions. Note rate constants marked with *, were data obtained from previous work (19). In this study the total HBCD concentration was 100 nM. The previous work total HBCD starting concentrations of 100 μM in 20% water/80% methanol and 1 μM total HBCD in 50% water/50% methanol, respectively.

<table>
<thead>
<tr>
<th>Solvent Temperature</th>
<th>20% water/80% methanol</th>
<th>50% water/50% methanol</th>
<th>80% water/20% methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced sulfur species</td>
<td>Sₙ⁻</td>
<td>Sₙ⁻</td>
<td>Sₙ⁻</td>
</tr>
<tr>
<td>kₐα[M⁻sec⁻¹]</td>
<td>0.008*</td>
<td>0.0023*</td>
<td>0.0007</td>
</tr>
<tr>
<td>kₐβ[M⁻sec⁻¹]</td>
<td>0.022*</td>
<td>0.063*</td>
<td>0.0063</td>
</tr>
<tr>
<td>kₐγ[M⁻sec⁻¹]</td>
<td>0.022*</td>
<td>0.041*</td>
<td>0.0067</td>
</tr>
</tbody>
</table>

51 | Page
**Glassware Cleaning method # 1**

![Pie chart showing percentage of clean and dirty glassware for method #1.]

**Figure 1a:** Percentage of Clean glassware recovered when using cleaning method # 1 using an aqua regia 24 hour soak followed by rinsing with deionized water.

**Glassware cleaning method # 2**

![Pie chart showing percentage of clean and dirty glassware for method #2.]

**Figure 1b:** Percentage of Clean glassware recovered when using cleaning method # 2 using soap and water to clean followed by an acetone rinse and deionized water.
Figure 2a. External calibration curve for $\alpha$-HBCD.

Figure 2b. External calibration curve for $\beta$-HBCD.
**Figure 2c.** External calibration curve for $\gamma$-HBCD.

**Figure 3a.** Plot of corrected concentration versus time of $\alpha$-HBCD versus time of 100 nM HBCD reacting with 1.58 mM $S_n^2$ at pH 9.02 and 25 °C in 80 % water/20% methanol (exp17).
Figure 3a1. Plot of ln [corrected concentration] versus time of $\alpha$-HBCD of 100 nM HBCD reacting with 1.58 mM $S_n$ at pH 9.02 and 25 $^\circ$C in 80 % water/20% methanol (exp17). Prior to statistical omission of any points $k'_{\alpha}=0.0015$ hr$^{-1}$.

Figure 3a2. Plot of ln [corrected concentration] versus time of $\alpha$-HBCD versus time of 100 nM HBCD reacting with 1.58 mM $S_n$ at pH 9.02 and 25 $^\circ$C in 80 % water/20% methanol (exp17). After statistical omission of all necessary points $k'_{\alpha}=0.0010$ hr$^{-1}$.
Figure 3b. Plot of corrected concentration versus time of β-HBCD versus time of 100 nM HBCD reacting with 1.58 mM S₅²⁻ at pH 9.02 and 25 °C in 80 % water/20% methanol (exp17).

Figure 3b1. Plot of ln(corrected concentration) versus time of β-HBCD versus time of 100 nM HBCD reacting with 1.58 mM S₅²⁻ at pH 9.02 and 25 °C in 80 % water/20% methanol (exp17). No points needed to be ommitted after statistical evaluation kₚ' = 0.0569 hr⁻¹.

y = -0.0569x + 4.6014
R² = 0.9905
Figure 3c. Plot of corrected concentration versus time of $\gamma$-HBCD versus time of 100 nM HBCD reacting with 1.58 mM $S_n^{2-}$ at pH 9.02 and 25 °C in 80 % water/20% methanol (exp17).

Prior to statistical omission of any points $k_{\gamma}' = 0.0940$ hr$^{-1}$.

Figure 3c1. Plot of ln [corrected concentration] versus time of $\gamma$-HBCD versus time of 100 nM HBCD reacting with 1.58 mM $S_n^{2-}$ at pH 9.02 and 25 °C in 80 % water/20% methanol (exp17). Prior to statistical omission of any points $k_{\gamma}' = 0.0940$ hr$^{-1}$. 
Figure 3c2. Plot of ln [corrected concentration] versus time of γ-HBCD versus time of 100 nM HBCD reacting with 1.58 mM S\textsubscript{n}\textsuperscript{2}\textsuperscript{−} at pH 9.02 and 25 °C in 80% water/20% methanol (exp17). After statistical omission of all necessary points $k'_\gamma$=0.0862 hr\textsuperscript{-1}.

Figure 4a. Plot of corrected concentration of γ-HBCD versus time of 100 nM HBCD reacting with 0.633 mM S\textsubscript{n}\textsuperscript{2}\textsuperscript{−} at pH 9.06 and 25 °C in 80% water/20% methanol (exp25). This was the worst experimental data collected in the batch of experiments conducted. This was provided to illustrate how well statistical analysis # 2 works for identifying and eliminating outlier points. See figures 4b-4f below.
**Figure 4b.** Natural logarithm of the corrected concentration of $\gamma$-HBCD versus time of 100 nM HBCD reacting with 0.633 mM $S_n^2$ at pH 9.06 and 25 °C in 80 % water/20% methanol. Prior to application of statistics analysis # 2 (exp25). This was the worst experimental data collected in the batch of experiments conducted. This was provided to illustrate how well statistical analysis # 2 works for identifying and eliminating outlier points. See figures 4c- 4f below.

**Figure 4c.** First application of statistical analysis # 2 to the plot of the Natural logarithm of the corrected concentration of $\gamma$-HBCD versus time of 100 nM HBCD reacting with 0.633 mM $S_n^2$ at pH 9.06 and 25 °C in 80 % water/20% methanol.
**Figure 4d.** Second application of statistical analysis # 2 to the plot of the Natural logarithm of the corrected concentration of $\gamma$-HBCD versus time of 100 nM HBCD reacting with 0.633 mM $S_n^2$ at pH 9.06 and 25 °C in 80% water/ 20% methanol.

**Figure 4e.** Third application of statistical analysis # 2 to the plot of the Natural logarithm of the corrected concentration of $\gamma$-HBCD versus time of 100 nM HBCD reacting with 0.633 mM $S_n^2$ at pH 9.06 and 25 °C in 80% water/ 20% methanol.
Figure 4f. Final application of statistical analysis # 2 to the plot of the natural logarithm of the corrected concentration of $\gamma$-HBCD versus time of 100 nM HBCD reacting with 0.633 mM $S_n^2$ at pH 9.06 and 25 °C in 80% water/20% methanol. $k'$ decreased by a factor of 1.6 between figure 4a and 4f.

Figure 5. $\alpha$-HBCD- Log of the first order rate constant versus Log of the polysulfide concentration at 25 °C and pH 9 in 80%water/20%water. The order of the reaction with respect to polysulfides is 1.12 +/- 0.933.
Figure 6. $\beta$-HBCD- Log of the first order rate constant versus Log of the polysulfide concentration at 25 °C and pH 9 in 80% water/20% water. The order of the reaction with respect to polysulfides is $1.37 \pm 0.953$.

Figure 7. $\gamma$-HBCD- Log of the first order rate constant versus Log of the polysulfide concentration at 25 °C and pH 9 in 80% water/20% water. The order of the reaction with respect to polysulfides is $1.01 \pm 0.891$. 
Figure 8. First order rate constant of α-HBCD versus polysulfide concentration. 100 nM α-HBCD reacting with various concentrations of $S_n^2$ at pH 9 and 25 °C in 80% water/20% methanol. The second order rate constant is the slope of this graph, $k_α'' = 2.37 \pm 2.08 \text{ M}^{-1}\text{hr}^{-1}$.

Figure 9. First order rate constant of β-HBCD versus Polysulfide. 100 nM β-HBCD reacting with various concentrations of $S_n^2$ at pH 9 and 25 °C in 80% water/20% methanol. The second order rate constant is the slope of this graph, $k_β'' = 22.63 \pm 18.43 \text{ M}^{-1}\text{hr}^{-1}$.
Figure 10. First order rate constant of $\gamma$-HBCD versus Polysulfide Concentration.
100 nM $\gamma$-HBCD reacting with various concentrations of $S_n^2$ at pH 9 and 25°C in 80% water/20% methanol. The second order rate constant is the slope of this graph, $k_\gamma = 24.2 \pm 33.4 \text{ M}^{-1}\text{hr}^{-1}$.

Figure 11a. Comparison of individual experiments carried out to study $\alpha$-HBCD degradation at 25°C in 80% water/20% methanol with hydrolysis (HO-) at pH 9.0, 3.38 mM bisulfide (HS-) at pH 9.5, and 0.226 mM polysulfide at pH 9.
Figure 11b. Comparison of individual experiments carried out to study $\alpha$-HBCD first order rate constants at 25 °C in 80% water/20% methanol by plotting the ln(corrected concentration) vs. time (hr). $\alpha$-HBCD reaction with hydrolysis (HO$^-$) at pH 9, 3.38 mM bisulfide (HS$^-$) at pH 9.5, and 0.226 mM polysulfide at pH 9 are compared.

Figure 12a. Comparison of individual experiments carried out to study $\beta$-HBCD degradation at 25 °C in 80% water/20% methanol with hydrolysis (HO$^-$) at pH 9, 3.38 mM bisulfide (HS$^-$) at pH 9.5, and 0.226 mM polysulfide at pH 9.
Figure 12b. Comparison of individual experiments carried out to study \( \beta \)-HBCD first order rate constants at 25 °C in 80% water/20% methanol by plotting the ln(corrected concentration) vs. time (hr). \( \beta \)-HBCD reaction with hydrolysis (HO\(-\)) at pH 9, 3.38 mM bisulfide (HS\(-\)) at pH 9.5, and 0.226 mM polysulfide at pH 9 are compared.

Figure 13a. Comparison of individual experiments carried out to study \( \gamma \)-HBCD degradation at 25 °C in 80% water/20% methanol with hydrolysis (HO\(-\)) at pH 9, 3.38 mM bisulfide (HS\(-\)) at pH 9.5, and 0.226 mM polysulfide at pH 9.
Figure 13b. Comparison of individual experiments carried out to study $\gamma$-HBCD first order rate constants at 25 °C in 80% water/ 20% methanol by plotting the ln(corrected concentration) vs. time (hr). $\gamma$-HBCD reaction with hydrolysis (HO-) at pH 9, 3.38 mM bisulfide (HS-) at pH 9.5, and 0.226 mM polysulfide at pH 9 are compared.
Figure 14. XIC MRM Chromatogram of the degradation product Dibromocyclodecene at a retention time of 5.627 minutes. The two transitions, 321/80.8 (purple line) and 321/78.9 (light blue line) overlap. α-HBCD RT = 4.24 minutes, β-HBCD RT = 4.52 minutes, and γ-HBCD RT = 4.69 minutes. All HBCD isomer peaks have the two transitions, 640.8/78.9 (dark blue line) and 640.8/80.8 (red line) that overlap. Two additional transitions entered were; 481/78.9 (green line) and 481/80.8 (brown line).
**Figure 14a:** Mass spectrum of the peaks for the observed degradation product dibromocyclodocene observed with both 321/80.8 and 321/78.9 transitions at 5.627 minutes. Illustrates the two transitions observed and the bromine isotope ratio, 321/80.0 = 680cps, and 321/78.9 = 170cps. This shows a 1(Br$_{78.9}$) : 4 (Br$_{80.8}$) ratio in the dibromocyclodocene product observed.
**Figure 14b:** Mass spectrum of a potential degradation product that does not contain bromine at RT = 4.186 minutes. Light blue peak in figure 14 above, only the 321/78.9 transition was observed. The possible bromine isotope ratio observed is 8:1 (Br$^{78.9}$:Br$^{80.8}$ respectively).
Figure 14c: Mass spectrum of a potential degradation product that does not contain bromine at RT = 3.75 minutes. Purple peak in figure 14 above, only the 321/80.8 transition was observed. The possible bromine isotope ratio observed is 1:34 ($\text{Br}^{78.9} : \text{Br}^{80.8}$ respectively).
Acknowledgements:

Professor Jans,

Thank you for taking the time out of your busy schedule, and for having the patience to guide me in the right direction with this research project. Thank you for giving me the opportunity to conduct research under your leadership at both the Undergraduate and Master’s Level. I learned a lot, and had a lot of fun. You are truly a great Professor in chemistry. You made a big difference in my life. Thank you Professor Urs Jans.

Dedication:

To my son Tyler,

Don’t always take the easy path through life. Follow your passion in life. Sometimes it is the path least traveled that is the most rewarding. There is no meaning behind the term I can’t, there is only I don’t want to. Never give up on the important things in life.

To my Mother,

I did my chemistry thing. I got my Master’s like you said. I know you were, and always will be proud of me. Thank you for always telling me I can, when I thought I couldn’t.