# Treatment of Cannabinoids in Wastewater

A Major Qualifying Project (MQP)
Submitted to the Faculty of
**WORCESTER POLYTECHNIC INSTITUTE**
in partial fulfillment of the requirements for the
degree of Bachelor of Science

Submitted by:

Madeline Barnard
Elisabeth Christ
Adrian Reddick

Date: March 19th, 2021

Advised By:
Dr. John Bergendahl
Dr. Stephen J. Kmiotek

# Abstract

Cannabis is the most commonly used drug in the United States and its use is causing cannabinoids such as THC and THC-COOH to enter the environment. Wastewater treatment plants are not designed to remove cannabinoids and removal rates are extremely variable. More research is needed to determine the chemical properties of cannabinoids and their behavior in treatment plants and the environment. Existing literature suggests a combination of activated sludge and oxidations through chlorination are effective. Part of why so much is unknown about cannabinoids in water is due to the illegality of research. Finding a synthetic substitute for research is a priority. Based on the parameters of legality, physical structure, and functional groups, CP-55940 is a feasible surrogate.

# Acknowledgments

We would like to take a moment to thank the following people for their contributions to the success of our project. In no particular order,

Wenwen Yao, Environmental Lab Manager at Worcester Polytechnic Institute.
Stephen Kmiotek, Advisor to our project.
John Bergendahl, Advisor to our project.

# Table of Contents

Cover Page ..... **Error! Bookmark not defined.**
Abstract ..... ii
Acknowledgments ..... iii
Table of Contents ..... iv
List of Tables ..... vi
Executive Summary ..... 1
Introduction ..... 3
Background ..... 5
Behavior of THC ..... 5
A Contaminant of Emerging Concern ..... 7
Scope of Problem ..... 7
Oxidation Using Chlorine ..... 9
UV/Chlorine Advanced Oxidation Process ..... 10
Environmental and Health Effects ..... 11
Legality and Synthetic Cannabinoids ..... 12
CP-55940 ..... 14
Proposed Methodology ..... 17
Analytical Methods ..... 17
Surrogacy Verification ..... 17
Chlorination Kinetics Determination ..... 17
Design ..... 20
Conclusion ..... 24
Recommendations ..... 24
Further Research ..... 24
Best Practices ..... 26
Activated Sludge ..... 26
Chlorination ..... 26
Works Cited ..... viii
Appendix A. Calculations for Chlorination Kinetics Tests ..... xv
Appendix B. TOC Procedure ..... xvi
Appendix C. Sodium Thiosulfate Dilution Calculations ..... xviii

# List of Figures

Figure 1: Solubility of Δ9-THC and CBN in supercritical CO2 at 327 K tested at different pressures. ..... 5
Figure 2: Chlorination byproducts ..... 9
Figure 3. Technical scheme of UV/chlorine AOP pilot system ..... 20

# List of Tables

Table 1: Extraction yields and THC contents of SFE extracts from Cannabis sativa L. plant…....6
Table 2. THC-COOH and CP-55940 Chemical Information…….……...14
Table 3: List of conditions to be tested……..18

# Executive Summary

Cannabis is the most used illicit drug in the United States and Europe (Zucatto et al, 2008). The most prevalent and well-studied cannabinoid is THC. The most common metabolite of THC is THC-COOH, which is commonly used as a biomarker because it has greater longevity in the environment. A recent study found that THC-COOH was present in 100% of wastewater influent and effluents and 50% of surface waters (Boix et al., 2013). The concentrations are not minor either: measured wastewater influent values were greater than 2000 ng/L and wastewater effluent values were greater than 700 ng/L.

The chemical and physical properties of cannabinoids are largely unknown because most of the research has been focused on biological and medicinal applications. Part of the reason that so little is known about THC and THC-COOH is due to the legality of these chemicals - both are Schedule I drugs. Therefore it is difficult for labs to purchase and possess these chemicals. To try and avoid these requirements pharmaceutical companies invented synthetic cannabinoids to do biological research. The most structurally similar, legally available, synthetic cannabinoid is CP-55940 and the objective of this project is to determine the feasibility of using it as a chemical surrogate for THC-COOH in further wastewater treatment studies.

Having a legal chemical surrogate will open up research to more institutions. Knowledge of the behavior of cannabinoids in the environment and water treatment processes is limited and often done in treatment plants that have less control over experimental conditions. The available literature on the effectiveness and ideal conditions of individual treatment methods for cannabinoids is limited. The current best practice for their removal is activated sludge and oxidation by chlorination. However, activated sludge removal is likely due to sorption which may allow the cannabinoids to continue to react and reenter the environment. Studies on THC-COOH's effect throughout the chlorination process have touted a range of removal rates and have found a variety of transformation byproducts to form, some being more toxic than the cannabinoids themselves (González-Mariño, 2013). Removal rates of CP-55940 during surrogacy verification and oxidation by chlorination treatment bench tests are to be analyzed in the laboratory using a TOC instrument.

A UV/chlorination advanced oxidation pilot-scale system was designed to treat spiked wastewater with CP-55940 based on a similar pilot system found in the literature. The addition of UV with chlorine creates more powerful oxidants and could help limit the creation of byproducts.

Experimentation varying chlorine concentration, CP-55940 concentration, and UV consumption are to be performed using this system.

# Introduction

Cannabis is the most used illicit drug in the United States and Europe (Zucatto et al., 2008). The most prevalent and well-studied cannabinoid is 11-Nor-9-carboxy-$\Delta^9$-tetrahydrocannabinol, also known as THC. The most common metabolite of THC is THC-COOH, which is commonly used as a biomarker because it has greater longevity in the environment. The chemical and physical properties of these chemicals are largely unknown because most of the research has been focused on biological and medicinal applications. The values used for the chemical properties are based on predictive models, not experimental values. THC-COOH can form complexes with glucuronic acid (in urine) and natural organic matter. These complexes further complicate estimated longevity in the environment and treatment processes.

A recent study found that THC-COOH was present in 100% of wastewater influent and effluents and 50% of surface waters (Boix et al., 2013). The concentrations are not minor either: measured wastewater influent values were greater than 2000 ng/L and wastewater effluent values were greater than 700 ng/L. Surface water concentrations were greater than 500 ng/L (Park, 2017). There are even some cases of THC-COOH in tap water (Carmona et al., 2014). The recent changes in state legislation regarding the growth and sale of marijuana has caused a significant increase in THC found in wastewater treatment plants and in local water bodies where treated materials are released. The presence of THC and THC-COOH in the surface water indicates that wastewater treatment plants are ineffective at removing THC and THC-COOH.

Conventional wastewater treatment plants are not designed to remove many common drugs such as THC, amphetamines, and opiates. Several studies on the disinfection process, in particular, have evaluated the reactions between chlorine and pollutants such as bactericides, pharmaceuticals, and THC metabolites (Mackie, 2017). There are 7 possible by-products of THC-COOH and chlorine during the disinfection process of wastewater treatments (González-Mariño, 2013). Disinfection by-products are more toxic than the original metabolite and pose a threat to environmental systems upon discharge (González-Mariño, 2013).

Few studies have quantified the effect of THC-COOH and its byproducts on living organisms or more generally the environment. This is due to legal regulations imposed by the United States federal government on all forms of THC which has caused limitations on research. For this purpose, scientists have created a family of synthetic cannabinoids to conduct research,

which function similarly to THC in terms of psychological effect on organisms but are molecularly different enough to bypass the regulations determining illegality. CP-55940 is one such synthetic cannabinoid that can be used as a surrogate for THC-COOH in wastewater treatment tests. CP-55940 is available for legal sale and its molecular similarities to THC-COOH make it a promising replacement for testing of THC metabolites through different treatment systems.

# Background

## Behavior of THC

Delta-9-Tetrahydrocannabinol, also known as THC, is the main psychoactive ingredient of cannabis ("Tetrahydrocannabinol"). THC is a hydrophobic oil that acts as the plant's defense system against ultraviolet radiation, pest infestation, and environmental stress (Pate, 1983; Pate, 1994). THC is a cannabinoid, which means it acts as an antagonist to both the $CB_1$ and $CB_2$ cannabinoid receptors. These receptors affect the nervous system and the cells of the immune system. Because of its interactions with these receptors, THC has a variety of medicinal uses including being used to alleviate neuropathic pain and spasticity. With over 400 ingredients, cannabis contains 66 cannabinoids which can show biological activity (Russo, 2003). A study done in 2006 found that THC can facilitate neuroregeneration and can prevent neural degradation from disorders such as MS and Parkinson's (Eubanks et al., 2006).

THC has a low solubility rate in water and a higher solubility rate in other nonpolar lipids. Because THC is a nonpolar molecule, it tends to be hydrophobic. In 2009, a study testing the solubility of cannabinol (CBN), a mildly psychoactive cannabinoid, in supercritical $CO_2$ determined the solubility values using a solubility cell. The samples were tested at temperatures 315, 327, 335, and 345, and pressures between 13.2 and 25.1 MPa as seen in Figure 1. Their selected values were based on the successful trials of previous studies of $\Delta^9$-tetrahydrocannabinol solubility.

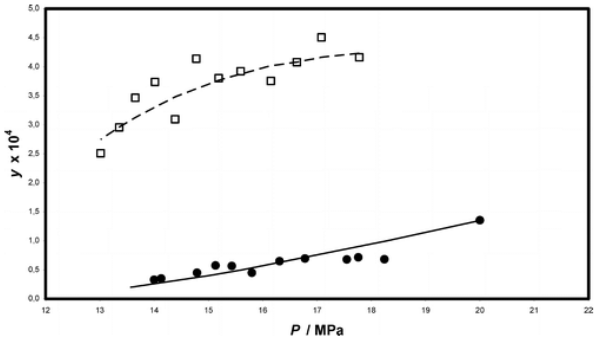



Figure 1: Solubility of Δ9-THC and CBN in supercritical CO2 at 327 K tested at different pressures. (H. Perrotin-Brunel et al. 2009).

In Figure 1, THC is represented by the dot, while CBN, the square. Molar solubilities ($y$) are shown on the x-axis. $\Delta^9$-THC was found to have lower solubility than that of CBN, which was thought to be due to its relatively higher polarity and higher molar mass. This gives an indication of a cannabinoid's solubility if its molar mass and polarity are known and compared to $\Delta^9$-THC.

THC is thermally unstable meaning it degrades quickly under heat but can be quickly managed when exposed to cold. This allows for the easy extraction process of THC from cannabis via thermal or pressurized methods. Another study which also used supercritical fluid extraction (SFE) found that extraction of THC tested at different pressures (15–33 MPa), temperatures (40–80 °C), and ethanol as co-solvent (0–5%), yielded up to 37.85% THC (Gallo-Molina, 2019).

Table 1: Extraction yields and THC contents of SFE extracts from Cannabis sativa L. plant. (Gallo-Molina, 2019).

| Extraction Parameters | | | | Extraction Yields (% wt d.b) | THC Contents | | |
|---|---|---|---|---|---|---|---|
| Extract | Pressure (MPa) | Temperature (C) | Cosolvent (% wt) | | %THC in dry extract | %THC in dry cannibis sample | THC Recovery |
| 1 | 15 | 40 | 0 | 4.83 | 32.25 | 1.56 | 4.58 |
| 2 | 15 | 80 | 0 | 6.32 | 31.08 | 1.96 | 4.41 |
| 3 | 15 | 80 | 5 | 21.17 | 24.88 | 5.27 | 3.53 |
| 4 | 15 | 40 | 5 | 23.36 | 30.14 | 7.04 | 4.28 |
| 5 | 33 | 80 | 0 | 10.41 | 24.73 | 2.57 | 3.51 |
| 6 | 33 | 80 | 5 | 26.36 | 15.52 | 4.09 | 2.2 |
| 7 | 33 | 40 | 5 | 21.49 | 20.34 | 4.37 | 2.89 |
| 8 | 33 | 40 | 0 | 6.32 | 25.81 | 1.63 | 3.67 |
| 9 | 33 | 60 | 2 | 16.01 | 37.85 | 6.06 | 5.38 |
| 10 | 24 | 80 | 2 | 17.43 | 29.54 | 5.15 | 4.2 |
| 11 | 15 | 60 | 2 | 13.97 | 36.18 | 5.05 | 5.14 |
| 12 | 24 | 40 | 2 | 15.57 | 25.1 | 3.91 | 3.57 |
| 13 | 24 | 60 | 5 | 18.27 | 22.04 | 4.03 | 3.13 |
| 14 | 24 | 60 | 0 | 13.06 | 28.38 | 3.71 | 4.03 |
| 15 | 24 | 60 | 2 | 16.39 | 24.09 | 3.95 | 3.42 |
| 16 | 24 | 60 | 2 | 17.18 | 26.15 | 4.49 | 3.71 |
| 17 | 24 | 60 | 2 | 16.13 | 23.84 | 3.85 | 3.39 |
| 18 | 24 | 60 | 2 | 18.61 | 24.53 | 4.57 | 3.48 |
| 19 | 24 | 60 | 2 | 15.48 | 28.28 | 4.38 | 4.02 |

[a] d.b. – dry basis.
[b] THC Recovery values were calculated as the ratio between the THC content in the raw extracts and the total THC in raw material ($\%THC_{extract}$ / $\%THC_{Total}$). The total THC observed in cannabis sample used was 7.04%, this was extracted followed the procedure described in Section 2.6.

Table 1 shows the extraction yields for the first eleven trials of THC extraction using SFE. Trial 9 contained the greatest THC percent yield which was found to be at 33 MPa and 60°C.

When metabolized in the body THC creates several byproducts. Currently, there are over 100 identified cannabinoid metabolites, the most common being 11-OH-THC and 11-nor-9-carboxy-THC (THC-COOH). Of these identified metabolites, 55% of THC is excreted as 11-OH-THC in feces and 0.6% of ingested THC is excreted as THC-COOH in a urine matrix where it is stable (González-Mariño, 2013) (Postigo, 2009). THC-COOH is often found in a higher concentration in urine and has a longer period of detection time, making it a better biomarker in urine (Postigo, 2009). Laboratory testing of THC metabolites found in urine is commonplace among drug tests for individuals.

## A Contaminant of Emerging Concern

Laboratory testing of THC metabolites found in urine is commonplace among drug tests for individuals. Testing for THC metabolites in drinking water, raw wastewater, and natural surface waters, as well as the chemical pathways for any reactions in those mediums, is far less common, as the monetary incentive for such basic research is usually low.

## Scope of Problem

THC-COOH and other drugs of abuse and their metabolites have been recently recognized as emerging organic contaminants. There are currently no state or federal regulations establishing an acceptable detection limit for THC metabolites discharged from water and wastewater treatment facilities (Cosenza, 2018). Increases in future consumption of THC, coupled with strong public opinion on the drug, could mean that regulations regarding its treatment and discharge are on their way. To evaluate THC-COOH's potential ecotoxicological effects and any subsequent treatment systems proposed thereafter, a literature review of THC-COOH concentrations found in wastewater, drinking water, and surface water must be completed (Postigo, 2009). The literature on the oxidation kinetics using chlorine of THC-COOH will help analyze bench tests while an established range of typical concentrations will help to inform a pilot treatment design.

THC-COOH is used as a biomarker for cannabis consumption of a population from its detection in different environmental compartments. Assessment of its concentration constitutes an indirect tool to estimate drug abuse by the population at the community level (Postigo, 2009).

This is because it is the metabolite most commonly excreted in urine and has greater longevity in the environment (Postigo, 2009).

Testing on wastewater effluents for THC-COOH have been used to track the illicit drug consumption of different populations. It has been proven through water quality tests worldwide that THC-COOH ends up in natural water systems (Postigo, 2009). Some surface waters exhibit concentrations of THC-COOH over 500 ng/L while others as low as 5.5 ng/L (Mackie et al., 2017).

In untreated wastewater, THC–COOH has been detected as high as 2500 ng/L and as low as 10.6 ng/L (Mackie et al., 2017). THC-COOH in raw influent wastewater samples from New York City were detected at 168.2–772.0 ng/L (Jacox, 2017). Another New York City study testing wastewater in different boroughs throughout 1 year found a range between 1854.9 ng/L and 101.8 ng/L of THC-COOH (Centazzo, 2019). In treated wastewater, it has been detected as high as 750 ng/L and as low as 5.2 ng/L (Mackie et al., 2017). A study of treated effluents in Sicily found a steady concentration of under 50 ng/L THC-COOH over 15 days (Cosenza, 2018). Reported removal rates in wastewater treatment plants vary significantly from; -18.3% to 100% (Mackie et al., 2017). Overall, total levels of the studied THC and other illicit drug metabolites observed in surface water (in the low ng/L range) were one and two orders of magnitude lower than those determined in effluent (in the ng/L range) and influent sewage water (in the μg/L range), respectively (Postigo, 2010).

In most instances, activated sludge processes were the most commonly studied methods of removal. When the sludge is removed or exposed to water with a lower concentration of THC it releases from the sludge and reenters the water stream (How, 2020). Also, THC continues to react and degrade into THC-OH and THC-COOH while attached to the sludge (Park, 2017). These compounds are much less likely to adsorb and have a higher solubility in water and thus often reenter the water stream (Park, 2017). Some levels in effluents are tested to be even higher than in influents. This increase in detection could be attributed to the hydrolysis of THC-COOH conjugates (González-Mariño, 2013). Some published treatment studies investigating the removal of THC-COOH from water have focused on chlorine or photo-degradation as the treatment method, (Mackie, 2017). Both chlorine or photo-degradation have been found to lead to transformation byproducts, rather than conclusive results of physical or chemical removal. One occurrence of THC–COOH in tap water has been reported at the detection limit of 1 ng/L

(Mackie, 2017), however, limited occurrences of detection in tap waters do not ensure safety. It could mean that other byproducts were formed through disinfection.

## Oxidation Using Chlorine

Oxidation using chlorine (chlorination) is fast and effective at the removal of THC and THC-COOH (Mackie, 2017). Chlorination is more effective at lower pH. This is likely due to the dissociation of THC-COOH complexes at lower pHs (How, 2020). The presence of natural organic matter drastically reduces the effectiveness of chlorination. It increases the needed contact time to an unfeasible duration (Mackie, 2017). This is partly because chlorine species vastly prefer reactions with natural organic matter. THC-COOH also forms complexes with natural organic matter that are less receptive to chlorination (Mackie, 2017). Thus chlorination works better as a pretreatment at high concentration sources such as marijuana growing facilities, hospitals, colleges, and sports arenas (Mackie, 2017).

Unfortunately, while chlorination is effective the oxidation is not complete. This causes the creation of a variety of byproducts. The transformation of THC-COOH into different by-products of unknown toxicity and stability raises concern. There are 7 known possible byproducts of THC-COOH and chlorine during the disinfection process of wastewater treatments (González-Mariño, 2013).

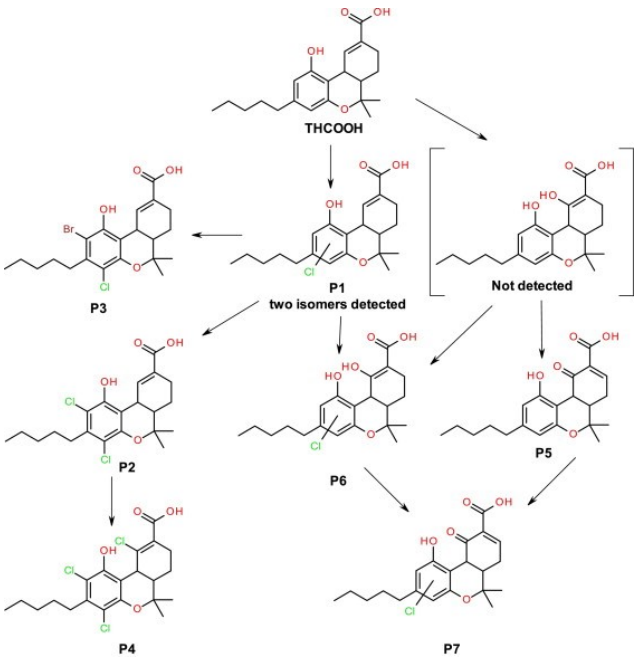



Figure 2: Chlorination byproducts (González-Mariño, 2013)

These chlorination byproducts are up to 15 times more toxic than THC-COOH. THC-COOH is 5 times as toxic as THC (González-Mariño, 2013). Concentrations as low as 13 ng/L of byproduct P3 are fatal to *Daphnia magna* in less than 48 hours (González-Mariño, 2013). Disinfection by-products made from THC-COOH have received growing attention. One study which examined the transformation of cannabinoids through engineered water systems found that halogenated cannabinoid disinfection byproducts were most likely to be formed. The study concluded that the destructive removal of cannabinoids via chlorination and other oxidation processes used in drinking water and wastewater treatment requires careful investigation. (Apul et al., 2020). At this time it is unknown what the chemical properties, prevalence, and longevity of these byproducts are. These byproducts have been detected in surface water and tap water. This indicates that they are stable enough to be present in the environment (Boix, 2014). There could be further chemicals caused by reactions with these chlorination byproducts. There could also be further byproducts from the chlorination of THC or THC-COOH not yet discovered.

## UV/Chlorine Advanced Oxidation Process

One way to improve rates of complete oxidation, thus further eliminating harmful organic pollutants such as disinfection byproducts, is with the use of a UV/chlorine advanced oxidation process (APO) (Rott, 2018).

Before meeting UV treatment, chlorine is added to the secondary effluent, starting the transformation of chlorine ($Cl_2$) into hypochlorous acid (HOCl) and hydrochloric acid (HCl) shown below:

$$Cl_2 + H_2O \rightarrow HOCl + Cl^- + H^+$$

The further dissociation of weak acid, HOCl, into hypochlorite anions ($ClO^-$) is pH dependent, shown in the next equation:

$$HOCl \rightarrow ClO^- + H^+ \qquad (pKa = 7.5)$$

The predominant species will be HOCl if pH values are kept around 7 (Rott, 2018). This is in line with operating conditions of other WWTPs; for example, studies of illicit drug removal efficiencies of two plants in Italy kept a pH of 7.4 and 7.5, and temperatures of 20C and 19.8C, respectively (Cosenza, 2018). Because $ClO^-$ is a less effective oxidant, increasing pH values will increase $ClO^-$ concentration, thus decreasing oxidation capability. In contrast, decreasing pH will shift the equilibrium towards HOCl, thus increasing oxidation capability. This effluent is

then treated with UV lamps that can be of low pressure, emitting one single wavelength (254 nm), or medium pressure, emitting a broader spectrum (200–400 nm). The use of UV in tandem with HOCl and ClO− creates chlorine and hydroxyl radicals that are reactive oxidants through the below reactions:

$$HOCl + UV\ photons \rightarrow {\bullet}OH + Cl{\bullet}$$

$$ClO^- + UV\ photons \rightarrow {\bullet}O^- + Cl{\bullet}$$

$${\bullet}O^- + H_2O \rightarrow {\bullet}OH + OH^-$$

These radicals are preferable because they can oxidize organic pollutants to $CO_2$ and $H_2O$, or at least render them biodegradable for subsequent natural degradation. UV/chlorine APOs are also more economically feasible by requiring less energy than other UV systems and have better removal rates than traditional chlorine treatment (Rott, 2018).

In the case of an incomplete reaction of FAC in the UV chamber, "residual free chlorine" (RFC) in the UV chamber effluent is quenched by sodium thiosulfate ($Na_2S_2O_3$) downstream of the UV chamber before entering the second static mixer.

## Environmental and Health Effects

Likely, THC-COOH is continuously being discharged into water systems by wastewater treatment plants worldwide in concentrations within the previously stated effluent and surface water ranges (Bijlsma, 2009). THC and its metabolites are contaminants of emerging concern due to the limited information on their environmental impacts. Releases to water systems and environments of concern are expected to increase greatly due to recent legalization and use (How, 2020). There is a potential impact on aquatic environments, as shown in studies directly testing the toxicity of THC-COOH on zebra mussels by measuring the oxidative stress. The highest concentration of THC-COOH tested on the mussels was 1000 ng/L, however, all concentrations tested resulted in increased DNA fragmentation but with no specific genetic damage (Parolini, 2016). A related study showed that significant oxidative stress to zebra mussels was observed after exposure to 500 ng/L of THC for 14 days (Parolini and Binelli, 2014). It was found in another study that THC at concentrations higher than 30 mg/L would result in increased anxiety behaviors in zebrafish (Stewart and Kalueff, 2014).

As mentioned earlier, several cannabis-compound-based drugs are being used to treat various illnesses and diseases. However, there is a significant point of contention when analyzing the negative effects of cannabis on the human body (Huestis, 2002). Due to the difference in experimental procedures, there is no accurate analysis of the negative effects. It has been theorized that the toxic effects of cannabis include panic attacks, lung damage and in severe cases can result in overdose (Huestis, 2002). Studies have also theorized that an increase in exposure to cannabinoid compounds can cause an increase in male factor infertility (Plessis et al., 2015).

## Legality and Synthetic Cannabinoids

Part of the reason that so little is known about THC and THC-COOH is due to the legality of these chemicals. Both are classed as Schedule I drugs meaning that the federal government views these chemicals as "having no currently accepted medical use and have a high potential for abuse ("Drug Scheduling")." To be able to research Schedule I drugs, researchers must go through an extensive application process that vets the researcher, institution, and individual project. First, the researcher must get DEA registration which requires filling out DEA forms 224, 225, 363, and 510 online and through registered mail (Corrigan). Then the research project must go through the research protocols set out in §1301.18 (Corrigan). The facilities that host Schedule I drugs must incur the extra expense for increased security that is compliant with §1301.75 including locked cabinets, vetting of people who have access to the lab, and secure mail procedures (Corrigan).

To try and avoid these requirements pharmaceutical companies invented synthetic cannabinoids to do biological research. These chemicals are designed to be structurally similar enough to be antagonists of CB1 and CB2 receptors ("Synthetic Cannabinoids", 2013). The first set of chemicals originated at Hebrew University (HU series) in 1988 and are the most structurally similar to THC ("Synthetic Cannabinoids", 2013). They are known as classic synthetic cannabinoids and are up to 100 times more potent than THC ("Synthetic Cannabinoids", 2013). However soon after its invention, the HU series was deemed a structural analog and therefore also Schedule I drugs under the Federal Analogue Act of 1986 (Abbate et al., 2018).

Next, the non-classical synthetic cannabinoids (CP series) were synthesized. These are bicyclic and tricyclic compounds that mimic the structure of THC without the tetrahydropyran (six-member hydrocarbon ring with one oxygen) (Spaderna et al., 2013). The next series was created by Alexandros Makriyannis (AM series) that are structurally based on anandamides which are a class of endocannabinoids that trigger the immunological effects of cannabinoids (Spaderna et al., 2013). The fourth series of synthetic cannabinoids were developed by John W Huffman (JWH series). They are modeled off of the A-G2 and AEA which are endocannabinoid hormones found in the human body (Wiley et al., 2011). Unfortunately, the JWH series is much easier to produce and led to a boom in the use of synthetic cannabinoids in the illegal drug market (Cha et al., 2014). They began being sprayed on organic material and marketed as incense and herbal blends marketed as "not for human consumption", but people began smoking them anyway (Wiley et al., 2011). Synthetic cannabinoids are much easier to traffic and have a lower overhead cost which lead to their increase in popularity - becoming the second most common drug used in the United States (Spaderna et al., 2013). The JWH series are 5 to 20 times more binding to the CB1 and CB2 receptors which increases the risk of addiction and overdose (Cha et al., 2014). They also have been determined to have severe health effects including neurotoxicity, strokes, heart attacks, and induced psychosis (Cha et al., 2014).

The increased availability and risk have led to increased legislation. However, due to the continued innovation and creation of synthetic cannabinoids, they are difficult to define and regulate (Spaderna et al., 2013). The most comprehensive federal regulation is the Synthetic Drug Abuse Prevention Act of 2012 which reclassified several types of synthetic cannabinoids as Schedule I drugs (Portman, 2012). This act defines synthetic cannabinoids as cannabimimetic agents that are CB1 agonists and fall within the following structural categories:

- 2-(3-hydroxycyclohexyl)phenol with substitution at the 5-position of the phenolic ring by alkyl or alkenyl. (Portman, 2012)
- 3-(1-naphthoyl)indole or 3-(1-naphthylmethane)indole by substitution at the nitrogen atom of the indole ring. (Portman, 2012)
- 3-(1-naphthoyl)pyrrole by substitution at the nitrogen atom of the pyrrole ring. (Portman, 2012)
- 1-(1-naphthylmethylene)indene by substitution of the 3-position of the indene ring. (Portman, 2012)

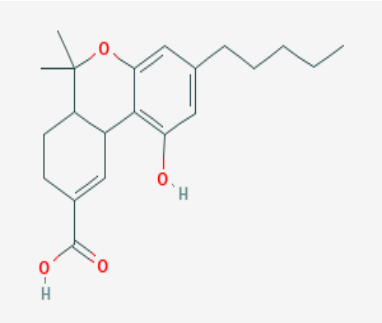
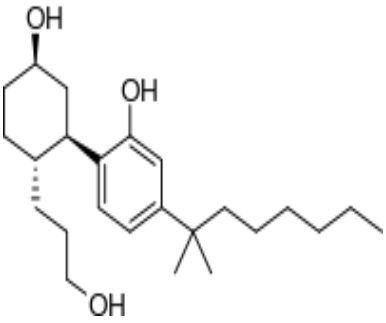
- 3-phenylacetylindole or 3-benzoylindole by substitution at the nitrogen atom of the indole ring. (Portman, 2012)

The first structural category refers to CP-47,497 while the rest refer to the JWH series of chemicals (Portman, 2012). These chemicals were selected for regulation because they were the most commonly used synthetic cannabinoids in DEA seized materials (Abbate et al., 2018).

## CP-55940

CP-55940 is not included in this ban as it is not a CB1 receptor antagonist making it an accessible substitution for THC-COOH. Because of the cognitive similarities to THC, it is worth studying the structural and chemical similarities of CP-55940 and THC-COOH to inform further experimentation of the treatment of these chemicals in wastewater. Information on both chemicals was gathered and represented in Table 2 below.

Table 2. THC-COOH and CP-55940 Chemical Information ("PubChem Compound Summary for CID 4412255", "PubChem Compound Summary for CID 107885, 11-Nor-9-carboxy-thc")

| | THC-COOH | CP-55940 |
|---|---|---|
| |  |  |
| Molecular Weight | 358.47g | 376.57g |
| Boiling Point | 429.9 C | 494.4 C |
| Polar Surface Area | 66.8 $A^2$ | 60.7 $A^2$ |

| | | |
|---|---|---|
| logP (a measure of partitioning) | 7.874 | 6.198 |
| H Acceptors and Donor Sum | 6 | 6 |
| Rotatable bonds | 6 | 13 |
| pKa | 3.32 | 10.25 |
| Mass Intrinsic Solubility @25C | 2.4E-4 g/L | 2.7E-3 g/L |
| Vapor Pressure @25C | 3.72E-8 Torr | 1.36E-10 Torr |

These molecules share several key features such as a benzene ring, a hydrocarbon ring, tetrahydropyran, two electronegative aspects attached to the rings including a hydroxyl group, a long hydrocarbon tail, and two methyl groups ("PubChem Compound Summary for CID 4412255", "PubChem Compound Summary for CID 107885, 11-Nor-9-carboxy-thc"). This makes the molecular weight, the number of bonds, logP, and polar surface area of these two chemicals very similar. The key differences between these two molecules are the rings - two of the rings are not fully fused like in THC-COOH because the tetrahydropyran ring is incomplete and in the form of a chain in CP-55940 ("PubChem Compound Summary for CID 4412255", "PubChem Compound Summary for CID 107885, 11-Nor-9-carboxy-thc"). That oxygen is on the end of a chain in the form of a hydroxyl group. This incomplete fusing makes the number of rotatable bonds increase and in turn, increases the pKa value ("PubChem Compound Summary for CID 4412255", "PubChem Compound Summary for CID 107885, 11-Nor-9-carboxy-thc"). The two methyl groups are attached to the hydrocarbon chain instead of the tetrahydropyran ring. CP-55940 also does not have the carboxyl group - it is replaced with a third hydroxyl group which gets rid of the double bond in that ring ("PubChem Compound Summary for CID 4412255", "PubChem Compound Summary for CID 107885, 11-Nor-9-carboxy-thc"). The pKa of the two compounds is different enough when starting in a neutral pH solution, one molecule might naturally turn the solution more acidic during ionization than would the other molecule.

CP-55940 and other nonclassical cannabinoids are normally used in drug therapy research settings and to study the endocannabinoid system. Most research thus far has been focused on the effects of these drugs on cannabinoid receptors found in the mammalian brain. CP-55940 is a selective, high-affinity cannabinoid agonist that binds to these receptors similarly to THC of the marijuana plant. CP-55940 is used in research as a substitute for THC when studying the endocannabinoid system. The endocannabinoid system is a biological system composed of endocannabinoids (endogenous lipid-based retrograde neurotransmitters) that bind to cannabinoid receptors (CBRs), and cannabinoid receptor proteins that are expressed throughout the nervous system. The endocannabinoid system may be involved in regulating physiological and cognitive processes including fertility and pregnancy, pre-and postnatal development, various activities of the immune system, appetite, pain-sensation, mood, memory, and in mediating the pharmacological effects of cannabis. However, the endocannabinoid system remains under preliminary research due to how regulated all cannabinoids are worldwide. Even less is known about how the molecules CP-55940 and THC-COOH act as they travel through our water treatment systems and affect our environment after being ingested and excreted by mammals. It is also known that THC-COOH reacts with chlorine to form chlorine byproducts that are orders of magnitude more toxic than THC-COOH (González-Mariño, 2013). More research is needed to determine the feasibility of water treatment systems as THC and THC-COOH are increasingly found in the environment with unknown effects. CP-55940 is legal for purchase and is a hopeful surrogate for THC-COOH in further research of wastewater treatment processes.

# Proposed Methodology

In the laboratory, experimentation to examine the effects of oxidation by chlorine on the synthetic cannabinoid CP-55940 is needed. Sodium hypochlorite (NaClO) will be added to samples spiked with CP-55940 and concentration will be determined by a Shimadzu TOC-L at various time increments. These tests are designed to treat CP-55940 similarly to how THC-COOH would be treated in bench-scale chlorination tests. Surrogacy verification tests will be first performed on CP-55940 to quantify its similarities to THC-COOH during treatment.

## Analytical Methods

To calibrate the TOC instrument potassium hydrogen phthalate (KHP) was used. First, the KHP must be dried and a stock primary standard solution of 1000 mg/L KHP prepared (see Appendix B). The stock primary standard solution has a known TOC value of one mg TOC per one mL. The stock primary solution can be kept in the refrigerator for a month and will be diluted to the intermediate standard of 100 mg/L which lasts for two days refrigerated. On the day of the test prepare three working samples containing the standard by diluting them to values that bracket the assumed test concentrations. The Shimadzu TOC-L was operated according to the manufacturer's instructions also located in Appendix B.

## Surrogacy Verification

To verify the validity of using CP-55940 as a surrogate for THC-COOH a procedure from a peer-reviewed article on chlorination kinetics was used (Mackie, 2017). A concentration of 1 µg/mL of CP-55940 and a chlorination value of 0.05 mg $Cl_2$/L at a pH of 7 were used. The source of chlorine is sodium hypochlorite. The reaction is run in a 200 mL beaker with magnetic stirring. The concentration was measured at time intervals of 0, 1, and, 2 minutes using the TOC instrument after quenching with sodium thiosulfate at a concentration of three times the initial chlorine dose to ensure complete quenching.

## Chlorination Kinetics Determination

To determine the kinetics of oxidation by chlorine, a variety of concentrations of CP-55940 and chlorine, in addition to time durations and pH values, must be used. See Appendix A for calculations for chlorination kinetics tests required dosages and dilution of CP-55940 and

NaOCl for test conditions, as well as $Cl_2$ conversion. Each condition should be repeated three times in addition to a control. The temperature should be held between 14.6-14.9°C to be consistent with the proposed pilot tests described in the design chapter. A hot plate is used to heat the distilled water to the desired temperature and routinely monitored via a thermometer before adding the appropriate chemical dosages for each trial. Sodium thiosulphate three times the concentration of the initial chlorination dose is used to ensure complete quenching of the reactions at the appropriate times. For the preliminary tests in distilled water, the TOC instrument can be used for quantification of the overall reaction.

| Table 3: List of conditions to be tested | | | |
|---|---|---|---|
| Conditions to be tested | | | |
| pH | [CP 55940] mg/L | [$Cl_2$] (times concentration of CP mg/L | Time [minutes] |
| 6 | 0.5 | 0.5 | 0 |
| 6.5 | 1 | 1 | 1 |
| 7 | 2 | 2 | 2 |
| 7.5 | 3 | 5 | 3 |
| | 0 | 10 | 4 |
| | | 20 | 5 |
| | | 0 | 10 |
| | | | 15 |
| | | | 20 |

After determining the kinetics in distilled water, additional testing of the same conditions is needed for spiked wastewater. A more exact method of detection for CP-55940 in pilot experiments should be used. Gas chromatography-mass spectrometry is overwhelmingly the

detection method of choice for the analysis of THC-COOH (Burgard, 2019) (Bijlsma, 2009). In the example pilot, determination of contaminants was performed via gas chromatography directly coupled with a mass selective spectrometer (GC Hewlett Packard 5890N Series II, Hewlett Packard 5972 Series detector, column: Varian VF-Xms, length: 30 m, diameter: 0.25 mm, film thickness: 0.25 µm) (Rott, 2018).

# Design

By analyzing the available literature on common removal practices of THC-COOH, we designed a pilot scale UV/chlorine (UV/HOCl) advanced oxidation process (AOP) to test removal efficiency of CP-55940 in wastewater. Our pilot design is based on a UV/chlorine AOP pilot within Stuttgart, Germany's Treatment Plant for Education and Research (LFKW, Lehr- und Forschungsklärwerk) (Rott, 2018). In line with LFKW's pilot system, our pilot will operate with a flow rate of 1 $m^3$/h for all experiments and will be equipped with a medium pressure UV lamp (200–400 nm) with an adjustable performance of up to 1 kW. Figure 3 below shows a configuration of our design developed from (Rott, 2018).

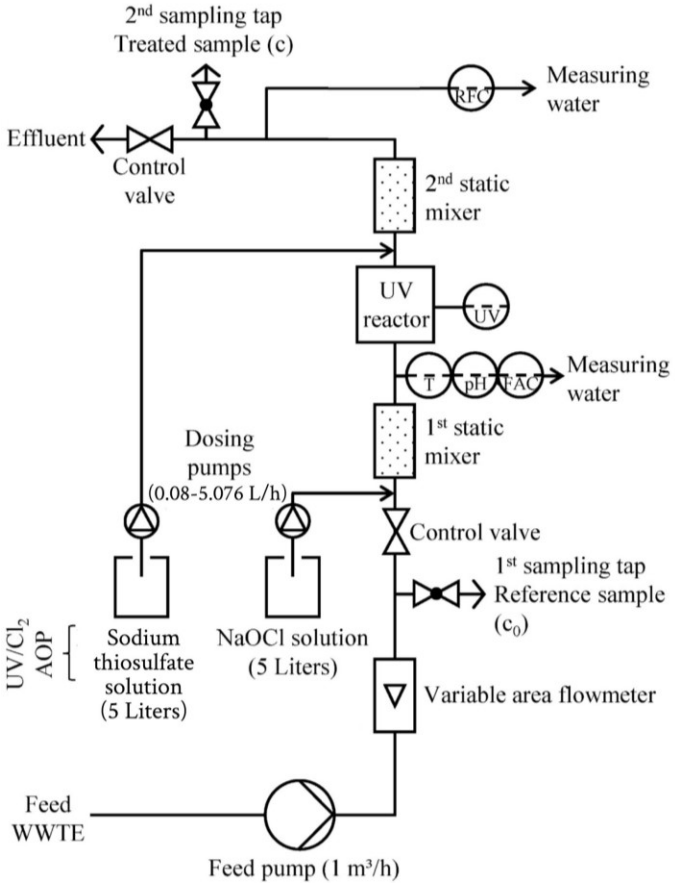



Figure 3. Technical scheme of UV/chlorine AOP pilot system

Spiked wastewater containing CP-55940 ranging from 0 to 1000ng/L is held in a 6000 L tank connected to the UV/chlorine AOP pilot system. Between the two is an inline totalizer used for establishing a running total of how much fluid is being discharged from the tank (capable of measuring flow between 30-290 L/h) (Dzombak, 2012), a normally closed solenoid valve,

followed by an Ultra HE Tankless Water Heater (8-100 psi) with capability to automatically adjust to the desired temperature of heating (EZ Tankless, 2020). Spiked wastewater is fed through the water heater and then through an eccentric screw pump (Moineau pump) both having a flow rate of 1 $m^3/h$ going into the pilot system. A variable area flowmeter is placed directly before the first of two sampling taps for influent flow rate and reference sample ($C_0$) collection, respectively. A control valve precedes the inlet of the first static mixer, where sodium hypochlorite (NaOCl) solution is added from a 5 liter tank using a peristaltic pump into a static mixer, guaranteeing extensive mixing through turbulence. The contact time of chlorine until reaching the UV chamber should be roughly 4.6–6.4 seconds (Rott, 2018). When NaOCl is dosed to the wastewater, free chlorine (HOCl and $ClO^-$) dissolves and partially or fully reacts with wastewater components. The remaining active free chlorine, also known also as "free available chlorine" (FAC), is important for subsequent reactions with UV. Therefore, FAC is brought to the desired concentrations by dosing NaOCl solution as needed. The spiked wastewater to be used in our pilot system will require different NaOCl doses than those of the example pilot study, which range from 0.08-4 L/h. Results of the preceding chlorination treatment bench tests for spiked wastewater will be used to inform the appropriate initial chlorination dose to be added before achieving desired FAC concentration of 1-5 mg/L in the UV chamber influent. Depending on initial testing, more than 5 mg/L FAC could be needed to see substantial elimination results.

FAC concentration (potentiostatic electrode amperometry sensor), pH (single junction, combination electrode sensor) and temperature are measured as wastewater enters the UV reactor via membrane sensors (Wallace & Tiernan). The immersion UV lamp (Wallace & Tiernan Barrier M35, type: WTL 1000) from Siemens Water Technologies, with 200 nm cut-off, is encased in a quartz sleeve with 1 mm thickness and installed in a stainless-steel chamber. Irradiance could be controlled by a visualized UV signal determined by a 4–20 mA UV sensor (signal in $W/m^2$). Contact time in the UV chamber was between 6–10 seconds (Rott, 2018). The example pilot study, nor the LFKW specify volume of the UV reactor or static mixers, however it can be assumed that each chamber will be about 1250-1750 L in volume. This is based on the LFKW's other pilot treatment designs of an aeration tank, sedimentation basin and anaerobic reactor which have similar volumes of 1430, 2150 and 250 L respectively (Maurer, 2020).

In the example pilot study, $H_2O_2$ was used as a quenching agent downstream of the UV chamber, with sodium thiosulfate ($Na_2S_2O_3$) used to quench additionally, after samples were taken from the sampling taps. However, for our pilot design, only $Na_2S_2O_3$ is used to quench excess free chlorine during the treatment process, with no extra doses added after samples are taken. Based on a FAC range of 1-6mg/L and supplied with a stock solution of 0.1 M sodium thiosulfate, a dosage of 0.846 - 5.076 L/hr would be needed (see Appendix C for calculations). This range could differ depending on the oxidation results by chlorine kinetics tests. Another peristaltic pump is used to deliver the quenching agent from a 5 L tank to the UV chamber effluent. The pump is operated in automatic mode controlled by means of an EMEC Chlorine Analyzer Control System from RealTech Controls for residual free chlorine concentration readings updated every second and process controller (MFC Analyzer/Controller) from Wallace & Tiernan, both placed before the junction of the wastewater and the $Na_2S_2O_3$ pipes. Contact time of the quenching agent from its dosage point before the second static mixer to the effluent of the pilot plant should be about 4.8–6.7 seconds (Rott, 2018).

The second static mixer's effluent is measured for RFC concentration in a measuring cell with a potentiostatic electrode amperometry sensor. A treated sample is also taken through the second sampling tap to be measured for CP-55940 concentration. A control valve is placed at the end of the system before treated wastewater would be discharged to a holding tank, or the next treatment process. Different experimental conditions are to be tested:

- Experiment 1: Variation of CP-55940 concentration (0, 250, 500, and 1000 ng/L) while 0.4 kWh/$m^3$ UV energy consumption and 1 mg/L FAC concentration held constant.
- Experiment 2: Variation of FAC concentration (0, 2, 4, and 6 mg/L) while 0.4 kWh/$m^3$ UV energy consumption and 1000 ng/L CP-55940 held constant.
- Experiment 3: Variation of UV energy consumption (0.0, 0.4, 0.7, and 1.0 kWh/$m^3$) while 0 mg/L FAC and 1000 ng/L CP-55940 held constant.
- Experiment 4: Variation of UV energy consumption (0.0, 0.4, 0.7, and 1.0 kWh/$m^3$) while 3 mg/L FAC and 1000 ng/L CP-55940 held constant.

Each experiment consists of 4 test variations. Each test variation requires the collection of three untreated and three treated samples to be taken from each sampling tap and tested for presence of CP-55940, totaling 24 samples taken per experiment. Unlike for surrogacy verification, use of

the TOC analyzer for sample testing would not yield any conclusive results as it would detect all the organic carbon in the wastewater rather than specifically measuring that of CP-55940. A more exact method such as gas chromatography-mass spectrometry would have to be used. Like the example pilot study, the pH will be held at 7 and temperature held between 14.6-14.9°C for all experiments. This requires use of the Ultra HE water heater as any incoming or stored water would assume a temperature of 13°C (room temperature) or lower. An estimated 25 to 29 seconds is needed for wastewater to travel through the entire system (Rott, 2018).

# Conclusion

Based on this group's research, CP-55940 is the most viable legal chemical surrogate for THC-COOH. It is the most similar structurally and has the requisite functional groups. The predicted values for polarity, weight, and partitioning are similar to THC-COOH. The number of rotatable bonds and kPa values differ enough that further oxidation tests are necessary. However, legal restrictions make CP-55940 the most viable candidate.

The most viable arrangement of treatment practices remains undetermined, as much is still unknown about the kinetic pathways of both contaminants during reactions with chlorine. Particularly troubling is the formation of harmful transformation byproducts in the wake of seemingly favorable removal rates. A pilot-scale UV/chlorine advanced oxidation process was designed for testing the relationship between varying concentrations of CP-55940 with levels of UV and chlorine oxidation treatment.

# Recommendations

## Further Research

If it can be determined that CP-55940 or another more accessible synthetic cannabinoid can be used as a surrogate for THC or THC-COOH, it would open up research opportunities previously infeasible due to strict cannabinoid regulation. Currently, these synthetic cannabinoids have only been tested for similarities in biological responses. By determining if they are a good chemical surrogate, research into water and wastewater treatment methods could be opened to more laboratories. Currently, most research is done by testing wastewater influents and effluents which are uncontrolled environments. Opening research in a controlled laboratory setting could help fill in the current gaps in knowledge such as chlorination reaction rates, transformation byproducts, and best mechanisms for removal.

Pilot systems which are used for the information of larger-scale water treatment plants must be able to accurately test concentrations of illicit drugs like THC-COOH like those in real life. Without current knowledge of THC-COOH concentration in the influent of a certain water treatment plant, one can use predicted or averaged drug consumption values and the population characteristics of residents served to back-calculate influent concentration. Future tests should seek to replicate hypothetical concentrations of wastewater-measured THC-COOH (ng/L) by multiplying the daily mass loads of illicit drugs (mg drug used/day) consumed by local

communities by the sewer-connected population, then further multiplying by the wastewater flow rate (L/day or $m^3$/day) (Yadav, 2017).

Further research in determining chlorination kinetics and ideal conditions are needed. Chlorination is being used under the assumption that it is safe, but research has been brought forth with contrary findings. What little research has been done has determined that chlorination kinetics decrease rapidly when in the presence of other organic material. Testing in the presence of other organics and in wastewater will help determine actual rates of removal. A variety of disinfection byproducts result from the chlorination of THC and THC-COOH. Preliminary tests show that these byproducts pose a greater risk to the environment and health. Further research is needed to determine the number of byproducts and their behavior in the environment. Depending on their effects and longevity chlorination could be an unfeasible or even dangerous treatment process.

If chlorination proves unfeasible, other oxidation processes such as UV treatment, Fenton's oxidation, and peroxide treatment should be investigated. These processes could have more favorable rates of removal or fewer byproducts. UV treatment, which was only used as a supplement to the chlorination AOP in our pilot, can be used alone without the presence of chlorine to test its sole effect on CP-55940. Further research into the best practices of UV treatment for emerging contaminants must first be studied. Other common treatment methods such as filtration, activated sludge, and activated carbon should be investigated. Activated sludge is often proposed as a potentially effective process. However, the mechanism of removal is unknown and preliminary tests show that adsorption is more likely than biological removal. This could limit the effectiveness of activated sludge as THC could continue to react and could be easily removed from the media. Further investigation on the mechanism, rate, and longevity of removal is needed. Research would also need to be done on what to do with the sludge after its removal from the plant. An activated sludge pilot design can therefore be incorporated into the current UV/chlorine AOP pilot to test some of these conditions.

The activated sludge pilot would be placed before the UV/chlorine pilot and would be directly connected to it via pipes with control valves and a holding tank potentially in between the two systems for sampling purposes. An overview of the parameters for wastewater treatment design and different technologies used can be found in the literature (Sarbu, 2017). This

activated sludge pilot design uses a $1m^3/h$ flow rate and is therefore conducive to tests with the current UV/chlorine pilot.

When carrying out controlled tests on pilot systems, a combination of different chemical loads in the raw wastewater and varying hydraulic retention/contact times along treatment processes (Yadev, 2017) can be tested and may result in increased or decreased removal capacities. Natural removal processes, which utilize the natural environment in both water and sediment, may over time facilitate further removal of compounds in receiving environments. Thus, they can be used as supplements to a treatment process and warrant further study (Yadev, 2017). However, based on the amount of data in the existing literature on each process, a combination of activated sludge and chlorination are still the best treatment processes to study in a controlled laboratory.

## Best Practices

*Activated Sludge*

The mechanism of removal for activated sludge is most likely sorption, not biological removal, as evidenced by a lack of change in the removal rate with temperature (How, 2020). THC adheres to media easier than THC-COOH due to THC's decreased polarity and increased hydrophobicity. It is unknown how well cannabinoids adhere to media on a long-term basis and if they continue to react. Treatment plans with a shorter solids retention time have better removal rates compared to longer times (Postigo, 2010). Membrane bioreactors that use ultrafiltration membranes submerged in activated sludge, maybe even more effective than conventional activated sludge, with results possibly due to some combination of higher operating MLSS concentrations, and/or improved effluent solids separation (Yadev, 2017). The membranes also provide more adhesion sites that are regularly removed. Much more research should be done to test these conditions and determine the behavior of cannabinoids attached to different media.

*Chlorination*

There is some evidence that cannabinoids form complexes for natural organic matter that could inhibit reactions with chlorine (González-Mariño, 2013). Preliminary tests have also shown that chlorination creates byproducts that are harmful to health and the environment (González-Mariño, 2013). UV/chlorine advanced oxidation processes can achieve higher rates of

complete oxidation, and when paired with $H_2O_2$ to quench excess chlorine from reacting further downstream, can limit byproducts (Rott, 2018). Further research and testing on THC-COOH's longevity in the environment, reactions in wastewater, and effect on the environment is needed, post-treatment. Further tests to compare the behavior of CP-55940 to THC-COOH in wastewater and environmental systems are needed.

# Works Cited

Abbate, V., Schwenk, M., Presley, B., & Uchiyama, N. (2018). The ongoing challenge of novel psychoactive drugs of abuse. Part I. Synthetic cannabinoids (IUPAC Technical Report). Pure and Applied Chemistry, 90(8), 1255–1282. https://doi.org/10.1515/pac-2017-0605

Aizpurua-Olaizola, O., Zarandona, I., Ortiz, L., Navarro, P., Etxebarria, N., & Usobiaga, A. (2017). Simultaneous quantification of major cannabinoids and metabolites in human urine and plasma by HPLC-MS/MS and enzyme-alkaline hydrolysis. Drug testing and analysis, 9(4), 626–633. https://doi.org/10.1002/dta.1998

Apul, O., Rowles, L., Khalid, A., Karanfil, T., Richardson, S., & Saleh, N. (2020, February 18). Transformation potential of cannabinoids during their passage through engineered water treatment systems: A perspective. Retrieved February 09, 2021, from https://www.sciencedirect.com/science/article/pii/S0160412019337055

Bijlsma, S. (2009). Simultaneous ultra-high-pressure liquid chromatography–tandem mass spectrometry determination of amphetamine and amphetamine-like stimulants, cocaine and its metabolites, and a cannabis metabolite in surface water and urban wastewater. Journal of Chromatography A, 1216(15), 3078–3089. https://doi.org/10.1016/j.chroma.2009.01.067

Boix, I. (2014). Investigation of cannabis biomarkers and transformation products in waters by liquid chromatography coupled to time of flight and triple quadrupole mass spectrometry. *Chemosphere (Oxford)*, *99*, 64–71. https://doi.org/10.1016/j.chemosphere.2013.10.007

Boleda, G. (2009). Monitoring of opiates, cannabinoids and their metabolites in wastewater, surface water and finished water in Catalonia, Spain. Water Research (Oxford), 43(4), 1126–1136. https://doi.org/10.1016/j.watres.2008.11.056

Burgard, D., Williams, J., Westerman, D., Rushing, R., Carpenter, R., LaRock, A., . . . Banta-Green, C. (2019, June 18). Using wastewater-based analysis to monitor the effects of Legalized retail sales on cannabis consumption in Washington State, USA. Retrieved March 17, 2021, from https://onlinelibrary.wiley.com/doi/abs/10.1111/add.14641

Cannabinoid Receptors. (n.d.). Retrieved October 30, 2020, from https://www.sigmaaldrich.com/technical-documents/articles/biology/rbi-handbook/non-peptide-receptors-synthesis-and-metabolism/cannabinoid-receptors.html

Carmona, E., Andreu, V., & Picó, Y. (2014). Occurrence of acidic pharmaceuticals and personal care products in Turia River Basin: From waste to drinking water. *Science of The Total Environment, 484*, 53-63. doi:10.1016/j.scitotenv.2014.02.085

Centazzo, N., Concheiro-Guisan, M., Cheng, S., Jacox, A., & Frederick, B. (2019). Wastewater analysis for Nicotine, cocaine, amphetamines, opioids and cannabis in New York City. Retrieved February 28, 2021, from https://pubmed.ncbi.nlm.nih.gov/31304444/

Cha, H., Lee, K., Song, M., Hyeon, Y., Hwang, J., Jang, C., . . . Jeong, H. (2014, July). Dependence Potential of the Synthetic Cannabinoids JWH-073, JWH-081, and JWH-210: In Vivo and In Vitro Approaches. Retrieved October 28, 2020, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4131522/

CP-55940. (n.d.). Retrieved October 26, 2020, from https://www.sigmaaldrich.com/catalog/product/sigma/c1112?lang=en®ion=US.

Corrigan, M. (n.d.). Controlled Substance Researchers' Guide. Retrieved October 27, 2020, from https://www.ehs.harvard.edu/sites/default/files/controlled_substance_researchers_guide.pdf

Cosenza, A., Maida, C., Piscionieri, D., Fanara, S., Gaudio, F., & Viviani, G. (2018, February 03). Occurrence of illicit drugs in two wastewater treatment plants in the south of Italy. Retrieved February 28, 2021, from https://www.sciencedirect.com/science/article/pii/S0045653518301759?casa_token=b1TN6SOxsUAAAAAA%3AHrmxdOcKNJeYT_W0UYfTl_1hC5lAI-0nJR-XZ2FOb_vkVw4qu05q-_DYoVgPXNqTIPTatS5F

Drug Scheduling. (n.d.). Retrieved October 26, 2020, from https://www.dea.gov/drug-scheduling

Eubanks, L. M., Rogers, C. J., Beuscher, A. E., 4th, Koob, G. F., Olson, A. J., Dickerson, T. J., & Janda, K. D. (2006). A molecular link between the active component of marijuana and Alzheimer's disease pathology. Molecular pharmaceutics, 3(6), 773–777. https://doi.org/10.1021/mp060066m

Feng, S., ElSohly, M. A., Salamone, S., & Salem, M. Y. (2000). Simultaneous analysis of Δ9-THC and its major metabolites in urine, plasma, and meconium by GC-MS using an immunoaffinity extraction procedure. Journal of analytical toxicology, 24(6), 395-402.

Gallo-Molina, A. C., Castro-Vargas, H. I., Garzón-Méndez, W. F., Ramírez, J. A. M., Monroy, Z. J. R., King, J. W., & Parada-Alfonso, F. (2019). Extraction, isolation and purification of tetrahydrocannabinol from the Cannabis sativa L. plant using supercritical fluid extraction and solid phase extraction. The Journal of Supercritical Fluids, 146, 208-216.

González-Mariño, I., Rodríguez, I., Quintana, J., & Cela, R. (2013, August 12). Investigation of the transformation of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol during water chlorination by liquid chromatography–quadrupole-time-of-flight-mass spectrometry. Retrieved November 04, 2020, from https://www.sciencedirect.com/science/article/pii/S0304389413005621

González-Mariño, I., Quintana, J., Rodríguez, I., & Cela, R. (2010, March 12). Determination of drugs of abuse in water by solid-phase extraction, derivatization and gas chromatography–ion trap-tandem mass spectrometry. Retrieved March 09, 2021, from https://www-sciencedirect-com.ezpxy-web-p-u01.wpi.edu/science/article/pii/S002196731000083X

How, Z., & Gamal El-Din, M. (2020, September 14). A critical review on the detection, occurrence, fate, toxicity, and removal of cannabinoids in the water system and the environment. Retrieved November 04, 2020, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7489229/

Jacox, A., Wetzel, J., Cheng, S., & Concheiro, M. (2017, January 30). Quantitative analysis of opioids and cannabinoids in wastewater samples. Retrieved February 28, 2021, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6197101/

Leishman, E., Murphy, M. N., Murphy, M. I., Mackie, K., & Bradshaw, H. B. (2018). Broad and region-specific impacts of the synthetic cannabinoid CP 55,940 in adolescent and adult female mouse brains. Frontiers in molecular neuroscience, 11, 436. Retrieved October 30, 2020 from https://www.frontiersin.org/articles/10.3389/fnmol.2018.00436/full.

National Center for Biotechnology Information (2020). PubChem Compound Summary for CID 107885, 11-Nor-9-carboxy-thc. Retrieved October 30, 2020 from https://pubchem.ncbi.nlm.nih.gov/compound/11-Nor-9-carboxy-thc.

National Center for Biotechnology Information (2020). PubChem Compound Summary for CID 16078, Dronabinol. Retrieved October 30, 2020 from https://pubchem.ncbi.nlm.nih.gov/compound/Dronabinol.

Mackie, A. (2017, August 14). Chlorination Kinetics of 11-Nor-9-carboxy-Δ9-tetrahydrocannabinol: Effects of pH and Humic Acid. Retrieved from https://pubs.acs.org/doi/full/10.1021/acs.est.7b02234#

Maurer, P. (2020). Services. Retrieved March 18, 2021, from https://www.iswa.uni-stuttgart.de/lehr-forschungsklaerwerk/klaerwerk/leistungen-00001/#id-a306e12f

Park, G. (2017). A critical review of the occurrence, detection, and treatment of Δ9-tetrahydrocannabinol in aquatic environments. *Environmental Reviews*, *25*(3), 255–268. https://doi.org/10.1139/er-2016-0061

Parolini, M., and Binelli, A. 2014. Oxidative and genetic responses induced by [DELTA]-9-tetrahydrocannabinol ([DELTA]-9-THC) to Dreissena polymorpha. Sci. Total Environ. 468: 68-76. doi:10.1016/j.scitotenv.2013.08.024. PMID:24008074.

Parolini, M., Castiglioni, S., Magni, S., Della Torre, C., and Binelli, A. 2016. Increase in cannabis use may indirectly affect the health status of a freshwater species [online]. Environ. Toxicol. Chem. doi:10.1002/etc.3575.

Pate, D. W. (1994). Chemical ecology of Cannabis. Journal of the International Hemp Association, 2(29), 32-37.

Pate, D. W. (1983). Possible role of ultraviolet radiation in evolution of Cannabis chemotypes. Economic Botany, 37(4), 396.

Politi, M., Peschel, W., Wilson, N., Zloh, M., Prieto, J. M., & Heinrich, M. (2008). Direct NMR analysis of cannabis water extracts and tinctures and semi-quantitative data on Δ9-THC and Δ9-THC-acid. Phytochemistry, 69(2), 562-570.

Postigo, C., Alda, M., & Barceló, D. (2009, November 13). Drugs of abuse and their metabolites in the Ebro River basin: Occurrence in sewage and surface water, sewage treatment plants removal efficiency, and collective drug usage estimation. Retrieved November 06, 2020, from https://www.sciencedirect.com/science/article/pii/S0160412009002104

Perrotin-Brunel, H., van Roosmalen, M. J., Kroon, M. C., van Spronsen, J., Witkamp, G. J., & Peters, C. J. (2010). Solubility of cannabinol in supercritical carbon dioxide. Journal of Chemical & Engineering Data, 55(9), 3704-3707.

Realtech Controls. (2020, October 20). Chlorine analyzer control system REALTECH Controls. Retrieved March 17, 2021, from https://realtechcontrols.com/water-treatment/chlorine-analyzer-control-system/

Rott, E., Kuch, B., Lange, C., Richter, P., Kugele, A., & Minke, R. (2018, May 07). Removal of emerging contaminants and estrogenic activity from wastewater treatment plant effluent with uv/chlorine and uv/h2o2 advanced oxidation treatment at pilot scale. Retrieved March 06, 2021, from https://www.mdpi.com/1660-4601/15/5/935/htm

Russo, E. (2003). Introduction to Cannabis: from Pariah to prescription J. Cannabis Ther., 3, 1–19.

Sarbu, J., & Filip, I. (2017, June). DESIGNING A PILOT SEWAGE TREATMENT PLANT FOR SMALL LOCALITIES. Retrieved 2021, from

https://www.researchgate.net/publication/320412679_DESIGNING_A_PILOT_SEWAGE_TREATMENT_PLANT_FOR_SMALL_LOCALITIES

Sharma, P., Murthy, P., & Bharath, M. M. (2012). Chemistry, metabolism, and toxicology of cannabis: clinical implications. *Iranian journal of psychiatry*, *7*(4), 149–156.

Spaderna, M., Addy, P., & D'Souza, D. (2013). Spicing things up: synthetic cannabinoids. Psychopharmacology (Berlin, Germany), 228(4), 525–540. https://doi.org/10.1007/s00213-013-3188-4

Stewart, A.M., and Kalueff, A.V. 2014. The behavioral effects of acute [DELTA]9-tetrahydrocannabinol and heroin (diacetylmorphine) exposure in adult zebrafish. Brain Res. 1543:109-119. doi:10.1016/j.brainres.2013.11.002. PMID: 24216135.

Synthetic Cannabinoids. (2013). Retrieved October 28, 2020, from https://www.unodc.org/LSS/SubstanceGroup/Details/ae45ce06-6d33-4f5f-916a-e873f07bde02

U.S. Environmental Protection Agency. CompTox Chemicals Dashboard. Retrieved October 28, 2020, from https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID6021327

U.S. Senate, Senate - Judiciary. (2012). *Congress.gov* (1019108446 783275610 R. Portman Sen., Author) [S. S.3190 from 112th Cong., 2nd sess.]. Retrieved October 30, 2020, from https://www.congress.gov/bill/112th-congress/senate-bill/3190

Wiley, J. L., Marusich, J. A., Huffman, J. W., Balster, R. L., & Thomas, B. F. (2011). Hijacking of basic research: The case of synthetic cannabinoids. RTI Press publication No. OP-0007-1111. Research Triangle Park, NC: RTI Press. Retrieved from http://www.rti.org/rtipress.

Yadav, M., Short, M., Aryal, R., Gerber, C., Akker, B., & Saint, C. (2017, July 26). Occurrence of illicit drugs in water and wastewater and their removal during wastewater treatment. Retrieved March 08, 2021, from https://www.sciencedirect.com/science/article/pii/S0043135417306413#bib94

Zuccato, E., Chiabrando, C., Castiglioni, S., Bagnati, R., & Fanelli, R. (2008). Estimating Community Drug Abuse by Wastewater Analysis. *Environmental Health Perspectives*, *116*(8), 1027–1032. https://doi.org/10.1289/ehp.11022

# Appendix A. Calculations for Chlorination Kinetics Tests

| Want | | | Have | | | Add | | |
|---|---|---|---|---|---|---|---|---|
| {CP} mg/L | $\{Cl_2\}$ mg/L | Volume mL | {CP} mg/L | % w/v NaClO | $\{Cl_2\}$ mg/L | {CP} mL | NaClO mL | Water mL |
| 1 | 7.18775 | 250 | 2 | 5.5000 | 52.3537 | 125 | 13.7292 | 111.2708 |
| 1 | 3.59387 | 250 | 2 | 5.5000 | 52.3537 | 125 | 0.0002 | 124.9998 |
| 1 | 1.79694 | 250 | 2 | 5.5000 | 52.3537 | 125 | 0.0001 | 124.9999 |
| 1 | 0.89847 | 250 | 2 | 5.5000 | 52.3537 | 125 | 0.0000 | 125.0000 |
| 1 | 0.05000 | 250 | 2 | 0.0055 | 0.0524 | 125 | 0.0023 | 124.9977 |

### Dilution Calculations

Multiple components do not contain CP-55940 and NaClO, they can be discounted in the volumetric calculations of these components.

$V_{wanted} = V_{CP55940}+V_{NaClO}+V_{water}$

$M_{wanted}V_{wanted} = M_{stock}V_{stock}$

### NaClO Calculations

Given 5.5% w/v

$$5.5\%\frac{w}{v} = \frac{5.5gNaClO}{100mL\ H20} * \frac{10ml}{10ml} * \frac{1000mL}{1L} * \frac{1molNaClO}{74.44g} = \frac{0.7388molNaClO}{L}$$

### Converting to $Cl_2$ Terminology

$$\frac{0.7388molNaClO}{L} * \frac{1molCl2}{1molNaClo} * \frac{70.906g}{molCl2} * \frac{10^3mg}{g} = \frac{52385.4mgCl2}{L}$$

# Appendix B. TOC Procedure

**Creating a Stock Standard (1000ppm OC)**
Dry about 0.75 g of Potassium Hydrogen Phthalate (KHP) in the oven at 103-110˚C for 30 min.

Cool in desiccator for 20min.

Weigh exactly 0.5314 g using an analytical balance.

Add to a 250ml volumetric flask to mark with DI water

Store in an amber glass bottle in the designated refrigerator.

Label well with the name, date, and "1000mg OC/L KHP standard".

Discard after 1 month

## Working Standards

Prepare working standards that bracket the sample concentrations

Use 100ml or 50ml volumetric flasks.

Fill halfway with DI water.

Add 1% v/v of 6N HCl (acid addition for NPOC analysis to bring pH around 2)

Add the desired volume of Stock Standard to each flask and fill to mark with DI water.

## NPOC Analysis

### Verify

Gas cylinder pressure is above 500psi

Regulator pressure is between 70-85 psi

Rinse the water bottle located behind the autosampler, and make sure it is full and the end of the tubing is at the bottom of the bottle. If it is not full, fill it with DI water.

The water level humidifier is above the "Lo" mark. If not, replenish by adding DI water through the port on top of the vessel until the "Hi" mark is reached.

### Turn on the Instrument

When the power button is orange, press the button to start up the instrument.

Place Standards and samples in the autosampler

Take off the sample cover and sample tray

Add standards and samples in sample tray and place sample tray back. Gently spin the tray until it sits in the right position.

Put sample cover back until it clips.

**Edit the Sample Table Editor**

Start the TOC-L Sample Table Editor on the desktop. Create a new sample table labeled with your initials. In the "Select H/W settings" window: select TOC-L HIGH SENS in system dropdowns.

**Calibration**

Click the Calibration Curve tab of the file viewer. Drag the right calibration file to the first line in the sample table

**Samples**

Click the Method tab of the file viewer. Drag the right method file to the following lines in the sample table. Copy and paste the line to insert multiple samples using the same method file.

**Vial Numbers**

Enter the standard and sample locations in the vial column.

**Connect**

Click the sample table to be used and connect. Wait approximately 40min for the instrument to warm up. When the status light at the upright corner shows "Ready". Click Start to start the analysis.

# Appendix C. Sodium Thiosulfate Dilution Calculations

$$Q = \frac{1\ m^3}{hr} = \frac{1000\ L}{hr}$$

$$\text{FAC} = \frac{1\text{ mg}}{\text{L}} * \frac{1000\text{ L}}{\text{hr}} * \frac{1\text{ mg FAC}}{\text{L}} * \frac{10^{-3}\text{g}}{\text{mg}} * \frac{1\text{ mols FAC}}{35.453\text{ g}} = 0.0282\ \frac{\text{mol FAC}}{\text{hr}}$$

$$\text{FAC} = \frac{6\text{ mg}}{\text{L}} * \frac{1000\text{ L}}{\text{hr}} * \frac{1\text{ mg FAC}}{\text{L}} * \frac{10^{-3}\text{g}}{\text{mg}} * \frac{1\text{ mols FAC}}{35.453\text{ g}} = 0.1692\ \frac{\text{mol FAC}}{\text{hr}}$$

**Using a thiosulfate dose 3 times that of FAC**

$$\text{FAC} = \frac{0.0282\text{ mol}}{\text{hr}} * \frac{3\text{ mol Thiosulfate}}{1\text{ mol FAC}} = 0.846\ \frac{\text{mol Thiosulfate}}{\text{hr}}$$

$$\text{FAC} = \frac{0.1692\text{ mol}}{\text{hr}} * \frac{3\text{ mol Thiosulfate}}{1\text{ mol FAC}} = 5.077\ \frac{\text{mol Thiosulfate}}{\text{hr}}$$

**Assuming a 0.1$\frac{mol}{L}$stock solution of Sodium Thiosulfate**

$$\frac{0.1\ mol}{L} = \frac{0.0846\ mol}{xL} = \frac{0.846\ L}{hr}$$

$$\frac{0.1\ mol}{L} = \frac{0.1692\ mol}{xL} = \frac{5.076 L}{hr}$$

**Range of Sodium Thiosulfate Dilution**

$$0.846\ \frac{\text{L}}{\text{hr}} - 5.076\ \frac{\text{L}}{\text{hr}}$$