The use of advanced oxidation processes in the degradation of the pesticide chlorpyrifos and reduction of microbial contamination on apples

By

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ABSTRACT

The use of advanced oxidation processes in the degradation of the pesticide chlorpyrifos and reduction of microbial contamination on apples

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A method based on Advanced Oxidation Process (AOP) was validated for the degradation of chlorpyrifos on apples and inactivating *Escherichia coli* O157:H7, along with *Aspergillus niger* spores. AOP generates free-radicals using UV-C light (at 254 nm), hydrogen peroxide, and ozone. The use of gaseous ozone alone was ineffective in chlorpyrifos degradation and was therefore excluded from AOP use. Response surface methodology (RSM) was used to find that the degradation of chlorpyrifos was primarily dependent on UV-C dose and hydrogen peroxide temperature but not hydrogen peroxide concentration. The maximal degradation of chlorpyrifos on apples was achieved through an AOP treatment applying 68.4 kJ/m² UV dose and 1.22% v/v H₂O₂ at 66°C that resulted in a 47% reduction (92µg) of the pesticide with < 0.004 µg accumulation of chlorpyrifos-oxon. The same treatment supported a >6.56 log CFU reduction in *E. coli* O157:H7 and >6.56 log CFU reduction of *A. niger* spores.
ACKNOWLEDGEMENTS

First, I’d like to thank my advisor Dr. Keith Warriner for taking me on as a graduate student, a cautionary tale I’m sure he would not repeat. Thanks for these two years, I’ve learned a lot in the field of food safety and enjoyed every minute of it. I also learned, more than I wanted, about how the world of academia works which is why I would hate to pursue a PhD degree! Too much politics and drama, although entertaining as a spectator, it doesn’t seem fun as a participant. I’d also like to thank my committee members Dr. Ryan Prosser and Dr. David Lubitz for helping me develop my presentation skills during my committee presentations as well as helping me edit this thesis. I hope you both well wishes and I couldn’t have asked for a better committee.

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LIST OF ABBREVIATIONS

2,4-D ................................................................. 2,4-Dichlorophenoxyacetic acid
ACH ................................................................. Acetylcholine
AChE ............................................................. Acetylcholinesterase
ANOVA ............................................................. Analysis of variance
AOP ................................................................. Advanced oxidation process
BBD ................................................................. Box-Behnkin design
CCD ................................................................. Central composite design
CFU ................................................................. Colony forming unit
CPY ................................................................. Chlordane
CPY-O ............................................................. Chlordane-oxon
DDT ................................................................. Dichlorodiphenyltrichloroethane
EHEC ............................................................ Enterohemorrhagic E.coli
EWG ............................................................... Environmental Working Group
FDA ................................................................. Food and Drug Administration
GRAS ............................................................. Generally recognized as safe
HPLC ............................................................... High performance liquid chromatography
HUS ................................................................. Hemolytic uremic syndrome
LOD ................................................................. Limit of detection
LOQ ................................................................. Limit of quantification
LPM ................................................................. Low pressure mercury
MDL ............................................................... Method detection limit
MPM ............................................................... Medium pressure mercury
MRL ............................................................... Maximum residue level
OP ................................................................. Organophosphate
PDA ................................................................. Potato dextrose agar
PON1 ............................................................... Paraoxonase 1
Q-ToF ............................................................... Quadrupole Time of Flight
Chapter 1: Introduction and Literature Review

1.1 Introduction

Pesticides are an important part of modern day farming and are necessary in the growth and production of fresh fruits and vegetables due to their ability to eliminate pests (Randall, Hock, Crow, Hudak-Wise, & Kasai, 2014). Despite their necessity in agriculture, they have been associated with numerous human health concerns as well as damage to the environment (Stoytcheva, 2011). One of the most controversial pesticides is chlorpyrifos (CPY), which could cause adverse effects on the nervous system of children (M. Bouchard, F. et al., 2011). Humans can be exposed to pesticides through a number of different routes, one of them being through ingestion of contaminated produce (Giesy et al., 2014).

The most common current approach to remaining CPY on produce is through washing, however, this process results in a redistribution of not only pesticides but also pathogenic microorganisms between produce batches and further dissemination within the environment via wastewater disposal (Burkul, Ranade, & Pangarkar, 2015; Köck-Schulmeyer et al., 2013). A more effective approach is to use water-free technologies to decontaminate fruits and vegetables thereby reducing pesticide levels entering down-stream washing processes. One potential approach is based on the advanced oxidation process (AOP) that has previously been shown to inactivate Listeria monocytogenes on apples (Murray et al., 2018). AOP involves generating hydroxyl radicals from the degradation of ozone and/or hydrogen peroxide. The generation of free radicals can be achieved through the interaction between ozone and hydrogen peroxide, or more rapidly via UV-C (254nm) mediated decomposition of the oxidants (Abramovic, Banic, & Sojic, 2010; Femia, Mariani, Zalazar, & Tiscornia, 2013; Marican & Durán-Lara, 2018).
In the following study the AOP originally developed to decontaminate apples was applied for CPY degradation as well as inactivate the pathogen *Escherichia coli* O157:H7 due to the previous links to foodborne illness outbreaks linked to apples (Kenney, Burnett, & Beuchat, 2001). *Aspergillus niger* was also selected a test microbe due to its inherent resistance to UV in the spore form, in addition to acting as a surrogate for potential mycotoxin producing molds (Taylor-Edmonds, Lichi, Rotstein-Mayer, & Mamane, 2015). A key part of the AOP is optimizing the hydrogen peroxide concentration and temperature along with UV-C dose. In the current study response surface methodology (RSM) was used to determine which factors and their settings affect the degradation of CPY.

**1.2 Size of the Fresh Produce Sector**

Fresh produce is important to Canadians, with about 28.6% of Canadians aged 12 and over (approximately 8.3 million people) reported to consume 5 or more fruits and vegetables per day (Statistics Canada, 2017). Fresh produce is also important to the Canadian economy, grossing approximately $2.24 billion in 2017 (Agriculture and Agri-Food Canada, 2017a, 2017b; Statistics Canada, 2017). The fruit industry makes up about $1.04 billion annually while vegetables make up the rest (Agriculture and Agri-Food Canada, 2017a). Of this value, apples have been the highest grossing fruit since 2013, making approximately $224.6 million in 2017 (Agriculture and Agri-Food Canada, 2017a). Apples are one of the most widely grown fruit species in the world with approximately 5.2 million hectares in production worldwide in 2016 (Agriculture and Agri-Food Canada, 2017a). According to Statistics Canada, apples are the most widely produced fruit in Canada with an estimated 424,709 tons being produced in 2018. In comparison, the second most widely produced fruit in Canada is cranberries with only 195,196 tons (Statistics Canada, 2018).
1.3 Climate change and fresh produce safety

Climate change is believed to be caused by an accumulation of various greenhouse gases, mostly carbon dioxide, which in effect is increasing a trend of warm and humid weather (Butler, 2018). Climate change has been shown to increase the prevalence of pests such as insects, pathogenic microorganisms, and weeds (Delcour, Spanoghe, & Uyttendaele, 2015; Gregory, Johnson, Newton, & Ingram, 2009; Roos, Hopkins, Kvarnheden, & Dixelius, 2011; Ziska, Teasdale, & Bunce, 1999). Although some crops are capable of growing faster at higher temperatures, they may be subjected to an increase in pests and pathogens that also benefit from higher temperatures and humidity (Gregory et al., 2009). Increased temperatures can be associated with an enhanced metabolic rate in various insects which would increase both their rate of food consumption and rate of reproduction (Delcour et al., 2015; Deutsch et al., 2018). Due to this, it has been predicted that yields of grain such as maize, rice and wheat will decrease by 10 to 25% per degree of global mean surface warming (Deutsch et al., 2018). This prediction is based on various models that show a 2 °C increase in global temperature is capable of causing an average yield loss of 46, 19, and 31% for wheat, rice and maize, respectively (Deutsch et al., 2018). These predictions differ from previous models which showed a rice yield reduction of 5% per °C rise above 32 °C (Walker, Steffen, Canadell, & Ingram, 1999) and a reduction of 10% maize yield (Jones & Thornton, 2003).

Along with a change in temperature, changes in precipitation, light exposure, and humidity can also affect the growth of pests and pathogens (Delcour et al., 2015; Holland & Sinclair, 2004; Keikotthaile, 2011). Higher moisture is also associated with various plant diseases since microorganisms are capable of thriving in wet conditions, promoting the growth of mold and bacteria as well as spore germination (Roos et al., 2011).
## 1.4 Pesticides

Pesticides are substances, either chemical or biological, used by humans for controlling pests that are capable of harming human health and crop yield (Randall et al., 2014). Pests can be weeds, insects, animals, and even pathogenic microorganisms that can cause harm to both humans and plants (Randall et al., 2014). Chemical pesticides were used as early as 2500 B.C. when sulphur compounds were used for insect control by ancient Sumerians (Randall et al., 2014). Nowadays, there are multiple types of pesticides including fungicides, herbicides, insecticides, repellents, and disinfectants which control fungi, plants, insects/invertebrates, animals, and pathogenic microorganisms respectively (Randall et al., 2014) (Table 1).

<table>
<thead>
<tr>
<th>Type of Pesticide</th>
<th>Target Organism</th>
<th>Examples</th>
</tr>
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<tr>
<td>Bactericides</td>
<td>Bacteria</td>
<td>Alcohols, disinfectants</td>
</tr>
<tr>
<td>Fungicides</td>
<td>Fungi</td>
<td>Tea tree oil, <em>Bacillus subtilis</em></td>
</tr>
<tr>
<td>Herbicides</td>
<td>Plants</td>
<td>Glyphosate, 2-4-D</td>
</tr>
<tr>
<td>Insecticides</td>
<td>Insects</td>
<td>Chlorpyrifos, imidacloprid</td>
</tr>
<tr>
<td>Larvicides</td>
<td>Larvae</td>
<td>Methoprene, temephos</td>
</tr>
<tr>
<td>Rodenticides</td>
<td>Rodents</td>
<td>Anticoagulants, metal phosphides</td>
</tr>
</tbody>
</table>

Many classes of synthetic chemical pesticides used in the modern era were originally produced researched for use in warfare during World War II, with some of these early synthetic pesticides still being used today for agricultural purposes (Sanborn, Cole, Helena Sanin, & Bassil, 2004). The most notable of those pesticides being 2,4-Dichlorophenoxyacetic acid (2,4-D), a phenoxy
herbicide that kills weeds by causing uncontrolled and unsustainable growth (Randall et al., 2014; Sanborn et al., 2004). Another noteworthy pesticide is dichlorodiphenyltrichloroethane (DDT), an insecticide which was used in war to prevent malaria and typhus (Beard, 2006). In the United States, it was approved for use in agriculture and households from 1945 until 1972 when it was eventually banned in 1972 (Beard, 2006). This ban was due to the fact that DDT was shown to be very persistent in the environment while maintaining its residual effects and it can biomagnify in the food chain (Figure 1) (Beard, 2006; Randall et al., 2014). To this day, DDT still persists in the environment and it is believed that every living organism on Earth has been exposed to DDT resulting in body fat storage (Nicolopoulou-Stamati, Maipas, Kotampasi, Stamatis, & Hens, 2016).

After DDT was banned from being used in the United States, farmers started to use more organophosphate (OP) insecticides which have a relatively high mammalian toxicity but do not persist in the environment and are effective for a wide spectrum of insects compared to other insecticides (Davis, 2014).

Figure 1. Bioaccumulation of the pesticide DDT throughout the food chain. Modified from Penn State Pesticide Education Manual.
OPs are effective at controlling insects and mammalian pests through their ability to inactivate acetylcholinesterase (AChE), an enzyme required for proper nervous system function (Williamson, Terry, & Bartlett, 2006). AChE breaks down acetylcholine (ACh), a neurotransmitter that stimulates nerve fibres and muscles, into choline and acetic acid (Figure 2) (Fukuto, 1990). If AChE is inhibited, ACh will continue to stimulate nerve fibres and muscles uncontrollably which in effect will cause muscle spasms and involuntary movement (Fukuto, 1990). Besides mammals and insects, AChE is also present in birds, fish and reptiles (Fukuto, 1990). Exposure to high enough concentrations of OPs can lead to unintentional deaths of these animals.

![Figure 2. Mechanism of action of acetylcholinesterase breaking down acetylcholine into choline and acetic acid.](image)

Although pesticides are crucial in food production, they are capable of causing harm to human health, especially insecticides (Ansari, Moraiet, & Ahmad, 2014). There are multiple types of insecticides that have mechanism of actions, that target the nervous system of insects (Casida & Durkin, 2013) (Table 2). Like DDT, residues of these insecticides can remain on produce after being harvested from the fields (Bajwa & Sandhu, 2014) and may also be found in water, soil, and/or air following field application (Ansari et al., 2014). It has been estimated that every year, over 26 million people, especially those working in the agriculture industry, are poisoned by
pesticides, resulting in over 220,000 deaths (Ansari et al., 2014). One of the major human health concerns associated with insecticides is neurotoxicity (Casida & Durkin, 2013).

Table 2. Different types of insecticides and their mechanism of action. Sourced from (NPIC, 2019).

<table>
<thead>
<tr>
<th>Type of Insecticide</th>
<th>Mechanism of Action</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamates</td>
<td>Acetylcholinesterase inhibitor</td>
<td>Carbaryl</td>
</tr>
<tr>
<td>Organochlorine</td>
<td>Opens nerve cell sodium channels</td>
<td>DDT</td>
</tr>
<tr>
<td>Organophosphates</td>
<td>Acetylcholinesterase inhibitor more toxic and longer lasting than carbamates</td>
<td>Chlorpyrifos</td>
</tr>
<tr>
<td>Phenylpyrazoles</td>
<td>Blocks glutamate-acitivated chloride channels</td>
<td>Fipronil</td>
</tr>
<tr>
<td>Pyrethroids</td>
<td>Modulates sodium channels</td>
<td>Cypermethrin</td>
</tr>
<tr>
<td>Neonicotinoids</td>
<td>Nicotinic Acetylcholine receptor agonist</td>
<td>Imidacloprid</td>
</tr>
<tr>
<td>Ryanoids</td>
<td>Binds to calcium channels to block nerve transmission</td>
<td>Chlorantraniliprole</td>
</tr>
</tbody>
</table>

Consumers are concerned about pesticides used in agriculture and in their food (Consumer Reports, 2015; Koch, Astrid Epp, Mark Lohmann, & Gaby-Fleur Böl, 2017). A survey by Consumer Reports in 2015 showed that of 1050 Americans surveyed, approximately 85% were concerned about pesticides in their food (Consumer Reports, 2015). The Environmental Working Group (EWG) states that in 2019, the top five fruits and vegetables linked to the highest end-user pesticide exposure are: strawberries, spinach, kale, nectarines, and apples (Table 3). The EWG also state that two or more pesticide residues were detected in about 90% of strawberries, apples, cherries, spinach, nectarines, and kale samples tested (EWG, 2019).
The CDC states that approximately 29 pesticides can be found in trace amounts in the average human body in the United States. When exposed to the same concentration of pesticides, children are more susceptible to danger than adults due to the difference in body sizes (Eskenazi, Bradman, & Castorina, 1999). Children also have a different metabolism than adults so their bodies are unable to remove toxins as quickly (Eskenazi et al., 1999). Due to these factors, maximum residue limits (MRLs) are put in place to ensure that pesticide levels are safe for children and infants. Studies have shown a correlation between pesticides and human health risks such as lower IQ in children, Alzheimer’s disease, Parkinson’s disease, and ADHD to name a few (M. Bouchard, F. et al., 2011; M. F. Bouchard, Bellinger, Wright, & Weisskopf, 2010; Brown, Rumsby, Capleton, Rushton, & Levy, 2006; Eskenazi et al., 1999; Kim, Lee, Lee, Jacobs, & Lee, 2015; Kuehn, 2010). Cancer development due to pesticide exposure is also a great concern for both children and adults. Multiple studies have shown a link between pesticide exposure and tumour growth (Bassil et al., 2007). Due to this, it has been suggested that a reduction of pesticide use, especially for non-commercial purposes, should be considered (Bassil et al., 2007).
et al., 2007). As a result of these concerns, organophosphate insecticide use in the United States has decreased from 70 million lbs in 2000 to 20 million lbs in 2012 (Atwood & Paisley-Jones, 2017). The use of all insecticides have also decreased from 99 million lbs in 2000 to 60 million lbs in 2012 (Atwood & Paisley-Jones, 2017). Although the use of insecticides has decreased, the sale and usage of all pesticides combined are increasing. In 2008, about 4850 million lbs of pesticides were being used but in 2012, an estimated 5821 million lbs were used (Atwood & Paisley-Jones, 2017). Despite the current effort to reduce the use of pesticides, the need for them may soon increase due to climate change (Delcour et al., 2015).

Increased temperature, humidity and CO₂ can induce a higher growth rate of plants which in effect will lead to a dilution of pesticide concentrations thus reducing residue levels which will require more frequent applications as the plant matures (Holland & Sinclair, 2004). Higher levels of precipitation and higher moisture levels can cause pesticides to dissipate faster which will also lead to reduced residue concentrations found on plants and require more applications (Delcour et al., 2015). Higher CO₂ levels have also been shown to increase weed tolerance to herbicides which may lead to the use of more potent herbicides (Ziska et al., 1999). Due to these factors decreasing the amount of pesticide residues found on crops, the frequency of pesticide application would increase which in effect leads to an increase in the risk of pesticide residue carriage on produce (Delcour et al., 2015). When pesticides are applied, they can be transported to different components of the environment, including: air, soil, ground water, and surface water (Figure 3).
Figure 3. Fate of pesticides after application and factors affecting them. Adapted from Declour et al. (2015).

1.4.1 Chlorpyrifos

One pesticide of great interest is chlorpyrifos (O,O-diethyl-O-3,5,6-trichloro-2-pyridylphosphorothioate), a broad spectrum OP insecticide that is widely used in the agricultural industry (Mladenova & Shtereva, 2009; K. R. Solomon et al., 2014; USEPA, 2006). In 2012, CPY was ranked as the 14th most used pesticide and the most widely used insecticide in the United States by the United States Department of Agriculture – National Agricultural Statistics Service (Atwood & Paisley-Jones, 2017). CPY is capable of controlling insects that commonly consume maize, fruit still on trees or vines, and root vegetables (Gomez, 2009; K. R. Solomon et al., 2014). It has been estimated that CPY is used to treat over 50 different crops (Gomez, 2009)
and fruits grown on trees such as apples account for approximately 10 percent of CPY usage (Eaton et al., 2008).

CPY has been shown to be a neurotoxin which can affect the mental development of children (Trasande, 2017). Longitudinal studies have shown that prenatal exposure to OPs is associated with neurological deficits in children (M. Bouchard, F. et al., 2011; Rauh et al., 2012). Bouchard et al. (2011) conducted a 7-year birth cohort study (n = 329) and reported that children exposed to higher levels of OPs had an average of 7 lower IQ points than children exposed to lower levels of OPs.

CPY exposure can occur in a number of ways including ingestion through food or water, skin contact, and inhalation through air (Giesy & Solomon, 2014). CPY can be used on crops throughout the year and its use is determined by the amount of pests present at a given time. It can be used in the winter season on tree crops and used in the summer season on field crops (Giesy & Solomon, 2014). The United States National Institutes of Health (NIH) has stated that the half-life of CPY is approximately 4.2 days in the summer and 9.7 days in the winter (Toxnet, 2014). CPY is capable of absorbing light in the UV range with a maximum absorbance of 290 nm (Zalat, Elsayed, Fayed, & Abd El Megid, 2014). The lower half-life in the summer is due to photolysis by sunlight because of this absorption (Toxnet, 2014). Photolysis requires the presence of water to occur (Etō, 1974). Under dry conditions, only an estimated 2% decomposition of CPY after 1200 hours of irradiation is predicted (Etō, 1974).

Some jurisdictions have decided the risk of CPY to human health and the environment is too great to allow continued use in agriculture. For example, the State of Hawaii has recently banned the use of CPY starting in 2022, being the first State to do so in the United States of America.
(State of Hawaii, 2017). New York has also proposed to ban all use of CPY by 2021 ("Assembly Bill A2477B," 2019). Although Hawaii and New York may have banned the use of CPY, other jurisdictions have not followed the same course of action. In 2017, a petition to ban the use of CPY was denied by the U.S. Environmental Protection Agency (EPA) stating that “the pesticide is crucial to U.S. agriculture.” (Environmental Protection Agency, 2017, March 29). As CPY continues to be used in agriculture, it is necessary to limit exposure to the public. Currently, the maximum residue limit (MRL) for CPY on apples in Canada is set at 0.01 µg/g (Health Canada, 2012, October 1). In the United States, the limit on apples is also set at 0.01 µg/g and due to its possible detrimental effects, produce such as spinach, squash and carrots cannot be treated using CPY as they are commonly fed to children (USEPA, 2006). If residue is found on these crops, they are considered to be adulterated which allows the FDA to consider enforcement actions (FDA, 1995).

If humans are exposed to high enough concentrations of CPY (LD50 in rats was determined to be 60 mg/kg bw (EXTOXNET, 1993), adverse effects including nausea, dizziness, respiratory paralysis and even death can occur (USEPA, 2006). It has also been shown recently that CPY exposure can promote the growth of mammary tumors and may be a risk factor for breast cancer development (Ventura et al., 2019). Various diseases that affect the nervous system such as Alzheimer’s and Parkinson’s disease have also been associated with, exposure to, insecticides but more research is required (Brown et al., 2006; Casida & Durkin, 2013; Kim et al., 2015). In response to this, it is important to have post-harvest techniques and treatments in place to minimize the amount of pesticide residue on produce to protect consumers.
1.4.2 Chlorpyrifos-Oxon

CPY can be degraded into two different metabolites, chlorpyrifos-oxon (CPY-O) and 3,5,6 – trichloro-2-pyridinol (TCP). CPY-O is the active form of CPY and is capable of irreversibly binding to AChE through phosphorylation (Giesy et al., 2014; Williamson et al., 2006). Structurally, CPY-O and CPY are similar but CPY-O contains a phosphorus-oxygen double bond (P=O) group whereas CPY contains a phosphothionate (P=S) group (Figure 4). Due to this structural difference, CPY-O is more efficient at binding to AChE than CPY (Giesy et al., 2014). The mechanism of action of CPY is to be transformed into CPY-O which prevents AChE action (Figure 5). This transformation has been shown to occur in the environment due to oxidative desulfonation which can be accelerated by photolysis (USEPA, n.d.-a) and in animals through multifunction oxidase (MFO) (Giesy et al., 2014). El-Merhibi et al (2004) showed in aquatic organisms, when MFO is inhibited, the toxicity of CPY can be reduced by up to six-fold (El-Merhibi, Kumar, & Smeaton, 2004; Giesy et al., 2014). Acute CPY poisoning will show symptoms when over 70% of AChE molecules have been bound (Connors et al., 2008). It has also been suggested by the USEPA that CPY-O can exert the same or greater levels of toxicity than CPY (Slotkin, Seidler, Wu, MacKillop, & Linden, 2009). They reported that CPY-O is 41 more times toxic than CPY when tested on aquatic animals (USEPA, n.d.-a). CPY-O can be detoxified in human bodies through hydrolysis facilitated by the enzyme paraoxonase 1 (PON1) (Costa, Giordano, Cole, Marsillach, & Furlong, 2013). When hydrolyzed, CPY-O will turn into the less harmful TCP metabolite and be excreted through urine (Costa et al., 2013).
Figure 4. Structures of chlorpyrifos and its active form chlorpyrifos-oxon as well as the inactive form TCP. Adapted from USEPA (n.d).

Figure 5. Mechanism of action of chlorpyrifos-oxon. It can phosphorylate AChE to permanently inactivate the enzyme or it can temporarily inactivate it. Adapted from Solomon (2014).
TCP is the inactive form of CPY and is filtered by the kidney without toxic effects. However, It has been reported in one study by Kashanian et al. (2012) that TCP has DNA binding effects implying that TCP may exert mutagenic effects (Kashanian, Shariati, Roshanfekr, & Ghobadi, 2012). The USEPA concluded however that TCP is significantly less toxic than CPY and is considered to be toxicologically insignificant (USEPA, n.d.-a). An animal study conducted by Hanley et al. (2000), reported a LD50 oral dose of 800 mg/kg for TCP. They concluded that because TCP lacks the phosphate group seen in CPY it cannot inhibit AChE and would therefore be less toxic than its parent compound (Hanley, Carney, & Johnson, 2000). They also stated that CPY is quickly broken down into TCP in mammalian systems through hydrolysis which would effectively detoxify CPY (Hanley et al., 2000).

1.5 Microbial Hazards

Besides chemical contamination via pesticides, microbial contamination can also be present on produce and some pathogens are capable of causing severe illness and even death for consumers (Rangel, Sparling, Crowe, Griffin, & Swerdlow, 2005). One pathogen of concern is *Escherichia coli* O157:H7 which has been implicated in numerous produce-linked foodborne outbreaks (Table 4). A recent outbreak occurred in Canada in 2018 when romaine lettuce imported from California was contaminated with *E.coli* O157:H7 which affected 29 individuals, 2 of which developed hemolytic uremic syndrome (Canada, 2019).
<table>
<thead>
<tr>
<th>Year</th>
<th>Source</th>
<th>Area(s) affected</th>
<th>Total cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>2019</td>
<td>Romaine lettuce</td>
<td>23 states in the USA</td>
<td>102</td>
</tr>
<tr>
<td>2018</td>
<td>Romaine lettuce</td>
<td>British Columbia, Alberta, Saskatchewan, Ontario, Quebec; 36 states in the USA</td>
<td>8 (Canada) + 210 (USA)</td>
</tr>
<tr>
<td>2018</td>
<td>Leafy greens</td>
<td>Ontario, Quebec, New Brunswick, Nova Scotia, and Newfoundland and Labrador; 15 states in the USA</td>
<td>67</td>
</tr>
<tr>
<td>2018</td>
<td>Alfalfa sprouts</td>
<td>Minnesota, Wisconsin (USA)</td>
<td>11</td>
</tr>
<tr>
<td>2017</td>
<td>Leafy greens</td>
<td>15 States in the USA</td>
<td>25</td>
</tr>
<tr>
<td>2015</td>
<td>Leafy greens</td>
<td>Alberta, Saskatchewan, Ontario, Newfoundland and Labrador (Canada)</td>
<td>13</td>
</tr>
<tr>
<td>2013</td>
<td>Shredded lettuce</td>
<td>Ontario, New Brunswick, Nova Scotia (Canada)</td>
<td>30</td>
</tr>
<tr>
<td>2013</td>
<td>Ready to eat salads</td>
<td>Washington, California, Arizona, Texas (USA)</td>
<td>33</td>
</tr>
<tr>
<td>2012</td>
<td>Organic spinach and spring mix</td>
<td>New York, Pennsylvania, Virginia, Maine, Connecticut (USA)</td>
<td>33</td>
</tr>
<tr>
<td>2012</td>
<td>Romaine lettuce</td>
<td>9 states in the USA</td>
<td>58</td>
</tr>
<tr>
<td>2006</td>
<td>Spinach</td>
<td>26 states in the USA</td>
<td>199</td>
</tr>
</tbody>
</table>

1.5.1 *Escherichia coli* O157:H7

*E. coli* O157:H7 is classified as an enterohemorrhagic *E.coli* (EHEC) which can cause gastroenteritis, enterocolitis, and bloody diarrhea in infected humans due to the production of shiga toxins (Stx), one of the most potent biological poisons (Lim, Yoon, & Hovde, 2010;
EHEC is a subgroup of Stx producing *E.coli* (STEC) that can induce apoptosis in cells (Figure 6) which causes bloody diarrhea and in worse cases, hemolytic-uremic syndrome (HUS), a condition involving kidney failure, haemolytic anemia, and thrombocytopenia (a reduction in blood platelets that help in blood clot formation) (Melton-Celsa, 2014). An estimated 8% of STEC infections leads to HUS (D. E. Thomas & Elliott, 2013).

Figure 6. Mechanism of action of STEC in causing cell death. 1.) STEC adheres to intestinal lumen, 2.) Shiga toxin (Stx) is produced, 3.) Stx adhering onto cell, 4.) Endocytosis of Stx into the cell, 5.) Stx in a vesicle to be transported into the Golgi complex, 6.) Stx in the Golgi complex, 7.) Stx breaks apart and the toxic A1 subunit is released, 8.) A1 subunit attaches to rRNA, 9.) RNA cannot be translated due to the A1 subunit so protein synthesis is inhibited, 10.) Lack of protein synthesis leads to cell death. Modified from V. Castro, Carvalho, Conte Junior, & Figueiredo (2017).
STEC can be transferred from person-to-person and contaminated food and drinking water (Figure 7) (Fairbrother & Nadeau, 2006; Lim et al., 2010; Salvadori et al., 2009). The most common route of STEC transmission is through consumption of contaminated food and beverages, commonly linked to undercooked ground beef and raw milk (CDC, 2016; Gally & Stevens, 2017; Lim et al., 2010). STEC has often been linked back to cattle manure runoff; approximately 30% of feed cattle shed *E. coli* O157:H7 in their manure (D. E. Thomas & Elliott, 2013). It has been estimated that less than 100 cells of *E. coli* O157:H7 can cause disease in susceptible hosts (Leach, Stroot, & Lim, 2010). The products most associated with human exposure are: ground meat, leafy greens, raw milk, and apple juice/cider (Lim et al., 2010).

Leafy greens such as lettuce can be contaminated through a number of ways including: human transfer, manure runoff, and irrigation water (E. B. Solomon, Yaron, & Matthews, 2002). It was demonstrated by Solomon, Yaron, and Matthews (2002) that *E. coli* O157:H7 can enter the root system of mature lettuce and be found in the edible leafy portions. They concluded that due to this route of entry, *E. coli* O157:H7 can avoid complete disinfection through surface sanitation of lettuce. In the 1990s, seven cases of *E. coli* O157:H7 outbreaks from contaminated apple products such as apple juice and apple cider were reported (Rangel et al., 2005). Due to being able to tolerate low pH levels, below 2, *E. coli* O157:H7 can grow in apple products without difficulty which is why nowadays, apple juices and ciders require pasteurization (Miller & Kaspar, 1994). One case occurring in 1996 determined that apples were contaminated in the orchard by contacting manure left by wildlife carrying *E. coli* O157:H7 as well as the use of rotten apples (Cody et al., 1999). From these events, it was suggested that washing apples could reduce the risk of transmission (Besser et al., 1993).
Figure 7. Transmission routes of STEC from farm cattle ingesting STEC (1) then contaminating the environment with fecal shedding (2) leading to contamination of food and water (3 and 4) consumed by humans. People can also transmit STEC from one to another (5). Adapted from Fairbrother and Nadeau (2006).

1.5.2 *Aspergillus niger* spores

Another organism of interest is *Aspergillus niger* (*A. niger*) which is commonly implicated in spoilage of produce. *Aspergillus niger* is a saprophytic filamentous fungus with a brown to dark brown colour that is commonly found in soil, compost and decaying plants (Schuster, Dunn-Coleman, Frisvad, & van Dijck, 2002). It is capable of growing at temperatures ranging from 25 – 40 °C, a pH range of 1.4 – 9.8 and a water activity down to 0.88 (Gautam, Sharma, Avasthi, & Bhadauria, 2011; Ladaniya, 2008). These conditions mean warm and humid environments are ideal for supporting growth on fruits (Gautam et al., 2011). *Aspergillus niger* reproduces asexually, resulting in the growth of vegetative mycelium and conidiophores that leads to spore formation (Krijgsheld et al., 2013). Once spores are formed they are able to survive in
environments outside of its usual growth parameters and then germinate once proper growth conditions occur (Krijgsfeld et al., 2013).

*Aspergillus niger* is labelled as “generally recognized as safe (GRAS)” by the FDA for use in citric acid and enzyme production (R. Sharma, 2012) however multiple *Aspergillus* species are capable of causing harm to human health (Al-hindi, Alnajada, & Mohamed, 2011). For example, *Aspergillus fumigatus* is capable of causing allergic reactions as well as life threatening infections in humans (Paulussen et al., 2017). *Aspergillus fumigatus* is responsible for 90% of aspergillus related infections followed by *Aspergillus flavus* and *A. niger* (Dagenais & Keller, 2009; Paulussen et al., 2017). *Aspergillus niger* has been found to be an opportunistic infectious organism to humans (R. Sharma, 2012). There have been rare cases of *A. niger* spores infecting patients with severe immunodeficiency (Schuster et al., 2002). One such case involved a patient with invasive pulmonary aspergillosis, a lung infection caused by aspergillus species which leads to necrosis of lung tissue (Person, Chudgar, Norton, Tong, & Stout, 2010). Various strains of *A. niger* are also capable of producing mycotoxins: aflatoxin, ochratoxin, and fumonisin B2 (Frisvad, Smedsgaard, Samson, Larsen, & Thrane, 2007; Gautam et al., 2011; Schuster et al., 2002). These mycotoxins are dangerous when consumed by humans and are capable of inducing hepatotoxicity, nephrotoxicity, immunotoxicity, and possible carcinogenicity (Gautam et al., 2011; Schuster et al., 2002).

*Aspergillus niger* is widely used in industry due to its ability to produce citric acid in copious amounts while also being economical (Gautam et al., 2011; Show et al., 2015). *Aspergillus niger* can also produce various other organic acids as well as food enzymes such as pectinase, proteases, and glucoamylase (Schuster et al., 2002). Due to its ability to produce organic acids and enzymes, *A. niger* can cause decay in nuts, legumes, cereals, and fresh produce such as
apples (Gautam et al., 2011). *Aspergillus niger* is one of the most prevalent spoilage microorganisms. One studied showed that *A. niger* was the most common fungi found in infected fruits such as pineapples, watermelon, and oranges with a 38% occurrence frequency (Mailafia, Okoh, Olabode, & Osanupin, 2017).

### 1.6 Current Treatments to Decrease Pesticide Residues Found on Produce

The most common current technology used to remove pesticide residues from produce is through post-harvest washing with clean, potable water (Table 5). Rawn and colleagues showed that washing and rubbing apples under running de-ionized water could effectively remove pesticide residues by approximately 50% (Rawn et al., 2008). Washing was shown to be effective, reducing CPY levels on fruits and vegetables by 30-50% (Mi-Gyung Lee, 2001; Rawn et al., 2008). It was also shown that washing removed approximately 60% of CPY on rice grains (Lee, Mourer, & Shibamoto, 1991). Another study showed that when shaken in water, red peppers that were artificially spiked with CPY had a 30-40% reduction after 5 minutes (Mi-Gyung Lee, 2001). Washing produce with different low concentration solutions of acetic acid, citric acid and potassium permanganate have also been shown to reduce pesticide concentrations in date fruits (Osman, Al-Humaid, Al-Redhaiman, & El-Mergawi, 2014). The problem with batch washing produce is that pesticides are removed from the surface of the product which in effect generates wastewater containing pesticides that can lead to cross contamination between batches (Burkul et al., 2015; Kayla Murray, Wu, Shi, Jun Xue, & Warriner, 2017). Although washing can reduce pesticide concentrations on produce, it does not degrade pesticides but rather redistributes it between batches and into the environment via the disposal of spent wash water (Burkul et al., 2015; Köck-Schulmeyer et al., 2013).
### Table 5. Reduction of pesticide residues on produce by current washing treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Reduction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washing apples using deionized water</td>
<td>50% Reduction of Captan</td>
<td>(Rawn et al., 2008)</td>
</tr>
<tr>
<td>Washing of red peppers using tap water</td>
<td>30-40% Reduction of CPY</td>
<td>(Mi-Gyung Lee, 2001)</td>
</tr>
<tr>
<td>Washing of red peppers using tap water then hot-air dried for 26 hrs</td>
<td>67% Reduction of CPY</td>
<td>(Chun &amp; Lee, 2006)</td>
</tr>
<tr>
<td>Washing of red peppers using tap water then hot-air dried and UV treated for 26 hrs</td>
<td>73% Reduction of CPY</td>
<td>(Chun &amp; Lee, 2006)</td>
</tr>
<tr>
<td>Washing of red peppers using 20% hydrogen peroxide solution</td>
<td>22% Reduction of CPY</td>
<td>(Lee et al., 1991)</td>
</tr>
<tr>
<td>Washing of rice using tap water</td>
<td>60% Reduction of CPY</td>
<td>(Lee et al., 1991)</td>
</tr>
</tbody>
</table>

Pesticides are also able to adhere onto soft skins and some may even be absorbed into the plant (Yang et al., 2017). Yang and colleagues showed that washing with a baking soda solution was more effective than with water or hypochlorite. They also found that the systemic pesticide thiabendazole is capable of penetrating the skin of apples which makes it harder to be removed through washing alone (Yang et al., 2017).
Approximately 6% of consumers do not wash their produce or choose to wash produce only when deemed necessary (Parnell & Harris, 2003). In 2016, the FDA conducted a survey asking consumers if they washed their produce before consumption. Only 54% replied “yes” when asked if they wash their lettuce before consumption and 56% washed their avocados (Lando, Verrill, Liu, & Smith, 2016). They also noted that when asked if they washed their strawberries or tomatoes before consumption, close to 100% responded “yes”. Washing has also been shown to be effective at removing microorganisms off the skin of produce (Parnell & Harris, 2003).

Parnell and Harris showed, in the lab, that a 3.2 log reduction of *Salmonella* on apples can be achieved through rubbing and rinsing with 200 mL of water for 5 seconds in combination with drying using a sterile paper towel. They also demonstrated that washing with a 5% vinegar or 200 µg/mL chlorine solution were better at reducing *Salmonella* than water with reductions ranging from 5.2 – 6.2 logs (Parnell & Harris, 2003). Another study also showed that chlorine bleach wash was more effective than running water at reducing *Salmonella* as well as *E.coli* O157:H7 (Fishburn, Tang, & Frank, 2012). Fishburn and colleagues (2012) showed that when washing lettuce under running water, log reductions of 1.58 and 1.69 can be observed for *Salmonella* and *E.coli* O157:H7 respectively while chlorine bleach reduced 2.05 and 2.34 logs.

In commercial testing, it was shown that washing only resulted in a 1 – 2 log reduction of *E.coli* (Murray et al., 2017, Barrera, 2012).

In-kitchen techniques and the application of heat (cooking and boiling) have also shown to be effective in reducing pesticide residues (Bajwa & Sandhu, 2014). It was also reported by Sharma and his colleagues in 2001, peeling in combination with cooking methods (boiling, steaming, etc.) to be the most effective method of removing the pesticide mancozeb from the surface of apples. They showed that washing alone reduced 20-52% of residues and when combined with
cooking led to 53-73% reduction (I. D. Sharma, Patyal, Nath, & Joshi, 2001). Cooking alone has been shown to not completely remove all pesticides in tea leaves and crops such as spinach, strawberries (made into jam), and oranges (Nagayama, 1996). Washing was also shown to be ineffective at reducing levels of CPY to below the maximum residue limit (MRL) (Nagesh & Verma, 1997). Lee and his colleagues also showed that approximately 30% of CPY residue remained on rice after cooking (Lee et al., 1991). Singh et al. (2017) conducted heat treatments such as boiling, pasteurization, and sterilization on CPY and found that after boiling for 5 minutes, 44% of CPY was degraded whereas sterilization at 121 °C for 15 minutes degraded 68% CPY (S. Singh, Krishnaiah, Rao, Pushpa, & Reddy, 2017). One possible explanation for CPY not being fully degraded may be because CPY decomposes at 160 °C, above the boiling point of water. These results suggests that heat may be a significant factor in CPY reduction.

1.6.1 Ultraviolet Radiation

The use of ultraviolet (UV) light has gained popularity over the years for food decontamination because it is cost effective, easy to use and monitor, and residue-free as no chemicals are required (Keklik, Krishnamurthy, & Demirci, 2012; National Environmental Services Center [NESC], 2000). The UV light spectrum ranges from 100-400 nm and can be generated by low or medium pressure mercury lamps (Keklik et al., 2012). This spectrum is categorized into four sections: UV-A (315-400 nm), UV-B (280-315 nm), UV-C (200-280 nm) and Vacuum UV (100-200 nm) (Figure 8). The region of interest is UV-C light at 254 nm which has been shown to be the most effective at inactivating microorganisms (Keklik et al., 2012). UV-C light can induce a germicidal effect on microorganisms through the absorption of UV light by living cells which damages DNA (Keklik et al., 2012). Due to this damage, bacterial cells are incapable of replicating DNA thus causing the cell to become inactivated (Keklik et al., 2012).
Low pressure mercury (LPM) lamps are capable of emitting this wavelength of 254 nm monochromatically whereas medium pressure mercury (MPM) lamps emit polychromatic light at wavelengths between 185-600 nm (Bohrerova, Shemer, Lantis, Impellitteri, & Linden, 2008). These lamps operate by using electricity to excite mercury vapor within a silica or quartz tube which then generates UV radiation when the mercury vapor returns to a lower energy level (Bohrerova et al., 2008; Keklik et al., 2012). Exact wavelengths emitted depend on temperature as well as the vapor pressure within the tube which is why LPM lamps produce a more monochromatic wavelength compared to MPM lamps (Keklik et al., 2012). Low pressure lamps have an operating temperature between 30 - 50 °C with vapor pressure between 0.1-10 Pa whereas medium pressure lamps operate at temperatures between 600 – 900 °C with a vapor pressure from 50 – 300 kPa (Keklik et al., 2012).

The Electromagnetic Spectrum

![The Electromagnetic Spectrum](https://www.evoqua.com/en/brands/ETS_UV/Pages/how-ets-uv-works.aspx)

Figure 8. The UV light spectrum of the electromagnetic spectrum. Modified from Evoqua Water Technologies, LLC. [https://www.evoqua.com/en/brands/ETS_UV/Pages/how-ets-uv-works.aspx](https://www.evoqua.com/en/brands/ETS_UV/Pages/how-ets-uv-works.aspx)
1.6.2 Ozone

The use of ozone for drinking water disinfection and oxidation has been around since the early 1900s in Europe (Langlais, Reckhow, & R. Brink, 1991). Nowadays it is not only employed in water treatment but also in the food industry for increasing produce shelf life and ensuring sanitation of surfaces (O'Donnell, Brijesh kumar, Cullen, & G. Rice, 2012; Wang, Wang, Wang, Li, & Wu, 2019). It has been shown that ozone treatment is capable of reducing cellular respiration and ethylene production which delays fruit ripening hence prolonging shelf life (Wang et al., 2019). For ozone to be generated, a diatomic oxygen molecule needs to be broken to create free radical oxygen which reacts with another diatomic oxygen thus forming a triatomic ozone molecule (O'Donnell et al., 2012). There are multiple ways to generate ozone, including the use of ultraviolet light at 188 nm which is capable of breaking the diatomic oxygen bond (O'Donnell et al., 2012). Another method to produce ozone is corona discharge, in which oxygen-containing gas is passed between two electrodes (Figure 9). These electrodes have a voltage applied to them which produces electrons that dissociate the oxygen molecules to form ozone (O'Donnell et al., 2012). It is estimated that if air is passed through the electrodes, only 1-3% ozone is produced but if high-purity oxygen is passed through, production can be as high as up to 16% (O'Donnell et al., 2012). In a contained environment, ozone concentration will reach equilibrium with its surroundings when the generation and degradation rate is the same. Once this point is reached, ozone concentrations cannot be increased any further (O'Donnell et al., 2012).

Ozone has multiple uses in the food industry including plant sanitation, pest reduction, packaging material disinfection as well as direct contact with food products to reduce microorganisms that negatively impact food quality and safety (Jegadeeshwar, Shankar, Kumar, & thottiam Vasudevan, 2017). In 2001, the FDA recognized ozone as an antimicrobial food additive and labelled it as GRAS (O'Donnell et al., 2012). The FDA states that ozone can be used as an additive in the “treatment, storage, and processing of foods” and currently has no limits set (Regulations, 2018). However, the FDA also states that any devices that generate ozone at a level over 0.05 ppm or accumulate ozone at a level over 0.05 ppm will be considered “adulterated and/or mislabelled” in animal products (FDA, 2018). This is because ozone can harm human health. It is capable of causing damage to the lungs, chest pain, coughing, shortness of breath and even throat irritations (USEPA, n.d). The National Institute of Occupational Safety and Health (NIOSH) recommends that ozone concentrations should not exceed 0.1 ppm at any time and the Occupational Safety and Health Administration (OSHA) states that workers should not be
exposed to an average concentration of more than 0.1 ppm for 8 hours (USEPA, n.d). Ozone must also be able to produce a minimum 5 log reduction for microorganisms commonly found in juices in order to comply with 21 CFR 101.17(g)(7) (FDA, 1977). Ozone can be applied in either an aqueous or gaseous form on produce to reduce the amount of pathogenic and spoilage microorganisms by direct oxidation (O'Donnell et al., 2012). Ozone disperses rapidly and decomposes into oxygen which leaves zero residues on food products (O'Donnell et al., 2012). Since ozone does not leave residues on products and surfaces it has grown in popularity in its use in the food industry (Jegadeeshwar et al., 2017). However, like UV treatments, ozone can degrade pesticides into more dangerous by-products than its parent compound such as the formation of oxons (Wang et al., 2019).

In the fruit and vegetable industry, ozone is generally used to inactivate pathogenic and spoilage-causing microorganisms as well as to reduce the amount of post-harvest pesticide and chemical residues (O'Donnell et al., 2012). Ozone is more effective when added to water due to increased contact between the surface being decontaminated leading to the formation of hydroxyl radicals which have a higher reaction rate and oxidizing power than ozone (Krishnan, Rawindran, Sinnathambi, & Lim, 2017). Ozone is capable of decomposing into hydroxyl radicals faster in water than air due to having a shorter half-life in water (LennTech, 2019). When in 20°C air, ozone has a half-life of 3 days whereas in 20°C water (pH = 7), the half-life is 20 minutes (LennTech, 2019). Achen and Yousef (2001) showed that apples treated with ozone being added during water washing inactivated *Escherichia coli* O157:H7 better than apples placed in pre-ozonated water with log reductions of 3.7 and 2.6, respectively. The time needed to generate hydroxyl radicals can also be shortened through increased temperature, higher pH, decreased concentrations of organic material, and increased dose of UV light (LennTech, 2019).
Maximizing the formation of hydroxyl radicals through optimizing the listed factors is known as an advanced oxidation process (Abramovic et al., 2010; LennTech, 2019).

1.7 Advanced Oxidation Process

A potential method to decrease the public’s exposure to CPY is the use of advanced oxidation processes (AOPs) combining UV-C light, hydrogen peroxide (H₂O₂), and ozone to degrade various organic contaminants (Abramovic et al., 2010; Femia et al., 2013; Marican & Durán-Lara, 2018). AOP has long been used in the treatment of wastewater (Andreozzi, Caprio, Insola, & Marotta, 1999; Gottschalk, Saupe, & Libra, 2010; Oppenländer, 2003) and has been shown to be effective in pesticide residue removal (Table 6) (Abramovic et al., 2010; Ikehata & Gamal El-Din, 2005).

Table 6. Degradation of pesticides using AOPs and individual processes.

<table>
<thead>
<tr>
<th>Target Pesticide</th>
<th>Treatment</th>
<th>Degradation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiacloprid</td>
<td>UV/H₂O₂ exposure for 120 minutes.</td>
<td>97% Degradation</td>
<td>(Abramovic et al., 2010)</td>
</tr>
<tr>
<td></td>
<td>UV exposure for 120 minutes</td>
<td>No significant degradation</td>
<td>(Abramovic et al., 2010)</td>
</tr>
<tr>
<td></td>
<td>H₂O₂ exposure for 120 minutes</td>
<td>No significant degradation</td>
<td>(Abramovic et al., 2010)</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>UV/H₂O₂ exposure for 20 minutes</td>
<td>93% Degradation (14 mg/L)</td>
<td>(Femia et al., 2013)</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>UV exposure for 300 minutes</td>
<td>100% Degradation (5 mg/L)</td>
<td>(Muhamad, 2010)</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>Ozone exposure</td>
<td>No significant degradation</td>
<td>(Ikehata &amp; Gamal El-Din, 2005)</td>
</tr>
<tr>
<td>Target Pesticide</td>
<td>Treatment</td>
<td>Degradation</td>
<td>Reference</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------------------------------------</td>
<td>------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>Lychee fruit – Gaseous ozone exposure</td>
<td>45% Degradation</td>
<td>(Whangchai, Uthaiibutra, Phyanalinmat, Pengphol, &amp; Nomura, 2011)</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>Lychee fruit – Aqueous ozone exposure</td>
<td>10% Degradation</td>
<td></td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>Apples – Aqueous ozone exposure for 15 minute</td>
<td>69% Degradation (0.36 µg/g)</td>
<td>(Swami et al., 2016)</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>Apples – Aqueous ozone exposure for 30 minute</td>
<td>95% Degradation (0.06 µg/g)</td>
<td></td>
</tr>
</tbody>
</table>

AOPs are chemical treatments that produce hydroxyl radicals (·OH) in copious amounts to oxidize organic and inorganic material in water and wastewater (Abramovic et al., 2010; Andreozzi et al., 1999; Femia et al., 2013; Glaze, Kang, & H. Chapin, 1987; Marican & Durán-Lara, 2018). Hydroxyl radicals are extremely reactive with little selectivity and a short life span of less than a second (Deng & Zhao, 2015). According to Parsons, the oxidation potential of hydroxyl radicals is +2.80 volts (V) which is almost as high as fluorine with a V of +3.06 (Table 7). The rate of oxidation is dependent on a number of factors including the concentrations of radicals, oxygen, and pollutants (Parsons, 2004). One of the main goals when utilizing an AOP is to elevate the production of hydroxyl radicals to increase oxidation of organic and inorganic micropollutants (Katsoyiannis, Canonica, & von Gunten, 2011). It should be noted however that due to the reactivity of hydroxyl radicals, a large number of reactions can occur and it would be difficult to predict every product generated through oxidation (Parsons, 2004). Also, due to hydroxyl reactivity, an equilibrium will be reached between the generation and dissipation of hydroxyl radicals (Parsons, 2004).
Table 7. Oxidation potential of reactive species. Adapted from Parsons (2004).

<table>
<thead>
<tr>
<th>Oxidant</th>
<th>Potential $E^0$ (V, 25 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoride</td>
<td>3.06</td>
</tr>
<tr>
<td>Hydroxyl radical</td>
<td>2.80</td>
</tr>
<tr>
<td>Atomic oxygen</td>
<td>2.42</td>
</tr>
<tr>
<td>Ozone</td>
<td>2.07</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>1.78</td>
</tr>
<tr>
<td>Permanganate</td>
<td>1.68</td>
</tr>
<tr>
<td>Chlorine dioxide</td>
<td>1.57</td>
</tr>
<tr>
<td>Hypochlorous acid</td>
<td>1.49</td>
</tr>
<tr>
<td>Chlorine</td>
<td>1.36</td>
</tr>
<tr>
<td>Oxygen</td>
<td>1.23</td>
</tr>
</tbody>
</table>

When AOPs were first introduced for industrial purposes, they were mainly used in the treatment of wastewater and were later utilized in the 1980s in the treatment of potable water (Deng & Zhao, 2015). These early uses of AOP technologies used combinations of ozone, hydrogen peroxide, and ultraviolet light to generate hydroxyl radicals (Glaze et al., 1987). Ozone and hydrogen peroxide are the most commonly used chemicals to generate hydroxyl radicals with ultraviolet light acting as a catalyst to start the reaction (Abramovic et al., 2010). Another early use of AOPs was in the treatment of wastewater due to its strong oxidation potential to remove both organic and inorganic pollutants such as benzene and iron (II) (Deng & Zhao, 2015).

Nowadays, AOP technologies are used to treat a number of different contaminants and wastes (Table 8) (Vogelpohl, 2003). AOPs are useful in treating wastewater due to their ability to reduce pathogens and pesticide concentrations (Abramovic et al., 2010; E. L. Thomas, Milligan, Joyner, & Jefferson, 1994).
Table 8. Contamination and waste products reduced by AOP technology treatments. Modified from Vogelpohl (2003).

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Parasites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics</td>
<td>Pesticide wastewater</td>
</tr>
<tr>
<td>Arsenic</td>
<td>pesticides</td>
</tr>
<tr>
<td><em>Coliforms</em></td>
<td>Phenolic wastewater</td>
</tr>
<tr>
<td><em>Cryptosporidium</em></td>
<td>Natural organic matter</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Municipal wastewater</td>
</tr>
<tr>
<td>Hospital wastewater</td>
<td>Taste and odour causing compounds</td>
</tr>
<tr>
<td>Insecticide</td>
<td>Trinitrotoluene (TNT)</td>
</tr>
</tbody>
</table>

1.7.1 UV-based Advanced Oxidation Process

When using UV light by itself to destroy contaminants, photons from UV light must be able to be absorbed and undergo degradation from their excited state (Parsons, 2004). This limits the application of direct UV photolysis in industry. In an AOP system however, UV light is generally used to initiate the production of hydroxyl radicals through decomposing ozone and hydrogen peroxide (Abramovic et al., 2010). Hydrogen peroxide and oxygen radicals are produced when UV light reacts with ozone and it is then cleaved into two hydroxyl radicals (equation 1 and 2) (Deng & Zhao, 2015). This is possible due to hydrogen peroxide being able to absorb the UV wavelength of 254 nm (Figure 10) (Gottschalk et al., 2010).

\[
O_3 + H_2O + hv \rightarrow H_2O_2 + O_2 \text{ (Equation 1)}
\]

\[
H_2O_2 + hv \rightarrow 2OH \text{ (Equation 2)}
\]

One study using thiacloprid, a neonicotinoid insecticide, found that this pesticide can be photodegraded when exposed to UV radiation between 200 – 280 nm with its maximum absorbance wavelength being 242 nm (Abramovic et al., 2010). This study also showed that when exposed to higher wavelengths, outside of the absorbance range (200 – 280 nm), pesticide degradation was minimal. When UV is combined with hydrogen peroxide however, a synergistic effect is observed due to the production of hydroxyl radicals (Abramovic et al., 2010; Krzemińska, Neczaj, & Borowski, 2015).
It has been shown that CPY can be degraded into its oxon form when exposed to UV-C light (Figure 4) (Savić et al., 2019). Other OPs have been observed to degrade under UV-C light (Bustos, Cruz-Alcalde, Iriel, Fernández Cirelli, & Sans, 2019).

### 1.7.2 Ozone-based Advanced Oxidation Process

Since ozone degrades into hydroxyl radicals, it is necessary for AOPs to optimize the factors listed in Section 1.6.2 affecting ozone degradation (Gottschalk et al., 2010). These processes can involve the use of hydrogen peroxide and/or UV light as stated previously to increase ozone degradation and hydroxyl radical formation (Deng & Zhao, 2015; EPA, 1999; O'Donnell et al., 2012).

When ozone reacts with H₂O₂, which is commonly referred to as the peroxone process, hydroxyl radicals are produced along with oxygen (Merényi, Lind, Naumov, & Sonntag, 2010). By adding hydrogen peroxide into ozonated water, the decomposition of ozone is increased, in effect elevating the concentration of hydroxyl radicals (Deng & Zhao, 2015; EPA, 1999).

\[
H₂O₂ → HO₂⁻ + H⁺ \text{(Equation 3)}
\]

\[
HO₂⁻ + O₃ → OH⁻ + O₂⁻ + O₂ \text{(Equation 4)}
\]

This process of generating hydroxyl radicals was optimized by Duguet et al. (1985) who concluded that hydrogen peroxide should be added in a controlled amount after the oxidation of highly reactive substances by ozone (P. Duguet, Brodard, Dussert, & Mallevalle, 1985). This mechanism is driven by hydrogen peroxide decomposing into hydroperoxide anions which react with ozone to produce hydroxyl radicals (Deng & Zhao, 2015). Due to improved oxidation through elevated concentrations of hydroxyl radicals, the peroxone process is considered superior to ozone on its own when degrading organic material and it has been shown to be able
to degrade difficult-to-treat organics such as geosmin, an organic compound with an earthy taste (EPA, 1999). However, peroxone is less effective at oxidizing inorganic material such as iron and manganese compared to ozone.

Peroxone is generally used for oxidizing taste and odor compounds, synthetic organic compounds, pathogens and pesticide degradation for examples, geosmin, 1,1 – dichloropropene, poliovirus, and atrazine (EPA, 1999). When dealing with pathogens and viruses in a peroxone system, ozone has been shown to be the main cause for reductions due to the combination of direct ozone contact and reactivity of hydroxyl radicals in the oxidation of pathogens (EPA, 1999).

When exposed to UV-C light, ozone undergoes a photolysis reaction (Figure 11) and hydroxyl radicals are formed. This synergistic reaction can help reduce dissolved organic matter better than UV light or ozone alone (USEPA, n.d.-b). It is estimated that the initial ozonation rate of organic substances can be increased up to 10,000 times when combined with UV irradiation which can help speed up the removal of total organic matter (USEPA, n.d.-b).

![Figure 11. Photolysis reaction of ozone when exposed to UV-C light at 254 nm. One oxygen molecule and hydroxyl radical is produced. Modified from Spartan Environmental Technologies, LLC.](image-url)
1.8 Response Surface Methodology

When determining whether a new technology such as AOP is capable of degrading pesticides, it is necessary to observe which operational factors are significant and if they can be optimized to achieve a maximal degradation. This study aimed to determine the significance of the following factors: temperature of hydrogen peroxide, concentration of hydrogen peroxide, ozone concentration, and UV dose. One way of determining the significance of these factors and their optimized settings for pesticide degradation is by using response surface methodology (RSM), a multivariable statistic technique that utilizes a set of mathematical and statistical techniques to explore the relationship between multiple variables and their response variables (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008). In this study, the multiple variables (temperature of hydrogen peroxide, concentration of hydrogen peroxide, ozone concentration, and UV dose) are set at different parameters (high, medium, and low) and the response variable is the amount of CPY degraded in micrograms (µg). By comparing these factors at different settings, RSM can determine their significance in CPY degradation and predict an optimized setting for each factor to achieve maximal response (Bezerra et al., 2008).

There are multiple types of RSM design, with central composite design (CCD) and Box-Behnkin design (BBD) being the most widely used (Pennsylvania State University, 2018). CCD consists of multiple parameter settings; a central point representing the medium setting of each factor, several axial (or “star”) points (Figure 12) which represent the highest or lowest settings, and a factorial design, either full or fractional, that observes several combinations of different factor settings (Bezerra et al., 2008). Full factorial design experiments consists of multiple factors, usually two or more, occurring in every possible combination with one another (Bezerra et al., 2008). Fractional factorial designs are similar to full factorial designs but only have a select few
combinations to test (Bezerra et al., 2008). BBDs are similar to CCDs but avoid testing star points and consists of fewer experiments which makes it more time efficient and less costly (Bezerra et al., 2008). In analytical chemistry, CCDs are used far more often than BBD as they provide more information and tests for star points. By testing for star points, a more complete data set is given which will provide more accurate predictive values (NIST, 2013).

![Figure 12. Response surface methodology design with center points and star points. Modified from Stat-Ease, Inc.](image)

The use of RSM for determining the optimal treatment for chemical degradation using AOPs has been shown to be effective (Ahmadi, Mohammadi, Igwegbe, Rahdar, & Banach, 2018; Behin & Farhadian, 2017). Ahmadi and colleagues found that reactive blue 19 dye removal using AOP can be optimized through using RSM and the experimental degradation values were close to the predicted values suggested by RSM. Behin and Farhadian (2017) found that the pesticide trifluralin can be fully degraded, into chemically inactive byproducts, by using optimal treatment values of: ozone flow rate, UV light exposure time, and hydrogen peroxide solution pH. These values were determined through a full factorial central composite design (Behin & Farhadian, 2017).
Hypothesis and Objectives

It is expected that an optimized AOP treatment that applies ozone, UV radiation and hydrogen peroxide in combination will degrade the pesticide CPY and inactivate microorganisms on the surface of apples without leaving toxic by-product residues such as CPY-O. The objectives of this study were to:

1.) Develop an analytical method to analyze CPY on apple skin.

2.) Develop a recovery method of CPY from apple skins that have been treated or not treated in the AOP reactor.

3.) Use RSM to model the degradation of CPY by AOP treatments with the following factors: Ozone concentration, UV dose, temperature of hydrogen peroxide, and concentration of hydrogen peroxide.

4.) Validate the model, determine which factors are significant, and optimize the AOP factors for maximal CPY degradation.

5.) Determine whether AOP treatment will degrade CPY into its more toxic by-product chlorpyrifos-oxon.

6.) Determine if the optimized parameters can inactivate the microorganisms *Escherichia coli* O157:H7 and *Aspergillus niger*.
2. Materials and Methods

2.1 Materials

Chlorpyrifos analytical standards (>99% purity, PESTANAL®), hydrogen peroxide solution (30% v/v), trifluoroacetic acid (>99% purity) and nalidixic acid sodium salt were obtained from Sigma Aldrich (St. Louis, MO, USA). Crispin apples were provided by Moyer’s Apple Products Ltd. (Beamville, ON, Canada). Acetonitrile (Optima™, HPLC grade) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Chlorpyrifos-oxon standards (>99% purity) were purchased from Toronto Research Chemicals (North York, ON, Canada). Trypic soy broth (TSB) and potato dextrose agar (PDA) were purchased from Oxoid (Hampshire, UK). MacConkey agar with sorbitol, cefixime, and tellurite (CT-SMac) was purchased from Thermo-Fisher Scientific.

2.2 Advanced Oxidation Process Reactor

The advanced oxidative process (AOP) reactor (Clean Works Corporation, Beamsville, ON, CA) (Figure 13) was constructed from stainless steel with frame dimensions of 122 cm × 38 cm × 20 cm (length × width × height). The reactor was fitted with a lightbox (61 × 35 × 15 cm) housing 4 × 25 W UV-C lamps (60 cm length) emitting at 254 nm (Sani-Ray, Hauppauge, NY, USA). The UV intensity was varied through altering the distance of the lamps from the samples from 10 cm – 16 cm). A radiometer (Trojan Technologies Inc, London, ON, CA) was placed in the middle of reactor and used to measure the UV-intensity with treatment times being used to deliver a defined UV-dose. The initial UV intensity (start of AOP treatment) and final UV intensity (end of AOP treatment) were recorded and the average was used along with treatment time to calculate the total UV dose. Prior to introducing the apple samples into the UV reactor, the
hydrogen peroxide (0.2 mL) of defined concentration (1-6% v/v) and temperature (23 – 70°C) was dispensed onto the sample.

Figure 13. Schematic diagram (A) and photograph (B) of the advanced oxidation process reactor used for chlorpyrifos degradation and microbial inactivation.
2.3 Response Surface Methodology

RSM was used in the experimental design to determine the effectiveness of AOP in degrading the pesticide CPY. Design Expert® Software 11.1.0 (Stat-Ease Inc, Minneapolis, MN, USA) was used to produce a fractional factorial central composite design investigating the effect of UV dose (kJ/m²), temperature of hydrogen peroxide (°C), and concentration of hydrogen peroxide (% v/v) on the degradation of CPY (Table 9). The experimental design of this study included a total of 20 runs with six replicate center points (Table 10). Runs were performed in triplicate involving the three previously mentioned factors. The response variable can be expressed as a function (Eqn. 5) of the independent process variables according to the following response surface quadratic model.

\[
Y = \alpha_0 + \sum_{i=1}^{3} \alpha_i x_i + \sum_{i=1}^{3} \alpha_{ii} x_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{3} \alpha_{ij} x_i x_j + \varepsilon
\]

(Equation 5)

where \(Y\) is the predicted response, \(x_i\) and \(x_j\) are factors influencing the predicted response, \(\alpha_i, \alpha_{ii},\) and \(\alpha_{ij}\) are the coefficients of the linear, quadratic, and interaction terms respectively. Lastly, \(\varepsilon\) is a random error.

Regression analysis for the quadratic equation model was determined using Design Expert® 11.1.0 software. First, the model fit was tested to determine the quality of the model using the coefficient of determination (\(R^2\)). ANOVA analysis was conducted to determine whether the test variables listed are significant or not. This was done using F-test and P-values with 95% confidence level.
Table 9. RSM trials (20) using various test parameters of UV-C dose (kJ/m²), temperature (°C) and concentration (%) of hydrogen peroxide.

<table>
<thead>
<tr>
<th>Trial</th>
<th>UV dose (kJ/m²) ($X_1$)</th>
<th>Temperature of H$_2$O$_2$ (°C) ($X_2$)</th>
<th>H$_2$O$_2$ (%) ($X_3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34.8</td>
<td>46.5</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>22.5</td>
<td>32.5</td>
<td>5.0</td>
</tr>
<tr>
<td>3</td>
<td>54.0</td>
<td>32.5</td>
<td>5.0</td>
</tr>
<tr>
<td>4</td>
<td>37.0</td>
<td>46.5</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>22.4</td>
<td>60.5</td>
<td>5.0</td>
</tr>
<tr>
<td>6</td>
<td>38.3</td>
<td>70.0</td>
<td>3.5</td>
</tr>
<tr>
<td>7</td>
<td>39.4</td>
<td>46.5</td>
<td>3.5</td>
</tr>
<tr>
<td>8</td>
<td>39.4</td>
<td>46.5</td>
<td>3.5</td>
</tr>
<tr>
<td>9</td>
<td>11.7</td>
<td>46.5</td>
<td>3.5</td>
</tr>
<tr>
<td>10</td>
<td>38.9</td>
<td>46.5</td>
<td>3.5</td>
</tr>
<tr>
<td>11</td>
<td>56.1</td>
<td>60.5</td>
<td>2.0</td>
</tr>
<tr>
<td>12</td>
<td>50.3</td>
<td>32.5</td>
<td>2.0</td>
</tr>
<tr>
<td>13</td>
<td>37.9</td>
<td>46.5</td>
<td>6.0</td>
</tr>
<tr>
<td>14</td>
<td>21.8</td>
<td>32.5</td>
<td>2.0</td>
</tr>
<tr>
<td>15</td>
<td>39.3</td>
<td>46.5</td>
<td>3.5</td>
</tr>
<tr>
<td>16</td>
<td>37.2</td>
<td>46.5</td>
<td>3.5</td>
</tr>
<tr>
<td>17</td>
<td>62.3</td>
<td>46.5</td>
<td>3.5</td>
</tr>
<tr>
<td>18</td>
<td>38.5</td>
<td>23.0</td>
<td>3.5</td>
</tr>
<tr>
<td>19</td>
<td>22.3</td>
<td>60.5</td>
<td>2.0</td>
</tr>
<tr>
<td>20</td>
<td>53.3</td>
<td>60.5</td>
<td>5.0</td>
</tr>
</tbody>
</table>
Table 10: Six center point replicate trials determining the replicability and precision of the experimental method. Arranged from lowest to highest reduction corrected with the average (n=177) positive control recovery of 89.4%. The standard deviation between reductions was 3.6 µg.

<table>
<thead>
<tr>
<th>Trial #</th>
<th>Temperature of H₂O₂ (°C)</th>
<th>Actual Temperature (°C)</th>
<th>Percent H₂O₂ (° v/v)</th>
<th>UV-C Dose (kJ/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46.5</td>
<td>45</td>
<td>3.5</td>
<td>34.8</td>
</tr>
<tr>
<td>15</td>
<td>46.5</td>
<td>46</td>
<td>3.5</td>
<td>39.3</td>
</tr>
<tr>
<td>8</td>
<td>46.5</td>
<td>46</td>
<td>3.5</td>
<td>39.4</td>
</tr>
<tr>
<td>7</td>
<td>46.5</td>
<td>47</td>
<td>3.5</td>
<td>39.4</td>
</tr>
<tr>
<td>10</td>
<td>46.5</td>
<td>45</td>
<td>3.5</td>
<td>38.9</td>
</tr>
<tr>
<td>16</td>
<td>46.5</td>
<td>47</td>
<td>3.5</td>
<td>37.2</td>
</tr>
</tbody>
</table>

2.4 AOP Treatment of Apple skins Spiked with Chlorpyrifos

Crispin apples were stored in a cold storage room at 4°C until required. Apples were taken out of cold storage when needed then washed under running municipal tap water followed with towel drying. The washed apples were then inspected for damage including spots, bumps, bruises and discolouration. Apples were then peeled vertically from the bottom to the top with a vegetable peeler. Peeled apple skins were then cut into two 2.5 cm × 3 cm pieces per fruit. The peel sections were then placed on individually labelled aluminum weigh dishes. Aliquots (0.2 mL) of a working 1000 µg/mL CPY stock solution, prepared by dissolving 10 mg of CPY standard into 10 mL of acetonitrile, were introduced onto the surface of the apple peel samples and allowed to
dry at room temperature (approximately 25 °C) in the dark until dried. Thus, 200 µg of CPY was deposited onto each piece of apple skin. One sample was treated with the AOP and the other was left in the dark, untreated (positive control). Each treatment involved three pieces of apple skin containing CPY that were treated with the AOP, three pieces of apple skin containing CPY that were not treated with the AOP (positive controls), and two negative controls. One piece of apple skin that did not contain CPY and was treated with the AOP (negative control 1) and one piece of apple skin that did not contain CPY and was not treated with the AOP (negative control 2). The spiked samples along with a negative control were lined up lengthwise on a tray with dimensions 31 x 23.5 cm (length x width) and set 5 cm apart from one another lengthwise.

Treatment with 1 of the 20 listed AOP treatment parameters were performed (Table 9), the tray was introduced into the reactor through its entrance and placed on top of an adjustable rack set so that samples were 16 cm away from the UV light source (Figure 13A).

After treatment, the tray containing samples was removed through the reactor entrance and all apple skins (treated, positive controls, and negative controls) were then placed inside individually labelled 50-mL centrifuge tubes. A 20-mL aliquot of acetonitrile was used to rinse the aluminum dish and was then placed inside the centrifuge tube containing the corresponding apple skin. Assuming no degradation, a final concentration of 10 µg/mL would be present in the rinsate. All centrifuge tubes with an apple skin and rinsate were sonicated (Branson 2510, Crystal Electronics, Newmarket, ON, CA) for 20 minutes at 40 kHz. Due to heat being generated during sonication (up to 30°C), water was replaced with cool tap water to keep sonicater water temperature constant at approximately 23°C ± 2°C. After sonication, 1 mL of rinsate was removed from each tube and placed into individually labelled high performance liquid chromatography (HPLC) vials. This process was repeated two more times to produce a triplicate
set of samples for each treatment (n = 3). A total of 24 samples were produced per trial (9 spiked and treated, 9 spiked and untreated (positive control), and 6 not spiked/untreated (negative controls). Sample vials were stored at 4 °C for a maximum period of 24 h before quantification using HPLC analysis. It should be noted in the current study, the spiked concentration of CPY, 10 µg/mL is 1000 times higher than the MRL of CPY on apples in Canada (0.01 µg/mL) (Health Canada, 2008).

2.5 Validation Trial

Once RSM was completed, results were analyzed using Design Expert® Software. A validation trial was suggested by the software in order to confirm the accuracy and validity of the model. The given parameters for this trial were as follows: 69 kJ/m² UV dose and 1.22% v/v H₂O₂ at 66°C. The predicted reduction for this trial was 92 µg (46% reduction of spiked chlorpyrifos). Samples were prepared and treated using the methodology as described above in Section 2.4.

2.6 UV Only Trial

Treatments with UV light only were conducted using the same sample preparation and HPLC analysis methods describe above in Section 2.4. Five trials at different UV doses (11, 23, 41, 53, and 66 kJ/m²) were tested. After these trials were completed, the UV lights were brought closer to the samples to be treated. By reducing the distance between UV lights and samples, UV dose per minute would be increased thus reducing treatment time. The trial 53 kJ/m² was re-conducted at this higher UV dose to compare the difference in treatment times between same UV doses. The positive controls between the higher and lower UV intensities were averaged and were used as the positive control corrections.
2.7 Ozone Treatment of Apple Skins Spiked with Chlorpyrifos

Crispin apples were prepared the same way as described above in Section 2.4. Nine apple skin sections were spiked with 0.2 mL of 1000 µg/mL CPY solution (200 µg) and dried in the dark before treatment. Twelve apple skins were left blank (no spike) to act as a negative control. Treatment occurred in a 30-L plastic container containing 2 holes, 1 for tubing connected to an ozone monitor (linear dynamic range of 0-100 mg/L) (Model 106-L, 2B Technologies, Colorado, USA) the other for tubing connected to a 2 g/h corona discharge ozone generator provided by Dr. Thomas Graham (University of Guelph, School of Environmental Science) (Figure 14). Three spiked skins along with 1 negative control was placed into the container with the lid closed. These samples were treated with ozone gas for 30 minutes then placed into 50-mL centrifuge tubes and filled with 20 mL of acetonitrile (ACN). These tubes were then sonicated (Branson 2510, Crystal Electronics, Newmarket, ON, CA) at 40 Hz for 20 minutes. After sonication, 1 mL of sample was removed from the tube and placed into a 2-mL HPLC vial. This was repeated for each sample and the extracts were then analyzed using an Agilent 1100 Series HPLC (Agilent, Fair Lawn, CA, USA).
Figure 14. Schematic (A) and photograph (B) of ozone chamber treatment of CPY spiked apple skins.
2.8 HPLC Analysis

High performance liquid chromatography was performed with an Agilent 1100 Series HPLC instrument equipped with a photodiode array detector (Agilent, Fair Lawn, CA, USA). An Agilent Zorbax Eclipse Plus C18 analytical column (4.6 x 150 mm, 5.0 µm) (Agilent, Santa Clara, CA, USA) equipped with an Agilent Zorbax Eclipse Plus C18 analytical guard column (4.6 x 12.5 mm, 5-micron, Agilent, Santa Clara, CA, USA) was used as a stationary phase with a flow rate of 1.0 mL/min and the column temperature was uncontrolled with an average room temperature of 23°C. The injection volume for samples and standards was 40 µL. The mobile phase was in isocratic mode with a composition of 85:15 (%, v/v) acetonitrile/water with 0.1% trifluoroacetic acid (Millipore Sigma, St Louis, MO, USA). The detection wavelength was set at 290 nm. The concentration of CPY samples were quantified by the use of an x-point calibration curve. Calibration standards for CPY were prepared in acetonitrile at concentrations of: 2.5, 5, 10, 20, and 40 µg/mL. The method detection limit (MDL) was determined by spiking apple skin pieces with 200 µg of CPY (resultant concentration of rinsate 10 µg/mL) and extracting the CPY to get a measure of the methods accuracy and precision (n = 7) (Shrivastava & Gupta, 2011). The average recovery was 81.1%. The LOD and LOQ were 0.5 µg/mL and 1.7 µg/mL, respectively. Replicate trials from the RSM table conducted on separate days were also compared to determine the replicability of trials.

2.9 Determination of Chlorpyrifos-Oxon Production by AOP Treatment using Q-ToF analysis

Apple skin samples were prepared as per Section 2.4 and were treated under the optimized conditions described in Section 2.5. Untreated spiked samples and treated samples prepared using the optimal treatment along with analytical standards of CPY, CPY-O and TCP were submitted to
the Mass Spectrometry Facility of the Advanced Analysis Centre at the University of Guelph for Quadrupole Time of Flight (Q-ToF) mass spectrometry analysis to determine the presence or absence of these compounds.

Liquid chromatography–mass spectrometry analyses was performed on an Agilent 1200 HPLC liquid chromatograph (Agilent, Fair Lawn, CA, USA) interfaced with an Agilent UHD 6530 Q-Tof mass spectrometer (Agilent, Fair Lawn, CA, USA). An Agilent Poroshell 120 C18 column (EC-C18 50 mm x 3.0 mm 2.7 µm) was used for chromatographic separation with the following solvents water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The mobile phase gradient was as follows: initial conditions were 10% B hold for 1 min then increasing to 100% B in 29 min followed by column wash at 100% B for 5 min and 20 min re-equilibration. The flow rate was maintained at 0.4 mL/min and sample injection volume was 10 µL. The mass spectrometer electrospray capillary voltage was maintained at 4.0 kV and the drying gas temperature at 250 ºC with a flow rate of 8 L/min. Nebulizer pressure was 30 psi and the fragmentor was set to 160 ºC. Nitrogen was used as both nebulizing and drying gas. The mass-to-charge ratio was scanned across the m/z range of 50-1500 m/z in 4GHz (extended dynamic range) positive and negative ion modes. The acquisition rate was set at 2 spectra/s. The instrument was externally calibrated with the ESI TuneMix (Agilent, Santa Clara, CA, USA). Mass spectrometer control, data acquisition and data analysis were performed with MassHunter® Workstation software (B.04.00).

The concentration of CPY-O samples were quantified using an x-point calibration curve. Calibration standards for CPY-O were prepared in acetonitrile at concentrations of 0.01, 0.02, 0.04, 0.08, 0.16, and 0.31 µg/mL. Concentrations of 0.16 and 0.31 µg/mL were removed due to low accuracy of the result which did not conform to the linearity of the lower concentrations below
0.16 µg/mL. The $R^2$ value of the remaining 4 standards was 0.9993 (Figure 15) and the LOD was below 0.01 µg/mL.

\[ y = 511134937.748587 x + 7736884.037739 \]
\[ R = 0.999632 \]

Figure 15. Calibration curve of chlorpyrifos-oxon standards ranging in concentrations from 0.01 – 0.31 µg/mL. Concentrations of 0.16 and 0.31 µg/mL were removed due to low accuracy. $R^2$ value = 0.9993.

2.10 Inactivation of *Escherichia coli* O157:H7 and *Aspergillus niger*

*Escherichia coli* O157:H7 PH1 (stx$_2$, eae, nalidixic acid resistant) originally isolated from a clinical case was provided by the Public Health Agency of Canada (Guelph, Ontario, Canada).

*Escherichia coli* O157:H7 was cultivated in TSB at 37°C for 16 h. The cells were harvested by centrifugation (Sorvall ST8, Thermo Fisher Scientific, Waltham, MA, USA) at 4000 rpm for 10 minutes and resuspended in saline and then optical density at 600 nm was measured and adjusted
to 0.2 to obtain approximately 8 log CFU/mL. The suspension was held at 4 °C until required for testing.

*Aspergillus niger* spores NU320 previously isolated from environmental sources was used in this study. *Aspergillus niger* spores were produced by inoculating the mold onto PDA and incubating for 14 days at 25 °C. The spores were harvested by flooding the plate with sterile distilled water and scraping with a spreader. The spore suspension was then harvested by centrifugation for 15 minutes at 4000 rpm followed by the pellet being resuspended in sterile distilled water prior to storing at 4 °C until required for testing.

Apple skin sections were inoculated with either *E. coli* O157:H7 or *A. niger* spores to give a final cell density of 8 and 7 log CFU/sample, respectively. The inoculated apple samples were then placed within a biosafety hood and allowed to dry at room temperature for 3.5 h. Three skin samples were treated with UV and 0.2 mL of 1.2 % v/v hydrogen peroxide (diluted from 30 % v/v hydrogen peroxide with milli-Q water; Sigma-Aldrich, Oakville, Ontario, Canada), three other samples were treated with only UV with a dose of 69 kJ/m² and another three samples were treated with hydrogen peroxide only (1.2 % v/v). Optimal treatment parameters were used to treat another three samples (1.2 % hydrogen peroxide heated to 69 °C and UV dose of 69 kJ/m²).

The treated samples and non-treated controls were placed in 20 mL of saline and vortexed for 10 seconds to release microbes from the apple skin samples. A dilution series was prepared in saline and plated onto agar. *Escherichia coli* O157:H7 was plated onto TSA supplemented with 50 µg/mL nalidixic acid that was subsequently incubated at 37 °C for 24 h. Colonies grown on plates were then swabbed onto CT-SMac agar and incubated for another 24 h at 37 °C to confirm
identification of *E. coli* 0157:H7. *Aspergillus niger* was enumerated on PDA plates incubated at 25 °C for 5 days.

### 2.11 Experimental plan and statistics

For RSM, trials were conducted with varying parameters of UV dose (kJ/m²), temperature (°C) and concentration (% v/v) of hydrogen peroxide. Each trial was conducted in triplicates with three samples per replicate. A total of 9 samples were produced per trial. Six trials were conducted around the central points with 54 samples produced under the same conditions. For the additional testing conditions (UV only, ozone fumigation), trials were performed at least twice. Analysis of variance (ANOVA) was performed on the data using Design Expert® Software 11.1.0. Statistical significance was determined at *P* > 0.05. Calculations of means and standard deviations were performed using Microsoft Excel.

For microbial trials, the log count reduction calculation was performed using Microsoft Excel 2013 and determined by comparing the log transformed CFU/sample of treated and positive control (inoculated but not treated) samples. Statistical significance between log CFUs or log count reductions were determined using a Welch’s t-test in Microsoft Excel 2013 at *P* > 0.05.

### 3. Results and Discussion

#### 3.1 Optimization of Recovery Method and HPLC Analysis

The method of recovering CPY from apples skins involved several steps in order to achieve a maximal recovery of ~89%. One step in the recovery method was the use of a sonicator which utilizes acoustic cavitation to generate bubbles which help in extracting deposited CPY from the apple skin thus facilitating higher recoveries of CPY. Without sonication, recovery rates of CPY were less than 80% but when sonication was utilized, recovery rates were consistently greater.
than 85%. Other studies have shown that ultrasonic solvent extraction (USE) is effective at improving recoveries of pesticides from produce (Pan, Xia, & Liang, 2008; Ramos, Rial-Otero, Ramos, & Capelo, 2008). Pan et al. (2008) showed that recoveries of the pesticides monocrotophos, dimethoate, carbendazim, carbaryl, imidacloprid and simazine could be optimized to over 90% when sonicated for 35 minutes at 28 kHz. In comparison to this result, they demonstrated that recovery rates were lower with decreased sonication time, for example, imidacloprid sonicated for 25 minutes at 28 kHz had a recovery of 71% (Pan et al., 2008). They also observed that increasing the volume of solvent was positively correlated with pesticide recovery with 40 mL per gram of sample proving optimal. For example, when comparing different volumes of ethyl acetate at 25 minutes of sonication with 5 g of pesticide-spiked spinach sample, a recovery of 40% of monocrotophos was observed using 10 mL of solvent whereas a recovery of 90% could be seen when using 40 mL (Pan et al., 2008). These factors were optimized in the current study and it was observed that a sonication time of 20 minutes and 20 mL of solvent (ACN) would support a sufficient and consistent recovery. Higher sonication times and solvent volume did not increase recovery rates above 85%.

Apple skin constituents had a negligible effect on the HPLC analysis of CPY with no major interfering peaks. One unknown peak derived from apple skins eluted around 3.9 min while CPY had an elution time of 2.3 min when using a 1 mL/min flow rate. The CPY peak had a tailing effect after elution resulting in it partially co-eluting with the peak associated with constituents of the apple skin. Water and hydrogen peroxide also initially reduced peak resolution with an elution time around 1.8 min. In order to separate these peaks, the HPLC column was changed from an initial 5 micron pore size stationary phase to 3.5 micron size. The mobile phase concentration ratios were also changed from 90:10 ACN/water with 0.1% TFA to 85:15. This
allowed for better resolution between peaks with no overlap due to an increase in CPY elution time to 4.6 min. This longer elution time also removed tailing due to eluting after the unknown apple skin peak. Because resolution was well established between peaks, there was no need for a clean-up procedure of extracts which is commonly employed when working with produce (Harshit, Charmy, & Nrupesh, 2017).

3.2 Response Surface Methodology

The average recovery (n = 177) of CPY from apple peels by using the applied methodology from Section 2.4 was 89.4% ± 3.8%. In comparison, Osmann et al. (2012) had recoveries of CPY between 87 – 92% from date fruits (n = 10) using GC-MS. Their method of extraction however used acetone as the extraction solvent rather than acetonitrile (Osman et al., 2014). Whangchai et al. (2011) also employed GC rather than HPLC and used acetone as the extraction solvent for removing CPY from lychee fruit but did not include % recovery in their study. Rawn et al. (2008) extracted captan, a fungicide, from apples and had an average recovery (n = 31) of 81% using GC-MS as well. Their recovery method may be lower due to involving a clean-up step which subjected the samples to gel permeation chromatography and then a Florisil column (Rawn et al., 2008).

The mean recovery was used as a recovery correction factor for AOP treated samples. The percent degradation of CPY across the various AOP treatments ranged from 28 % to 92 % (Table 11). The extent of CPY degradation was analyzed using Design Expert® 11.1.0 software and was determined to fit a linear model (Table 13). ANOVA analysis showed the linear terms X₁ (UV dose) and X₂ (H₂O₂ temperature) were significant (P < 0.05) in CPY degradation whereas X₃ (H₂O₂ concentration) was not significant (Table 13).
The RSM approach demonstrated that AOP could degrade CPY via a process that was primarily dependent on the UV-C dose and temperature of hydrogen peroxide. It has been reported that CPY in aqueous solution is relatively stable to UV-C light with the addition of hydrogen peroxide or iron (Fe$^{3+}$) required to support degradation of the pesticide (Gandhi, Lari, Tripathi, & Kanade, 2016). Further studies have confirmed the finding that UV-C degradation enhances the degradation of CPY when applied as part of an advanced oxidation process (Gandhi et al., 2016; Savic et al., 2019; Thind, Kumari, & John, 2018; Utzig et al., 2019). Thind et al. (2018) reported that hydrogen peroxide within the range of 0.5 – 1.5 % v/v supported a 42 % decrease in CPY levels but could be increased to 53 % reduction when used in combination with UV-C. A further increase in CPY degradation up to 74 % was obtained by using a combination of hydrogen peroxide, titanium dioxide and UV-C therefore suggesting a synergistic effect. Oliveria et al. (2014) also showed that low concentrations of hydrogen peroxide between concentrations of 0.025 % to 0.2 % had an effect on the degradation of CPY ranging from 49.2% to 73.9% reduction. In contrast to Oliveria et al. (2014), this study used concentrations ranging from 1.0 – 6.0%. Another study concluded that 0.16 mg of chlorpyrifos was degraded per mg of H$_2$O$_2$ consumed and this relationship was observed up to a maximum concentration 0.09% H$_2$O$_2$ (Femia et al., 2013). Utzig et al (2019) reported the degradation of CPY in acetonitrile with UV supporting complete degradation of 200 µg/L within 120 min treatment and the process was accelerated to 30 minutes with the inclusion of 12 mg/L hydrogen peroxide. The authors determined that at a UV-C radiation intensity of 0.75 mW/cm$^2$ caused the half- life of CPY utilizing UV-C radiation alone would be 12.9 min whereas when combined with hydrogen peroxide, the half-life decreased to 5.8 min.
Previous reports on the AOP mediated degradation of CPY have mainly focused on aqueous or ethanolic solutions. In the current study, where the AOP process was performed on the surface of apples, it was evident that hydrogen peroxide had a minor role on the overall degradation process compared to when applied in an aqueous phase. The presence of increasing levels of hydrogen peroxide in the AOP reduced the degree of CPY degradation by UV-C light. It has been stated that higher concentrations of hydrogen peroxide may form water and a hydroperoxyl radical (HO$_2$) which is capable of interfering with chlorpyrifos degradation (Oliveira et al., 2014).

Another issue with using hydrogen peroxide and UV light is that hydrogen peroxide does not absorb 254 nm light well due to its low molecular extinction coefficient of 19 L/mol·cm (Figure 10) (Gottschalk et al., 2010) so OH radical production is low which will require longer UV exposure times and a surplus of hydrogen peroxide (Gottschalk et al., 2010).

There could be several reasons underlying the differences observed in the degradation of CPY on the surface of apples compared to in aqueous solution. For example, it is known that the concentration of hydrogen peroxide has to be balanced with generating sufficient free-radicals to react with CPY and an excess peroxide concentration can impede CPY degradation (Oliveira et al., 2014). In the case of aqueous solutions, diffusion of hydrogen peroxide and generation of hydroxyl radicals can prevent the reaction from being impeded. It is also conceivable that in air the UV-C transmission to the surface of apples would be greater than in water, especially if absorbing contaminants are present. Therefore, in aqueous solutions the hydrogen peroxide would constitute the primary element of the AOP given the absorption of UV-C by the water constituents. By directly illuminating the surface of apples, the generation of radicals can occur but CPY degradation would be primarily due to UV-C photon exposure rather than hydrogen peroxide. Additional possibilities are the aqueous phase forms a relative constant matrix to
support the reaction of radicals with CPY. For example, high alkaline pH and presence of Fe can promote the CPY degradation but which could be less controlled on the apple surface (Behin & Farhadian, 2017). Regardless of this fact, the results indicate that AOP studies performed in the aqueous phase do not always translate to treating whole fruit in air.

In the current study the degradation of CPY was positively correlated to temperature which is in agreement with others (Cengiz, Catal, Erler, & Bilgin, 2015; Oliveira et al., 2014). A study conducted by Oliveria et al. (2014) investigating the degradation of CPY in an aqueous system using AOP also showed that an increase in temperature slightly influenced CPY degradation. They suggested that higher temperatures used in combination with UV radiation supported higher degradation compared to elevated temperatures used in combination with AOP involving UV exposure for 8 hours (no dose or intensity given) and hydrogen peroxide (0.1%) (Oliveira et al., 2014). The same result was observed in this study, with AOP treatments producing a lower reduction than UV used alone at the same dose. This may be due to hydrogen peroxide scavenging UV-C radiation, which may be more significant in CPY degradation, to generate minimal amounts of hydroxyl radicals (Gottschalk et al., 2010). In a different study conducted by Cengiz et al. (2015), it was observed that the reduction of chlorpyrifos-ethyl could be as high as 0.56 µg/kg when treated for 60 minutes at 90°C whereas a lower heat treatment of 60°C at the same time produced a reduction of 0.37 µg/kg (Cengiz et al., 2015). The mechanism of thermal decomposition of CPY involves a series of stepwise elimination reactions that cleaves ethylene residues to leave 3,5,6-trichloro-2-pyridinol as the main degradation product (Kennedy & Mackie, 2018). Although greater temperatures have been shown to increase degradation of CPY, the temperature sensitivity of fruits would make higher temperatures non relevant in the context of commercial food processing.
Table 11. RSM trials with amount of CPY reduction (µg) observed using various test parameters of UV-C dose (kJ/m²), temperature (°C) and concentration (%) of hydrogen peroxide. Reduction from each trial was corrected using the average positive control recovery of 89.4% (n=177).

<table>
<thead>
<tr>
<th>Trial</th>
<th>UV dose (kJ/m²) ($X_1$)</th>
<th>Temperature of H₂O₂ (°C) ($X_2$)</th>
<th>H₂O₂ (%) ($X_3$)</th>
<th>CPY Reduction (µg) ($Y$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34.8</td>
<td>46.5</td>
<td>3.5</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>22.5</td>
<td>32.5</td>
<td>5.0</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>54.0</td>
<td>32.5</td>
<td>5.0</td>
<td>52</td>
</tr>
<tr>
<td>4</td>
<td>37.0</td>
<td>46.5</td>
<td>1.0</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>22.4</td>
<td>60.5</td>
<td>5.0</td>
<td>54</td>
</tr>
<tr>
<td>6</td>
<td>38.3</td>
<td>70.0</td>
<td>3.5</td>
<td>58</td>
</tr>
<tr>
<td>7</td>
<td>39.4</td>
<td>46.5</td>
<td>3.5</td>
<td>52</td>
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<td>8</td>
<td>39.4</td>
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<td>54</td>
</tr>
<tr>
<td>9</td>
<td>11.7</td>
<td>46.5</td>
<td>3.5</td>
<td>28</td>
</tr>
<tr>
<td>10</td>
<td>38.9</td>
<td>46.5</td>
<td>3.5</td>
<td>56</td>
</tr>
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<tr>
<td>13</td>
<td>37.9</td>
<td>46.5</td>
<td>6.0</td>
<td>54</td>
</tr>
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<td>14</td>
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<td>32.5</td>
<td>2.0</td>
<td>30</td>
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<tr>
<td>15</td>
<td>39.3</td>
<td>46.5</td>
<td>3.5</td>
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<td>16</td>
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<td>3.5</td>
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<td>18</td>
<td>38.5</td>
<td>23.0</td>
<td>3.5</td>
<td>58</td>
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<td>19</td>
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<td>60.5</td>
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<tr>
<td>Max</td>
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<td>70.0</td>
<td>6.0</td>
<td>92</td>
</tr>
<tr>
<td>Min</td>
<td>11.7</td>
<td>23.0</td>
<td>1.0</td>
<td>28</td>
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</tbody>
</table>
Table 12: Six center point replicate trials determining the replicability and precision of the experimental method. Arranged from lowest to highest reduction corrected with the average (n=177) positive control recovery of 89.4%. The standard deviation between reductions was 3.6 µg.

<table>
<thead>
<tr>
<th>Trial #</th>
<th>Temperature of H₂O₂ (°C)</th>
<th>Actual Temperature (°C)</th>
<th>Percent H₂O₂ (%)</th>
<th>UV-C Dose (kJ/m²)</th>
<th>Correction using Average Pos Control (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46.5</td>
<td>45</td>
<td>3.5</td>
<td>34.8</td>
<td>50</td>
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<tr>
<td>15</td>
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<tr>
<td>8</td>
<td>46.5</td>
<td>46</td>
<td>3.5</td>
<td>39.4</td>
<td>54</td>
</tr>
<tr>
<td>7</td>
<td>46.5</td>
<td>47</td>
<td>3.5</td>
<td>39.4</td>
<td>52</td>
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<tr>
<td>10</td>
<td>46.5</td>
<td>45</td>
<td>3.5</td>
<td>38.9</td>
<td>56</td>
</tr>
<tr>
<td>16</td>
<td>46.5</td>
<td>47</td>
<td>3.5</td>
<td>37.2</td>
<td>62</td>
</tr>
</tbody>
</table>

Table 13. ANOVA analysis table of the linear model indicating that values lower than P < 0.05 are significant. In this case, UV-C dose and H₂O₂ temperature are significant whereas H₂O₂ concentration was not significant. * indicates P < 0.05.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df*</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>7.70</td>
<td>3</td>
<td>2.57</td>
<td>23.72</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>A-UV Dose</td>
<td>6.48</td>
<td>1</td>
<td>6.48</td>
<td>59.93</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>B-H₂O₂ Temperature</td>
<td>0.8333</td>
<td>1</td>
<td>0.8333</td>
<td>7.70</td>
<td>0.0135*</td>
</tr>
<tr>
<td>C-H₂O₂ Concentration</td>
<td>0.3080</td>
<td>1</td>
<td>0.3080</td>
<td>2.85</td>
<td>0.1109</td>
</tr>
<tr>
<td>Residual</td>
<td>1.73</td>
<td>16</td>
<td>0.1082</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>1.73</td>
<td>15</td>
<td>0.1150</td>
<td>23.01</td>
<td>0.1623</td>
</tr>
<tr>
<td>Pure Error</td>
<td>0.0050</td>
<td>1</td>
<td>0.0050</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*df is the degrees of freedom.
Surface response plots generated by Design Expert® software illustrates the effects of UV-C dose, hydrogen peroxide concentration and temperature in degrading CPY (Figure 16). A synergistic effect between hydrogen peroxide temperature and UV dose on the degradation of CPY was observed (Figure 16A). In contrast, there was no significant (P > 0.05) interaction between hydrogen peroxide concentration and the applied UV-C dose (Figure 16B). In a similar manner, there was a relatively negligible interaction between the concentration and temperature of hydrogen peroxide on CPY degradation (Figure 16C).
**B**

Factor Coding: Actual

Reduction (ug)
- • Design points above predicted value
- ○ Design points below predicted value

X1 = A: UV Dose
X2 = C: H$_2$O$_2$ Conc

Actual Factor
B: H$_2$O$_2$ Temp = 46.5

**C**

Factor Coding: Actual

Reduction (ug)

X1 = B: H$_2$O$_2$ Temp
X2 = C: H$_2$O$_2$ Conc

Actual Factor
A: UV Dose = 43
Figure 16. 3D surface graph of different interaction effects of an advanced oxidation process for degrading CPY on apples. CPY was introduced onto apple peel and treated with various combinations of hydrogen peroxide and UV dose with the decrease in pesticide levels being recorded. The plots illustrate UV dose and H$_2$O$_2$ temperature (A), UV dose and H$_2$O$_2$ concentration (B) & H$_2$O$_2$ concentration and H$_2$O$_2$ temperature (C).

From the interaction between the three parameters it was possible to generate a predictive equation (Eqn. 6) used to describe the observed reduction of CPY. Reduction of CPY from the six center points ranged from 50 – 62 µg (25 – 31% of initial concentration) with a standard deviation of 3.6 µg, demonstrating reproducibility of the predictive model (Table 12). This shows that the data is replicable with minimal variation between samples. In addition, through verification, a 94 µg (47%) degradation of CPY was achieved using an optimal AOP treatment of 68.4 kJ/m$^2$ UV dose and 1.22% v/v H$_2$O$_2$ at 66°C. This reduction agreed with a predictive value of 92 µg suggested by Design Expert software (Eqn. 6) and was within a ± 5% standard deviation (87 - 97 µg). It can also be seen from the predicted vs actual degradation graph that the model is capable of accurately predicting CPY degradation following specified parameters (Figure 17).

$$Y = 5.742 + 1.071 X_1 + 0.390 X_2 - 2.241 X_3 @ 36 \frac{J}{s \cdot m^2}$$

(Equation 6)
### Ultraviolet light mediated degradation of CPY

The average positive control samples recovery was $90.5 \pm 1.8\%$ ($n = 42$) and was used as the correction factor in subsequent trials. The UV-C mediated degradation of CPY followed linear kinetics with ($r^2 = 0.982$) with 66 kJ/m$^2$ dose supported a 140 µg (70%) reduction of CPY (Figure 18).

When the distance between the sample and the UV light was altered from 16 cm to 10 cm, the average UV intensities changed from 36 J/s·m$^2$ to 43 J/s·m$^2$, respectively. At this higher intensity (10 cm, 43 J/s·m$^2$), the average ($n=17$) positive control recovery of $87.7\% \pm 5.0\%$ was used to correct reduction from treatments. Treatments at higher and lower UV intensities were compared.
with UV exposure times remaining the same which resulted in different UV doses. UV exposure times of 25 minutes were compared. An average (n=15) positive control recovery of 87.8% ± 5.2% CPY was used for the reduction correction. After 25 minutes of UV exposure, the lower UV intensity treatment (16 cm between UV light and sample) was subjected to a total UV dose of 53 kJ/m$^2$ which achieved a CPY reduction of 106 µg (53%). In comparison, the higher UV intensity treatment (10 cm between UV light and sample) produced a total UV dose of 64 kJ/m$^2$. CPY reduction of 141 µg (71%) was observed at this higher UV dose. When comparing the same UV dose of 53 kJ/m$^2$ at different UV exposure treatment times of 20.5 and 25 minutes, no significant difference was observed with both producing CPY reductions of 106 µg (53%). These results showed that by increasing UV intensity and by decreasing the distance between the UV source and sample, treatment times could be shortened.

![Graph of CPY reduction using only UV radiation with a linear trend line (orange line). Reductions were calculated using HPLC analysis. A $R^2$ value of 0.982 was calculated using 5 data points of varying UV doses (kJ/m$^2$) and CPY reduction (µg).](image)
3.4 CPY-O production from AOP exposure

The positive control apple skins (spiked with CPY but not treated) showed concentrations of CPY-O below the limit of detection (0.001 µg). CYP-O was not detected in negative control apple skins (not spiked with CPY) by Q-ToF analysis. Of the 9 treated apple skin samples spiked with CPY, a single sample showed levels of CPY-O below the limit of detection. The other 8 samples showed concentrations ranging from 0.002 – 0.004 µg. These results indicate that CPY-O was generated when treated with AOP technologies (Table 14 and Figure 4). This result agreed with other studies that also observed the generation of CPY-O due to AOP exposure (Bavcon, Franko, & Trebše, 2007; Savić et al., 2019; Utzig et al., 2019). CPY, like other OP pesticides, contains a P=S group which has been known to be converted to its oxon derivative group (P=O) by metabolic oxidation (Savić et al., 2019). Bavcon et al. (2007) demonstrated using GC-MS that CPY-O was the main degradation product of CPY from photoexcitation via xenon lamp emitting below 400 nm. They observed, after 60 minutes of exposure, CPY-O generation did not exceed 1% of the initial CPY concentration (Bavcon et al., 2007). Savic et al. (2019) concluded that UV-C irradiation of CPY resulted in the generation of CPY-O with maximum generation of >1% of the initial CPY concentration after 80 minutes of exposure. Utzig et al. (2019) reported that although CPY degradation could be observed when using UV and hydrogen peroxide, the generation of CPY-O was also observed when the AOP was applied, but not when UV-C was applied alone. These studies however did not state the UV dose but rather included UV exposure time instead.
Table 14. Q-ToF analysis of CPY-O on apple skins spiked with CPY. It can be observed that apple skin samples treated with AOP produced the by-product CPY-O while samples spiked with CPY but not treated with AOP had concentrations below the limit of detection.

<table>
<thead>
<tr>
<th>Positive Control (µg)</th>
<th>Treated Sample (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.000</td>
<td>0.002</td>
</tr>
<tr>
<td>&lt; 0.000</td>
<td>0.004</td>
</tr>
<tr>
<td>&lt; 0.000</td>
<td>0.004</td>
</tr>
<tr>
<td>&lt; 0.000</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>&lt; 0.000</td>
<td>0.004</td>
</tr>
<tr>
<td>&lt; 0.000</td>
<td>0.004</td>
</tr>
<tr>
<td>&lt; 0.000</td>
<td>0.002</td>
</tr>
<tr>
<td>&lt; 0.000</td>
<td>0.004</td>
</tr>
<tr>
<td>&lt; 0.000</td>
<td>0.004</td>
</tr>
</tbody>
</table>

3.5 Ozone Treatment

In the first replicate, the average % recovery for positive controls (spiked, untreated) apple skins was 66% (n=8), and the treated samples had a recovery of 65% (n=9). HPLC analysis showed that ozone gas treatment for 30 minutes (~ 1 g of gaseous ozone) had no effect on CPY degradation. The concentration of ozone was above the linear dynamic range of the ozone monitor of 100 mg/L. This experiment was repeated but with a higher relative humidity (>70% RH) and the % recovery for positive controls was 82% (n=6) compared to treated samples with 85% (n=6). Recovery may have been lower in the first experiment due to sample preparation and storage errors. Another repeat of this experiment was conducted and showed an 82% (n=5) recovery of positive controls and 85% (n=6) for treated samples. These results showed that the average recoveries for both positive controls and treated samples were not significantly different.
when compared using Welch’s t test ($P <0.05$). This suggests that the ozone treatment did not have a measureable effect on CPY on the surface of apple skins.

In other studies, gaseous ozone was seen to be successful at degrading pesticides. Whangchai et al. (2011) concluded that gaseous ozone fumigation for 1 hour at 240 mg/L could degrade 45% (4.5 mg/L) of CPY on lychee fruit. This result however was not supported in the current study. One possible reason for this may be due to the difference in the concentration of ozone generated and exposure time. Karaca et al (2012) demonstrated that 3 out of 5 tested fungicides stored in ozone enriched air degraded residues faster than storage in ambient air conditions (2°C and 95% RH). They observed reductions of 34.7%, 51.6%, and 64.5% in cyprodinil, pyrimethanil, and fenhexamid respectively (Karaca, Walse, & Smilanick, 2012). Research conducted on using ozone in reducing pesticides dissolved in water has been shown to be effective (Ikehata & Gamal El-Din, 2005; Swami et al., 2016; Wu, Luan, Lan, Lo, & Chan, 2007). Swami et al (2016) demonstrated that CPY degradation of 95% (0.06 µg/g) could be achieved when aqueous samples were treated for 30 minutes using a 200 mg/h ozone generator Wu et al. (2006) observed different reductions of the insecticide cypermethrin on vegetables (Brassica rapa) such as bok choy treated with differing concentrations of ozonated water and exposure times. When treated for 15 minutes with tap water or ozonated water at 1.4 mg/L, and 2.0 mg/L, reductions of 26%, 33%, and 54% was observed, respectively (Wu et al., 2007). After 30 minutes of exposure, reductions of 31%, 54%, and 61% were observed (Wu et al., 2007). They concluded that higher concentrations and longer exposure times increased removal of cypermethrin on vegetables. They also saw the same results when using other pesticides including methyl-parathion, parathion, and diazinon.
3.6 Microbial Inactivation

The initial loading of *E. coli* O157:H7 and *A. niger* were determined to be 8.59 ± 0.52 and 6.98 ± 0.35 log CFU/sample, respectively (Figure 19). The maximal CPY degradation reduction treatment with a UV-C dose of 69 kJ/m² and 1.22% v/v H₂O₂ at 66°C supported a >7 log CFU reduction of *E. coli* O157:H7 and a >4.68 log CFU/sample reduction of *A. niger* (Table 15). These reductions were determined to be statistically significant when assessed using Welch’s t-test. When determining individual factors, 1.22% v/v hydrogen peroxide alone applied at 66°C for 29 minutes (approximate equivalent amount of time for UV-C dose to reach 69 kJ/m²) also supported >8 log CFU reduction of *E. coli* but only supported a 1.65 log CFU/sample reduction of *A. niger* spores (Table 15). According to Welch’s t-test, both of these reductions using heated hydrogen peroxide were determined to be statistically significant (*P* < 0.05). It was suspected that the use of heat alone could significantly reduce *E. coli* O157:H7, perhaps more than the hydrogen peroxide itself. To test this theory, triplicate trials were conducted with sterile, room temperature or heated (~66°C) milli-Q water. The initial load of *E. coli* O157:H7 was 5.92 ± 0.09 log CFU/sample. After treatments, the average log CFU/sample for the room temperature and heated water treatments were 5.86 ± 0.06 and 5.81 ± 0.06 for room temperature and heat treatment, respectively, and they were not significantly different from the initial load (*P* > 0.05). Consequently, it can be assumed that heat alone played a minor role in *E. coli* O157:H7 reduction. This could be due to *E. coli* having the ability to survive in temperatures over 66°C for an extended period of time (Droffner & Brinton, 1995; R. Singh, Kim, Shepherd, Luo, & Jiang, 2011). Droffner and Brinton (1995) showed that *E. coli* could survive in temperatures between 60 – 70 °C for 9 days under dry conditions. Singh et al. (2011) showed that *E. coli* O157:H7 could survive in compost at 50% moisture for 1 day at 60 °C. In this study, heated solution was applied
onto room temperature apple skins which would in theory, begin cooling down the heated solution when it comes in contact with surface of the apple. This may explain why a heated solution did not be significantly reduce *E. coli* O157:H7. In comparison, hydrogen peroxide treatments were re-conducted but with both room temperature and heated (~66°C) solutions and an initial load of 5.84 ± 0.14 log CFU/sample of *E. coli* O157:H7. The resultant load of *E. coli* O157:H7 was < 2.3 log CFU/sample for both room temperature and heated treatments. This result showed that hydrogen peroxide could exert a germicidal effect on *E. coli* O157:H7 without the addition of heat. A UV-C dose of 69 kJ/m² alone was also determined to be statistically significant, resulting in a >6.56 log CFU/sample decrease in *E. coli* O157:H7. In comparison, *A. niger* spores with UV-C alone resulted in a >4.68 log CFU/sample reduction.

A UV-C process alone could be used to degrade CPY but for inactivation of *E. coli* O157:H7 there was a significant contribution from hydrogen peroxide exposure. Although hydrogen peroxide does not have as large of a contribution to *A. niger* reduction relative to *E. coli* O157:H7, it is still considered to be statistically significant.

The results are in agreement with other reports on the inactivation of microbes on fresh produce and egg shell surfaces by AOP treatment (Hadjok, Mittal, & Warriner, 2008; K. Murray, Moyer, Wu, Goyette, & Warriner, 2018; Rehkopf, Byrd, Coufal, & Duong, 2017; Xie, Hajdok, Mittal, & Warriner, 2008). It is thought that the free radicals generated on the surface of the sample can access microbes shielded from UV-C by surface structures. It is possible that the proportion of free radicals required to inactive microbes is less than that needed for CPY oxidation. The results suggest that hydrogen peroxide makes a greater contribution to antimicrobial action of AOP, while photo-oxidation by UV-C makes a greater contribution to the degradation of CPY, and it can be enhanced by temperature. In this respect, the optimum AOP treatment parameters
identified in this study can collectively act to reduce microbial and chemical hazards in fresh produce.

Figure 19. Log counts of Escherichia coli O157:H7 and Aspergillus niger spores. *<2.3 CFU/mL is the limit of enumeration. All treatments are determined to be statistically significant for both organisms.

Table 15. Log count reduction of Escherichia coli O157:H7 and Aspergillus niger spores inoculated onto apple peel sections then treated with UV-C (at 254 nm), hydrogen peroxide (1.22% v/v at 66°C) or a combination of UV-C and hydrogen peroxide.

<table>
<thead>
<tr>
<th>Microbe</th>
<th>Treatment</th>
<th>Log CFU/Sample</th>
<th>Initial Loading</th>
<th>Post-Treatment</th>
<th>LCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli O157:H7</td>
<td>Control (Non-treated)</td>
<td>8.59±0.52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H₂O₂ (1.22% v/v)</td>
<td>&lt;2.30*</td>
<td>&gt;6.56a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UV-C (69 kJ/m²)</td>
<td>2.69±0.41</td>
<td>5.89±0.41b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Treatment</th>
<th>Microbe</th>
<th>Mean ± SD</th>
<th>UV-C (69 kJ/m²)</th>
<th>H₂O₂ (1.22% v/v)</th>
<th>UV-C + H₂O₂ (69 kJ/m² and 1.22% v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Non-treated)</td>
<td>Aspergillus niger</td>
<td>6.98±0.35</td>
<td>&lt;2.30*</td>
<td>5.32±0.40</td>
<td>1.65±0.40a</td>
</tr>
<tr>
<td>UV-C (69 kJ/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;2.30*</td>
</tr>
<tr>
<td>UV-C + H₂O₂ (69 kJ/m² and 1.22% v/v)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;2.30*</td>
</tr>
</tbody>
</table>

Mean values for each of the test microbes, followed by the same letter are not significantly (P>0.05) different.

*Limit of enumeration = 2.30 log CFU/Sample

### 4. Conclusions

This study found that the use of AOPs involving UV radiation at 254 nm and hydrogen peroxide were successful in degrading the pesticide chlorpyrifos (CPY) on the surface of apple skins. An HPLC analysis method and recovery method for determining residual CPY levels on apples was shown to be effective and accurate with an average positive control recovery of 89.4% ± 3.8% (n=177). Through multiple ozone fumigation trials, it was shown that ozone gas was incapable of degrading CPY on apple skins after 30 minutes of exposure in an enclosed space. A set of experiments designed using RSM showed that hydrogen peroxide concentration is an insignificant factor in degrading CPY while UV radiation and hydrogen peroxide temperature were significant factors. When using the optimized treatment parameters of 68.4 kJ/m² UV dose and 1.22% v/v H₂O₂ at 66°C, an average degradation of 94 µg (47% of the initial concentration) (n = 9) was observed. However, it was shown that UV radiation on its own was more effective than when used in combination with hydrogen peroxide; a UV dose of 64 kJ/m² alone produced
an average CPY degradation of 141 µg (n = 9). Although degradation of CPY was observed, Q-ToF analysis showed that CPY degradation resulted in the production of chlorpyrifos-oxon (CPY-O), the active and more toxic form of CPY. Treatment of 10 µg/mL CPY with optimized degradation parameters resulted in the production of 0.004 µg (0.02 µg/mL) CPY-O. The spike amount used in this study was 1000 times the MRL for CPY on apples in Canada of 0.01 µg/mL which suggests that the production of CPY-O from treated apples with CPY concentrations near the MRL would be minimal and not pose a significant risk to human health. Decreasing the contact distance between the UV lamp source and apple skin surface resulted in the UV dose being increased due to an increased UV intensity, thus reducing the treatment time required for the same effect. AOP treatment was also successful in reducing microbial colonies of both the pathogen *E.coli* O157:H7 and *A. niger*. Despite being insignificant in degrading CPY, the addition of hydrogen peroxide is useful for treating microbial contamination, and was significant in reducing *E.coli* O157:H7 to below the limit of enumeration (2.30 log CFU/sample).

**Future work**

This study focused only on the treatment of apples, which have a relatively smooth surface compared to most other fruits and vegetables. Different types of produce should be tested with the AOP to determine its effectiveness in reducing CPY and microbial contaminants. Other types of insecticides besides CPY should be tested as well including: carbamates, organochlorides, phenylpyrazole, pyrethroids, neonicotinoids, and ryanoids. Other types of pesticides can also be tested such as fungicides, bactericides, and especially herbicides, which are the most widely used type of pesticide in the world. Modern day pesticide analysis involves testing for multiple pesticide residues at the same time. A known concentration pesticide solution containing multiple pesticides should be spiked onto apple skin samples and analyzed using triple-quad LC-
MS-MS in order to determine the degradation of multiple pesticides at the same time rather than using HPLC to determine the degradation of a single pesticide as performed in the current study. Other future work of interest may be to use AOP to treat lower CPY spike concentrations, down to 0.01 µg/mL (maximal residue limit on apples in Canada). It may also be worth investigating how to obtain a higher UV dose in order to decrease treatment time, which will impact the rate of food production.

Different microbial organisms can also be tested on apples or different types of produce including: *salmonella* and if possible, viruses. UV-C radiation has been known to inactivate viruses as well as bacteria and molds (Tseng & Li, 2007; Watanabe et al., 2010).
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