Comparing the in vitro human hepatic metabolism of α-cypermethrin (αCM) and λ-cyhalothrin (λ-CH), and the impact of co-exposure to an organophosphate pesticide on biotransformation

By
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Dedication

I dedicate this thesis to my mother and daughters Dima and Jana who gave me their unconditional support during my journey and made this possible. I also want to dedicate this work to my son Hashem who passed away five years ago; you will always be in my heart whenever you are. I want to thank my friends and family for being part of my life and supporting me in the last few years and giving me faith in myself to face all kind of challenges and difficulties during the past few years.

“In the middle of difficulty lies opportunity." Albert Einstein
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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>3PBA</td>
<td>3-Phenoxybenzoic Acid</td>
</tr>
<tr>
<td>3PB Alcohol</td>
<td>3-Phenoxy benzyl Alcohol</td>
</tr>
<tr>
<td>AChE</td>
<td>Acetylcholinesterase</td>
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<tr>
<td>αCM</td>
<td>Alpha cypermethrin</td>
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<tr>
<td>BChE</td>
<td>Butyrylcholinesterase</td>
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<tr>
<td>CE</td>
<td>Carboxylesterase</td>
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<tr>
<td>cis-DCCA</td>
<td>cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid</td>
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<tr>
<td>CPF</td>
<td>Chlorpyrifos</td>
</tr>
<tr>
<td>CPF-O</td>
<td>Chlorpyrifos-oxon</td>
</tr>
<tr>
<td>CYP</td>
<td>Cytochrome P450</td>
</tr>
<tr>
<td>EPA</td>
<td>Environmental Protection Agency</td>
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<tr>
<td>HLMs</td>
<td>Human liver microsomes</td>
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<tr>
<td>HPLC</td>
<td>High pressure liquid chromatography</td>
</tr>
<tr>
<td>iso-OMPA</td>
<td>Tetraisopropyl pyrophosphoramide</td>
</tr>
<tr>
<td>λCH</td>
<td>Lambda cyhalothrin</td>
</tr>
<tr>
<td>LC Acid</td>
<td>Lambda Cyhalothric Acid</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide adenine dinucleotide phosphate-oxidase</td>
</tr>
<tr>
<td>OP</td>
<td>Organophosphate pesticide</td>
</tr>
<tr>
<td>TCPy</td>
<td>3,4,5- trichloropyridinol</td>
</tr>
</tbody>
</table>
# Table of Contents

Title Page i
Dedication ii
Acknowledgements iii
Abbreviations iv
Table of Contents v
Abstract vii

## Introduction

- **Figure 1.1** Representative pictures illustrating different methods of pesticide application in industrial, agricultural, household, and aquatic setting. 2
- **Figure 1.2** Structure difference between Type I and Type II pyrethroids. 4
- **Figure 1.3** Metabolic pathway of alpha-cypermethrin in humans 5
- **Figure 1.4** Estimated usage of Alpha-cypermethrin in the U.S by years and crop 6
- **Figure 1.5** Metabolic pathway of lambda-cyhalothrin 7
- **Figure 1.6** Estimated usage of lambda-cyhalothrin in the U.S. by year and crop 8
- **Figure 1.7** Metabolic scheme for organophosphate insecticide chlorpyrifos 10
- **Figure 1.8** Estimated usage of chlorpyrifos in U.S. by year and crop 10
- **Figure 1.9** Michaels-Menten model for calculating enzyme kinetic 12
- **Figure 1.10** Illustrate the type of inhibition mechanism 13

## Aims of Thesis

13

## Methods

- Method validation for analysis of α-CM and λ-CH metabolites 14
- In vitro metabolism assay for α-CM and λ-CH 15
- High-performance liquid chromatography analysis 15
- The impact of NADPH on the metabolism of lambda-cyhalothrin and alpha-cypermethrin 16
- Interactive effects of iso-OMPA, a cholinesterase inhibitor, on the metabolism of lambda-cyhalothrin and alpha-cypermethrin 16
- Interactive effects of OPs (Chlorpyrifos-Oxon) on the metabolism of lambda-cyhalothrin and alpha-cypermethrin 17
Results

Figure 2.1 Time-dependent formation of 100 μM αCM and λ-CH metabolites by HLMs. 19
Figure 2.2 Time-dependent formation of 20 μM αCM and λ-CH metabolites by HLMs. 19
Figure 2.3: Biotransformation of lambda-cyhalothrin by pooled human liver microsomes. 20
Figure 2.4: Biotransformation of alpha-cypermethrin by pooled human liver microsomes 21
Table 2.1: Comparison of Vmax and Km of alpha-cypermethrin and lambda-cyhalothrin metabolites. 22
Figure 2.5: Biotransformation of lambda-cyhalothrin by human liver microsomes in the presence and absence of NADPH 23
Figure 2.6: alpha-cypermethrin biotransformation by HLMs in the presence and absence of NADPH 24
Figure 2.7: Metabolism of 20 μM alpha-cypermethrin and lambda-cyhalothrin with iso-OMPA by HLMs. 25
Table 2.2: The rate of formation of metabolites of lambda-cyhalothrin and alpha-cypermethrin (20 μM) by HLMs in the presence and absence of iso-OMPA 26
Figure 2.8: Metabolism of 100 μM alpha-cypermethrin and lambda-cyhalothrin with iso-OMPA by HLMs. 26
Table 2.3: The rate of formation of metabolites of lambda-cyhalothrin and alpha-cypermethrin (100 μM) by HLMs in the presence and absence of iso-OMPA 26
Figure 2.9 Impact of chlorpyrifos oxon (CFP-O) on the biotransformation of α-CM by HLMs. 27
Table 2.4: Kinetic values (Km, Vmax) for the in vitro metabolism of alpha-cypermethrin in presence and absence of CPF-O 28
Figure 2.10 Impact of chlorpyrifos oxon (CFP-O) on the biotransformation of λ-CH by HLMs. 29
Table 2.5: Kinetic values (Km, Vmax) for the in vitro metabolism of lambda-cyhalothrin in the presence and absence of CPF-O 30

Discussion

References
Abstract:

Pyrethroids (PYRs), including lambda-cyhalothrin (λCH) and alpha-cypermethrin (αCM), are insecticides used in both agricultural and residential settings in the United States and other parts of the world. PYR usage has increased during the past decade with the decline of organophosphate (OP) pesticide usage. Although, OPs, such as chlorpyrifos, are still commonly used throughout the world, they are often applied with type II pyrethroid to increase the effectiveness of insect control. Type II pyrethroid such as λCH and αCM are neurotoxic, acting by prolonging the open time of voltage-gated sodium channels. While little data are available on the human metabolism of λCH and αCM, rodent studies suggest that PYRs are metabolized by cytochrome P-450 (CYP) and carboxylesterase (CE) enzymes, resulting in the formation of inactive metabolites that serve as biomarkers of exposure. The aims of this thesis were to address this data gap by comparing the metabolism of αCM and λ-CH by human liver microsomes and the impact of co-exposure to an OP pesticide on pyrethroid biotransformation. Pooled human liver microsomes were used to assess kinetic parameters (Km and Vmax) for λCH and αCM metabolism, through the formation of non-specific metabolites, 3-phenoxybenzyl alcohol (3PB Alcohol), 3-phenoxybenzoic acid (3-PBA), and the specific metabolites, cis-3-(2,2- dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (cis-DCCA) from αCM and lambda cyhalothric acid (LC acid) from λCH. The formation of 3PB alcohol, 3-PBA and LC acid from λCH had Vmax values of 523, 218, and 923 pmol/min/mg protein, and Km values of 54, 16, and 35 μM, respectively. Similarly, the formation of 3PB alcohol, 3-PBA and cis DCCA from αCM had Vmax values of 700, 333, and 1377 pmol/min/mg protein, and Km values of 35, 17, and 23 respectively. Incubation in the absence of NADPH resulted in a marked reduction in the formation of only 3-PBA and 3-PB alcohol, suggesting that their formation was CYP mediated,
while supporting the role of carboxylesterase in mediating the formation of cis-DCCA and lambda cyhalothric acid. Iso-OMPA, a potent esterase inhibitor, was used to assess the impact of carboxylesterase inhibition on the biotransformation reactions. High concentration of iso-OMPA (500 µM) resulted in a reduction in the formation of all metabolites of λCH and αCM, suggesting that carboxylesterase catalyzes the initial step in the biotransformation of λCH and αCM. To assess the impact of co-exposure of αCM and λCH with an OP pesticide, incubations were conducted with chlorpyrifos-Oxon (CPF-O), the active metabolite of chlorpyrifos, which is a potent esterase inhibitor. CPF-O at 100 and 200 nM produced a concentration dependent inhibition in the formation of all metabolites of αCM and λCH. This indicates that combined exposures to PYR and OP insecticides results in a reduced rate of αCM and λCH biotransformation, which can result in slower in vivo clearance and increased toxic potency of the PYR pesticides. These metabolites were also detected in urine samples of Egyptian adolescent agriculture workers that applied λCH and αCM to the cotton fields, thus serving as biomarkers of pesticide exposure. Together, in vivo exposure and in vitro metabolism data can be used in risk assessment for occupational and residential exposures and to assist efforts to assess risk and reduce exposures.

**Introduction:**

The four major classes of insecticides that are currently approved in the United States, including organophosphates, pyrethroids, neonicotinoids, and carbamates. Organochlorine pesticides (OCs) are no longer approved for use in the US due to their toxicity and persistence in the environment and subsequent potential to bio-accumulate in wildlife and humans. The use of organophosphate pesticides (OPs) in U.S. has declined in recent years. due to their greater potential toxic effects on humans and wildlife. While OPs remain the most widely used insecticide
worldwide, pyrethroids are the second most commonly used insecticide in the world and are included in over 3,500 registered products.

While less toxic than OPs, synthetic pyrethroid insecticides are also considered a potential human health concern due to their widespread use in innumerable applications in agriculture, urban and rural communities, and household. Pesticides can be applied in many ways, including handheld equipment by low-pressure hand wand sprayers, backpack sprayers, and hose-end sprayers, as shown in Fig. 1.1. In addition, pesticides can be applied by many different methods including, band, basal, broadcast, crack and crevice, directed-spray, foliar, rope or wiper treatments, and many other methods. For that, proper method should be used while applying pesticides to have maximum effectivity and minimum toxicity.

![Figure 1.1](image_url) Representative pictures illustrating different methods of pesticide application in industrial, agricultural, household, and aquatic settings.
Increased risk for human exposures and the potential for adverse human health effects are related to the increase in worldwide pyrethroid usage during the last decade. Pyrethroid insecticides are classified into two types, type I is characterized by the absence of α-cyano group, while type II have an α-cyano group (Fig 1.2). In addition, other alteration can be added to type II pyrethroids, including alcohol moieties, variation in the category of halogen and hydrophobic groups, and its stereochemical arrangement (Romero, et al. 2015; Wolansky and Harrill 2008). Symptoms associated with high acute exposures to natural pyrethin’s and synthetic type I pyrethroid insecticides include the induction of a whole-body tremor known as (T syndrome). High acute exposures to type II pyrethroids produces sinuous writhing convulsions accompanied by salivation, known as (CS syndrome) (Burr and Ray 2004).

Alpha-cypermethrin (αCM) and lambda-cyhalothrin (λCH) are two common commercially manufactured synthetic type II pyrethroid insecticides, which are very similar in structure to pyrethrin, but are often more toxic to insects, and last longer in the environment than pyrethrin (ATSDR September 2003). Humans can be exposed to pyrethroid insecticides in many ways including ingesting contaminated food, dermal exposure due to pyrethroid skin contact after spaying, and breathing contaminated air, especially after the insecticide has been sprayed (Abass and Pelkonen 2013). Pyrethroid insecticides are quickly broken down by enzymes in the body into non-toxic metabolites (Kaneko 2010), that are mainly excreted in urine and serve as biomarkers of exposure. The phenoxybenzyl group is a common structure in most synthetic pyrethroids. The biotransformation of pyrethroids often includes hydrolysis by carboxylesterase followed by oxidation, giving 3-phenoxybenzoic acid (3PBA), which is considered a metabolite that is common to many pyrethroids and serves as a general, non-specific biomarker of exposure (Chang, et al. 2016).
In previous studies, the main metabolites of pyrethroids have been frequently detected at low levels in urine samples from general population in the U.S., demonstrating widespread low-level exposures of children and adults to one or more pyrethroids (Saillenfait, et al. 2015). In these studies, it was shown that the concentrations of detected urinary metabolites increased as the level of exposure increased (ATSDR September 2003).

**Alpha-cypermethrin** is a pyrethroid insecticide that is similar chemically to pyrethrins in prethrum extract. Cypermethrin is a type II pyrethroid derived from a mixture of 8-stereoisomers (Liu, et al. 2009; Singleton, et al. 2014), having a structure that makes it more effective and last longer in the environment than pyrethrin (NPTN). Alpha-cypermethrin is a racemic mixture of (S)-a-cyano-3-phenoxybenzyl-(1R,3R)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate and (R)-a-cyano-3-phenoxybenzyl-(1S,3S)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate (Singleton, et al. 2014) (WHO 2002), with a very low volatility and water solubility. Cypermethrin is registered for agricultural use (Taju, et al. 2014) as a foliar application on food and feed crops including cotton, pecans, peanuts, soybeans, wheat, and sweet corn. It was first registered through the U.S. EPA in 1989 (NSCEP 1989) for industrial, commercial, and residential usage.
In previous studies, 3-phenoxybenzoic acid (3PBA) was identified as a common metabolite of many pyrethroid insecticides as a result of oxidation of pyrethroid insecticides (Barr, et al. 2010; Singleton, et al. 2014). Cis- and trans- (2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (cis- and trans-DCCA) were identified as specific metabolites of cypermethrin (Barr, et al. 2010) (Fig 1.3). In previous studies in the U.S. general population in 1999 and 2001, five urinary metabolites of pyrethroid insecticides were reported, including 3PBA and cis-DCCA. (Barr, et al. 2010).

![Figure 1.3 Metabolic pathway of alpha-cypermethrin in humans (Singleton et. al., 2015)](image)

Urinary levels of 3PBA and cis-DCCA were used in a previous study to assess occupational and environmental exposures of Egyptian agriculture workers to alpha-cypermethrin (Singleton et al., 2014). However, to our knowledge, limited information is available regarding potential human exposures and health risks in the United States, where the use of alpha-cypermethrin has been declining in more recent years (Fig 1.4). The acceptable daily dose (ADI) and no observable effect level (NOEL) for αCM were evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and reported as 0-0.2 and 1.5 mg/kg body weight/day, respectively. (WHO 2014)
Lambda-cyhalothrin is a type II pyrethroid comprised of equal quantities of (S)-a-cyano-3-phenoxybenzyl (Z)-(1R,3R)-3-(2-chloro3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate and (R)-a-cyano-3-phenoxybenzyl (Z)-(1S,3S)-3-(2chboro-3,3,3-trifluoroprop-1-enyl)-2dimethylcyclopropanecarboxylate (Colombo, et al. 2013; WHO 2003). It was initially registered for use in the U.S. in 1988 (NSCEP 1988). Lambda-cyhalothrin has low water-soluble and lipophilic, allowing it to build up in tissues and produce oxidative damage in biological membranes (El-Demerdash 2012). Lambda-cyhalothrin is highly effective and widely used pesticide in different crops such as cotton and corn (He, et al. 2008). In contrast to alpha cypermethrin, lambda-cyhalothrin use in the U.S. has been increasing in more recent years (Fig 1.6). λ-CH was evaluated by an international expert scientific group administered jointly by the Food and Agriculture Organization of the United Nations and WHO (JMPR), which reported ADI and NOAEL values of 0-0.02 and 2 mg/kg body weight per day, respectively (JMPR 2005).
Lambda-cyhalothrin undergoes metabolism mainly through ester hydrolysis and hydroxylation (figure 1.5) (Schettgen, et al. 2016). As a result, 3PBA which is a common metabolite to pyrethroid insecticides having equivalent alcohol moiety in their structure (Krieger and ScienceDirect 2010) and cis-3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2 dimethylcyclopropanecarboxylic acid (lambda – cyhalothric acid) are the general (non-specific) and specific metabolites of lambda-cyhalothrin, respectively. Therefore, these metabolites are suitable urinary biomarkers of human exposure and can assist with assessing health risks associated with exposure to this pyrethroid insecticide (Khemiri, et al. 2017).
**Pyrethroid mechanism of action:**

Pyrethroids general mechanism of action is through the nervous system of insects, mainly at voltage-gated sodium channel (Burr and Ray 2004; Romero, et al. 2015; Schettgen, et al. 2016). These channels are incorporated in transmembrane proteins, which are essential for exciting cells due to their role in cell membrane depolarization (Zhao, et al. 2014). Therefore, synthetic pyrethroids target voltage-gated sodium channels in insects by locking the open state, leading to the paralysis and death of the insect (Dong 2007; Soderlund 2010). In previous studies, it was also shown that pyrethroids primary target mammalian voltage-gated sodium channels, but other studies report that in addition, it may affect different types of voltage gated ion channels (Romero, et al. 2015; Soderlund 2010). Moderate to high levels of exposure to these pesticides without proper occupational protection may result in some reversible symptoms including cough, respiratory irritation, headache, dizziness, nausea, vomiting, and paresthesia (Chen, et al. 2015; Saillenfait, et al. 2015).
**Chlorpyrifos** (O, O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate) is an organophosphate insecticide, that is one of the most common active ingredients used as an agricultural pesticide around the world (Darwiche, et al. 2017; Farag, et al. 2010; Sanchez-Hernandez and Sandoval 2017). It was originally registered in the United States in 1965 (NPIC April 2010). While chlorpyrifos (CPF) remains the most commonly used OP insecticide, its’ use in the U.S. has declined in recent years (Fig 1.8). Chlorpyrifos is bioactivated by cytochrome P450, replacing the sulfur atom bonded to a phosphorus atom with an oxygen atom, yielding chlorpyrifos-oxon (O, O-diethyl O-3, 5, 6-trichloro-2-pyridyl phosphate, CPF-O) (Crane, et al. 2012; Sanchez-Hernandez and Sandoval 2017) (Fig 1.7). The mechanism of action of CPF- oxon is through inhibition of the acetylcholinesterase enzyme (Catalina Rodríguez, et al. 2013; Sanchez-Nogue, et al. 2013; Zhu, et al. 2015). The main role of acetylcholinesterase is to catalyze the hydrolysis of acetylcholine (Arora, et al. 2017; Farag, et al. 2010), and thus removing this neurotransmitter from the synaptic clef in the central nervous system in humans and insects (Catalina Rodríguez, et al. 2013). Inhibition of this enzyme by CPF-oxon leads to accumulation of this neurotransmitter in the synapse. It has been noticed that acute and chronic exposures may lead to similar outcomes, including impaired memory, disorientation, severe depressions, irritability, confusion, headache, speech difficulties, delayed reaction times, nightmares, sleepwalking and drowsiness or insomnia. Influenza-like condition with headache, nausea, weakness, and loss of appetite are also observed with exposure to OPs and can make the diagnosis of pesticide intoxication in populations more difficult (Colovic, et al. 2013). In addition, OPs, including the active metabolite CPF-oxon, inhibit other β-esterases, including acetylcholine esterase (AchE), butyryl esterase (BuChE), and carboxyl esterase (CaE). In recent
studies, it was indicated that chlorpyrifos exposure may also affect nitric oxide synthase leading to the increase in nitric oxide levels (Darwiche, et al. 2017; Yildirim, et al. 2013).

**Figure 1.7** Metabolic scheme for organophosphate insecticide chlorpyrifos (CPF) (Foxenberg et al, 2011). Chlorpyrifos-oxon is the active metabolite produced by bioactivation of chlorpyrifos through hepatic enzymes (cytochrome P450, CYP2B6), chlorpyrifos-oxon result in inhibition of β-esterase’s, including acetylcholine esterase (AchE), butyryl esterase (BuChE), and carboxyl esterase (CaE).

**Figure 1.8** Estimated usage of chlorpyrifos in the U.S. by year and crop (2015 U.S. Geological Survey)

**Organophosphate and pyrethroid co-exposure and its effect on pyrethroid biotransformation**

Pyrethroids and organophosphates are two of the most commonly applied insecticides for commercial and residential use. Co-exposure to insecticide mixtures are commonly used to increase the efficacy of insecticides, delay the development of insecticide resistance, or
overcome current resistance (Ahmad 2004). Due to the difference in mechanism of action, type II pyrethroids mainly inhibit voltage-gated channels while organophosphate pesticides inhibit AChE and BuChE, in addition to carboxylase enzyme which is important in pyrethroid biotransformation in humans. To detect organophosphate exposure, erythrocyte AChE enzyme activity can be evaluated as a biological biomarker (He, et al. 2002). These insecticides remain a human health concern due to their widespread use and the increasing use of combined and/or serial application of OPs and PYRs. Consequently, studies of these interactions are critically significant in understanding the potential human health effects associated with occupational and environmental exposures due to co-exposure or serial exposure of organophosphates and pyrethroids.

**Pesticide Metabolism and Michaelis-Menten Kinetics**

In vitro studies of pesticide metabolism kinetics by human liver microsomes (HLMs) is a beneficial method to evaluate the relative potential of human liver to detoxify and/or bioactivate pesticides. The main function of enzymes is to improve the reaction rate. In the presence of enzymes and necessary cofactors, the rate of biotransformation increases linearly as substrate concentration increases and then begins to reach a steady, maximum rate at higher substrate concentrations (Pollard, 2003). Kinetic data ($K_m$, $V_{max}$) on pesticide metabolism are utilized to better inform exposure and risk assessment in humans, including the development of physiologically based pharmacokinetic (PBPK) models.
Figure 1.9 Michaelis-Menten is a model for calculating enzyme kinetics. The rate is directly proportional to the substrate concentration at very low substrate concentration, where \( [S] \) is much less than \( K_M \), \( V_0 = \left( \frac{V_{\text{max}}}{K_M} \right) [S] \). At high substrate concentration, when \( [S] \) is much greater than \( K_M \), the rate is maximum \( V_0 = V_{\text{max}} \), independent of substrate concentration.

The Michaelis-Menten equation (fig 1.9) illustrates the rate of an enzymatic reaction by describing the relationship of reaction rate to the concentration of substrate in the reaction.

According to this equation where \( R = \frac{V_{\text{max}} [S]}{K_M + [S]} \), the \( V_{\text{max}} \) on the Y-axis represent the maximum velocity in the reaction, where a large \( V_{\text{max}} \) indicates a higher velocity to convert substrate to product. The Michaelis constant (\( K_M \)) on the x-axis represents the concentration of the substrate at \( \frac{1}{2} V_{\text{max}} \), where enzymes with low \( K_M \) have a high affinity and high efficiency to reach the \( V_{\text{max}} \) at low substrate concentration. Inhibition occurs by reduction of enzyme activity via binding of an inhibitor at a catalytic or regulatory site on the enzyme. Inhibition can be through a covalent bond (irreversible) or non-covalent bond (reversible). Types of inhibition occur through competitive, the substrate and the inhibitor cannot be simultaneously bound to the enzyme. Non-competitive, inhibitor binding site is unaffected with substrate binding. Mixed, inhibitor binding site is affected with substrate binding. Finally, uncompetitive
inhibition, the inhibitor binding site is dependent on substrate binding to the enzyme.

![Graph illustrating inhibition mechanisms](image)

**Figure (1.10):** Illustrate types of inhibition mechanism. (A) Normal Enzyme kinetics (V_max, Km). (B) Competitive inhibition. The apparent value of Km is increased, whereas V_max remains unchanged. (C) Noncompetitive inhibition, the Km remains the same and the V_max decreased. (D) Uncompetitive inhibition, the value of Km increased while V_max decreased.

**Aims of Thesis:**

1) Investigate the in vitro metabolism and kinetics (Km, V_max) of lambda-cyhalothrin (λCH) and alpha-cypermethrin (αCM) by pooled human liver microsomes (HLMs).

2) Assess the impact of NADPH on lambda-cyhalothrin and alpha-cypermethrin metabolism.

3) Assessing the impact of iso-OMPA, a potent esterase inhibitor, on the biotransformation of lambda-cyhalothrin and alpha-cypermethrin.

4) Assess the impact of co-exposure to chlorpyrifos-oxon, active metabolite of the OP pesticide, chlorpyrifos, on the in vitro metabolism of lambda cyhalothrin and alpha-cypermethrin by HLMs.
Methods

Materials and reagents and standards:

Alpha-cypermethrin and lambda-cyhalothrin working stock were prepared by dissolving in dimethyl sulfoxide (DMSO) and stored when it was not used at -20°C. Human liver microsomes (HLMs, 20 mg/ml stock, contains liver microsomes pooled from 50 donors of mixed sex) were purchased from XenoTech (Lenexa, KS). Lambda-cyhalothrin (CAS 91465-08-6), lambda cyhalothric acid (CAS 72748-35-7), alpha-cypermethrin (CAS 67375-30-8), 3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropane carboxylic acid (CAS 55701-05-8), chlorpyrifos-oxon (CAS 5598-15-2) were purchased from ChemService Inc (West Chester, PA). 3-phenoxybenzoic acid (CAS 3739-38-6), 3-phenoxy benzyl alcohol (CAS 13826-35-2), and tetraisopropyl pyrophosphoramide (iso-OMPA; CAS 513-00-8) were purchased from Sigma-Aldrich (St. Louis, MO). Magnesium chloride (MgCl₂) and EDTA were purchased from JT Baker (Phillipsburg, NJ). β-glucuronidase/sulfatase was purchased from Sigma Aldrich, (St. Louis, MO). NADPH (CAS 2646-71-1) was purchased from Calbiochem (Billerica, MA).

Method validation for analysis of α-CM and λ-CH metabolites

Two reverse phase HPLC columns, Zorbax SB-C18 (25 cm x 4.6mm, 5 μm particle size) and Ascentis Express C18 (15cm x 3mm, 5 μm particle size) were evaluated for best separations of the PYR metabolites. A gradient elution with changes in mobile phase composition of water, acetonitrile, and methanol with the presence of 0.1% H₃PO₄ was developed to provide optimal
peak separation for quantification and metabolite detection. Ascentic Express C18 had optimal performance and was chosen for further method development.

**In vitro metabolism assay for α-CM and λ-CH**

For kinetic studies, pooled human liver microsomes and 1mM NADPH were added to buffer (100mM Tris-HCl, 5mM MgCl₂, 1mM EDTA; pH 7.4) and incubating for 15 minutes at 37°C in a shaking water bath with a final volume of reaction mixture of 0.5 ml. All incubations were initiated by adding the corresponding parent pesticide compound. Initially, time course studies were performed to assure that the reaction (metabolite formation) was linear with time in incubations with HLMs (0.5 mg/ml protein concentration). Lambda-cyhalothrin and alpha-cypermethrin at two different concentrations (5μM, and 20 μM) were incubated from 0-30 minutes. Optimal, linear metabolite formation was found at 15-minute incubation.

For kinetic studies, either lambda-cyhalothrin or alpha-cypermethrin was dissolved in dimethyl sulfoxide (DMSO) to generate the desired final concentration in the incubation mixture (1.0, 2.5, 5.0, 10, 20, 50, 100, and 150 μM). Incubation reactions were quenched after 15 minutes of reaction initiation with 0.5 mL ice-cold MeOH/ 0.1 % phosphoric acid, samples were placed on ice and 5 ul of 500 μg/ml 2-phenoxybenzoic acid (2PBA) was added as internal standard and kept on ice for at least 15 minutes and centrifuged at 2500 rpm for 15 minutes to precipitate the protein. The supernatant was transferred into HPLC sample vial for HPLC analysis.

**High-performance liquid chromatography analysis**

Chromatographic analysis was performed using a Hewlett Packard HPLC 1100 series with degasser, quaternary pump, autosampler, and diode array detector. Twenty μl samples
were injected into an analytical column (Ascentis Express C18, 15cm x 3mm, 5 μm particle size) connected with a guard column (Supelco LC-18, 2 cm x 4.5 mm, 5 μm particle size) to analyze lambda-cyhalothrin and alpha-cypermethrin metabolites. The injected sample eluted at a flow rate of 0.430 ml/min. The solvent system consists of, acetonitrile w/0.1% phosphoric acid (H₃PO₄) (solvent B), 95% H₂O/5% acetonitrile with 0.1% H₃PO₄ (solvent C), methanol with 0.1% H₃PO₄ (solvent D). All solvents were filtered before use through a 0.2 μm WHATMAN nylon filter (Healthcare Bio-Sciences, Marlborough, MA). The gradient started with initial mobile phase 60% C: 40% D, at time 12 minutes the solvent changed to 30% B: 30% C: 40% D, at time 25 the solvent changed to 60% B: 40% D for five minutes, then returned back to initial mobile phase and stayed at initial mobile phase for 20 minutes to re-equilibrate. The signal was monitored at wavelengths 215nm and 230nm. Samples were quantified by calibration curve established by authentic metabolite standards (2.5, 6.25, 12.5, 25, 50, 125, 250, 500, and 1,000 ng / mL).

The impact of NADPH on the metabolism of lambda-cyhalothrin and alpha-cypermethrin

Pooled human liver microsomes were incubated in the presence or absence of NADPH, a required cofactor for all reactions catalyzed by cytochrome P-450 enzymes. The reaction was initiated with lambda-cyhalothrin or alpha-cypermethrin as described above, and the supernatant was collected for HPLC analysis.

Interactive effects of iso-OMPA, a cholinesterase inhibitor, on the metabolism of lambda-cyhalothrin and alpha-cypermethrin

Iso-OMPA, a potent carboxylesterase inhibitor, was used to assess the relative impact of carboxylesterase on lambda-cyhalothrin and alpha-cypermethrin metabolism. Iso-OMPA was
dissolved in the buffer in various final concentrations (0, 50, 500 μM). The buffer, HLMs, and NADPH were incubated at 37°C in a final volume of 0.5 ml. Five minutes after incubation with iso-OMPA, lambda-cyhalothrin or alpha-cypermethrin (20, 100 μM) was added to initiate the reaction and incubated at 37°C for 15 minutes. The reactions were then carried out as described above followed by HPLC analysis.

**Interactive effects of OPs (Chlorpyrifos-Oxon) on the metabolism of lambda-cyhalothrin and alpha-cypermethrin**

The active metabolite of chlorpyrifos, chlorpyrifos-oxon (CPF-O; 0, 100 and 200 nM) was added after 15-minute incubation at 37°C of the buffer, NADPH, and HLMs (0.5 mg protein/mL). The mixture was incubated for 5 minutes prior to initiating the reaction with different concentrations of lambda-cyhalothrin or alpha-cypermethrin. The reactions were then carried out as described above followed by HPLC analysis.

**Data Analysis**

Acquired data and quantification of metabolites were processed by Agilent HPLC software (OPENLAB CDS Chemstation, version 11). The kinetic values $V_{\text{max}}$ and $K_m$ were determined by non-linear regression analyses (enzyme kinetics module of Sigma Plot, SyStat Software Inc, V11) of hyperbolic plots obeying Michaelis-Menten kinetics.
Results:

**Alpha-cypermethrin and lambda-cyhalothrin in vitro Metabolism Assay Optimization Using Pooled Human Liver Microsomes (HLMs)**

It is important to better understand factors which regulate the biotransformation of pyrethroids in humans since metabolism detoxifies these agents and thus regulates their relative persistence and resulting risk to human health. Experimental conditions have been optimized to assess the in vitro biotransformation of αCM and λCH by using a sensitive and specific analytical method that has been developed for quantifying the formation of various αCM and λ-CH metabolites. Analysis of the time-dependent formation of metabolites was performed using an Ascentis Express C18 (15cm x 3mm, 5 µm particle size) HPLC column. Alpha-cypermethrin and lambda-cyhalothrin (5 and 20 µM) were incubated with HLMs over a 30-minute period to identify the time dependent formation of metabolites. Figure 2.1 and 2.2 illustrate the concentration of metabolites formed from 0-30 minutes. The results show that a linear rate of metabolite formation was maintained for an incubation period of 15 min for both alpha-cypermethrin and lambda-cyhalothrin at both concentrations. A 15 min incubation period was chosen for all metabolism assays to assure a linear, constant rate of biotransformation of αCM and λ-CH in order to determine kinetic parameters (Km and Vmax) for αCM and λ-CH metabolism by HLMs.
Figure 2.1: Time-dependent formation of αCM and λ-CH metabolites by HLMs incubated with (A) 5 µM αCM and, (B) 5 µM λ-CH. The plots represent the formation of 3PBA and 3PB alcohol (non-specific PYR metabolites). In addition, formation of cis-DCCA and lambda-cyhalothric acid, specific metabolites, was assessed over 30 min.

Figure 2.2: Time-dependent formation of metabolites of (A) 20 µM αCM and, (B) 20 µM λ-CH incubated with HLMs.

In Vitro Metabolism kinetics for lambda-cyhalothrin

Figure 2.3 illustrates the in vitro biotransformation of lambda-cyhalothrin by pooled HLMs. The y-axis shows the rate of formation of metabolites 3PB alcohol, 3PBA, and lambda-cyhalothric acid as the concentration of lambda-cyhalothrin increase (x-axis). The Michaelis-Menten equation was used to calculate Vmax and Km. Table 2.1 shows the kinetic parameters.
The Km values for 3PB alcohol, 3PBA, and lambda-cyhalothric acid were 54, 16, and 35 µM respectively. The Vmax for 3PB alcohol, 3PBA, and lambda-cyhalothric acid were 523, 218, and 923 pmol/min/mg HLM respectively.

![Graphs](image)

**Figure 2.3**: Biotransformation of lambda-cyhalothrin by pooled human liver microsomes. The plots illustrate the relationship between the rate of metabolites formation and lambda-cyhalothrin concentration. Sigma Plot 11 enzyme kinetic software was used to measure Michaels-Menten kinetic parameters Vmax, and Km. (A) 3PB alcohol, and (B) 3PBA, are non-specific PYR metabolite (C)lambda-cyhalothric acid, is a lambda-cyhalothrin specific metabolite.
In Vitro Metabolism kinetics for alpha-cypermethrin

Figure 2.4 illustrates the rate of formation of nonspecific PYR metabolites 3PB alcohol, 3PBA, and the specific metabolite cis-DCCA in pmol/min/mg protein, following incubation of alpha-cypermethrin with pooled HLMs. The Michaelis-Menten equation was used to calculate the kinetic parameters (Vmax and Km), which are summarized in Table 2.1. The calculated Km values for 3PB alcohol, 3PBA, and cis-DCCA were 35, 17, and 23 μM respectively. Vmax values for 3PB alcohol, 3PBA, and cis-DCCA acid were 700, 333, and 1377 pmol/min/mg HLM respectively.

Figure 2.4: Biotransformation of alpha-cypermethrin by pooled human liver microsomes. The plots illustrate the relationship between the rate of metabolites formation and alpha-cypermethrin concentration. Sigma Plot 11 enzyme kinetic software was used to measure Michaelis-Menten kinetic parameters Vmax, and Km. (A) 3PB alcohol, and (B) 3PBA, are non-specific PYR metabolite (C) cis-DCCA, specific alpha-cypermethrin metabolite.
Comparison of kinetics for in vitro microsomal metabolism of lambda-cyhalothrin and alpha-cypermethrin with HLMs

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Vmax (pmol/min/mg HLM)</th>
<th>Km (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>αCM</td>
<td>λCH</td>
</tr>
<tr>
<td>LC Acid</td>
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</tr>
<tr>
<td>cis-DCCA</td>
<td>1377.2±100.1</td>
<td>ND</td>
</tr>
<tr>
<td>3-PB alcohol</td>
<td>700.3±53.2</td>
<td>522.9±25.5</td>
</tr>
<tr>
<td>3PBA</td>
<td>333.4±16.0</td>
<td>218.1±9.2</td>
</tr>
</tbody>
</table>

Table 2.1: Comparison of Vmax (pmol/min/mg HLM) and Km (µM) values for metabolites of alpha-cypermethrin and lambda-cyhalothrin by HLMs. 3PBA and PB alcohol are non-specific metabolites produced by alpha-cypermethrin and lambda-cyhalothrin. Lambda-cyhalothric acid and cis-DCCA are specific metabolites of λ-CH and α-CM respectively. Values represent the mean of αCM and λCH ± SD of 10 and 4 experiments, respectively.

Assess the impact of NADPH on lambda-cyhalothrin and alpha-cypermethrin metabolism.

To study the impact of NADPH, a required cofactor for CYP-mediated reactions, on the biotransformation of alpha-cypermethrin and lambda-cyhalothrin, HLMs were incubated with λ-CH and α-CM in the presence and absence of NADPH. As shown in fig 2.5 and 2.6, the rate of formation of the specific metabolites, lambda-cyhalothric acid, and cis-DCCA were similar in the presence and absence of NADPH. However, the formation of the non-specific metabolites of λ-CH and α-CM, 3PB alcohol and 3PBA decreased with the absence of NADPH, indicating that the formation of these metabolites was catalyzed by CYP enzymes. In addition, there was a marked increase in the 3PB aldehyde peak area as the concentration of lambda-cyhalothrin and alpha-cypermethrin increases.
The impact of NADPH on the rate of formation of lambda-cyhalothrin metabolites

**3-PB alcohol**

![Graph showing the rate of formation of 3-PB alcohol vs. conc CH (µM).]

**3-PBA**

![Graph showing the rate of formation of 3-PBA vs. conc CH (µM).]

**lambda cyhalothric acid**

![Graph showing the rate of formation of lambda cyhalothric acid vs. conc CH (µM).]

**3PB aldehyde**

![Graph showing the peak area of 3PB aldehyde in µg/ml vs. CH conc (µM).]

**Figure 2.5**: Biotransformation of lambda-cyhalothrin (n=3) by human liver microsomes in the presence and absence of NADPH (mean +/- SD). The rate of formation of lambda-cyhalothrin metabolites (pmol/min/mg HLM) and the peak area of 3PB aldehyde in µg/ml were calculated for different concentrations of lambda-cyhalothrin (1, 10, 20, 50, 100, and 150 µM)
The impact of NADPH on the rate of formation of alpha-cypermethrin metabolites

![Graphs showing the impact of NADPH on the rate of formation of alpha-cypermethrin metabolites](image)

**Figure 2.6:** alpha-cypermethrin (n=3) biotransformation by HLMs in the presence and absence of NADPH (mean +/- SD). The rate of formation of alpha-cypermethrin metabolites (pmol/min/mg HLMs) and the peak area of 3PB aldehyde in µg/ml were calculated for different concentration of lambda-cyhalothrin (1, 10, 20, 50, 100, and 150 µM).

**Impact of iso-OMPA, a potent esterase inhibitor, on the biotransformation of lambda-cyhalothrin and α-cypermethrin**

Pyrethroid compounds such as λ-CH and α-CM are metabolized via carboxylase and cytochrome P450 hydrolysis. The impact and the role of each pathway can be assessed by pre-incubation with iso-OMPA, a potent cholinesterase inhibitor. Pooled human liver microsomes...
where incubated with iso-OMPA (50, 500 μM) prior to initiation the biotransformation reaction with either lambda-cyhalothrin or alpha-cypermethrin (20, 100 μM). Iso-OMPA had a slight effect on the rate of formation of metabolites of lambda-cyhalothrin and alpha-cypermethrin when 50 μM of iso-OMPA was added compared to the vehicle for both 20 and 100 μM of either alpha-cypermethrin or lambda-cyhalothrin. However, pre-incubation with a high concentration of iso-OMPA (500 μM) was needed to inhibit the rate of formation of all metabolites. Even though, the metabolites were still detected when inhibited with high concentration of cholinesterase inhibitor as it is illustrated fig 2.7 and 2.8. According to table 2.2 and 2.3, the rate of formation of lambda-cyhalothric acid and cis-DCCA was nearly double the rate of formation of 3PBA or 3PB alcohol.

**Impact of iso-OMPA on the biotransformation of 20 μM lambda-cyhalothrin and alpha-cypermethrin by HLMs**

![Graph](image)

**Figure 2.7:** Metabolism of alpha-cypermethrin and lambda-cyhalothrin (20 μM) by HLMs in the presence of a vehicle, and 50 μM and 500 μM of iso-OMPA, an esterase inhibitor.
Table 2.2: The rate of formation of metabolites of lambda-cyhalothrin and alpha-cypermethrin (20 μM) by HLMs in the presence and absence of iso-OMPA (0, 50, and 500 μM). Values represent the mean ± SD.

Impact of iso-OMPA on the biotransformation of 100 μM lambda-cyhalothrin and alpha-cypermethrin by HLMs

Figure 2.8: Metabolism of alpha-cypermethrin and lambda-cyhalothrin (100 μM) by HLMs in the presence of a vehicle, and 50 μM and 500 μM of iso-OMPA, an esterase inhibitor.

Table 2.3: The rate of formation of metabolites of lambda-cyhalothrin and alpha-cypermethrin (100 μM) by HLMs in the presence and absence of iso-OMPA (0, 50, and 500 μM). Values represent the mean ± SD.
Assessing the impact of co-exposure with the organophosphate pesticide, chlorpyrifos-oxon, on the biotransformation of alpha-cypermethrin by HLMs.

Chlorpyrifos (CPF) is an organophosphate pesticide which is activated by cytochrome P 450 through desulfuration reaction to form chlorpyrifos-oxon, a potent esterase inhibitor. CPF-O (100, and 200 nM) was pre-incubated with HLMs before initiation of the reaction with alpha-cypermethrin. Figure 2.9 illustrates the impact of co-exposure of CPF-O with alpha-cypermethrin and its effect on the rate of formation of non-specific metabolites; 3 PB alcohol, and 3 PBA. The rate of formation of metabolites was measured in pmol/min/mg protein (y-axis) when a range of concentrations of alpha-cypermethrin (x-axis) was added. In the presence of CPF-O, the rate of formation of metabolites decreased as the concentration of the inhibitor increased. The data in table 2.4 summarize the Vmax value for formation of 3PBA of 333, 295, 227 pmole/min/mg protein and the Km value of 17, 23, 62 µM when chlorpyrifos-oxon (0, 100, 200 nM) was added, respectively. 3PB alcohol had a Vmax value of 700, 512, 316 pmol/min/mg protein and Km value of 35, 42, 107 µM Km when chlorpyrifos-oxon (0, 100, 200 nM) was added, respectively. The rate of formation of cis-DCCA, specific metabolite of alpha-cypermethrin had a Vmax value of 1377, 1009, 579 pmol/min/mg protein, and Km value of 23, 71, 33 µM when chlorpyrifos-oxon (0, 100, 200 nM) was added, respectively.
Impact of chlorpyrifos oxon (CFP-O) on the biotransformation of α-CM by HLMs

![Graphs showing the biotransformation of 3PB alcohol, 3PBA, and Cis DCCA in the presence of chlorpyrifos oxon (CFP-O).]

**Figure 2.9** Impact of chlorpyrifos oxon (CFP-O) on the biotransformation of α-CM by HLMs. Rate of formation of 3PB alcohol (A), 3PBA (B) and cis DCCA (C) in the presence and absence of chlorpyrifos oxon (CFP-O).

**Comparison of kinetics for in vitro microsomal metabolism of alpha-cypermethrin with HLMs**

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<thead>
<tr>
<th></th>
<th>Vmax</th>
<th>Km</th>
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<tbody>
<tr>
<td></td>
<td>0nM</td>
<td>100nM</td>
</tr>
<tr>
<td><strong>CPF-O</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cis-DCCA</strong></td>
<td>1377.2±100.1</td>
<td>1008.9±86.7</td>
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<td><strong>3-PB alcohol</strong></td>
<td>700.3±53.2</td>
<td>512.3±53.7</td>
</tr>
<tr>
<td><strong>3-PBA</strong></td>
<td>333.4±16.0</td>
<td>295.1±17.7</td>
</tr>
</tbody>
</table>

**Table 2.4**: Comparison of kinetic values (Km, Vmax) for the in vitro metabolism of alpha-cypermethrin in the presence and absence of CPF-O. 3PBA and 3PB alcohol are non-specific metabolite produced by αCM. Cis-DCCA is specific metabolite for αCM. Km and Vmax values were determined using non-linear regression analysis with the enzyme kinetics module of Sigma Plot, SyStat Software Inc, V11.
**Assessing the impact of co-exposure with the organophosphate pesticide, chlorpyrifos-oxon, on the biotransformation of lambda-cyhalothrin by HLMs.**

In comparison to lambda-cypermethrin, CPF-O (100, and 200 nM) was pre-incubated with HLMs before initiation of the reaction with lambda-cyhalothrin. Figure 2.10 illustrates the impact of co-exposure of CPF-O with lambda-cyhalothrin and its effect on the rate of formation of non-specific metabolites; 3 PB alcohol, and 3 PBA. The rate of formation of metabolites was measured in pmol/min/mg protein (y-axis) when a range of concentrations of lambda-cyhalothrin (x-axis) was added. In the presence of CPF-O, the rate of formation of metabolites decreased as the concentration of the inhibitor increased. The data in table 2.4 summarize the Vmax for the formation of 3PBA of 218, 168, 113 pmol/min/mg protein and Km values of 16, 15, 39 μM when chlorpyrifos-oxon (0, 100, 200 nM) was added, respectively. 3PB alcohol had a Vmax value of 523, 317, 129 pmol/min/mg protein and Km value of 54, 42, 33 μM when chlorpyrifos-oxon (0, 100, 200 nM) was added, respectively. Furthermore, lambda-cyhalothric acid had Vmax value of 923, 593, 34.6 pmol/min/mg protein, and Km value of 35, 27, 35 μM when chlorpyrifos-oxon (0, 100, 200 nM) was added, respectively. In conclusion, the rate of formation of metabolite decrease as the concentration of the inhibitor increase similarly to alpha-cypermethrin.
Figure 2.10 Impact of chlorpyrifos oxon (CFP-O) on the biotransformation of λ-CH by HLMs. Rate of formation of 3PB alcohol (A), 3PBA (B) and lambda-cyhalothric acid (C) in the presence and absence of chlorpyrifos oxon (CFP-O).

Comparison of kinetics for in vitro microsomal metabolism of lambda-cyhalothrin with HLMs

<table>
<thead>
<tr>
<th></th>
<th>Vmax</th>
<th>Km</th>
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<tr>
<td></td>
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<td>100nM</td>
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<tr>
<td>CPF-O</td>
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<td>LC Acid</td>
<td>923.0±43.6</td>
<td>593.1±23.5</td>
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<td>3-PB Alcohol</td>
<td>522.9±25.5</td>
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<tr>
<td>3-PBA</td>
<td>218.1±9.2</td>
<td>168.5±5.3</td>
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</table>

Table 2.5: Comparison of kinetic values (Km, Vmax) for the in vitro metabolism of lambda-cyhalothrin in the presence and absence of CPF-O. 3PBA and 3PB alcohol are non-specific metabolite produced by λCH. LC-Acid is specific metabolite for λCH. Km and Vmax values were determined using non-linear regression analysis with the enzyme kinetics module of Sigma Plot, SyStat Software Inc, V11.
It is important to emphasize that in contrast to the experimental esterase inhibitor, iso OMPA, very low concentrations of CPF-O can reduce the formation of all metabolites of these PYRs by HLMs. This suggests that co-exposures to OPs has the potential to reduce the metabolism and slow the clearance of PYRs, resulting in higher levels of PYRs and greater potential risk for adverse effects.

**Discussion:**

The global use of pesticides for agricultural and residual settings amplifies the need to assess the risk of short and long-term exposure of these agents and its effect on human health. With the increasing use of pyrethroid pesticides, it is important to understand the biotransformation of these synthetic chemicals in the human body. This is especially important since metabolism regulates the rate of detoxification leading to the formation of inactive metabolites that can be used as a biomarker of exposure. Lambda-cyhalothrin and alpha-cypermethrin are examples of type II pyrethroid that are commonly used all around the world to control insect pests. Thus, the toxicity of these pesticides is strongly influenced by their biotransformation in the human body.

The in vitro metabolism of λCH by HLMs results in the formation of lambda cyhalothric acid (LC Acid), 3PBA, and 3PB alcohol. LC Acid is a specific metabolite produced by the hydrolysis of λCH. In contrast, 3PBA and 3PB alcohol are general metabolites that are common metabolites with other pyrethroids. Thus, LC Acid is considered as a specific biomarker of
exposure to λCH. However, 3PBA and 3PB alcohol are general biomarkers of exposure of λCH and a number of other pyrethroid pesticides.

Alpha-cypermethrin is another structurally related, potent type II pyrethroid. The in vitro metabolism of αCM by HLMs results in the formation of cis-DCCA, 3PBA, and 3PB alcohol. Cis-DCCA is considered as a specific metabolite for αCM, while 3PBA and 3PB alcohol are general metabolites. In the case of λCH, αCM, and other pyrethroids, 3PBA and 3PB alcohol are general biomarkers of exposure. However, cis-DCCA and LC Acid are specific biomarkers that can be used to distinguish between λCH and αCM exposure. Following systemic absorption and biotransformation, these metabolites are readily excreted into urine, which provides a convenient bio-specimen for measuring metabolite levels, which corresponds directly to in vivo exposures to these pyrethroids.

It is important to use the kinetic parameter (Vmax, Km) values to compare lambda-cyhalothrin and alpha-cypermethrin metabolism in HLMs which serve as an accepted in vitro model to predict in vivo human biotransformation reactions. The Vmax values of the specific metabolites of each PYR were almost twice the Vmax values of the general metabolites, indicating that specific metabolites, formed by the initial hydrolysis reaction, directly reflect the rate of biotransformation on a molar basis of each PYR (table 2.1). Thus, the daily urinary concentration of the specific metabolites are suitable biomarkers to assess human exposure to these PYRs. Km represents the affinity of the substrate for the enzyme or the concentration of substrate that is needed to reach half of the Vmax. According to table 2.1, Vmax and Km values for the metabolites were within the same range for both λCH and αCM, suggesting that these structurally similar PYRs are bio-transformed in humans in a similar manner.
NADPH is a necessary cofactor that plays a role in donating electrons and a hydrogen to the reaction catalyzed by cytochrome P450 enzymes. Conducting in vitro incubations with pooled HLMs in the presence and absence of NADPH was used to analyze the role of CYP P450 on the metabolism of αCM and λCH. In the absence of NADPH, the metabolism of αCM and λCH by HLMs resulted in a marked reduction in the formation of 3-PBA and 3-PB alcohol, suggesting that these metabolites resulted from CYP-mediated oxidative metabolism. In contrast, the absence of NADPH had no effect on the formation of lambda-cyhalothric acid and cis-DCCA, suggesting that this hydrolysis reaction was mediated by carboxyl esterases.

Iso-OMPA, a potent esterase inhibitor, was used to assess the initial step of λCH and αCM hydrolysis. In vitro incubations of HLMs were pre-incubation with 50 µM iso-OMPA didn’t show an impact on metabolite formation at 20 or 100 µM of either λCH or αCM. However, 500 µM of iso-OMPA inhibited the rate of formation of all metabolites of alpha-cypermethrin and lambda-cyhalothrin by HLMs at 20, and 100 µM. This suggests, that carboxylesterases represent the initial rate-limiting step in the biotransformation of αCM and λCH. Furthermore, the high concentration of iso-OMPA didn’t completely block the rate of formation of metabolites. These results support the suggestion that the cytochrome P 450 hydrolysis pathway has an important impact on the hepatic metabolism of lambda-cyhalothrin and alpha-cypermethrin.

Organophosphate agents are commonly used as insecticides, but also originated as part of the nerve gas family that was developed during World War II. One of the most commonly used organophosphates is chlorpyrifos. Nevertheless, chlorpyrifos usage has declined in the past decade, due to its toxic effect on human health through increasing the risk on behavioral, neurological, and developmental delays. The U.S. Environmental Protection Agency (EPA)
estimates that more than 20 million pounds of chlorpyrifos is applied yearly, and more than half of that amount is for non-agricultural applications. The widespread usage is due to its efficacy and its wide spectrum of activity compared to other products. Chlorpyrifos activation is through CYP P450, mainly CYP 2B6, forming chlorpyrifos-Oxon by replacing the sulfur atom bonded to phosphorus atom with an oxygen atom. The main mechanism of action of chlorpyrifos-Oxon is through non-specific cholinesterase inhibition. Since OPs are commonly used with PYRs, it was important to assess the impact of co-exposure of αCM and λCH with CPF-Oxon. Very low concentrations of CPF-O (100, and 200 nM) inhibited the formation of αCM and λCH metabolites. Consequently, lowering the Vmax of both lambda-cyhalothrin and alpha-cypermethrin metabolites as the concentration of CPF-O increased (table 2.4). According to fig 2.9 and 1.10 it is suggested that CPF-O competitively inhibit the biotransformation of alpha-cypermethrin and lambda-cyhalothrin. Consequently, prior human exposures to OP pesticides may slow the metabolism and clearance of pyrethroids and thus potentially enhance their toxicity.

In conclusion, the results suggest that CYP enzymes mediate the formation 3-PBA and 3-PB alcohol, while carboxylase esterase enzyme mediates the initial step hydrolysis to cis DCCA and lambda-cyhalothric acid from αCM and λ-CH, respectively. These metabolites were also detected in urine samples of Egyptian adolescent agriculture workers that applied λCH and αCM to the cotton fields. Together, in vivo exposure and in vitro metabolism data can be used in risk assessment for occupational and residential exposures and to assist efforts to reduce exposures.
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