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IMPACT DE LA COEXPOSITION SUR LES BIOMARQUEURS D'EXPOSITION AUX PESTICIDES PYRÉTHRINOÏDES

Études animales et chez des travailleurs agricoles

Par

Marc Bossou

Département de santé environnementale et santé au travail,

École de Santé Publique

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IMPACT DE LA COEXPOSITION SUR LES BIOMARQUEURS D'EXPOSITION AUX PESTICIDES PYRÉTHRINOÏDES :

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Présenté par

Yélian Marc Bossou

A été évaluée par un jury composé des personnes suivantes

Ludwig Vinches
Président-rapporteur

Michèle Bouchard
Directrice de recherche

Claude Emond
Membre du jury

Brice Appenzeller
Examinateur externe

Résumé

La surveillance biologique de l'exposition est reconnue comme approche privilégiée pour évaluer l'exposition aux pesticides pyréthrinoïdes en milieu de travail. Néanmoins, les niveaux d'exposition peuvent être influencés par plusieurs facteurs, dont la coexposition. L'objectif général du projet de thèse était d'évaluer l'impact de la coexposition sur les biomarqueurs d'exposition aux pyréthrinoïdes, par une étude animal in vivo, d'une part, et chez les travailleurs agricoles, d'autre part. En utilisant le pyréthrinoïde lambda-cyhalothrine (LCT) et le fongicide captane comme pesticides sentinelles pour l'étude de cette coexposition, trois objectifs spécifiques ont été établis et ont fait l'objet de trois articles.

Dans le premier article, des groupes de rats ont été exposés par gavage à la LCT seule (2,5 ou 12,5 mg/kg p.c.) ou à un mélange binaire de LCT et de captane (2,5/2,5 ou 2,5/12,5 ou 12,5/12,5 mg/kg p.c.). Des collectes de sang et d'excrétas (urine et fèces) ont été effectuées à des intervalles prédéterminés jusqu'à 48 heures après dosage, afin d'établir les profils temporels des principaux métabolites de la LCT (CFMP, 3-PBA et 4-OH3-PBA). Les profils temporels du CFMP et 3-PBA dans le plasma, l'urine et les fèces étaient similaires après exposition à la dose de 2.5 mg/kg pc de LCT seule ou en combinaison avec le captan. Cependant, les niveaux plasmatiques de 3-PBA étaient plus faibles dans le groupe coexposés à la dose élevée. L'excrétion urinaire du 4-OH3PBA était également plus elevée dans le groupe coexposé à cette dose.

Dans le deuxième article, les profils temporels individuels des biomarqueurs d'exposition à la LCT chez des travailleurs appliquant des pesticides dans des champs de fraises ont été comparés après un épisode d'application de la LCT seule ou en coexposition avec le captane. Les participants ont fourni toutes leurs urines sur une période de trois jours suivant une application d'une formulation de pesticide contenant de la LCT seule (E1) ou de la LCT mélangée à du captane (E2), et dans certains cas après être retournés dans le champ traité (E3). Les métabolites de pyréthrinoïdes ont été mesurés dans tous les échantillons d'urine, en particulier le CFMP, 3-PBA et 4-OH3BPA. Il n'y avait pas de différences évidentes, attribuables à la coexposition, dans les profils individuels des concentrations en fonction du temps et dans l'excrétion cumulative des métabolites (CFMP, 3-PBA, 4-OH3BPA) après une exposition à la LCT seule ou en combinaison avec le captane.

Dans le troisième article, une étude a été menée sur 87 travailleurs agricoles affectés à différentes tâches agricoles (application, désherbage, cueillette). Ces travailleurs ont fourni des échantillons d'urine avant et après l'application de LCT seule ou en combinaison avec du captane, ainsi qu'après des tâches dans les champs traités, avec également un échantillon de contrôle. Les concentrations des métabolites CFMP et le 3-PBA ont été mesurées dans les échantillons. À l'aide d'un questionnaire, les déterminants potentiels d'exposition incluant la tâche effectuée et les facteurs personnels ont également été documentés. Les analyses statistiques ont montré que la coexposition au captane n'induisait pas de changement dans les concentrations urinaires observés de 3-PBA et de CFMP. Seule la tâche professionnelle principale montrait une association avec les niveaux urinaires de ces métabolites. Comparativement aux tâches de désherbage ou de cueillette, la tâche d'application de pesticides était associée à des concentrations urinaires plus élevées de 3-PBA et de CFMP.

En résumé, bien qu'un impact de la coexposition LCT-captan a été démontré expérimentalement à de fortes doses, l'étude chez les travailleurs a révélé que la coexposition ne contribuait pas significativement aux variations dans les concentrations des biomarqueurs d'exposition, aux niveaux d'exposition observés chez les travailleurs de la culture de la fraise par rapport aux autres facteurs qui contribuent à cette variabilité. Cette étude a également confirmé les données antérieures suggérant que les applicateurs étaient plus exposés que les travailleurs affectés à des tâches telles que le désherbage et la cueillette. Cette recherche a le potentiel d'améliorer la compréhension de l'impact de la coexposition par rapport à d'autres facteurs sur les niveaux de biomarqueurs d'exposition aux pyréthrinoïdes ; elle contribue ainsi à mieux interpréter les données de biosurveillance.

Mots-clés : biosurveillance, pesticides, biomarqueurs, pyréthrinoïdes, Coexposition, travailleur agricole, Lambda-cyhalothrin · Captan, Toxicocinétique.

Abstract

Biological exposure monitoring is recognized as the preferred approach for assessing exposure to pyrethroid pesticides in the workplace. Nevertheless, exposure levels can be influenced by several factors, including coexposure. The overall aim of the thesis project was to assess the impact of coexposure on biomarkers of exposure to pyrethroids, through an *in vivo* animal study on the one hand, and in agricultural workers on the other. Using the pyrethroid lambda-cyhalothrin (LCT) and the fungicide captan as sentinel pesticides for the study of this coexposure, three specific objectives were established and were the subject of three published articles.

In the first paper, groups of rats were exposed by gavage to LCT alone (2.5 or 12.5 mg/kg bw) or to a binary mixture of LCT and captan (2.5/2.5 or 2.5/12.5 or 12.5/12.5 mg/kg bw). Blood and excreta (urine and faeces) were collected at predetermined intervals up to 48 hours after dosing, to establish the temporal profiles of the main LCT metabolites (CFMP, 3-PBA and 4-OH3-PBA). The temporal profiles of CFMP and 3-PBA in plasma, urine and feces were similar after exposure to 2.5 mg/kg bw of LCT alone or in combination with captan. However, plasma levels of 3-PBA were lower in the high-dose co-exposure group. Urinary excretion of 4-OH3PBA was also higher in the high-dose coexposure group.

In the second paper, individual temporal profiles of LCT exposure biomarkers in workers applying pesticides in strawberry fields were compared after an episode of LCT application alone or in coexposure with captan. Participants provided all their urine over a three-day period following application of a pesticide formulation containing LCT alone (E1) or LCT mixed with captan (E2), and in some cases after returning to the treated field (E3). Pyrethroid metabolites were measured in all urine samples, in particular CFMP, 3-PBA and 4-OH3BPA. No differences were observed in individual concentration-time profiles or in the cumulative excretion of metabolites (CFMP, 3-PBA, 4-OH3BPA) after exposure to LCT alone or in combination with captan.

In the third article, a study was carried out on 87 farm workers assigned to different agricultural tasks (application, weeding, picking). These workers provided urine samples before and after the application of LCT alone or in combination with captan, as well as after tasks in treated fields, with also a control sample. Concentrations of the metabolites CFMP and 3-PBA were measured in the

samples. Using a questionnaire, potential determinants of exposure including the task performed and personal factors were also documented. Statistical analyses showed that coexposure to captan did not lead to any change in the observed urinary concentrations of 3-PBA and CFMP. Only the main occupational task showed an association with urinary levels of these metabolites. Compared with weeding or picking tasks, the pesticide application task was associated with higher urinary concentrations of 3-PBA and CFMP.

In summary, although an impact of LCT-captan coexposure has been demonstrated experimentally at high LCT doses, the field study revealed that coexposure did not contribute significantly to variations in exposure biomarker concentrations, at the exposure levels observed in strawberry crop workers compared to other factors contributing to this variability. This study also confirmed previous data suggesting that applicators were more exposed than workers assigned to tasks such as weeding and picking. This research has the potential to improve understanding of the impact of coexposure versus other factors on pyrethroid exposure biomarker levels, and thus contribute to better interpretation of biomonitoring data.

Keywords: Biomonitoring, pesticides, biomarkers, pyrethroids, coexposure, farm worker, lambda-cyhalothrin - captan, toxicokinetics.

Table des matières

Liste des figures

Liste des tableaux

Chapitre 3

Article 1

Article 2

Article 3

Liste des sigles et abréviations

3-PBA	Acide 3-phénoxybenzoïque
4F-3PBA	Acide 4-fluoro-3-phénoxybenzoïque
4-OH-3-PBA	Acide 4-hydroxy-3-phénoxybenzoïque
i.a	ingrédient actif
AIC	Critère d'information d'Akaike
AJS ESI	Source d'ionisation par électrospray « Jet Stream »
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail
AOEL	Acceptable Operator Exposure Level
AOEL	Acceptable Operator Exposure Level
ARLA	Agence de Réglementation de la Lutte Antiparasitaire
AUC	Aire sous la courbe (area under the curve)
AUMC	Aire sous la courbe du premier moment (area under the first moment curve)
CFMP	Acide cis-3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2 diméthylcyclopropanecarboxylique
ECMS	Enquête canadienne sur les mesures de santé
EFSA	Autorité européenne de sécurité des aliments
IRSST	Institut de recherche Robert-Sauvé en santé et en Sécurité du travail (IRSST)
LCT	Lambda cyhalothrine
LOAEL	Lowest Observed Adverse Effect Level
LOD	Limit of detection
MAPAQ	Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec
MDDELCC	Ministère du Développement durable, Environnement et Lutte contre les changements climatiques
MIXM	Modèle linéaire à effets mixtes
MRT	Temps de résidence moyen (mean residence time)
MS	Spectrométrie de masse
NOAEL	No Observed Adverse Effect Level
OECD	Organisation de Coopération et de Développement Économiques
RfD	Dose de reference
trans-DCCA	Acide trans-3-(2,2-dichlorovinyl)-2,2-diméthyl cyclopropane carboxylique
U.S. EPA	United States Environmental Protection Agency

Dédié avec amour et reconnaissance

À

Dieu, le créateur:

Mes parents, mon épouse et mes enfants, pour leur soutien sans faille qui m'a permis de surmonter les défis de mon doctorat.

Ma directrice de thèse, pour avoir été une source d'inspiration et d'orientation tout au long de cette aventure.

Tous ceux qui travaillent pour minimiser les expositions chimiques en milieux de travail.

Avant-propos

Cette thèse a été rédigée selon le modèle de thèse par article au lieu du format traditionnel des chapitres. Elle comprend donc une introduction générale, des objectifs spécifiques, et une description des méthodologies employées pour répondre à chaque objectif. Les résultats de la thèse sont présentés sous la forme de trois articles, détaillés dans le troisième chapitre de ce manuscrit. Ensuite, la discussion générale intègre les résultats globaux des trois articles, l'interprétation des résultats, leurs portées et limites, ainsi que les recommandations pour les recherches futures.

CHAPITRE PREMIER :

INTRODUCTION GÉNÉRALE

1. Problématique de santé et de sécurité du travail

1.1. Contexte

1.1.1. La production agricole au Québec et l'utilisation de pesticides

Au Québec, l'agriculture est un secteur d'activité essentiel pour le developpement économique. D'après les données du recensement 2021 du ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, il y avait environ 27 930 exploitations agricoles qui emploient 56 600 travailleurs (MAPAQ 2021). Selon le dernier rapport régional de l'industrie bioalimentaire du Québec, la région de la Montérégie possède le plus grand nombre de fermes, totalisant 6 767 exploitations (MAPAQ 2021). Dans ce milieu, une proportion considérable des employés sont engagés sur une base saisonnière, tandis que d'autres sont des travailleurs étrangers temporaires. En 2021, le nombre des travailleurs étrangers temporaires employés dans le secteur agricole au Québec s'élevait à 18 216 (Statistique Canada, 2022).

Dans le milieu de travail agricole, les pesticides (insecticides, fongicides, herbicides) demeurent largement utilisés malgré la volonté des autorités à réduire leur utilisation. En effet, le gouvernement du Québec a mis en place un plan d'agriculture durable dont l'un des objectifs était de réduire l'usage des pesticides et leurs risques pour la santé et l'environnement d'ici l'année 2030 (MAPAQ 2020). Selon le dernier rapport du Ministère du Développement durable, Environnement et Lutte contre les changements climatiques, les ventes de pesticides destinés à la production agricole représentaient (3,6 millions de kg d'ingrédient actif i.a) 72 % des ventes totales en 2021 (Gouvernement du Québec, 2023). Les ouvriers agricoles sont donc exposés de façon répétée à

plusieurs pesticides en milieu de travail, généralement sur une courte période chaque année, et ce de manière séquentielle ou concomitante. L'utilisation des pesticides représente ainsi un facteur de risque pour la santé des travailleurs.

1.1.2. Les risques associés à l'utilisation de pesticides et l'évaluation de l'exposition

Bien que les pesticides soient soumis à des réglementations strictes pour minimiser les risques, des préoccupations subsistent concernant l'exposition professionnelle aux pesticides et la présence de résidus dans les aliments. Ainsi, l'évaluation des risques associés à l'exposition aux pesticides à integrer les priorités des grands organismes gouvernementaux comme Santé Canada, le U.S. EPA et l'ANSES. En effet, plusieurs études animales ont montré que l'exposition à de fortes doses répétées de ces produits chimiques induisait des altérations biologiques précoces, telles que des stress oxydants, des altérations immunitaires et des perturbations endocriniennes (Ansari *et al.*, 2012; Aouey *et al.*, 2017; Righi *et al.*, 2009).

Des cas d'intoxications aiguës ou d'incidents chez des travailleurs exposés aux pesticides ont aussi été rapportés, incluant des symptômes respiratoires et neurologiques (Burns et Pastoor, 2018; Mamane *et al.*, 2015; Ratanachina *et al.*, 2020; Saillenfait *et al.*, 2015). Une étude américaine a par ailleurs rapporté 6 692 cas de maladies/blessures liées aux expositions professionnelles aux pesticides, dont 24% sont imputables aux formulations à bases de pyréthrinoïdes/pyréthrines, excluant les désinfectants (Hudson *et al.*, 2014). Il est donc important de développer et d'appliquer

des outils pour bien évaluer et contrôler l'exposition à ces contaminants encore peu évalués au Québec en milieu de travail.

1.2 Origine du projet

1.2.1. L'importance du suivi biologique de l'exposition aux pesticides chez les travailleurs agricoles

Pour une meilleure caractérisation de l'exposition des travailleurs aux pesticides, il est nécessaire de développer des méthodes qui permettent de déterminer les doses réellement absorbées. La surveillance biologique de l'exposition par la mesure de métabolites de pesticides dans les urines est reconnue comme un outil adapté (Angerer *et al.*, 2007; Hardt et Angerer, 2003). Cette approche a pour principale avantage de fournir des informations sur l'exposition interne agrégée et réelle, en tenant compte de différentes sources d'exposition combinées, de toutes les voies d'exposition et des différences interindividuelles, par exemple dans le métabolisme, le régime alimentaire et le mode de vie (Angerer *et al.*, 2007; Linda, 2022). Néanmoins, l'interprétation des données de surveillance biologique nécessite de bien comprendre les facteurs qui influencent le devenir (comportement toxicocinétique) de la substance d'intérêt dans l'organisme humain afin de pouvoir faire les liens appropriés entre les niveaux de biomarqueurs d'exposition chez les travailleurs et les doses réellement absorbées.

1.2.2. Les facteurs influençant les niveaux de biomarqueurs d'exposition aux pesticides

Les travaux menés par notre équipe en culture maraîchère ont montré, à partir de mesures de métabolites de pesticides dans les urines, que les applicateurs présentaient souvent les niveaux d'exposition les plus élevés en comparaison avec des travailleurs affectés à des tâches telles que la cueillette ou le désherbage dans des zones traitées (Ratelle *et al.*, 2016). Ces travaux ont aussi montré que l'exposition orale par inadvertance, en milieu de travail, devrait faire l'objet d'une évaluation plus détaillée (Côté et Bouchard, 2018). Ils ont également soulevé la question de l'exposition multiple à plusieurs pesticides et l'impact que cette coexposition (exposition concomitante) ou exposition séquentielle pourrait avoir sur l'interprétation des données de biosurveillance. L'impact de la coexposition sur les niveaux de biomarqueurs utilisés pour évaluer leur exposition, par rapport à d'autres facteurs tels que l'hygiène et les pratiques de travail, est encore très peu évalué. Par ailleurs, pour la prise en compte de la multiexposition aux pesticides (exposition par différentes voies à des agents multiples, incluant la coexposition), le plan quinquennal 2018-2022 de l'IRSST prévoit le développement d'une programmation thématique sur les pesticides et la mise à jour de celle portant sur l'expologie. Il est donc nécessaire de comprendre l'influence de la coexposition sur les niveaux de biomarqueurs d'exposition d'intérêt, afin de bien interpréter les résultats de biosurveillance chez les travailleurs agricoles.

2. État des connaissances

2.1. Les pyréthrinoïdes comme pesticides prioritaires et l'importance de la lambda-cyhalothrine

Les pesticides de type pyréthrinoïdes figurent parmi les insecticides les plus utilisés en milieu agricole (Gorse et Balg, 2013). Ces derniers sont abondamment épandus, notamment en culture maraîchère, de sorte que les travailleurs y sont exposés de façon régulière, habituellement sur une courte période chaque année. Après la perméthrine et la cypermthrine qui ont longtemps figuré parmi les pyréthrinoïdes les plus appliqués, la lambda cyhalothrine (Figure 1) constitue maintenant un autre pyréthrinoïde encore plus abondement utilisé sur ces cultures. Ce pyréthrinoïde d'ingestion et de contact à action rapide est efficace sur un vaste éventail de ravageurs. Il est aussi largement utilisé pour contrôler les ravageurs et les parasites dans les bâtiments et leurs périmètres (Syngenta, 2022). Lambda cyhalothrine est toutefois très peu soluble dans l'eau et donc s'adsorbe facilement sur les sédiments. Sa demi-vie dans le sol est de 31,5 jours en moyenne (Santé Canada, 2021).

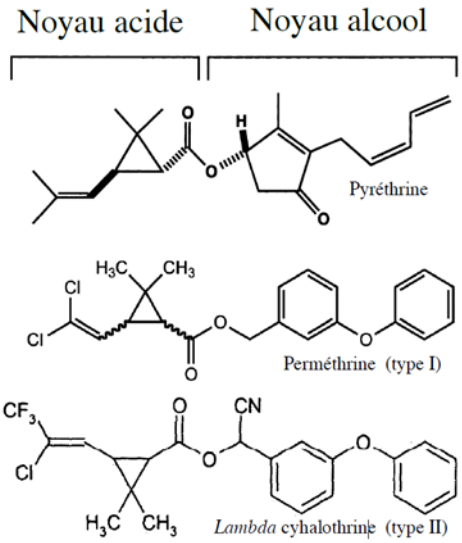

Figure 1 - Structure chimique de la pyréthrine et des pyréthrinoïdes synthétiques (type I et II)

Afin de prévenir les effets néfastes qui pourraient être reliés à l'exposition aux pyréthrinoïdes lambda cyhalothrine en milieu de travail, seul l'EFSA semble avoir établi une valeur de référence. Un Acceptable Operator Exposure Level (AOEL) systémique de 0,63 µg/kg pc/jour (EFSA, 2014a). Selon une étude précédente effectuée chez des travailleurs exposés à la cyperméthrine et la perméthrine, mais potentiellement aussi à la lambda-cyhalothrine (Bouchard *et al.*, 2016; Ferland *et al.*, 2015; Ratelle *et al.*, 2016), les doses absorbées reconstruites atteignaient 2,4 µg/kg pc/jour. Cette valeur maximale dépasse la valeur limite AOEL de 0,63 µg/kg pc/jour pour la lambda-cyhalothrine. On peut donc s'attendre à ce que les travailleurs puissent être exposés à des niveaux de lambda-cyhalothrine qui dépassent cette valeur limite.

2.1.1. La surveillance biologique de l'exposition aux pesticides pyréthrinoïdes

À la suite d'une exposition, les pyréthrinoïdes sont rapidement absorbées, biotransformés et excrétées de l'organisme et génèrent des métabolites retrouvés en grande partie dans l'urine (Anadon *et al.*, 1991; Anadon *et al.*, 2006; Ratelle *et al.*, 2015a, 2015b). La mesure de ces métabolites urinaires constitue donc un outil privilégié pour évaluer les doses de pyréthrinoïdes réellement absorbées dans l'organisme. Dans le cas de la lambda-cyhalothrine, les études disponibles chez le rat et les données humaines de notre laboratoire ont montré qu'elle était très rapidement scindée dans l'organisme, par les carboxylestérases et les cytochromes P450, pour générer plusieurs métabolites. Ces derniers sont excrétés rapidement dans l'urine et les fèces après une exposition. L'acide cis-3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-diméthylcyclopropanecarboxylique (CFMP), l'acide 3-phénoxybenzoïque (3-PBA) et l'acide 4-hydroxy-3-phénoxybenzoïque (4-OH-3-PBA), étaient les trois métabolites majeurs identifiés dans

l'urine (Figure 2). Le métabolite 3-PBA est commun à au moins dix autres pyréthrinoïdes, dont la perméthrine, cyperméthrine, deltaméthrine, tandis que le CFMP quantifiable dans le sang, les tissus et les excrétas est spécifique à la lambda cyhalothrine (Bouchard *et al.*, 2019; Khemiri *et al.*, 2017, 2018). Bien que ce métabolite semble être majeur et spécifique de la lambda-cyhalothrine, il n'existe pas à ce jour d'études publiées ayant mesurées le CFMP chez les travailleurs agricoles afin d'évaluer leurs expositions à lambda cyhalothrine. La seule étude disponible chez les travailleurs a été réalisée au Pakistan par Chester *et al.* (1992), et a examiné l'exposition professionnelle des pulvérisateurs dans le cadre du programme de lutte antipaludique. À considérer l'importante variabilité observée dans le niveau d'exposition des travailleurs aux pyréthrinoïdes en fonction des tâches et des quarts de travail (Ratelle *et al.*, 2016), il est fort probable que le profil d'exposition chez les travailleurs agricoles diffère de celui rapporté par Chester *et al.* (1992).

Bien que la mesure des métabolites urinaires soit utile pour évaluer l'exposition aux pyréthrinoïdes, les niveaux urinaires de ces biomarqueurs d'exposition peuvent varier largement d'un individu à l'autre ou chez un même individu en fonction du temps et être influencés par plusieurs facteurs (Fortin *et al.*, 2008; Ratelle *et al.*, 2016).

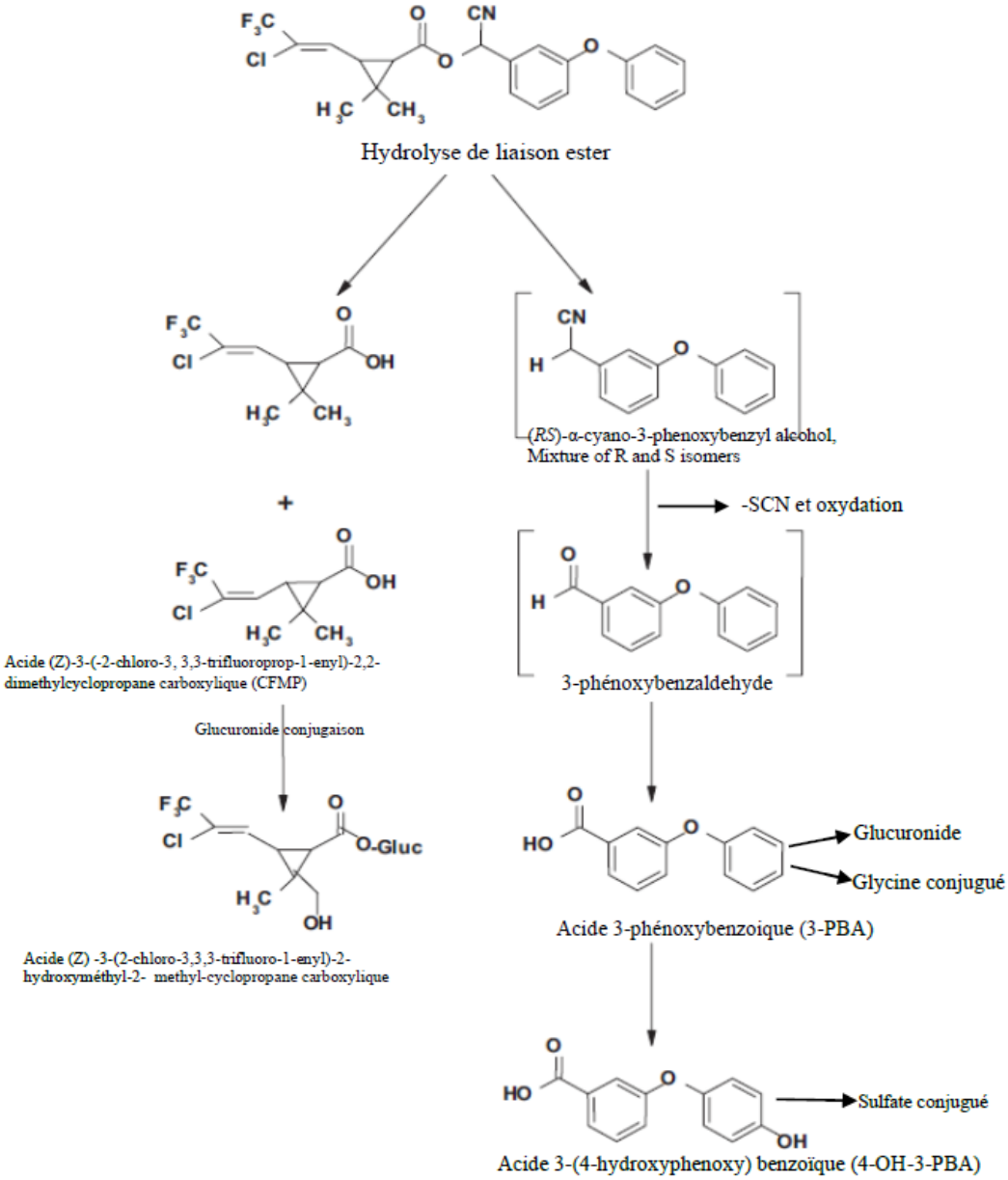

Figure 2 - Principales étapes de biotransformation de lambda cyhalothrine chez les mammifères, adapté de (EFSA 2014b; Kaneko, 2011).

2.1.2. Influence de différents facteurs, y compris la coexposition à plusieurs pesticides, sur la variabilité des niveaux de biomarqueurs d'exposition

Certaines études publiées en milieu agricole ont tenté de mieux comprendre l'influence de divers facteurs (déterminants) sur les données de biosurveillance de l'exposition aux pesticides, mais pas spécifiquement aux pyréthrinoïdes. Ces études se limitent à l'évaluation de l'impact des tâches de travail et des différentes méthodes de manipulation et d'application de pesticides (Coronado *et al.*, 2004; Grover *et al.*, 1986; Ratelle *et al.*, 2016) ou encore à l'efficacité d'équipements de protection individuelle (Krieger et Dinoff, 2000; Panuwet *et al.*, 2008).

Récemment, l'impact de facteurs tels que les tâches de travail sur les niveaux et la variabilité biologique de biomarqueurs d'exposition aux pyréthrinoïdes perméthrine et cyperméthrine a été évalué chez des travailleurs agricoles (Bouchard et al. 2016; Ratelle et al. 2016; Ferland et al. 2015). Cette recherche, a été, à notre connaissance, la première publiée sur le suivi biologique de l'exposition aux pesticides pyréthrinoïdes en milieu agricole au Québec. Les résultats ont montré que les profils temporels urinaires de biomarqueurs d'exposition à la perméthrine et à la cyperméthrine chez des agriculteurs de culture légumière au Québec variaient selon les tâches effectuées (épandage, inspection, cueillette, désherbage). Ils ont également soulevé la question de l'exposition multiple à plusieurs pesticides et l'impact que cette coexposition pourrait avoir sur l'interprétation des données de biosurveillance (Bouchard *et al.*, 2016; Khemiri *et al.*, 2017; Ratelle *et al.*, 2015a, 2015b). En effet, Ferland et al. 2015 ont observé chez certains travailleurs (superviseurs) des pics précoces du métabolite urinaire 3-PBA non compatible avec le temps passé dans le champ traité à la perméthrine. Aussi, plusieurs travailleurs exposés à la cypermethrine, avaient des concentrations urinaires du métabolite 3-PBA plus élevée que celle du trans-DCCA

(Ratelle *et al.*, 2016) alors que les données chez les volontaires obtenu en conditions contrôlées ont montré des niveaux similaires entre ces deux métabolites (Woollen et al. 1992 ; Ratelle et al. 2015). Ces constats suggèrent que les travailleurs ont probablement été coexposés à des pyréthrinoïdes précurseurs de 3-PBA utilisés dans des exploitations voisines ou à d'autres familles de pesticides pouvant moduler la cinétique du métabolite 3-PBA, largement utilisé dans le suivi biologique des pyréthrinoïdes. À l'heure actuelle, il manque de données visant à documenter l'influence de déterminants majeurs des niveaux de biomarqueurs d'exposition à des pesticides fréquemment utilisés en milieu agricole, incluant l'influence de la coexposition à plusieurs pesticides.

2.1.3. Données disponibles pour comprendre l'influence de la coexposition sur la cinétique des biomarqueurs d'expositions aux pesticides pyréthrinoïdes.

De façon globale, les données publiées sur l'impact de la coexposition sur le comportement biologique de pesticides et en particulier de leur toxicocinétique dans l'organisme sont très limitées. Elles proviennent essentiellement d'études *in vitro* ou *in vivo* chez l'animal. Quelques études *in vitro* sur des cultures cellulaires ont montré que la coexposition à des pesticides peut avoir un impact sur la vitesse de métabolisme des substances (Abass et Pelkonen 2013; Joo et al. 2007; Tang et al. 2002). D'autres études ont rapporté que les principales enzymes impliquées dans la biotransformation des pyréthrinoïdes, notamment les CYP450s (CYP1A1, 1A2, 2A1, 2B1, 2B2, 2E1, 3A1, 3A2, 3A4, 3A5, 4A1, 2C8, 2C9, 2C19), sont aussi impliqués dans la biotransformation de plusieurs autres pesticides employés de façon concomitante en agriculture (Scollon et al. 2009; Yang et al. 2009; Joo et al. 2010 ; Martínez et al. 2018). Une étude *in vivo* chez l'animal a aussi

montré que la coexposition à des insecticides organophosphorés en mélange binaire avait un impact sur leur vitesse d'absorption et d'élimination (Timchalk *et al.*, 2005).

Concernant les pyréthrinoïdes spécifiquement, seulement deux études animales ont été publiées. Ces deux études ont rapporté une diminution de l'excrétion des métabolites urinaires de pyréthrinoïdes chez des rats coexposés avec des insecticides organophosphorés (Hirosawa *et al.*, 2011; Wielgomas et Krechniak, 2007). Dans la première étude, des rats prétraités avec l'insecticide organophosphoré dichlorvos, à différentes doses (1,5 mg/kg ou 0,3 mg/kg), ont ensuite été exposés à une dose unique du pyréthrinoïdes cis-perméthrine (20 mg/kg). Une semaine après l'administration de la cis-perméthrine, les quantités totales du métabolite 3-PBA dans l'urine étaient significativement plus faibles dans le groupe prétraité à forte dose de dichlorvos comparativement au groupe témoin (Hirosawa *et al.*, 2011). Dans la seconde étude, des rats ont été exposés oralement, de façon répétée, sept jours/semaine pendant 28 jours, soit à une dose 10 mg/kg pc du pesticide organophosphoré chlorpyrifos ou du pyréthrinoïde α-cyperméthrine ou soit à 5 mg/kg de chacun de ces composés en mélange. Les auteurs ont rapporté une réduction de 30% de l'excrétion du métabolite 4-OH-3PBA dans le groupe des rats coexposés (exposé au mélange de pesticides) comparativement au groupe exposé uniquement à α-cyperméthrine (Wielgomas et Krechniak, 2007). La seule donnée humaine disponible concerne une étude chez des volontaires exposés oralement dans des conditions contrôlées au pyréthrinoïde deltaméthrine seul ou en mélange avec le chlorpyrifos-méthyle (Sams et Jones, 2011). À partir des profils temporels de 3-PBA dans l'urine, les auteurs ont rapporté une demi-vie d'élimination apparente en moyenne plus longue après administration en mélange (7,1 et 10,1 h, respectivement, pour l'exposition à la deltaméthrine seule et en mélange). Cette demi-vie d'élimination apparente étant déterminée à

partir de la pente d'attrition des niveaux urinaires, sa valeur dépend de plusieurs processus physiologiques, incluant le métabolisme, le stockage et le transfert du sang vers l'urine et les fèces. Toutes ces études ont donc rapporté une diminution de l'excrétion urinaire des biomarqueurs d'exposition aux pyréthrinoïdes chez des animaux ou des volontaires coexposés avec des insecticides organophosphorés. Cependant, les doses administrées étaient relativement élevées par rapport aux niveaux d'exposition des travailleurs et les scénarios d'exposition (coexposition pyréthrinoïdes/organophosphorés) étaient peu représentatifs du contexte d'exposition des travailleurs du Québec.

2.1.4. Déficits de connaissance identifiés et besoins cernés

En situation d'exposition réelle chez des travailleurs, il n'existe pas au Canada, ni ailleurs d'étude ayant évalué systématiquement l'impact de la coexposition à plusieurs pesticides sur le comportement cinétique de ces molécules dans l'organisme et donc sur le profil des biomarqueurs d'exposition. Il n'y a pas d'études non plus qui ont évalué l'impact de la coexposition chez les travailleurs sur la variabilité dans les niveaux biologiques, par rapport à d'autres facteurs. Tel que mentionné précédemment, afin de bien interpréter la signification d'une mesure de ces biomarqueurs d'exposition, il devient nécessaire de bien comprendre leur comportement toxicocinétique dans l'organisme (c'est-à-dire leur vitesse d'absorption, de distribution, de métabolisme et d'excrétion) et l'influence de paramètres comme la coexposition à d'autres pesticides.

Objectifs et hypothèses de recherche

L'objectif général de ce projet de recherche était d'évaluer l'impact de la coexposition sur les biomarqueurs d'exposition aux pesticides pyréthrinoïdes chez les travailleurs agricoles.

De façon plus spécifique, il s'agit de :

1) Documenter par un modèle animal, l'impact de la coexposition sur le devenir de biomarqueurs d'exposition aux pyréthrinoïdes dans des conditions expérimentales contrôlées (Article 1)

2) Étudier l'influence de la coexposition sur le profil temporel des biomarqueurs d'exposition aux pyréthrinoïdes dans un contexte semi-contrôlé (Article 2).

3) Vérifier, dans des conditions d'exposition réelles chez les travailleurs, si la coexposition est un déterminant majeur de la variabilité dans les niveaux de biomarqueurs d'exposition (Article 3).

Ce projet de recherche a pour première hypothèse que l'impact de la coexposition sur la cinétique des biomarqueurs d'exposition aux pyréthrinoïdes serait un déterminant majeur à considérer pour comprendre et mieux interpréter les mesures de surveillance biologique chez les travailleurs agricoles. Le projet a pour seconde hypothèse que les données recueillies pour des pesticides sentinelles seront extrapolables à d'autres pesticides et d'autres scénarios de coexposition.

CHAPITRE DEUXIÈME :

MÉTHODOLOGIE DE RECHERCHE

MÉTHODOLOGIE DE RECHERCHE

La méthodologie utilisée dans ce projet de recherche s'appuie sur les méthodes des projets passés portant sur le développement de biomarqueurs d'exposition aux pesticides pyréthrinoïdes et de modèles toxicocinétiques spécifiques à ces molécules. Le pyréthrinoïde lambda-cyhalothrine et le fongicide captane ont été choisis comme pesticides sentinelles pour étudier la coexposition. Ce projet de thèse de doctorat comprend trois volets. Chaque volet correspond à un des trois objectifs spécifiques susmentionnés et recourt à une méthodologie distincte. Ainsi, une étude expérimentale a été réalisée chez l'animal pour vérifier l'impact de la coexposition dans des conditions contrôlées, en comparant des rats exposés à la lambda-cyhalothrine seule ou en coexposition avec le captan à différentes doses. Ensuite, une étude cinétique des biomarqueurs d'exposition au pyréthrinoïde lambda-cyhalothrine chez des travailleurs potentiellement les plus exposés, suite à une pulvérisation de la lambda-cyhalothrine seule ou en coexposition avec le captan. Enfin, une étude transversale chez des travailleurs, dans leurs conditions de travail usuelles, pour étudier l'impact de la coexposition au captan par opposition à d'autres facteurs de variabilités pouvant moduler les niveaux de biomarqueurs d'exposition à la lambda-cyhalothrine.

2.1. Volet 1 - Expérimentation animale

À défaut de pouvoir contrôler certaines variables de l'étude sur terrain, le volet expérimental a permis de contrôler le plus possible de variables comme les conditions d'expositions. De plus, les animaux ont été exposés dans les mêmes conditions à des doses élevées afin de minimiser l'influence des facteurs alimentaires et environnementaux autres que la coexposition pouvant influencer le niveau urinaire des biomarqueurs d'exposition aux pyréthrinoïdes.

Les objectifs spécifiques de ce volet sont :

1) tester la coexposition au pyréthrinoide de type lambda-cyhalothrine (insecticide) et captan (fongicide) par voie orale chez des rats mâles;

2) documenter l'impact de la dose d'exposition sur le devenir de biomarqueurs d'exposition à lambda-cyhalothrine.

2.1.1. Traitement des animaux

L'ensemble de l'expérimentation est réalisé conformément aux recommandations de l'Organisation de Coopération et de Développement Économiques (guide OECD-417). Au total, 30 rats mâles Sprague-Dawley répartis au hasard en 6 groupes (n = 5 rats/groupe) ont été exposés par voie orale (gavage) à différentes doses de lambda-cyhalothrine (LCT) seule (2,5 mg/kg pc ou 12,5 mg/kg pc) ou en coexposition avec le captan selon les doses suivantes : i) 2,5 mg/kg pc + 12,5 mg/kg pc ($NOAEL_{LCT}$ + $NOAEL_{captan}$); ii : 2,5 mg/kg pc + 2,5 mg/kg pc ($NOAEL_{LCT}$ + dose massique équivalente de captan); iii : 12,5 mg/kg pc + 12,5 mg/kg pc ($LOAEL_{LCT}$ + $NOAEL_{captan}$). Pour les

deux pesticides d'intérêts, les doses d'expositions ont été ajustées après une étude préliminaire. Un groupe de rats témoins ont aussi été exposés au véhicule pour servir de contrôle à la voie d'administration. Afin de suivre dans le temps, les niveaux d'excrétion de chacun des biomarqueurs d'exposition et d'effectuer un bilan massique, les animaux ont été hébergés individuellement dans des cages métaboliques permettant d'effectuer des prélèvements d'urine et de fèces.

Les rats mâles de souche Sprague-Dawley ont été sélectionnés pour permettre la comparaison avec les données cinétiques disponibles dans la littérature (Anadon et al. 2005). La LCT a été choisie, car elle fait partie de la nouvelle génération de pyréthrinoïdes (encore peu étudiée) et est très largement utilisée. La combinaison avec le fongicide captan est justifiée par le fait que ces deux pesticides sont employés sur le même type de cultures, et font l'objet d'applications multiples par saison. De plus, selon la littérature, ces pesticides sont métabolisés par les mêmes enzymes, en particulier les cytochromes P450s (Paolini, 1999; Kolukisaoglu et al. 2009). Quant aux différents niveaux de doses de la LCT ciblés, ils correspondent aux doses critiques (NOAEL, LOAEL) déterminées dans des études subchroniques chez des rats (US EPA 2004). Finalement, la voie orale a été choisie, puisque l'ingestion par inadvertance serait une voie d'exposition principale chez les travailleurs (Côté et Bouchard, 2017).

2.1.2. Prélèvements biologiques

Des prélèvements d'urine et de fèces ont été réalisés sur chaque groupe de rats exposés (n= 5 par dose) au temps 0-24 heure avant exposition et à des intervalles de temps réguliers sur une période

de 48 heures soit : 0-3, 3-6, 6-9, 9-12, 12-24, 24-30, 30-48 h après traitements (8 temps). Tout de suite après les collectes, les volumes d'urine ont été mesurés à l'aide de cylindres gradués et les fèces pesées puis congelées à -20ºC.

Afin de suivre, dans le temps, les niveaux sanguins de biomarqueurs d'exposition, des prélèvements séquentiels de sang veineux (400 µl par temps de collecte) par la veine saphène (incision par la veine de la cuisse) au temps 12 h avant exposition puis à intervalles rapprochés durant les premières 30 h post-exposition, soit : 0.5, 1, 2, 4, 8, 12, 24, 30 heures (9 temps). Les échantillons sanguins ont été prélevés dans des « microtainers » contenant du K2-EDTA afin d'éviter la coagulation. Les échantillons de sang ont été ensuite centrifugés 15 min à 3700 rpm, 4ºC. Le surnageant (plasma) est transféré dans des tubes de 8 mL en verre et conservés à -20º C jusqu'au jour de l'analyse.

2.1.3 Sacrifice des rats et prélèvements biologiques

Au temps 48 h après exposition, les rats ont été anesthésiés au CO_2 puis sacrifiés par ponction cardiaque (correspondant au 10^{e} temps). Le sang total a été prélevé et transféré dans des tubes de prélèvements contenant du K2-EDTA. Ces échantillons de sang ont ensuite été centrifugés 15 min à 3700 rpm, 4ºC et le plasma est repris dans des tubes de 8 mL en verre.

Les rats ont été ensuite disséqués et les principaux organes (foie, tractus gastro-intestinal, reins, cerveau) ont été excisés, nettoyés avec une solution saline physiologique (excepté : TGI et cerveau) avant d'être pesés et conservés à -20°C jusqu'à l'analyse des biomarqueurs d'exposition à la

lambda-cyhalothrine. Les rats témoins étaient également sacrifiés aux mêmes temps que les rats traités.

2.1.4. Analyse du profil cinétique des biomarqueurs d'exposition chez le rat pour les matrices biologiques (plasma, fèces, urine)

Les niveaux de métabolites de la lambda-cyhalothrine soit: CFMP, 3-PBA et 4-OH-3-PBA, ont été mesurés dans le plasma, les tissus, les fèces et l'urine des rats. La méthode d'analyse des échantillons a été adaptée de celle déjà développée par notre équipe pour des biomarqueurs dans les matrices biologiques de rats et de volontaires exposés à lambda-cyhalothrine (Aouey *et al.*, 2017; Khemiri *et al.*, 2017). Les métabolites ont été analysés par chromatographie liquide à ultra haute performance couplée à la spectrométrie de masse à temps de vol quadripolaire (UHPLC/ Q-ToF MS).

2.1.5 Analyse des données

2.1.5.1. Analyse toxicocinétique des données

Les données collectées dans ce volet expérimentale ont permis d'établir le profil temporel des biomarqueurs d'exposition à LCT seule ou en coexposition avec le captan dans le plasma, les fèces et les urines.

À partir du profil des concentrations plasmatiques des biomarqueurs d'exposition à LCT en fonction du temps, plusieurs paramètres toxicocinétiques sont déterminés. Il s'agit notamment des temps de demi-vie d'éliminations ($t_{1/2}$), aire sous la courbe (AUC) des concentrations plasmatiques

des métabolites de LCT en fonction du temps, l'aire sous la première courbe du moment (AUMC), le temps moyen de résidence (MRT).

2.1.5.2 Analyse statistique

Les niveaux matriciels de biomarqueurs de même que les paramètres toxicocinétiques sont exprimés sous la forme de moyenne ± écart type. Les profils d'élimination urinaire des biomarqueurs ont été comparés entre les différents scénarios d'expositions. L'analyse des variances (ANOVA) a été utilisée pour déterminer les différences statistiquement significatives entre les différents groupes expérimentaux.

2.2. Volet 2 – Étude de l'impact de la coexposition sur la cinétique détaillée de biomarqueurs chez un groupe restreint de travailleurs

Dans le volet 2 du projet de thèse, une étude des profils urinaires détaillés de biomarqueurs d'exposition au pyréthrinoïde lambda-cyhalothrine a été réalisée chez un groupe de travailleurs, suivant un épisode d'exposition à la lambda-cyhalothrine seule ou en coexposition avec le fongicide captan.

Population, cultures et molécules cibles

Les personnes ciblées sont des travailleurs agricoles exposés à la lambda-cyhalothrine seule, ou encore, en coexposition avec le captan. Les travailleurs de la culture de fraises sont ciblés a priori,

car cette culture représente une production importante au Québec, implique un grand nombre de travailleurs et les pyréthrinoïdes et fongicides sont largement utilisés dans ces champs (MAPAQ 2015, 2016; ARLA 2018). D'autres cultures où ces produits sont appliqués de façon concomitante avait aussi été considérées, comme la culture de la patate, tomates et la pomiculture (ARLA 2018).

L'étude a ciblée principalement la région de la Montérégie en raison de son importance en tant que région agricole (MAPAQ 2015), mais d'autres régions, telles que l'Île d'Orléans, où la culture de fraises est également significative, ont été considérées. Les applicateurs ou pulvérisateurs, qui ont été identifiés comme présentant un risque accru dans des études antérieures menées par notre équipe de recherche (Bouchard *et al.*, 2016), ont été particulièrement visés.

Pour le recrutement des participants, une stratégie similaire à celle utilisée dans une étude antérieure chez des travailleurs exposés aux pesticides avait été utilisée (Bouchard et al. 2016). Avec l'aide des membres de notre comité de suivi, les responsables de fermes utilisant la lambda-cyhalothrine et le captan dans leur milieu de travail et intéressés à participer à notre étude étaient sollicités. Les producteurs agricoles ont notamment été contactés par téléphone, grâce à un accès au répertoire des producteurs maraîchers du Québec et plus spécifiquement des producteurs de fraises. Les travailleurs ont également été invités à participer lors de journées agricoles.

2.2.1. Collectes urinaires et suivi biologique de l'exposition

Dans ce volet 2 du projet, les profils temporels des métabolites urinaires de la lambda-cyhalothrine ont été évalués chez des applicateurs, selon trois scénarios d'exposition, qui ont été adaptés à la suite des discussions avec les producteurs et responsables agricoles. Ces scénarios tiennent compte du nombre d'applications permis, de chaque pesticide ciblé et des délais avant récolte (ARLA 2018).

Il avait été planifié de recruter un petit nombre de travailleurs (n = 5-7 comme objectif minimum, basé sur une étude précédente) et de documenter le profil détaillé de l'excrétion de biomarqueurs d'exposition suivant trois épisodes d'exposition préétablis à la lambda-cyhalothrine seule ou en coexposition. Pour l'épisode 1, la lambda-cyhalothrine était appliquée seule et les profils temporels de ses métabolites urinaires, utilisés comme biomarqueurs d'exposition, ont été établis chez les applicateurs sur une période de 3 jours après le début de l'application (considérant la vitesse d'élimination rapide de ce pesticide (Khemiri *et al.*, 2017, 2018). Pour l'épisode 2, le captan et la lambda-cyhalothrine était appliqués de façon concomitante et les profils temporels des biomarqueurs d'exposition à la lambda-cyhalothrine sont établis chez les applicateurs sur une période de 3 jours après le début de l'application. Pour l'épisode 3 (7 jours après l'épisode 2), correspondant au délai de 7 jours avant récolte, les profils temporels de biomarqueurs d'exposition seront documentés chez des cueilleurs affectés aux tâches dans la zone où le captan et la lambda-cyhalothrine auront été appliqués simultanément.

Pour chaque participant, toutes les urines durant les 3 jours suivant le début de la période d'application de la lambda-cyhalothrine seule ou en coexposition ou encore d'une cueillette dans une zone traitée avec le captan et la lambda-cyhalothrine ont collectées, dans des pots distincts, ainsi qu'une urine contrôle préexposition. Plus précisément, les travailleurs recrutés ont été invités à effectuer une collecte urinaire (première urine complète du matin) avant un épisode d'exposition professionnelle à la lambda-cyhalothrine seule ou en coexposition avec le captan afin d'établir le niveau de base d'exposition. L'urine était recueillie dans un seul contenant. Ensuite, toutes les mictions durant une période d'application ou encore de manipulation dans une zone traitée ont été collectées ainsi que toutes les urines durant les 3 jours suivant le début de cette période d'exposition. Chaque miction (ou collecte d'urine) de la journée était recueillie dans un contenant différent et clairement identifié (heure, individu). Ceci a permis de déterminer l'élimination des pyréthrinoïdes chez les travailleurs après une période d'exposition et l'impact de la coexposition sur les profils temporels, de même que les quantités excrétées. Chaque travailleur avait rempli une feuille de temps de collecte sur laquelle il indiquait la date et l'heure de la miction pour chaque contenant correspondant qui avait été préalablement identifié par un numéro. Les contenants ont ensuite été ramassés directement sur le lieu de travail.

Puisque ce protocole implique des mesures répétées chez les travailleurs et donc la possibilité d'environ 30 échantillons par individu (selon notre étude précédente (Bouchard *et al.*, 2016)) pour chacun des trois scénarios prévus par travailleur, il était planifié de recruter 5 à 7 travailleurs pour ce volet a priori, ce qui pourrait totaliser environ 600 échantillons d'urine de travailleurs. De plus, puisque les travailleurs agricoles sont majoritairement des latino-américains dans les plus grosses fermes et de québécois d'origine dans les petites fermes familiales, ces différentes origines

ethniques ont été prises en compte, en ce qui concerne la variabilité possible dû notamment au polymorphisme enzymatique.

Le CFMP (numéro CAS : 72748-35-7) et le 3-PBA (numéro CAS 3739-38-6), deux métabolites majeurs de la lambda-cyhalothrine excrétés dans l'urine ont été mesurés comme biomarqueurs d'exposition. Les échantillons d'urine ont été traités selon une méthode développée dans notre laboratoire (Khemiri *et al.*, 2017).

2.2.2. Questionnaires et observations sur le terrain

Les travailleurs ont été invités à remplir un questionnaire portant sur le type de travail effectué et les pratiques de travail ainsi que les habitudes de vie, afin de documenter les facteurs pouvant influencer les résultats. Il leur était également demandé de ne pas changer leurs pratiques de travail ou habitudes de vie lors de la réalisation de l'étude afin de minimiser les facteurs de variabilités. Le questionnaire était rédigé dans leur langue maternelle (française ou espagnole). Des renseignements personnels ont été recueillis (sexe, âge, origine ethnique, scolarité, état de santé général) ainsi que des informations sur les habitudes de vie (activité physique, usage domestique de produits à base de pesticides pyréthrinoïdes comme documenté à partir d'une liste préétablie, tabagisme ou exposition à la fumée secondaire, consommation d'alcool, de médicaments ou d'aliments pouvant contenir des résidus de pesticides). Des questions sur les conditions d'application des pesticides ciblés et les pratiques de travail étaien aussi incluses : formulation utilisée lors de l'application de l'ingrédient actif ciblé dans l'étude; moyen d'application; heures de début et de fin de quart de travail; temps consacré à la préparation, au mélange et à la

pulvérisation des pesticides ciblés dans le cas de l'application ou temps passé dans une zone traitée pour la cueillette; décontamination du matériel par les applicateurs; équipements de protection individuelle (survêtement en Tyvek, port ou non de gants, de masque…); hygiène personnelle après le travail (douche et lavage, changement ou manipulation des vêtements de travail). En plus de la coexposition au captan, la possibilité d'exposition concomitante à d'autres pesticides avait été également documentée. Le protocole initial prevoyait aussi une équipe présente sur le terrain pendant la collecte des échantillons d'urine, pour enregistrer en parallèlle les doses d'exposition aux pesticides, les concentrations de pesticides utilisées, l'ingrédient actif, le volume préparé et appliqué, la superficie de la zone traitée, ainsi que le nombre d'employés dans la ferme. Les questionnaires et les observations du terrain (lorsque réalisé) ont été utilisés pour recueillir des informations sur les facteurs personnels et les paramètres qui pourraient affecter les niveaux d'urine. L'équipe a documenté également les tâches et les pratiques de travail à l'aide d'un journal de bord, afin de comprendre les éléments pouvant influencer les résultats des échantillons d'urine.

2.2.3. Analyse des profils temporels

Les profils temporels des métabolites de la lambda-cyhalothrine ont été comparés selon les trois scénarios d'exposition. Les niveaux de métabolites ont été exprimées en concentrations ajustées par la créatinine (µmol/mol créat) pour tenir compte du degré de dilution variable des urines (Sarazin et al., 2022) ainsi qu'en excrétion cumulative (en pmol/kg pc). Pour chaque travailleur, les formes des profils temporels détaillés (vitesse d'augmentation et d'attrition dans les concentrations biologiques) et les quantités excrétées selon les trois scénarios, en absence ou en présence de coexposition ont servi à établir l'impact de la coexposition sur la cinétique urinaire des

biomarqueurs. Les autres facteurs pouvant influencer les niveaux urinaires et documentés par questionnaire et observation de terrain ont été également évalués. Puisque le nombre d'individus est faible dans ce volet 2 du projet, ces données ont été utilisées pour une évaluation au cas par cas (travailleur par travailleur).

2.3. Volet 3 – Étude de l'impact de la coexposition par rapport à d'autres facteurs sur la mesure de biomarqueurs chez des travailleurs

Dans le volet 3 du projet, une étude transversale de l'impact de la coexposition par rapport à d'autres facteurs sur la mesure de biomarqueurs chez des travailleurs a été réalisée. Ce volet implique un échantillonnage chez un plus grand nombre de travailleurs, par rapport au volet 2, mais avec moins de mesures biologiques par travailleur. Des modèles statistiques multivariés ont servi à évaluer la contribution de la coexposition par rapport à d'autres facteurs dans la variabilité dans les niveaux biologiques.

2.3.1. Population, cultures et molécules cibles

Le milieu de travail et la culture ciblés sont identiques au volet 2 du projet. Les travailleurs étaient évalués dans leurs conditions de travail usuelles. Contrairement au volet 2 qui ciblait surtout les applicateurs/pulverisateurs, il avait été planifié de recruter 60 travailleurs affectés à différentes tâches (application, cueillette, inspection), sur la base d'une étude précédente, en visant une homogénéité entre travailleurs exposés à la lambda-cyhalothrine seule *versus* coexposés au captan. Cette taille d'échantillon avait été ciblée en se basant sur l'effectif utilisé pour une étude précédente pour évaluer, par analyse statistique multivariée, l'impact de divers facteurs personnels et déterminants de l'exposition sur la cinétique de biomarqueurs d'exposition à la cyperméthrine chez des travailleurs de la culture maraichère (Ratelle *et al.*, 2016) .

2.3.2. Collectes urinaires et mesure de biomarqueurs d'exposition

Les travailleurs recrutés ont été invités à fournir une première urine complète du matin avant un épisode d'exposition afin d'établir les niveaux de base d'exposition. Ils ont fourni également deux collectes urinaires de 24 h consécutives à une des conditions suivantes : épisode d'application de lambda-cyhalothrine seule, en coexposition avec le captan ou après un travail dans les champs traités. Les concentrations de biomarqueurs d'exposition à la lambda-cyhalothrine, soit le CFMP et 3-PBA, ont été analysées dans les urines suivant la même méthode d'analyse du volet 2.

2.3.3. Questionnaires et observations sur le terrain

De la même façon que le volet 2, les déterminants potentiels des niveaux de biomarqueurs d'exposition, incluant la coexposition à la lambda-cyhalothrine et au captan, les tâches, les pratiques et l'hygiène de travail et les équipements de protection, ainsi que les facteurs de confusion potentiels (facteurs personnels, habitudes de vie) ont été documentés par questionnaire et par observations sur le terrain.

2.3.4. Analyse des données

L'impact de la coexposition sur les variations du niveau urinaire des biomarqueurs d'exposition a été établi à l'aide de modèles statistiques linéaires à effets mixtes (linear mixed effects models). Les niveaux de métabolites ont été exprimées en concentrations ajustées par la créatinine (µmol/mol créat) pour tenir compte du degré de dilution variable des urines (Sarazin et al., 2022).

Les déterminants potentiels de l'exposition documentés, incluant la tâche effectuée, certaines mesures d'hygiène et pratiques de travail, ont été ainsi considérés dans les modèles en plus du scenario d'exposition. Seules les variables prédictives contribuant à l'ajustement aux données avaient été retenues dans les modèles multivariés finaux (Zuur *et al.*, 2009).

Plus précisément, la variable « sujet » avait été retenue comme un effet aléatoire (random effect) et une structure de covariance à symétrie composée était considérée pour les mesures répétées (compound symmetry covariance structure among repeated measurements). Les niveaux de métabolites CFMP et 3-PBA exprimés en concentrations (µmol/mol créat.) étaient considérés comme variable dépendante dans les modèles.

Les déterminants potentiels des niveaux biologiques suivants ont été considérés : 1) la coexposition au captan; 2) tâche effectuée (application (incluant la préparation du mélange), cueillette ou desherbage); 3) le temps depuis le début de l'application de pesticide lambda-cyhalothrine seule ou en combinaison avec le captane (exprimée en variable dichotomique ≤ ou > 7 jours); 4) la taille de la ferme (exprimée en variable dichotomique ≤ ou > 10 employés). Les variables potentiellement confondantes qui ont été considérées dans les modèles étaient, l'âge (variable continue en années log-transformée), l'indice de masse corporelle (IMC en log-transformée), le tabagisme (oui/non), la consommation d'alcool (oui/non au cours de la période d'étude), la scolarité (primaire/secondaire ou collégial/universitaire), l'origine ethnique (caucasienne ou latine), la prise d'ibuprofène ou d'acétaminophène, la prise d'autres médicaments.

Les associations entre les déterminants potentiels d'exposition, tels que la coexposition ou les facteurs potentiellement confondants, et les biomarqueurs d'exposition ont été évaluées de manière approfondie. Initialement, des modèles univariés ont été utilisés, considérant chaque variable explicative individuellement. Ensuite, des modèles multivariés ont été construits en incluant toutes les variables, puis en les réduisant séquentiellement en utilisant le critère d'information d'Akaike (AIC) selon l'approche de (Zuur *et al.*, 2009). Seuls les facteurs prédictifs et variables de confusion contribuant à l'ajustement des modèles multivariés aux données ont été retenus dans les modèles finaux. Les analyses statistiques ont été réalisées avec SPSS plus (SPSS Inc., Chicago). Le niveau de signification statistique pour les modèles multivariés finaux était fixé à $p \leq 0,05$.

Ces renseignements ont permis ainsi de documenter certains déterminants des niveaux de biomarqueurs d'exposition observés, dont l'influence de la coexposition, ainsi que des facteurs de confusion potentiels, chez les travailleurs œuvrant dans leurs conditions de travail usuelles.

2.3.4. Considérations éthiques

Le volet 2 et 3 de ce projet repose sur une participation volontaire des individus. Les participants ont signé un formulaire de consentement libre et éclairé, après avoir reçu toute l'information nécessaire concernant le projet. Chaque participant était libre de se retirer à tout moment s'il le désirait, sans aucun préjudice. Le protocole d'étude, le formulaire de consentement, ainsi que les autres documents pertinents ont été approuvés par le comité d'éthique de la recherche de l'Université de Montréal. Aucune autre analyse que celles prévues n'a été réalisée sur les échantillons; l'anonymat des sujets a aussi été respecté par une codification des échantillons. Les

volontaires ont reçu une petite compensation financière pour le temps consacré et autres préjudices éventuels qui pourrait leur être causé.

CHAPITRE TROISIÈME :

PRÉSENTATION DES ARTICLE

ARTICLE 1

ARTICLE 1

Impact of pesticide coexposure: an experimental study with binary mixtures of lambda-cyhalothrin (LCT) and captan and its impact on the toxicokinetics of LCT biomarkers of exposure

Yélian Marc Bossou[a], Jonathan Côté[a], Marc Mantha[a], Sami Haddad[a], Sophie Achard[b], Michèle Bouchard[a,*]

[a] Department of Environmental and Occupational Health, Chair in Toxicological Risk Assessment and Management, and Public Health Research Center (CReSP), University of Montreal, Roger-Gaudry Building, U424, P.O. Box 6128, Main Station, Montreal, Quebec, Canada, H3C 3J7

[b] HERA team (Health Environment Risk Assessment), INSERM UMR1153-CRESS (Research Center in Epidemiology and StatisticS), Faculty of Health - Pharmacy, University of Paris, 4 Avenue de l'Observatoire, 75006 Paris, France.

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Abstract

This study aimed at gaining more insights into the impact of pesticide coexposure on the toxicokinetics of biomarkers of exposure. This was done by conducting an *in vivo* experimental case-study with binary mixtures of lambda-cyhalothrin (LCT) and captan and by assessing its impact on the kinetic profiles of LCT biomarkers of exposure. Groups of male Sprague-Dawley rats were exposed orally by gavage to LCT alone (2.5 or 12.5 mg/kg bw) or to a binary mixture of LCT and captan (2.5/2.5 or 2.5/12.5 or 12.5/12.5 mg/kg bw). In order to establish the temporal profiles of the main metabolites of LCT, serial blood samples were taken, and excreta (urine and feces) were collected at predetermined intervals up to 48 h post-dosing. Major LCT metabolites were quantified in these matrices: 3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-cyclopropane carboxylic (CFMP), 3-phenoxybenzoic acid (3-PBA), 4-hydroxy-3-phenoxybenzoic acid (4-OH3PBA). There was no clear effect of coexposure at the low LCT dose on the kinetics of CFMP and 3-PBA metabolites, based on the combined assessment of temporal profiles of these metabolites in plasma, urine and feces; however, plasma levels of 3-PBA were diminished in the coexposed high-dose groups. A significant effect of coexposure on the urinary excretion of 4-OH3PBA was also observed while fecal excretion was not affected. The temporal profiles of metabolites in plasma and in excreta were further influenced by the LCT dose. In addition, the study revealed kinetic differences between metabolites with a faster elimination of 3-PBA and 4-OH3BPA compared to CFMP. These results suggest that the pyrethroid metabolites CFMP and 3-PBA, mostly measured in biomonitoring studies, remain useful as biomarkers of exposure in mixtures, when pesticide exposure levels are below the reference values. However, the trend of coexposure effect observed in the benzyl metabolite pathway (in particular 4-OH3BPA) prompts further investigation.

Key words: Toxicokinetics; biomarkers; coexposure; pyrethroids; lambda-cyhalothrin; captan.

Résumé

Cette étude visait à mieux comprendre l'impact de la coexposition aux pesticides sur la toxicocinétique des biomarqueurs d'exposition. Pour ce faire, une étude de cas expérimentale *in vivo* a été réalisée avec des mélanges binaires de lambda-cyhalothrine (LCT) et de captane, et son impact sur les profils cinétiques des biomarqueurs d'exposition à la LCT a été évalué. Des groupes de rats mâles Sprague-Dawley ont été exposés par voie oral au LCT seul (2,5 ou 12,5 mg/kg p.c.) ou à un mélange binaire de LCT et de captane (2,5/2,5 ou 2,5/12,5 ou 12,5/12,5 mg/kg p.c.). Afin d'établir les profils temporels des principaux métabolites du LCT, des échantillons de sang ont été prélevés en série et les excréments (urine et fèces) ont été collectés à des intervalles prédéterminés jusqu'à 48 heures après l'administration de la dose. Les principaux métabolites du LCT ont été quantifiés dans ces matrices : 3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-diméthyl-cyclopropane carboxylique (CFMP), acide 3-phénoxybenzoïque (3-PBA), acide 4-hydroxy-3-phénoxybenzoïque (4-OH3PBA). La coexposition à la faible dose de LCT n'a pas eu d'influence clair sur la cinétique des métabolites CFMP et 3-PBA, d'après l'évaluation combinée des profils temporels de ces métabolites dans le plasma, l'urine et les fèces ; cependant, les niveaux plasmatiques de 3-PBA ont été diminués dans les groupes coexposés à la dose élevée. Un effet significatif de la coexposition sur l'excrétion urinaire du 4-OH3PBA a également été observé, tandis que l'excrétion fécale n'a pas été affectée. Les profils temporels des métabolites dans le plasma et dans les excréments ont également été influencés par la dose de LCT. En outre, l'étude a révélé des différences cinétiques entre les métabolites, avec une élimination plus rapide du 3-PBA et du 4-OH3BPA par rapport au CFMP. Ces résultats suggèrent que les métabolites des pyréthrinoïdes, CFMP et 3-PBA, principalement mesurés dans les études de biosurveillance, restent utiles comme biomarqueurs de l'exposition dans les mélanges, lorsque les niveaux d'exposition aux pesticides sont inférieurs aux

valeurs de référence. Toutefois, la tendance à l'effet de coexposition observée dans la voie des métabolites benzylés (en particulier le 4-OH3BPA) incite à poursuivre les recherches.

Mots-clés : Toxicocinétique, biomarqueurs, coexposition, pyréthrinoïdes, lambda-cyhalothrine, captane.

INTRODUCTION

Insecticides belonging to the pyrethroid family are widely used worldwide because of their broad spectrum of action (Katsuda, 2012; Matsuo, 2019; 2016). The most largely used pyrethroids include permethrin, cypermethrin, deltamethrin, lambda-cyhalothrin (LCT) (Matsuo, 2019). Pyrethroids represent more than a quarter of global insecticide use and are integrated in agricultural, residential and public health programs to control pests (Aznar-Alemany et Eljarrat, 2020). Although pyrethroid insecticides are not persistent in the environment, their frequent use contributes to maintain background levels in the human body as shown by measurements of metabolites in urine and blood samples collected from various populations (Barr *et al.*, 2010b; Channa *et al.*, 2012; Choi *et al.*, 2017; Li *et al.*, 2020; Valcke *et al.*, 2020; Wielgomas *et al.*, 2013; Wielgomas et Piskunowicz, 2013). In addition to this background exposure, agricultural workers are also exposed in the workplace, making their levels of exposure higher than those of the general population (Ratelle, Mylène *et al.*, 2016). In these environments, workers are repeatedly or simultaneously exposed to several chemical pesticides.

Numerous efforts have been made in recent years to assess and take into account interactions that could exist between pesticides in coexposure situations. Toxicodynamic and metabolic interactions following exposure to mixtures of pesticides containing pyrethroids were previously studied. The type of interaction varied depending on the molecules and the mechanisms of action. Additive type interactions were evidenced in *in vivo* experiments *i*) looking at motor activity in rodents acutely exposed to mixtures of several pyrethroids (Starr *et al.*, 2014; Starr *et al.*, 2012; Wolansky *et al.*, 2009) and *ii*) assessing biochemical markers of cytotoxicity in serum of subchronically exposed rodents pyrethroids (cypermethrin) in combination with imidazole and benzimidazole fungicides

(imazalil, carbendazime) (Dikic *et al.*, 2012). In *in vitro* experiments, additive interactions on oestrogen/androgen receptor activity and aromatase activity were also documented when exposing human cell lines to cypermethrin pyrethroid in combination with triazine and triazole fungicides as well as an organophosphorus (OP) insecticide (terbuthylazine, bitertanol, propiconazole, malathion) (Kjeldsen *et al.*, 2013). Another *in vitro* study reported synergistic inhibition of acetylcholinesterase activity following mixed exposure to pyrethroids (deltamethrin and cypermethrin) in combination with OPs (triazophos, malathion, chlorpyrifos) (Arora *et al.*, 2017). An *in vivo* study in rodents repeatedly exposed to deltamethrin pyrethroid in combination with thiacloprid also showed a synergistic effect on thyroid hormone levels (Sekeroglu *et al.*, 2014). Metabolism studies further showed that CYP 450-actived OPs decreased the ability to detoxify pyrethroids due to inhibition of esterase activity, leading to potentiation effects (Hernández *et al.*, 2013).

Coexposure to various pesticides can also have an impact on the toxicokinetics of the parent compounds and their metabolites. An *in vivo* study in rats has shown that coexposure to OP insecticides in binary mixture had an impact on their rate of absorption and elimination (Timchalk *et al.*, 2005b). With regard to coexposure with pyrethroids more specifically, only a limited number of *in vivo* rodent studies have compared the toxicokinetics of pyrethroid metabolites after exposure to the compound in mixture and alone (Hirosawa *et al.*, 2011b; Wielgomas, B et Krechniak, J, 2007). The latter studies relate to permethrin and cypermethrin exposures, and report a decrease in the excretion of urinary pyrethroid metabolites in rats after coexposure with OP insecticides (Hirosawa *et al.*, 2011b; Wielgomas, B et Krechniak, J, 2007).

Some *in vitro* studies on cell cultures have further shown that coexposure to pesticides can have an impact on the rate of metabolism of substances (Abass, Khaled et Pelkonen, Olavi, 2013; Joo *et al.*, 2007b; Tang *et al.*, 2002b). Other *in vitro* studies have reported that the main enzymes involved in the biotransformation of pyrethroids, in particular CYP450s (CYP1A1, 1A2, 2A1, 2B1, 2B2, 2E1, 3A1, 3A2, 3A4, 3A5, 4A1, 2C8, 2C9, 2C19), are also implicated in the biotransformation of several other pesticides used concomitantly in agriculture (Joo *et al.*, 2007b; Martinez *et al.*, 2018b; Scollon, Edward J *et al.*, 2009; Yang, Dongfang *et al.*, 2009). In particular, pyrethroids and phthalimides such as captan are metabolized by common CYP450 enzymes, namely CYP3A, CYP1A1, CYP1A2, CYP2A1, CYP2B1 in the liver (Paolini *et al.*, 1999a). Phthalimide inhibition of these liver enzymes was noted in this latter study.

The objective of the current study was to gain more insights into the potential impact of pesticide coexposure on the toxicokinetics of biomarkers of exposure. This was done by conducting an *in vivo* experiment assessing the toxicokinetics of LCT biomarkers of exposure in rats exposed to binary mixtures of LCT and captan compared to LCT alone. LCT was chosen as a key pyrethroid largely used in agriculture and captan because it is used abundantly and concomitantly on crops. The focus was placed on the metabolites of LCT used as biomarkers of exposure in biomonitoring studies, 3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-cyclopropane carboxylic (CFMP), 3-phenoxybenzoic acid (3-PBA) and 4-hydroxy-3-phenoxybenzoic acid (4-OH3PBA) (Anadon *et al.*, 2006; Khemiri *et al.*, 2017a, 2018a; Schettgen, Thomas *et al.*, 2016). The chosen LCT doses were based on the No-Observed Adverse Effect Level (NOAEL) of 2.5 mg/kg bw/day and Lowest-Observed Adverse Effect Level (LOAEL) of 12.5 mg/kg bw/day reported by the EPA (2004b) and established from a two-year chronic feeding/carcinogenicity study in rats (Alpk/AP strain) exposed to cyhalothrin and an observed decrease in body weight and food consumption. In an acute

neurotoxicity study in Wistar rats orally exposed to LCT at doses of 2.5, 10 or 35 mg/kg bw, the NOAEL for LCT was reported to be the same as that of cyhalothrin, based on clinical signs of neurotoxicity at highest doses and increased breathing rate at the intermediate dose (EFSA, 2014). To prevent adverse effects that may be related to acute and chronic ingestion of pesticides in the general population, acute and chronic reference doses (RfDs) of 0.0025 and 0.001 mg/kg bw/day, respectively, were established for LCT based on a chronic neurotoxicity study in dogs exposed orally (NOEL 0.25 and 0.1 mg/kg bw, respectively, divided by an uncertainty factor of 100) (EPA, 2004b). These NOEL were not retained in our study given they are based on a dog study rather than a rat study. A RfD has also been derived for captan fungicide. It was established at 0.1 mg/kg bw/day in a three-generation reproduction study in rats based on a NOAEL of 12.5 mg/kg bw to which a safety factor of 100 was applied (EPA, 2004a).

MATERIELS AND METHODS

Chemicals and reagents

Lambda-cyhalothrin (98.7% purity) and captan (99.2% purity) were purchased from Sigma Aldrich (Saint Louis, USA). Reference standards of 3-PBA, 4-OH3BPA and the internal standards of $^{13}C_2$ 1D *trans*-DCCA and $^{13}C_6$-3-PBA (97–98% purity) were obtained from Cambridge Laboratories Inc. (Andover, MA, USA); reference standard of CFMP (> 95% purity) was acquired from ArkPharm. MS grade methanol (MeOH) was purchased from Honeywell. Glacial HPLC grade acetic acid, ethyl acetate and sodium acetate were obtained from Fisher Scientific (Ottawa, ON, Canada). β-Glucuronidase/arylsulfatase (100,000 Fishman U/mL and 800,000 Roy U/mL from Helix Pomatia) was obtained from Roche Diagnostics (Laval, Quebec, Canada).

Animal acclimation, food and housing

Male Sprague-Dawley rats (10 weeks old, 275 ± 50 g each) were purchased from Charles River Canada (St-Constant, Quebec, Canada). Before the experiment, animals were kept in plastic cages in groups of two to three rats and had free access to food (*Teklad Global Diets*® #2018 from Envigo, Canada) and tap water *ad libitum*. Prior to dosing, rats were acclimatized to the metabolic cages designed for separate urine from feces. The individual acclimatization of the rats in metabolic cages was done over three days in a gradual manner, *i.e.* for 1, 2 and 4 h per day on days -3, -2 and -1 before the experiment, respectively. Twelve hours before the experiment, rats were individually housed in stainless steel metabolic cages designed to separate urine from feces, and they were

supplied with drinking water containing glucose (D-Glucose: 40 g/L) to induce a physiologic polyuria allowing for frequent urine collections.

Immediately after exposure, rats were put back in their respective metabolic cage for the duration of the experiment. They had continued access to water with glucose for the first 12 h post-exposure and then to tap water for the rest of the experiment. In addition, rats had access to food *ad libitum* throughout the experiment period. Lighting was maintained on a 12-h light-dark cycle; the ambient temperature was maintained at 22 ± 3°C and humidity values ranged from 40 to 70%. The experiment was carried out in accordance with OECD Guideline 417. The protocols were approved by the Ethics Committee for Animal Experimentation of the University of Montreal (CDEA approval #17-044).

Throughout the experimental period, animals were observed at least once a day for clinical signs of treatment-related toxicity. The body weight (bw) of each animal, the mass of food and the volume of water consumed were monitored and recorded daily.

Animal treatment

Thirty Sprague-Dawley rats were randomly divided into six groups of five rats each (n = 5 rats per group). Two groups of rats were exposed orally (by gavage) to a single dose of LCT alone (2.5 or 12.5 mg/kg bw) (corresponding to groups G1 and G3, respectively). These doses are equivalent to the No-Observed Adverse Effect Level (NOAEL) and the Lowest Observed Adverse Effect Level (LOAEL) for LCT, respectively (EPA, 2004b). Three other groups were each exposed by gavage to a binary mixture of LCT and captan prepared as follows: [$NOAEL_{LCT}$ + equivalent mass dose

of captan]: 2.5 mg/kg bw + 2.5 mg/kg bw; [$NOAEL_{LCT}$ + $NOAEL_{captan}$]: 2.5 mg/kg bw + 12.5 mg/kg bw; [$LOAEL_{LCT}$ + $NOAEL_{captan}$]: 12.5 mg/kg bw + 12.5 mg/kg bw (corresponding to G2, G4 and G5, respectively). The pesticides were dissolved in biological olive oil (*Irresistible brand)*. Ten milliliters of solution were administered per kilogram of body weight for all doses. A control group of five rats was exposed only to the vehicle used for administration. Prior to this experiment, a pilot study was conducted to verify that the lowest dose of LCT administered to rats was enough to allow quantification of metabolites.

To prepare LCT dosing solutions, appropriate mass of LCT was weighed and premixed in olive oil. For the mixture of LCT and captan solution, each pesticide was weighed separately, mixed together in the same flask, and dissolved in a single volume of olive oil. All dose solutions were sonicated thrice successively over periods of 10 min until dissolution. The solutions were also vortexed during the preparation and just before preparing the syringes for gavage. All dose solutions were prepared the day before administration and were stored in a dark container at room temperature until usage.

Biological sampling

Blood sampling and isolation of plasma

Blood samples of approximately 400 µL per sampling were collected by the saphenous vein 20 h prior to exposure and sequentially at 0.5, 1, 2, 4, 8, 12, 24, 30 h after exposure. At 48 h postexposure, rats were anesthetized with CO_2, then sacrificed by cardiac puncture and whole blood was withdrawn. Blood samples were collected in microtainers or tubes containing K2-EDTA

to prevent coagulation. Samples were kept on ice until the plasma was isolated. To separate plasma from red blood cells, blood samples were centrifuged for 15 min at 1500 g, 4°C. The plasma supernatant were transferred into clearly identified glass tubes serving for extraction of the metabolites and stored at -20°C until analysis. Remaining plasma was pooled and then aliquots were prepared to serve as positive controls.

Collection of urine and feces

Urine and fecal samples were collected from each rat at predetermined intervals over a 48 h period, *i.e.* 0-3, 3-6, 6-9, 9-12, 12-24, 24-30 and 30-48 h after treatment. Control samples were collected during the 12 h before exposure. Once collected, urine volumes were measured using graduated cylinders, and aliquots were prepared in polypropylene Sarstedt tubes; feces were weighed and transferred in glass tubes. Samples were frozen at -20°C until analysis.

Tissue sampling

At the time of sacrifice, main organs were excised, including brain, liver, kidneys and gastrointestinal tract (GI). Tissues (except GI) were rinsed with physiological saline solution (to remove blood), blotted dry before being weighed, transferred in vials and stored at -20°C until analysis. Control rats were also sacrificed at the same time as the treated rats.

Sample treatment and analysis

Before extraction, all samples were thawed. All matrices were subjected to an enzymatic hydrolysis to obtain the sum of free and conjugated metabolites of LCT. Hydrolysis was followed by solid phase extraction (SPE) or liquid-liquid extraction before analysis by ultra-high-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UHPLC-MS-Q-ToF).

Plasma samples (an exact measurement of 150 or 200 µL depending on available volumes and treated directly in the preservation tubes) were spiked with 200 pmol of internal standard mix ($^{13}C_2$ 1D-*trans*-DCCA and $^{13}C_6$-3-PBA). Enzymatic hydrolysis was carried out by adding 200 µL of sodium acetate buffer (0.1 M, pH 5.0), 4 µL of β-glucuronidase/arylsulfatase enzyme (100,000 Fishman U/ml and 800,000 Roy U/ml from Helix pomatia) and leaving samples for 16 h in a shaking bath at 37°C. Analytes were then extracted twice with 3 mL of ethyl acetate saturated in water after centrifugation for 20 min at 1500 g, 4°C. The organic extracts were evaporated under a gentle nitrogen flow, in a water bath at 40°C. Residues were dissolved in 250 µL of MeOH (100%). Samples were transferred to Eppendorf microtubes, centrifuged for 60 s at 604 g and transferred to HPLC vials for UHPLC-MS-Q-ToF analysis.

Urine samples (1 mL) were spiked with 200 pmol of internal standard mix ($^{13}C_2$ 1D-*trans*-DCCA and $^{13}C_6$-3-PBA). A volume of 1 mL of sodium acetate buffer (0.1 M, pH 5.0) and 20 µL of β-glucuronidase/arylsulfatase were added to allow hydrolysis for 16 h in a shaking bath at 37°C. Samples were subjected to solid phase extraction (SPE) with SEP-PAK C18 cartridges (Waters, Milford, MA, USA). Cartridges were conditioned with 4 mL of MeOH and 8 mL of water; samples were loaded on the cartridges, which were then cleaned with 8 mL of water; analytes were eluted

from the cartridges with 8 mL of methanol into 10 mL glass tubes. The organic extracts were evaporated until dryness under a gentle nitrogen flow in a water bath at 40°C. Residues were dissolved in 1 mL of MeOH. Samples were centrifuged 60 s at 604 g to remove remaining solid residues. The methanolic extract was then transferred into HPLC vials for analysis by UHPLC-MS-Q-ToF.

Tissue and fecal samples were individually homogenized using a polytron after adding sodium acetate buffer solution (0.1 M pH 5.0) so as to have a concentration of 500 mg liver, 250 mg kidney, 400 mg brain, 500 mg GI and 250 mg feces in 4 mL. The samples were then spiked with 200 pmol of internal standard mix ($^{13}C_2$ 1D-*trans*-DCCA and $^{13}C_6$-3-PBA). Liver samples were heated in a water bath at 90°C for 5 min and then cooled at room temperature. Enzymatic hydrolysis was performed by adding 20 µL of β-glucuronidase/arylsulfatase and shaking in a water bath for 16 h, at 37°C. The analytes were then extracted with ethyl acetate as described for plasma. After evaporation of the organic extracts, the residues were resuspended in 1 mL of MeOH.

UHPLC-MS-Q-ToF analysis of CFMP, 4-OH3PBA and 3-PBA was performed exactly with the conditions described in Khemiri et al. (2017). The analytical limit of detection (LOD) was 0.6 and 1.2 pmol/mL of methanolic plasma extract for CFMP and 3-PBA metabolites, respectively. The LODs were 3.9, 0.7 and 2.3 pmol/mL of methanolic urinary extract for CFMP, 3-PBA and 4-OH3PBA, respectively. Depending on the tissue, the LODs varied between 0.4 and 2 pmol/mL of methanolic tissue extract for CFMP between 0.5 and 3 pmol/mL for 3-PBA and 4-OH3PBA.

Data analysis

The time courses of CFMP, 3-PBA and 4-OH3BPA in plasma, urine and feces were reported as a molar percentage of the administered dose. From plasma concentration (C) - time profiles after oral exposure, toxicokinetic parameters were calculated and include the discrete version of the area under the concentration-time curve (AUC), the area under the first moment of concentration-time curve (AUMC), the mean residence time (MRT), the apparent global elimination rate (k_{elim}) constant and elimination half-life (t1/2), with the values for the terminal elimination phase (Gibaldi et Perrier, 1982; Hayes, 2007). Comparison of toxicokinetic parameter values between groups (G1, G2, G3, G4, G5) was performed by analysis of variance (ANOVA) followed by parametric Bonferroni post tests for comparison between two groups. A value of $p < 0.05$ was considered significant (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

RESULTS

Effect of coexposure on the time courses of lambda-cyhalothrin metabolites in plasma

Figures 1A and 1B respectively depict the temporal profiles of CFMP and 3-PBA in the plasma of rats over a 48-h period following ingestion of 2.5 or 12.5 mg LCT/kg bw alone or as a binary mixture with 2.5 or 12.5 mg captan/kg bw. A significant increase in concentrations compared to controls, for which values were below the LOD, was observed in all cases. The plasma profile of CFMP and 3-PBA in rats exposed to the low dose of 2.5 mg LCT/kg bw alone (G1) was similar to that observed in rats exposed to the binary mixtures of this low LCT dose in combination with both the low and high doses of 2.5 and 12.5 mg captan/kg bw (G2 and G4, respectively); this suggests the absence of interaction at this NOAEL dose of LCT (Fig. 1). However, for the high dose groups (12.5 mg/kg bw of LCT alone or in combination with this equivalent mass dose of captan), plasma levels of 3-PBA at time < 24 h were higher in rats exposed to LCT alone (G3) compared to those exposed to the binary mixture (G5); this suggests a possible effect of coexposure on the benzyl metabolite pathway at that dose. More evidently, Figure 1 shows an apparent effect of the LCT dose (2.5 versus 12.5 mg/kg bw) on the temporal profiles of CFMP and 3-PBA ([G1, G2, G4] vs [G3, G5]).

The basic toxicokinetic parameters calculated from the plasma profiles of CFMP and 3-PBA for the groups of rats exposed to LCT alone at the low dose (G1 of 2.5 mg LCT/kg bw) or in binary mixture with captan (at 2.5 or 12.5 mg captan/kg bw; G2 and G4, respectively) confirmed the absence of coexposure effect at these doses; the various mean parameter values were not

significantly different between groups (G1 vs G2 vs G4) (Table 1). For the groups receiving the high dose of 12.5 mg LCT/kg bw alone (G3) or in combination with the equivalent massic dose of captan (G5), it was not possible to determine the appropriate toxicokinetic parameters and clearly observe elimination, because some animals showed clinical signs of toxicity. These include feeding cessation, diarrhea, jumping in cage, tremor and mobility impairment. Therefore, these groups of animals were sacrificed at 24 or 30 h after exposure to avoid animal suffering.

Comparison of the kinetic time courses of CFMP and 3-PBA in plasma

Regardless of the exposure group (G1, Fig. 2A; G2, Fig. 2B; G4, Fig. 2C), the comparison of the kinetic time courses of CFMP and 3-PBA in plasma also shows a difference in the kinetics of the two metabolites, with a faster elimination of 3-PBA compared to CFMP. Table 1 highlights that the maximum residence time (MRT) and apparent global plasma elimination half-life ($t_{1/2}$ derived from k_{elim}) was close to two times higher for CFMP compared to 3-PBA, confirming a longer residence time of CFMP in plasma. 4-OH3PBA levels in plasma were not detectable in blood with the developed method.

Effect of captan coexposure on the urinary and fecal excretion of lambda-cyhalothrin metabolites

Figure 3 and Table 2 show that there was no clear effect of coexposure with captan (at 2.5 and 12.5 mg/kg bw) on the total percentage of dose recovered as CFMP and 3-PBA in urine over the 0-24 or 0-48 h post-treatment (*i.e.* G1 vs G2 vs G4 or G3 vs G5). The total percentage of dose excreted

as 3-PBA in 0-24 h and 0-48 h urine collections after administration of the binary mixture of 2.5 mg LCT/kg bw LCT and 2.5 mg captan/kg bw (G2) was significantly higher than corresponding values observed in the groups of rats exposed to 2.5 mg LCT/kg bw alone (G1). However, this does not clearly indicate a coexposure effect given that urinary excretion values in rats exposed to the low LCT dose alone (G1) were not significantly different from those observed in rats exposed to the binary mixture of 2.5 mg LCT/kg bw and 12.5 mg captan/kg bw (G4). This is further supported by the lack of significant difference in urinary excretion values between rats exposed to the high LCT dose of 12.5 mg/kw bw alone (G3) and to the binary mixture of 12.5 mg LCT/kg bw and 12.5 mg captan/kg bw (G5). There was also no clear effect of the LCT dose (2.5 versus 12.5 mg/kg bw) on the total percentage of CFMP and 3-PBA recovered in urine.

Conversely, there was a significant difference in the total percentage of dose recovered as 4-OH3BPA in urine over the 0-24 or 0-48 h post-treatment between the different exposure groups (*i.e.* G1 vs G2 vs G4 or G3 vs G5) with higher values in coexposure groups than groups exposed to LCT alone (G1 vs G4 and G3 vs G5 in 0-24 h urine samples; G1 vs G2 and G1 vs G4 in 0-48 h urine samples) (Table 2). There was also a significant effect of the LCT dose (2.5 versus 12.5 mg/kg bw) on the total percentage of 4-OH3PBA recovered in urine ([G1, G2, G4] vs [G3, G5] except for G1 vs G5 and G2 vs G5).

Figure 4 and Table 2 show that, when comparing exposure groups, there was no significant effect of coexposure with captan (at 2.5 and 12.5 mg/kg bw) on the total percentage of dose recovered as CFMP, 3-PBA or 4-OH3PBA in feces over the 0-24 or 0-48 h (*i.e.* G1 vs G2 vs G4 or G3 vs G5). However, there was a non-significant lower percentage of dose recovered as CFMP, 3-PBA and 4-OH3BPA in feces at the higher dose compared to the lower dose (G1 of 2.5 mg LCT/kg bw vs G3

of 12.5 mg LCT/kg bw; G1 of 2.5 mg LCT/kg bw vs G5 of 12.5 mg LCT/kg bw + 12.5 mg captan/kg bw).

Similar to plasma results, the urinary and fecal excretion (Figs. 3 and 4 and Table 2) also highlighted differences in the elimination rate of CFMP compared to 3-PBA and 4-OH3BPA. The time courses of CFMP in urine and feces clearly show that elimination was incomplete 48 h after exposure (for groups G1, G2 and G4, which were not sacrificed earlier). Conversely, excretion of 3-PBA and 4-OH3PBA appeared almost complete at that time period. Combined urinary and fecal results further indicate that excreted CFMP and 3-PBA only represented a small percentage of the administered dose with urinary levels being higher than fecal levels for both metabolites (at the group level, up to 8% on average for CFMP in urine and 2% for CFMP in feces; up to 5.6% on average for 3-PBA in urine and 0.15% for 3-PBA in feces) and that 4-OH3PBA was a major metabolite excreted in the urine of rats (representing up to 46% of dose on average in urine and 0.5% of dose in feces).

Tissue residues of lambda-cyhalothrin metabolites

The residual amounts of CFMP, 3-PBA and 4-OH3PBA remaining in tissues of rats 48 h (30 h for the high dose groups) after oral administration of LCT alone or as a binary mixture with captan are presented in Table 3, and show no marked differences between groups (although values were too close to the limit of detection to allow statistical comparison between groups). For all dose groups, CFMP, 3-PBA and 4-OH3PBA levels in tissues were in the following descending order: gastrointestinal tract (GI) > liver > kidneys > brain. The slower elimination of CFMP as compared to 3-PBA was also evident from the assessment of GI levels at 48 h post-dosing (for rats sacrificed

at 48 h (G1, G2, G4), up to 2.6% of dose on average was found as CFMP versus 0.007% as 3-PBA).

DISCUSSION

Effect of coexposure on the time courses of the biomarkers of exposure

This study assessed the effect of pesticide coexposure on the toxicokinetics of LCT biomarkers of exposure, using an experimental *in vivo* case-study with binary mixtures of LCT and captan. The *a priori* hypothesis was that captan could interfere with CYP450 metabolism pathway of pyrethroids (Paolini *et al.*, 1999a) or excretion mechanisms. Our study showed that when the dose of LCT tested corresponded to the NOAEL, the time profiles of the metabolites CFMP and 3-PBA in plasma were not affected by coadministration of captan. Our data are in line with those of a recent study that assessed the toxicokinetics of 17 pesticides largely measured in biomonitoring studies and their major metabolites in the plasma of female Long-Evans rats following a single oral dose of the complex mixture (Chata *et al.*, 2019). In the latter study, eight families of pesticides were measured, including anilines, carbamates, carboxamides, organochlorines, organophosphates, oxadiazines, phenylpyrazoles and pyrethroids (LCT, permethrin, cypermethrin); captan was not included in the mixture. Metabolites assessed were CFMP and 3-PBA. The average terminal elimination half-life of CFMP estimated in plasma by these authors and the MRT were similar to those obtained in the current study (respective mean $t_{1/2}$ of 11.0 versus 9.2-12.1 h and MRT of 19.0 versus 21.6 - 24.1 h). Although cypermethrin and permethrin were present in the mixture in addition to LCT in the study of Chata *et al.* (2019), both of which form 3-PBA metabolites, the mean terminal elimination half-life of 3-BPA in plasma and the MRT were also similar to those reported in our study, both in the groups of rats exposed to LCT alone or as a mixture with captan (respective mean $t_{1/2}$ of 7.0 versus 7.4 - 7.9 h and MRT of 10.0 versus 11.3 - 12.5 h). This suggests that our results may be valid for low-dose complex mixtures. Chata *et*

al. (2019) also concluded that metabolic enzymes were not saturated at their 0.4 mg/kg bw dose of the mixture.

Nonetheless, at the high LOAEL dose of LCT of 12.5 mg/kg bw and high dose of captan in our study, corresponding to its NOAEL of 12.5 mg/kw bw, a possible effect of coexposure on the benzyl metabolite pathway leading to 3-PBA formation could not be excluded. More specifically, plasma levels of 3-PBA at time < 24 h were higher in rats exposed to the high dose of LCT alone (G3) compared to those exposed to the binary mixture of LCT and captan (G5). However, there was no clear effect of coexposure with captan (at 2.5 and 12.5 mg/kg bw) on the total percentage of dose recovered as CFMP or 3-PBA in urine or feces over the 0-24 or 0-48 h period post-dosing. On the other hand, a significant effect of coexposure on 4-OH3BPA excretion in urine, but not in feces was observed at both the low and high doses. A potential effect of coexposure was reported in the available animal studies on permethrin and cypermethrin pyrethroids at high doses in binary mixture with the organophosphorus insecticides dichlorvos and chlorpyrifos (Hirosawa *et al.*, 2011b; Wielgomas, B et Krechniak, J, 2007). In the study of Hirosawa et al. (2011), male Wistar rats pretreated intraperitonealy with the organophosphorus insecticide dichlorvos, at a low or high dose (0.3 or 1.5 mg/kg bw), were then intravenously injected with a single high dose of the pyrethroid cis-permethrin (20 mg/kg bw). The time course of 3-PBA in the urine over a 48-h period in rats pretreated with the high-dose of dichlorvos showed a significantly lower excretion compared to the non-preteated control group (Hirosawa *et al.*, 2011b). In the study of Wielgomas and Krechniak (2007), rats were repeatedly exposed by gavage, seven days/week for 28 days, either to 10 mg/kg bw of the organophosphorus pesticide chlorpyrifos or to the pyrethroid α-cypermethrin or to 5 mg/kg bw of each of these compounds as a mixture. The authors reported a 30% reduction in the excretion of the metabolite 4-OH3BPA in the group of coexposed rats (exposed to the

pesticide mixture) compared to the group exposed only to α-cypermethrin (Wielgomas, B et Krechniak, J, 2007). In our study, the effect of coexposure on 4-OH3BPA in urine and plasma results at the high LCT and captan doses prompts further assessment of the potential impact of coexposure on the benzyl metabolite pathway.

In humans, the only controlled data available on the impact of coexposure on the kinetics of pyrethroids and their metabolites concerns a study in volunteers exposed orally to deltamethrin alone at the Acceptable daily intake (ADI) level of 0.01 mg/kg bw or in mixture with the organophosphorus insecticide chlorpyrifos-methyl at an equivalent massic dose (Sams et Jones, 2011a). From the temporal profiles of 3-(2,2-dibromovinyl)-2,2-dimethyl-(1-cyclopropane)carboxylic acid (DVBA) and 3-PBA metabolites of deltamethrin in urine, the authors reported that there was no statistically significant difference in the apparent excretion half-life and cumulative excretion in urine during the 0-24 h period following mixed exposure as compared to administration of deltamethrin alone (on average 7.1 and 10.4 h, respectively, for exposure with deltamethrin alone and as a mixture). This apparent elimination half-life was determined from the attrition slope of urinary levels; its value thus depends on several physiological processes, including metabolism, storage and transfer of blood to urine and feces. Overall, similar to our rat results on the benzyl pathway, but with a different coexposure scenario (pyrethroid/organophosphorus coexposure) from the one studied in the current work, published high dose studies in animals indicate an effect of coexposure (Hirosawa *et al.*, 2011b; Wielgomas, B et Krechniak, J, 2007) while the low dose in humans did not concur to reveal such mixture effect (Sams et Jones, 2011a).

Comparison of the kinetic time courses of metabolites in plasma

For all exposure scenarios, the current study further showed a difference in the kinetic time courses of CFMP and 3-PBA, with a slower elimination of CFMP from the body compared to 3-PBA. This was confirmed by the longer residence time (MRT) of CFMP in plasma compared to 3-PBA and the incomplete urinary and fecal excretion of CFMP 48 h after exposure (lack of asymptote) contrary to 3-PBA. Upon repeated exposure, CFMP is thus more likely to accumulate in the body; it appears to be the case when comparing CFMP and 3-PBA levels in the plasma on days 7, 30, 45 and 60 in Wistar rats subchronically exposed to a daily oral dose of 6.2 and 31.1 mg LCT /kg bw (Aouey, B. *et al.*, 2019). In the latter study, a dose-dependent increase in levels of LCT metabolites in plasma was also observed following repeated dosing in rats while there were little variations in levels from week to week for a given dose, suggesting that steady-state equilibrium was rapidly reached within the first week of exposure for both metabolites. CFMP was also found in higher concentrations than 3-PBA in liver of the rats subchronically exposed to a daily oral dose of 6.2 and 31.1 mg LCT/kg bw (Aouey, Bakhta *et al.*, 2017). However, given that Wistar rats were exposed in the latter studies (Aouey, Bakhta *et al.*, 2017; Aouey, B. *et al.*, 2019) while Sprague-Dawley rats were used in the current work, differences in metabolite formation and kinetics between strains of rats cannot be excluded (Kishida *et al.*, 2008; Saito *et al.*, 2004).

On the other hand, in volunteers exposed to LCT by the oral route, Khemiri *et al.* (2017a) showed similar time courses for CFMP and 3-PBA in plasma, with respective average peak values at 3.1 and 4.0 h post-dosing and mean elimination half-lives of about 5.3 and 6.4 h. Urinary rate time courses were also similar to the plasma concentration-time curves. This highlights species differences in the kinetics of LCT and its metabolites. Moreover, in the assessed volunteers of the

study of Khemiri *et al.* (2017a), on average 21% of LCT dose were excreted as CFMP in urine in the 84-h period post-treatment as compared to 30% as 3-PBA. In the rats of the current study, cumulative excretion of CFMP and 3-PBA in urine respectively amounted to up to 8 and 5.6% on average and corresponding values in feces were 2% and 0.15%; 4-OH3PBA in urine represented a much higher percent of the dose (on average 18 to 48% in the groups exposed to the low LCT dose alone or in mixture with captan) while its fecal excretion was limited (0.5%).

Our results are in line with the unpublished toxicokinetic studies reviewed in the European Commission (EC) report (2014), and conducted by Syngenta according to the OECD guidelines 417 in rats. In a toxicokinetic study in male and female Han Wistar rats exposed orally to a single dose of 1 or 12.5 mg/kg bw of cyclopropyl- or phenoxy- ^{14}C-labeled LCT, oral absorption of labeled LCT was estimated between 7 and 23%. Labeled LCT was recovered mainly in feces (on average 93-95% by 4-days postdosing of 1 and 12.5 mg/kg bw of cyclopropyl-^{14}C-LCT; on average 83-90% by 4-days postdosing of 1 and 12.5 mg/kg bw of phenoxy-^{14}C-LCT) and mostly in the unchanged parent compound form. Urinary levels of ^{14}C-equivalents represented on average 0.8 to 1.5% and 5.8 to 9.7% of the cyclopropyl-^{14}C and phenoxy-^{14}C LCT dose, respectively. In the EC report, it is further indicated that the fraction of LCT dose absorbed in the body was completely metabolized and eliminated mainly *via* the kidney in the tested animals, that metabolites were excreted mainly as conjugates in urine and bile of rats, and that 4-OH3PBA was the main excreted metabolite (up to 9.3% of dose found in urine and bile). They further reported that there were differences in the kinetics between animals and humans, although the main metabolism steps appear the same (cleavage of the ester bond of LCT and biotransformation products). In rats, 3-PBA was found mainly conjugated to sulfates (EC, 2014) while it is considered to be mainly conjugated to glucuronides in humans (Khemiri *et al.*, 2017a; Marsh *et al.*, 1994). Oral absorption

also appeared much higher in humans based on CFMP excretion in urine (Khemiri *et al.*, 2017a; Marsh *et al.*, 1994) when compared to results of this study and rats and those reported in EC (2014).

Effect of dose on the time courses of metabolites

In our study, although there was no clear effect of coexposure on the kinetics of CFMP and 3-PBA in plasma and excreta (urine and feces), the plasma time courses of CFMP and 3-PBA observed revealed an effect of the LCT dose. This was most evident for 3-PBA with a higher retention in plasma at the LOAEL dose of 12.5 mg LCT /kg bw compared to the NOAEL dose of 2.5 mg/kg bw. While there was no clear effect of the dose on the cumulative percentage of CFMP and 3-PBA recovered in urine, a non-significantly lower cumulative percentage of dose was recovered as CFMP and 3-PBA in feces. A significant effect of the LCT dose (2.5 versus 12.5 mg/kg bw) on the total percentage of 4-OH3PBA recovered in urine was also observed and there was a non-significant trend on the fecal excretion.

Similar to our findings, in the unpublished study conducted by Syngenta and reported in EC (2014), after administration of phenoxy-^{14}C LCT in Wistar Han rats, somewhat lower average cumulative urinary excretion values of ^{14}C equivalents were found both in male and female at the high dose of 12.5 mg/kg bw (5.8 and 6.7%, respectively) compared to the low dose of 1 mg/kg bw (9.7 and 8.1%, respectively). This was also apparent in bile with cumulative excretion values representing on average 3.3% in both male and female rats at the high dose compared to 6.3 and 12 % of dose in males and females, respectively at the low dose. Such difference in urinary and biliary excretion was not found after administration of cyclopropyl-^{14}C LCT.

Other studies on pyrethroid metabolites failed to show an effect of dose on their toxicokinetic parameters. In a study looking at the effect of dose on the kinetics of transfluthrin metabolites, Yoshida (2014) reported that values of the fraction of metabolites excreted in urine, the mean residence time in body (MRT) and half-life period of the urinary excretion rate ($t_{1/2}$) were dose-independent for each metabolite. In studies on the kinetics of deltamethrin in Sprague-Dawley rats, Mortuza *et al.* (2018) also showed that plasma toxicokinetic parameters of deltamethrin (MRT, elimination half-life $t_{1/2}$ from plasma, clearance Cl, oral absorption rate constant k_{abs}, bioavailability F) were independent of the dose, and maximum concentration C_{max} and area under the plasma concentration time-course (AUC) values were directly proportional to the dose.

Furthermore, in the current study, the LOAEL dose of LCT administered alone or as a mixture with captan caused toxicity in some of the rats (tremor, impairment of locomotion), such that they had to be euthanatized. The toxicity thus appeared to be related to LCT administration. Similarly, in an experiment in male Sprague-Dawley rats acutely exposed by gavage to 10 or 20 mg/kg bw of LCT (dissolved in corn oil), Weiner *et al.* (2009) observed signs of toxicities 4 h after exposure (*i.e.* the time of peak effect) at both doses. The authors reported a disappearance of the signs of toxicity in the surviving subjects 24 h after treatment.

CONCLUSION

Our study showed that exposure to LCT alone at the NOAEL dose or as a binary mixture with captan, which are metabolized by common enzymes (including CYP450), did not alter the toxicokinetics of the metabolites CFMP and 3-PBA commonly used in biomonitoring studies to assess exposure to LCT. These findings confirm the usefulness of these pyrethroid metabolite measurements to assess absorbed doses in biomonitoring studies in individuals exposed to low levels of pesticide mixtures. However, the trend of coexposure effect observed for 3-PBA at the LOAEL dose of LCT and for 4-OH3BPA prompts further investigation. The impact of coexposure on the toxicokinetics of LCT biomarkers of exposure is nonetheless limited to binary mixtures rather than complex mixtures, and high doses that may not be relevant to humans; there is also evident biological variability that may render interpretation more difficult. The method for the measurement of 4-OH3BPA in plasma should also be optimized in order to further document the effect of coexposure on the benzyl pathway leading to the formation of this metabolite. Future perspectives include verifying coexposure effect in potentially highly exposed individuals, such as workers.

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CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

CAPTIONS TO FIGURES

Fig 1. Effect of lambda-cyhalothrin (LCT) coexposure with captan on the time courses of CFMP (A) and 3-PBA (B) in the plasma of rats (expressed as a molar percentage of the administered dose) following oral exposure. ●: G1 = administration of LCT alone (2.5 mg/kg bw); ■: G2 = coadministration of LCT + captan (2.5 mg/kg bw each); ▲: G3 = administration of LCT alone (12.5 mg/kg bw); ×: G4 = coadministration of LCT + captan (2.5 mg/kg bw and 12.5 mg/kg bw respectively); ♦: G5 = coadministration of LCT + captan (12.5 mg/kg bw each). Symbols represent mean values and bars are standard deviations.

Fig 2. Comparison of the kinetic time course of CFMP (▲) and 3-PBA (●) in plasma (expressed as pmol/mL/kg bw) in rats following oral administration of LCT alone at a dose of 2.5 mg/kg bw (A) or in combination with captan at a dose of 2.5 mg/kg bw (B) or 12.5 mg/kg bw (C). Symbols represent mean values and bars are standard deviations.

Fig. 3. Effect of captan coexposure on the cumulative excretion time course of lambda-cyhalothrin biomarkers of exposure, CFMP (A), 3-PBA (B) and 4-OH3PBA (C), in rat urine (expressed as a molar percentage of the administered dose) following oral administration. ●: G1 = administration of LCT alone (2.5 mg/kg bw); ■: G2 = coadministration of LCT + captan (2.5 mg/kg bw each); ▲: G3 = administration of LCT alone (12.5 mg/kg bw); ×: G4 = coadministration of LCT + captan (2.5 mg/kg bw and 12.5 mg/kg bw, respectively); ♦: G5 = coadministration of LCT + captan (12.5 mg/kg bw each). Symbols represent mean values and bars are standard deviations.

Fig 4. Effect of captan coexposure on the cumulative excretion time course of lambda-cyhalothrin biomarkers of exposure, CFMP (A), 3-PBA (B) and 4-OH3PBA (C), in rat feces (expressed as a molar percentage of the administered dose/h/kg bw) following oral exposure. ●: G1 = administration of LCT alone (2.5 mg/kg bw); ■: G2 = coadministration of LCT + captan (2.5 mg/kg bw each); ▲: G3 = administration of LCT alone (12.5 mg/kg bw); ×: G4 = coadministration of LCT + captan (2.5 mg/kg bw and 12.5 mg/kg bw, respectively); ♦: G5 = coadministration of LCT + captan (12.5 mg/kg bw each). Symbols represent mean values and bars are standard deviations.

Fig. 1.

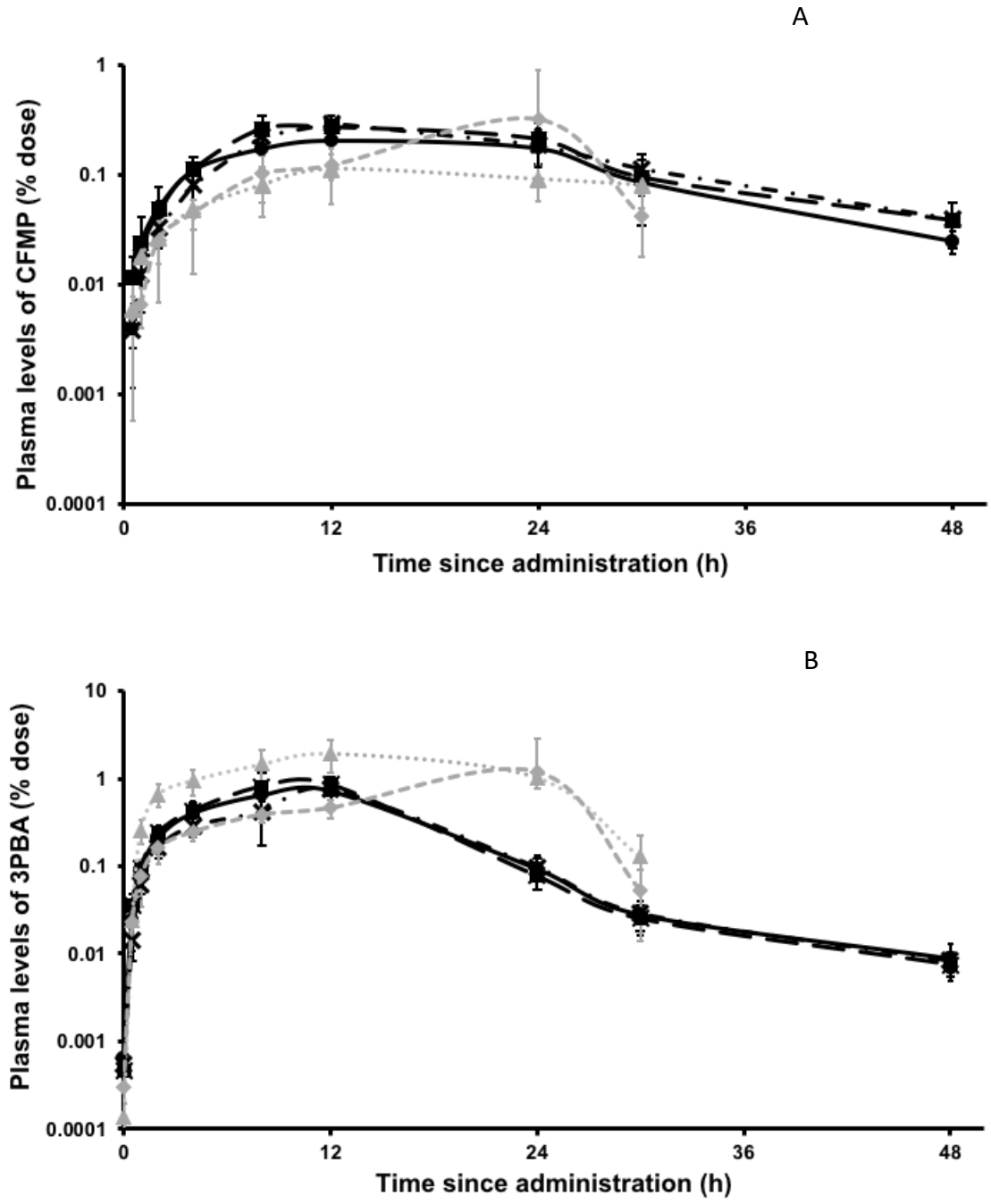
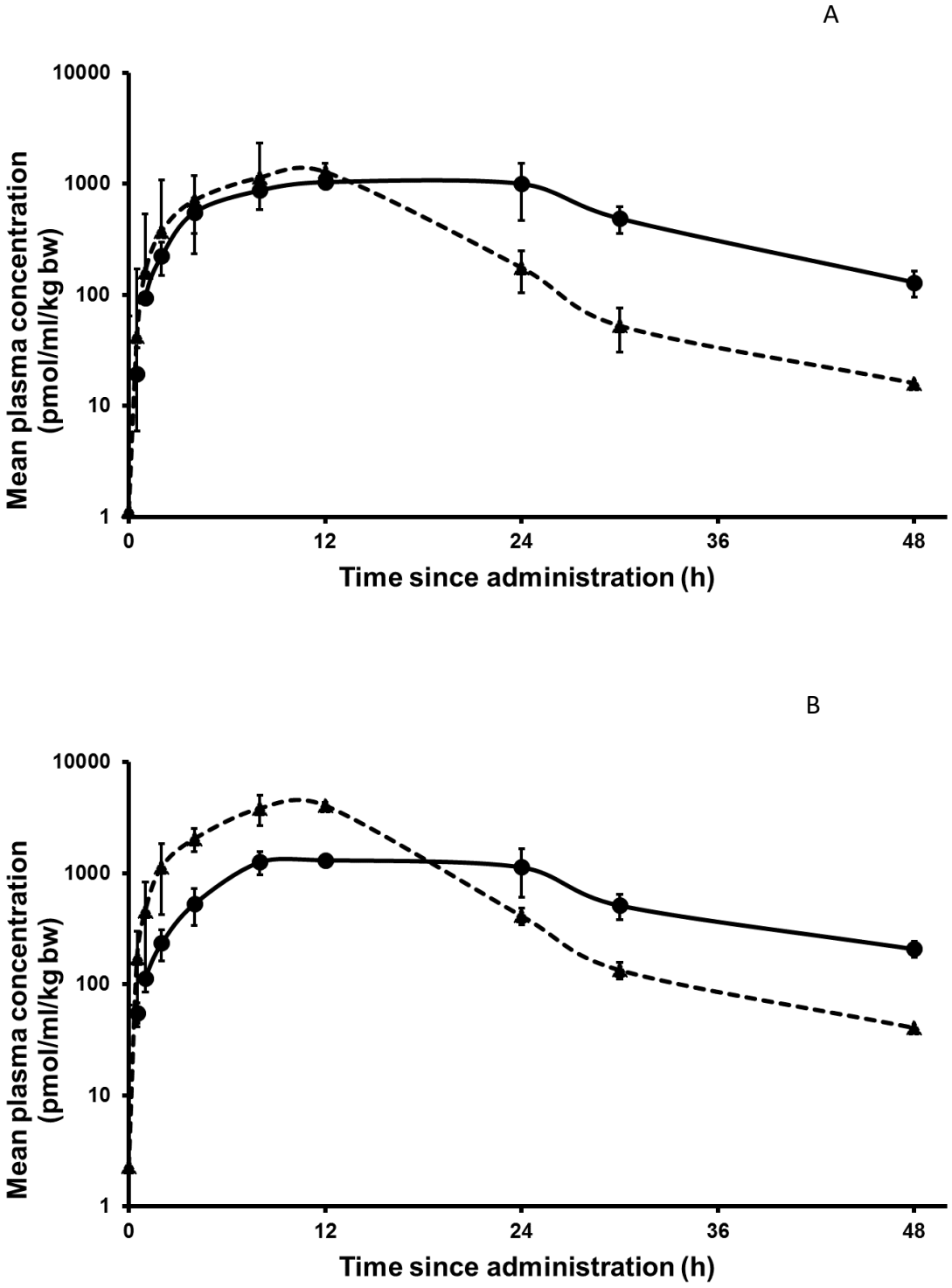

A
Plasma levels of CFMP (% dose)
1
0.1
0.01
0.001
0.0001
0
12
24
36
48
Time since administration (h)
B
Plasma levels of 3PBA (% dose)
10
1
0.1
0.01
0.001
0.0001
0
12
24
36
48
Time since administration (h)

Fig. 2.


A
10000
1000
100
10
1
Mean plasma concentration (pmol/ml/kg bw)
0
12
24
36
48
Time since administration (h)
B
10000
1000
100
10
1
Mean plasma concentration (pmol/ml/kg bw)
0
12
24
36
48
Time since administration (h)

C

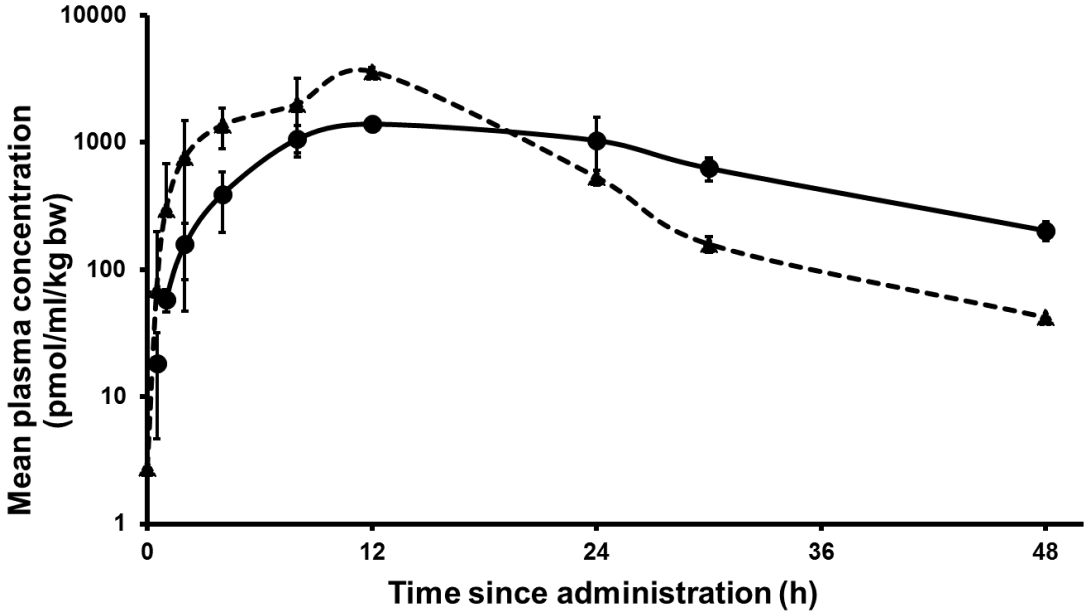
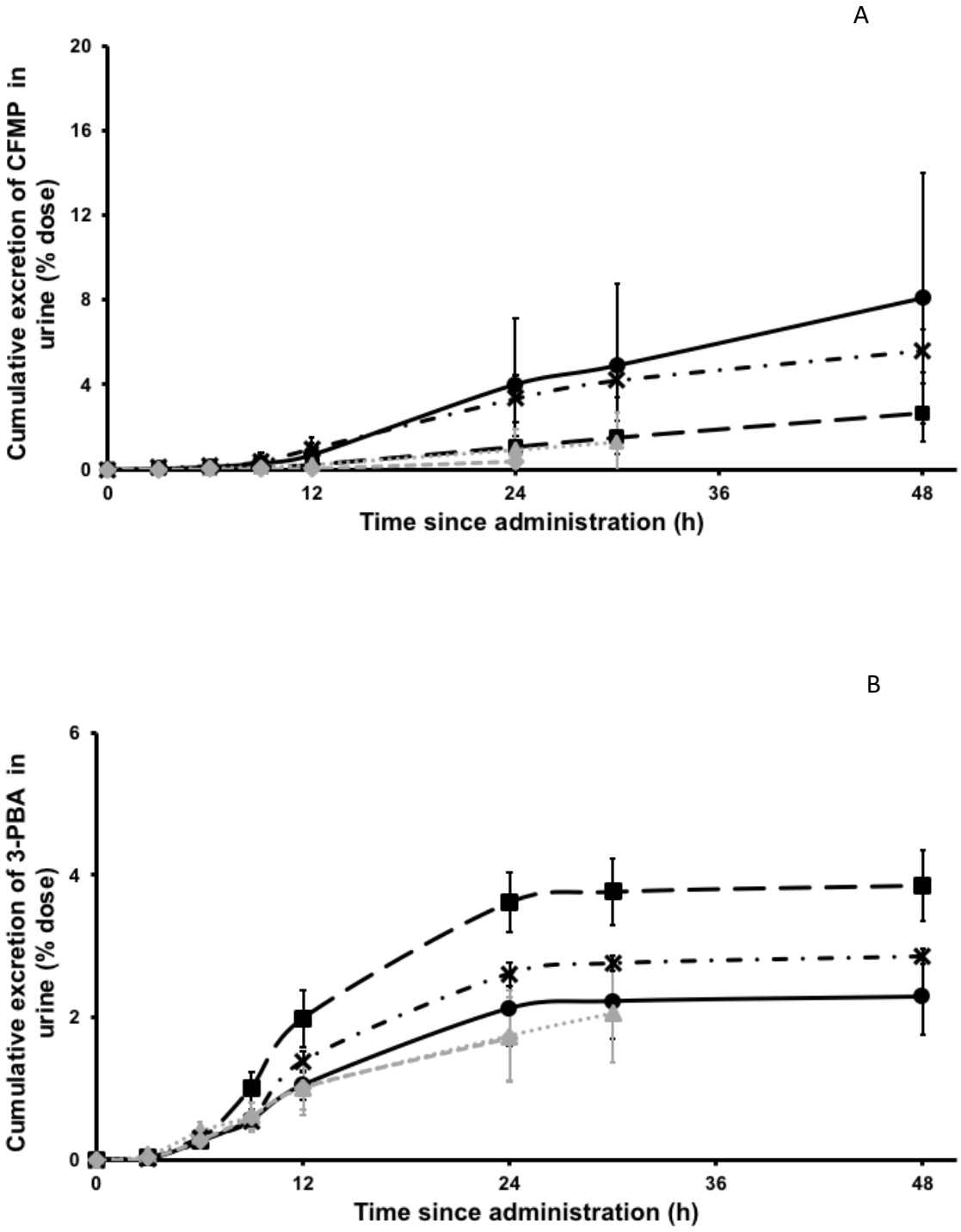

10000
1000
100
10
1
Mean plasma concentration (pmol/ml/kg bw)
0
12
24
36
48
Time since administration (h)

Fig 3.


A
Cumulative excretion of CFMP in urine (% dose)
20
16
12
8
4
0
0
12
24
36
48
Time since administration (h)
B
Cumulative excretion of 3-PBA in urine (% dose)
6
4
2
0
0
12
24
36
48
Time since administration (h)

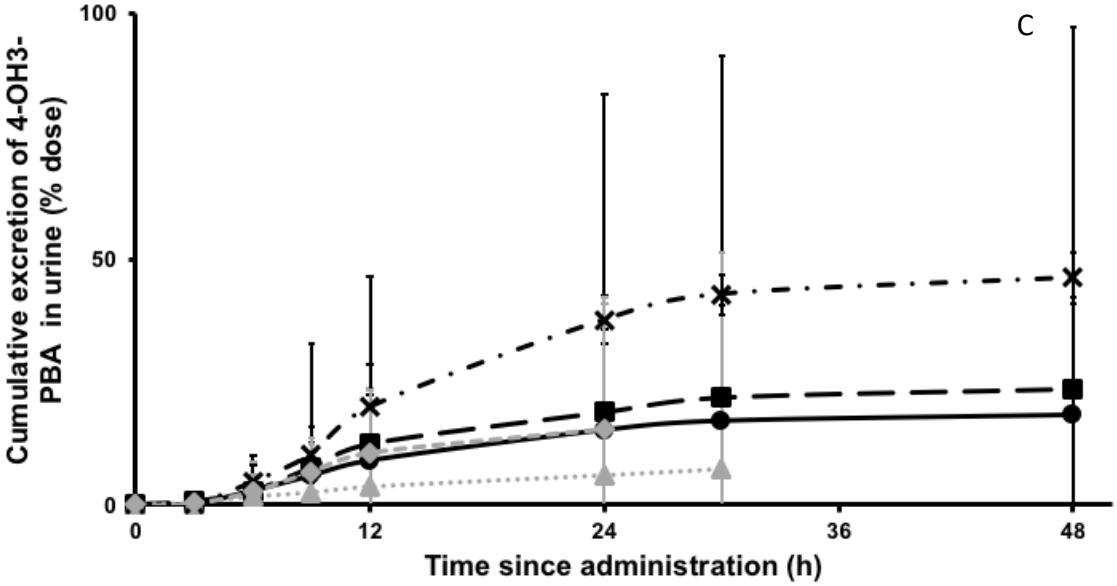
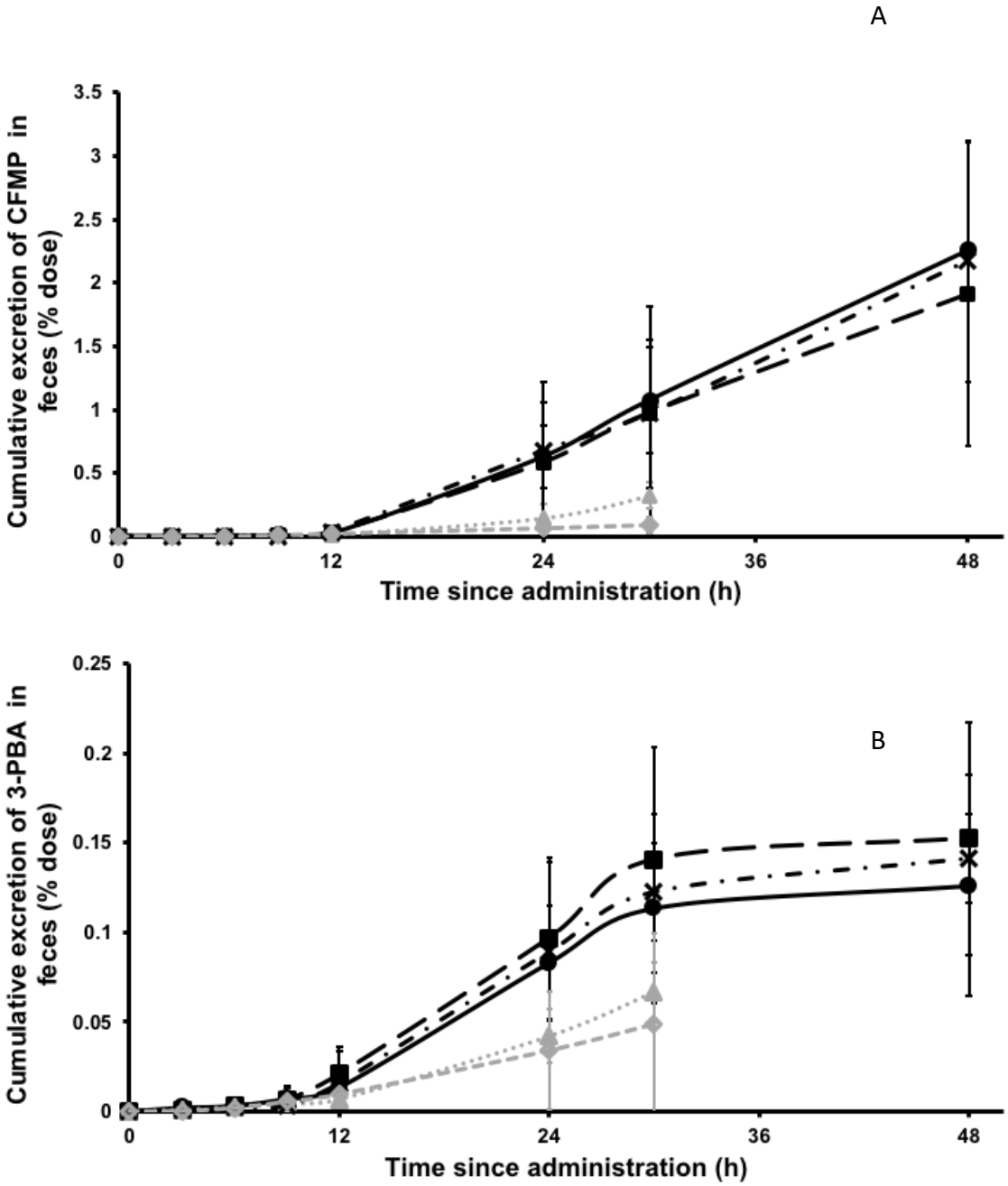

C
Cumulative excretion of 4-OH3-PBA in urine (% dose)
100
50
0
0
12
24
36
48
Time since administration (h)

Fig. 4.


A
Cumulative excretion of CFMP in feces (% dose)
3.5
3
2.5
2
1.5
1
0.5
0
0
12
24
36
48
Time since administration (h)
B
Cumulative excretion of 3-PBA in feces (% dose)
0.25
0.2
0.15
0.1
0.05
0
0
12
24
36
48
Time since administration (h)

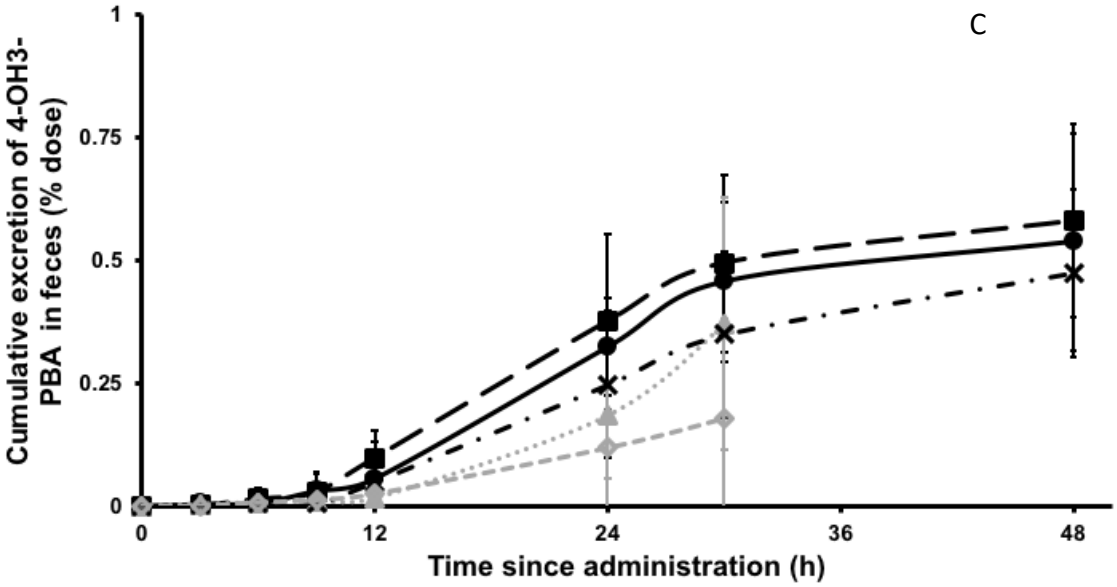

C
Cumulative excretion of 4-OH3-PBA in feces (% dose)
1
0.75
0.5
0.25
0
0
12
24
36
48
Time since administration (h)

Table 1.

Toxicokinetic parameters (mean ± SD) calculated from concentration-time profiles of CFMP and 3-PBA in rats orally exposed to lambda-cyhalothrin alone at a dose of 2.5 mg/kg bw (G1) or as a binary mixture with captan at a dose of 2.5 mg/kg bw or 12.5 mg/kg bw (G2 and G4, respectively).

Parameters		**Exposure group**		
		G1	**G2**	**G4**
AUC (pmol*h/mL/kg bw) (mean ± SD)	AUC_{CFMP}	31521 ± 8001	39623 ± 7207	38976 ± 8496
	AUC_{3PBA}	57706 ± 14103	62623 ± 12530	49779 ± 3352
AUMC (pmol*h²/mL/kg bw) (mean ± SD)	$AUMC_{CFMP}$	682069 ± 185665	940364 ± 269169	946770 ± 303468
	$AUMC_{3PBA}$	680147 ± 180193	706438 ± 128529	623723 ± 67225
MRT (h) (mean ± SD)	MRT_{CFMP}	21.6 ± 1.2	23.5 ± 3.3	24.1 ± 3.6
	MRT_{3PBA}	11.8 ± 0.6	11.3 ± 0.7	12.5 ± 0.8
Overall apparent plasma k_{elim} (h^{-1})[a] (mean ± SD)	k_{elim_CFMP}	0.046 ± 0.003	0.04 ± 0.01	0.04 ± 0.01
	k_{elim_3PBA}	0.085 ± 0.004	0.09 ± 0.01	0.08 ± 0.01
Overall apparent plasma elimination half-life (h) (mean ± SD) [a]	$t_{1/2\ CFMP}$	15.0 ± 0.8	16.3 ± 2.3	16.7 ± 2.5
	$t_{1/2\ \ 3PBA}$	8.1 ± 0.4	7.9 ± 0.5	8.7 ± 0.6
Apparent terminal elimination k_β (h^{-1}) (mean ± SD)	$k_{\beta\ CFMP}$	0.08 ± 0.01	0.06 ± 0.02	0.06 ± 0.02
	$k_{\beta\ 3PBA}$	0.09 ± 0.02	0.09 ± 0.01	0.09 ± 0.01
Apparent terminal elimination half-life (h) (mean ± SD)	$t_{1/2\beta\ CFMP}$	9.2 ± 1.4	12.1 ± 5.1	12.0 ± 4.4
	$t_{1/2\beta\ 3PBA}$	7.9 ± 1.4	7.8 ± 0.9	7.4 ± 0.7

Note: ANOVA group comparison not significant (G1 vs G2 vs G4).

[a] An overall elimination rate and half-life were calculated although it is apparent that elimination is biphasic.

Table 2.

Cumulative excretion of CFMP, 3-PBA and 4-OH3PBA in urine and feces expressed as percentage of administered lambda-cyhalothrin dose.

Matrix	Time interval post-dosing (h)	Metabolite	Cumulative excretion (mean ± SD % dose) Exposure group				
			G1	**G2**	**G3**	**G4**	**G5**
Urine	0 - 24	CFMP*	**4.02 ± 3.15^{e}**	1.08 ± 0.55	0.93 ± 0.98	3.35 ± 1.10	**0.37 ± 0.34^{a}**
		3-PBA***	**2.13 ± 0.51^{b}**	**3.62 ± 0.42^{ace}**	**1.74 ± 0.65^{b}**	2.61 ± 0.16	**1.71 ± 0.56^{b}**
		4-OH3PBA***	**15.2 ± 0.39^{cd}**	**18.9 ± 2.22^{cd}**	**5.97 ± 0.87^{abde}**	**37.6 ± 4.14^{abce}**	**15.3 ± 3.77^{cd}**
	0 – 48	CFMP^{NS}	8.1 ± 5.91	2.68 ± 1.37		5.62 ± 1.02	
		3-PBA**	**2.29 ± 0.53^{b}**	**3.86 ± 0.5^{ad}**		**2.85 ± 0.11^{b}**	
		4-OH3PBA***	**18.3 ± 1.38^{bd}**	**23.7 ± 3.01^{ad}**		**46.1 ± 3.33^{ab}**	
Feces	0 – 24	CFMP*	0.63 ± 0.24	0.58 ± 0.48	0.15 ± 0.11	0.67 ± 0.55	0.064 ± 0.05
		3-PBA*	0.08 ± 0.03	0.097 ± 0.05	0.04 ± 0.02	0.09 ± 0.05	0.03 ± 0.03
		4-OH3PBA^{NS}	0.32 ± 0.10	0.38 ± 0.18	0.18 ± 0.13	0.25 ± 0.15	0.12 ± 0.13
	0 – 48	CFMP^{NS}	2.26 ± 0.96	1.91 ± 1.20		2.17 ± 0.95	
		3-PBA^{NS}	0.12 ± 0.06	0.15 ± 0.07		0.14 ± 0.17	
		4-OH3PBA^{NS}	0.54 ± 0.22	0.58 ± 0.20		0.47 ± 0.18	

G1 = LCT alone (2.5 mg/kg bw); G2 = coadministration of LCT + captan (2.5 mg/kg bw each); G3 = administration of LCT alone (12.5 mg/kg bw); G4 = coadministration of LCT + captan (2.5 mg/kg bw and 12.5 mg/kg bw. respectively); G5 = coadministration of LCT + captan (12.5 mg/kg bw each).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for ANOVA group comparison (G1 vs G2 vs G3 vs G4 vs G5); NS = non-significant with $p > 0.05$.

[a,b,c,d,e] Mean with different exponent (letters) are statistically different with Bonferroni post-hoc test ($p < 0.05$).

[a] Group mean are significantly different from G1
[b] Group mean are significantly different from G2
[c] Group mean are significantly different from G3
[d] Group mean are significantly different from G4
[e] Group mean are significantly different from G5

Table 3.

Levels of CFMP, 3-PBA and 4-OH3PBA remaining in tissues 48 h after oral administration of lambda-cyhalothrin alone or as a binary mixture with captan (expressed as a percentage of administered dose).

Tissue	Metabolite	Metabolite levels (mean % dose ± SD)[a]				
		G1	G2	G3[b]	G4	G5[b]
		48 h	48 h	30 h	48 h	30 h
Brain	CFMP	ND	ND	0.0003 ± 0.0001	ND	0.0002 ± 0.0001
	3-PBA	ND	ND	0.0003 ± 0.0002	ND	0.0002 ± 0.0001
	4-OH3PBA	0.00007 ± 0.00001	0.00007 ± 0.00001	0.001 ± 0.0001	0.00008± 0.00001	0.0003 ± 0.0002
GI	CFMP	2.6 ± 1.3	2.6 ± 0.64	4.1 ± 1.0	2.7 ± 1.4	3.2 ± 2.1
	3-PBA	0.002 ± 0.001	0.002 ± 0.001	0.12 ± 0.02	0.007 ± 0.006	0.10 ± 0.07
	4-OH3PBA	0.03 ± 0.01	0.03 ± 0.02	0.87 ± 0.55	0.05 ± 0.02	0.65 ± 0.25
Kidneys	CFMP	0.006 ± 0.0006	0.008 ± 0.004	0.02 ± 0.005	0.007 ± 0.004	0.01 ± 0.003
	3-PBA	0.0008 ± 0.0001	0.001 ± 0.0001	0.007 ± 0.004	0.0009 ± 0.0004	0.004 ± 0.003
	4-OH3PBA	0.001 ± 0.001	0.001 ± 0.001	0.02 ± 0.01	0.002 ± 0.001	0.03 ± 0.02
Liver	CFMP	0.13 ± 0.05	0.19 ± 0.10	0.27 ± 0.10	0.12 ± 0.07	0.17 ± 0.05
	3-PBA	0.003 ± 0.001	0.002 ± 0.001	0.04 ± 0.02	0.004 ± 0.001	0.02 ± 0.01
	4-OH3PBA	0.002 ± 0.001	0.002 ± 0.001	0.03 ± 0.01	0.003 ± 0.002	0.04 ± 0.03

G1 = LCT alone (2.5 mg/kg bw); G2 = coadministration of LCT + captan (2.5 mg/kg bw each); G3 = administration of LCT alone (12.5 mg/kg bw); G4 = coadministration of LCT + captan (2.5 mg/kg bw and 12.5 mg/kg bw. respectively); G5 = coadministration of LCT + captan (12.5 mg/kg bw each).

ND: Values were below the LOD.

[a] Values were too close to the LOD to allow statistical comparison.

[b] Values were calculated with $n < 5$ animals because some rats were sacrificed at 24 h due to observed signs of suffering.

REFERENCES

Abass K, Pelkonen O (2013) The inhibition of major human hepatic cytochrome P450 enzymes by 18 pesticides: Comparison of the N-in-one and single substrate approaches. Toxicology in Vitro 27(5):1584-1588. doi:10.1016/j.tiv.2012.05.003

Anadon A, Martınez M, Martınez MA, Dıaz MJ, Martınez-Larranaga MR (2006) Toxicokinetics of lambda-cyhalothrin in rats. Toxicology Letters 165:47-56. doi:10.1016/j.toxlet.2006.01.014

Aouey B, Derbali M, Chtourou Y, Bouchard M, Khabir A, Fetoui H (2017) Pyrethroid insecticide lambda-cyhalothrin and its metabolites induce liver injury through the activation of oxidative stress and proinflammatory gene expression in rats following acute and subchronic exposure. Environmental Science and Pollution Research:1-16. doi:10.1007/s11356-016-8323-4

Aouey B, Fares E, Chtourou Y, Bouchard M, Fetoui H (2019) Lambda-cyhalothrin exposure alters purine nucleotide hydrolysis and nucleotidase gene expression pattern in platelets and liver of rats. Chem Biol Interact 311:108796. doi:10.1016/j.cbi.2019.108796

Arora S, Balotra S, Pandey G, Kumar A (2017) Binary combinations of organophosphorus and synthetic pyrethroids are more potent acetylcholinesterase inhibitors than organophosphorus and carbamate mixtures: An in vitro assessment. Toxicol Lett 268:8-16. doi:10.1016/j.toxlet.2016.12.009

Aznar-Alemany Ò, Eljarrat E (2020) Introduction to Pyrethroid Insecticides: Chemical Structures, Properties, Mode of Action and Use The Handbook of Environmental Chemistry. Springer, Berlin, Heidelberg ppp 1-16.

Barr DB, Olsson AO, Wong LY, et al. (2010) Urinary concentrations of metabolites of pyrethroid insecticides in the general U.S. population: National Health and Nutrition Examination Survey 1999-2002. Environ Health Perspect 118(6):742-8. doi:10.1289/ehp.0901275

Channa KR, Rollin HB, Wilson KS, et al. (2012) Regional variation in pesticide concentrations in plasma of delivering women residing in rural Indian Ocean coastal regions of South Africa. J Environ Monit 14(11):2952-60. doi:10.1039/c2em30264k

Chata C, Palazzi P, Grova N, et al. (2019) Blood pharmacokinetic of 17 common pesticides in mixture following a single oral exposure in rats: implications for human biomonitoring and exposure assessment. Arch Toxicol 93(10):2849-2862. doi:10.1007/s00204-019-02546-y

Choi W, Kim S, Baek YW, et al. (2017) Exposure to environmental chemicals among Korean adults-updates from the second Korean National Environmental Health Survey (2012-2014). Int J Hyg Environ Health 220(2 Pt A):29-35. doi:10.1016/j.ijheh.2016.10.002

Dikic D, Landeka I, Knezevic F, et al. (2012) Carbendazim impends hepatic necrosis when combined with imazalil or cypermethrin. Basic & clinical pharmacology & toxicology 110(5):433-40 doi:10.1111/j.1742-7843.2011.00831.x

EC (2014) Final addendum to the Renewal Assessment Report (RAR) on the active substance lambda-cyhalothrin provided by the rapporteur Member State Sweden and co-rapporteur Member State Spain, in the framework of Commission Regulation (EU) No 1141/2010. European Commission.

EFSA (2014) Conclusion on the peer review of the pesticide risk assessment of the active substance lambda-cyhalothrin. EFSA Journal 12(5):3677

EPA (2004a) Amendment to the 1999 captan Registration Eligibility Decision (RED). Captan: cancer reclassification. U.S. Environmental Protection Agency, Washington. Report No Fed. Reg. 68357-68360, p 1-281

EPA (2004b) Lambda-cyhalothrin and an isomer gamma-cyhalothrin; Tolerances for residues. vol 69. U.S. Environmental Protection Agency, p 10

Gibaldi M, Perrier D (1982) Drugs and the pharmaceutical sciences. In: Dekker M (ed) Pharmacokinetics. vol 15, 2 edn, New York, p 445-449

Hayes AW (2007) Principles and methods of toxicology. Crc Press

Hernández A, Parrón T, Tsatsakis A, Requena M, Alarcón R, López Guarnido O (2013) Toxic effects of pesticide mixtures at a molecular level: their relevance to human health. Toxicology 307:136-145

Hirosawa N, Ueyama J, Kondo T, et al. (2011) Effect of DDVP on urinary excretion levels of pyrethroid metabolite 3-phenoxybenzoic acid in rats. Toxicol Lett 203(1):28-32. doi:10.1016/j.toxlet.2011.02.016

Joo H, Choi K, Rose RL, Hodgson E (2007) Inhibition of fipronil and nonane metabolism in human liver microsomes and human cytochrome P450 isoforms by chlorpyrifos. J Biochem Mol Toxicol 21(2):76-80. doi:10.1002/jbt.20161

Katsuda Y (2012) Progress and future of pyrethroids. Topics in current chemistry 314:1-30. doi:10.1007/128_2011_252

Khemiri R, Cote J, Fetoui H, Bouchard M (2017) Documenting the kinetic time course of lambda-cyhalothrin metabolites in orally exposed volunteers for the interpretation of biomonitoring data. Toxicol Lett 276:115-121. doi:10.1016/j.toxlet.2017.05.022

Khemiri R, Cote J, Fetoui H, Bouchard M (2018) Kinetic time courses of lambda-cyhalothrin metabolites after dermal application of Matador EC 120 in volunteers. Toxicol Lett 296:132-138. doi:10.1016/j.toxlet.2018.08.008

Kishida T, Muto SI, Hayashi M, et al. (2008) Strain differences in hepatic cytochrome P450 1A and 3A expression between Sprague-Dawley and Wistar rats. J Toxicol Sci 33(4):447-457. doi:DOI 10.2131/jts.33.447

Kjeldsen LS, Ghisari M, Bonefeld-Jørgensen EC (2013) Currently used pesticides and their mixtures affect the function of sex hormone receptors and aromatase enzyme activity. Toxicology and applied pharmacology 272(2):453-464. doi:10.1016/j.taap.2013.06.028

Li AJ, Chen Z, Lin TC, Buck Louis GM, Kannan K (2020) Association of urinary metabolites of organophosphate and pyrethroid insecticides, and phenoxy herbicides with endometriosis. Environ Int 136:105456. doi:10.1016/j.envint.2019.105456

Marsh J, Woollen B, Wilks M (1994) The Metabolism and Pharmacokinetics of Lambda-cyhalothrin in Man. Unpublished CTL/P/4208

Martinez MA, Ares I, Rodriguez JL, et al. (2018) Pyrethroid insecticide lambda-cyhalothrin induces hepatic cytochrome P450 enzymes, oxidative stress and apoptosis in rats. Sci Total Environ 631-632:1371-1382. doi:10.1016/j.scitotenv.2018.03.030

Matsuo N (2019) Discovery and development of pyrethroid insecticides. Proceedings of the Japan Academy Series B, Physical and biological sciences 95(7):378-400. doi:10.2183/pjab.95.027

Mortuza T, Chen C, White CA, et al. (2018) Toxicokinetics of Deltamethrin: Dosage Dependency, Vehicle Effects, and Low-Dose Age-Equivalent Dosimetry in Rats. Toxicological sciences : an official journal of the Society of Toxicology 162(1):327-336. doi:10.1093/toxsci/kfx260

Paolini M, Barillari J, Trespidi S, Valgimigli L, Pedulli GF, Cantelli-Forti G (1999) Captan impairs CYP-catalyzed drug metabolism in the mouse. Chem Biol Interact 123(2):149-70.

Perkins A, Walters F, Sievert J, Rhodes B, Morrissey B, Karr CJ (2016) Home Use of a Pyrethroid-Containing Pesticide and Facial Paresthesia in a Toddler: A Case Report. International Journal of Environmental Research and Public Health 13(8):829. doi:10.3390/ijerph13080829

Ratelle M, Côté J, Bouchard M (2016) Time courses and variability of pyrethroid biomarkers of exposure in a group of agricultural workers in Quebec, Canada. International archives of occupational and environmental health 89(5):767-783 doi:10.1007/s00420-016-1114-x

Saito K, Sakai N, Kim HS, Ishizuka M, Kazusaka A, Fujita S (2004) Strain differences in diazepam metabolism at its three metabolic sites in sprague-dawley, brown norway, dark agouti, and wistar strain rats. Drug metabolism and disposition: the biological fate of chemicals 32(9):959-65.

Sams C, Jones K (2011) Human volunteer studies investigating the potential for toxicokinetic interactions between the pesticides deltamethrin; Pirimicarb and chlorpyrifos-methyl following oral exposure at the acceptable daily intake. Toxicology Letters 200(1-2):41-45. doi:10.1016/j.toxlet.2010.10.012

Schettgen T, Dewes P, Kraus T (2016) A method for the simultaneous quantification of eight metabolites of synthetic pyrethroids in urine of the general population using gas chromatography-tandem mass spectrometry. Analytical and bioanalytical chemistry 408(20):5467-5478 doi:10.1007/s00216-016-9645-2

Scollon EJ, Starr JM, Godin SJ, DeVito MJ, Hughes MF (2009) In vitro metabolism of pyrethroid pesticides by rat and human hepatic microsomes and cytochrome p450 isoforms. Drug metabolism and disposition: the biological fate of chemicals 37(1):221-228. doi:10.1124/dmd.108.022343

Sekeroglu V, Sekeroglu ZA, Demirhan E (2014) Effects of commercial formulations of deltamethrin and/or thiacloprid on thyroid hormone levels in rat serum. Toxicology and industrial health 30(1):40-6. doi:10.1177/0748233712448114

Starr JM, Graham SE, Ross DG, et al. (2014) Environmentally relevant mixing ratios in cumulative assessments: A study of the kinetics of pyrethroids and their ester cleavage metabolites in blood and brain; and the effect of a pyrethroid mixture on the motor activity of rats. Toxicology 320:15-24. doi:10.1016/j.tox.2014.02.016

Starr JM, Scollon EJ, Hughes MF, et al. (2012) Environmentally Relevant Mixtures in Cumulative Assessments: An Acute Study of Toxicokinetics and Effects on Motor Activity in Rats Exposed to a Mixture of Pyrethroids. Toxicological Sciences 130(2):309-318. doi:10.1093/toxsci/kfs245

Tang J, Cao Y, Rose RL, Hodgson E (2002) In vitro metabolism of carbaryl by human cytochrome P450 and its inhibition by chlorpyrifos. Chem Biol Interact 141(3):229-41. doi:10.1016/s0009-2797(02)00074-1

Timchalk C, Poet TS, Hinman MN, Busby AL, Kousba AA (2005) Pharmacokinetic and pharmacodynamic interaction for a binary mixture of chlorpyrifos and diazinon in the rat. Toxicology and applied pharmacology 205(1):31-42 doi:10.1016/j.taap.2004.09.004

Valcke M, Karthikeyan S, Walker M, Gagne M, Copes R, St-Amand A (2020) Regional variations in human chemical exposures in Canada: A case study using biomonitoring data from the Canadian Health Measures Survey for the provinces of Quebec and Ontario. Int J Hyg Environ Health 225:113451. doi:10.1016/j.ijheh.2020.113451

Weiner ML, Nemec M, Sheets L, Sargent D, Breckenridge C (2009) Comparative functional observational battery study of twelve commercial pyrethroid insecticides in male rats following acute oral exposure. NeuroToxicology 30:S1-S16. doi:10.1016/j.neuro.2009.08.014

Wielgomas B, Krechniak J (2007) Toxicokinetic interactions of alpha-cypermethrin and chlorpyrifos in rats. Polish Journal of Environmental Studies 16(2):267.

Wielgomas B, Nahorski W, Czarnowski W (2013) Urinary concentrations of pyrethroid metabolites in the convenience sample of an urban population of Northern Poland. Int J Hyg Environ Health 216(3):295-300. doi:10.1016/j.ijheh.2012.09.001

Wielgomas B, Piskunowicz M (2013) Biomonitoring of pyrethroid exposure among rural and urban populations in northern Poland. Chemosphere 93(10):2547-53. doi:10.1016/j.chemosphere.2013.09.070

Wolansky MJ, Gennings C, DeVito MJ, Crofton KM (2009) Evidence for dose-additive effects of pyrethroids on motor activity in rats. Environ Health Perspect 117(10):1563-70. doi:10.1289/ehp.0900667

Yang D, Wang X, Chen Y-t, Deng R, Yan BJT, pharmacology a (2009) Pyrethroid insecticides: isoform-dependent hydrolysis, induction of cytochrome P450 3A4 and evidence on the involvement of the pregnane X receptor. Toxicology and applied pharmacology 237(1):49-58. doi:10.1016/j.taap.2009.02.012

Yoshida T (2014) Biomarkers for monitoring profluthrin exposure: Urinary excretion kinetics of profluthrin metabolites in rats. Environmental toxicology and pharmacology 37(3):1123-1128. doi:10.1016/j.etap.2014.03.019

ARTICLE 2

ARTICLE 2

Excretion time courses of lambda-cyhalothrin metabolites in the urine of strawberry farmworkers and effect of coexposure with captan

Yélian Marc Bossou[a], Jonathan Côté[a], Louiza Mahrouche[b], Marc Mantha[a], Naïma El Majidi[a], Alexandra Furtos[b], Michèle Bouchard[a]

[a] Department of Environmental and Occupational Health, Chair in Toxicological Risk Assessment and Management, and Public Health Research Center (CReSP), University of Montreal, Roger-Gaudry Building, U436, P.O. Box 6128, Main Station, Montreal, Quebec, Canada, H3C 3J7

[b] Department of Chemistry, University of Montreal, MIL Building, P.O. Box 6128, Main Station, Montreal, Quebec, Canada, H3C 3J7

* Correspondence to:
Michèle Bouchard
Department of Environmental and Occupational Health
University of Montreal
Roger-Gaudry Building, U436
P.O. Box 6128, Main Station, Montreal, Quebec, Canada, H3C 3J7.
E-mail: michele.bouchard@umontreal.ca
Telephone number: (514) 343-6111 ext 1640
Fax number: (514) 343-2200

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ABSTRACT

There is limited literature data on the impact of coexposure on the toxicokinetics of pesticides in agricultural workers. Using the largely employed pyrethroid lambda-cyhalothrin (LCT) and fungicide captan as sentinel pesticides, we compared individual temporal profiles of biomarkers of exposure to LCT in strawberry field workers following an application episode of LCT alone or in coexposure with captan. Participants provided all urine voided over a three-day period after an application of a pesticide formulation containing LCT alone (E1) or LCT mixed with captan (E2), and in some cases following re-entry in treated field (E3). Pyrethroid metabolites were measured in all urine samples, in particular 3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethyl-cyclopropanecarboxylic acid (CFMP), 3-phenoxybenzoic acid (3-PBA) and 4-hydroxy-3-phenoxybenzoic acid (4-OH3PBA). There were no obvious differences in individual concentration-time profiles and cumulative excretion of metabolites (CFMP, 3-PBA, 4-OH3BPA) after exposure to LCT alone or in combination with captan. For most workers and exposure scenarios, CFMP was the main metabolite excreted, but time courses of CFMP in urine did not always follow that of 3-PBA and 4-OH3BPA. Given that the latter metabolites are common to other pyrethroids, this suggests that some workers were coexposed to pyrethroids other than LCT. For several workers and exposure scenarios E1 and E2, values of CFMP increased in the hours following spraying. However, for many pesticide operators, other peaks of CFMP were observed at later times, indicating that tasks other than spraying of LCT-containing formulations contributed to this increased exposure. These tasks were mainly handling/cleaning of equipment used for spraying (tractor or sprayer) or work/inspection in LCT-treated field according to questionnaire responses. Overall, this study provided novel excretion time course data for LCT metabolites valuable for interpretation of biomonitoring data in

workers, but also showed that coexposure was not a major determinant of variability in exposure biomarker levels. Our analysis also pointed out the importance of measuring specific metabolites.

Keywords: Biomonitoring, toxicokinetics, coexposure, pyrethroids, lambda-cyhalothrin, captan

INTRODUCTION

Pyrethroids are among the most widely used insecticides in agriculture (Barr *et al.*, 2010a; Ravula et Yenugu, 2021a; Thatheyus et Selvam, 2013). Repeated exposure to these molecules in humans, as encountered in agricultural settings, could represent a health risk for workers. Epidemiological studies have reported associations between occupational exposure to several pyrethroids and neurotoxic effects, endocrine disruption, reproductive, and sexual development impairment (Burns et Pastoor, 2018; Saillenfait *et al.*, 2015). Therefore, it is important to assess exposure to pyrethroids using reliable exposure assessment tools.

To assess exposure to pyrethroids and evaluate health risks in exposed workers, biomonitoring of exposure is used and consists of measuring metabolites in accessible biological matrices such as urine (Buchholz *et al.*, 2021; Maule *et al.*, 2019). In the specific case of the largely used pyrethroid insecticide lambda-cyhalothrin (LCT), controlled studies in animals and humans have shown that it is rapidly metabolized and excreted predominantly in urine as 3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethyl-cyclopropanecarboxylic acid (CFMP), 3-phenoxybenzoic acid (3-PBA) and 4-hydroxy-3-phenoxybenzoic acid (4-OH3PBA) (Bossou *et al.*, 2020; Khemiri *et al.*, 2017b, 2018b). 3-PBA is a metabolite common to several pyrethroids (permethrin, cypermethrin, deltamethrin and cyfluthrin) while CFMP is generated by a limited number of pyrethroids (LCT, bifenthrin). Although CFMP appears to be a major and rather specific metabolite of LCT, there are no published studies to date that have measured CFMP in agricultural workers to assess exposure to LCT. The only available biomonitoring study in workers exposed to LCT was conducted by Chester *et al.* (1992), and examined occupational exposure in sprayers as part of a malaria control program.

Although the measurement of urinary metabolites is useful for assessing internal exposure to pyrethroids, urinary levels of these biomarkers of exposure can vary widely among individuals or within the same individual over time and be influenced by several factors (Fortin, M.C. *et al.*, 2008; Fortin, M. C. *et al.*, 2008; Jones, 2020). Studies in agricultural settings have attempted to better understand pesticide exposure and the influence of various factors on biomonitoring data, but not specifically for pyrethroid formulations. These studies were limited to assessing the impact of tasks and various pesticide handling and application methods (Coronado *et al.*, 2004; Grover *et al.*, 1986; Ratelle, M. *et al.*, 2016) or the effectiveness of personal protective equipment (Krieger et Dinoff, 2000). None of these studies addressed the impact of coexposure on biological levels of metabolites in workers used as biomarkers of exposure. Previous studies also raised the difficulty of interpreting biomonitoring data in workers suspected of being co-exposed to pesticides other than those under studies (Bouchard *et al.*, 2019).

Overall, the data published in the scientific literature on the impact of coexposure on the biological behavior of pesticides and in particular their toxicokinetics in the animal and human body is very scarce. These data originate mainly from *in vitro* or *in vivo* studies in animals. *In vitro* studies on cell cultures have shown that coexposure to multiple pesticides could impact the rate of metabolism of substances (Abass, K. et Pelkonen, O., 2013; Joo *et al.*, 2007a; Tang *et al.*, 2002a). Other studies have reported that key enzymes involved in the biotransformation of pyrethroids – including CYP450s (CYP1A1, 1A2, 2A1, 2B1, 2B2, 2E1, 3A1, 3A2, 3A4, 3A5, 4A1, 2C8, 2C9, 2C19) – are also involved in the biotransformation of several other pesticides used concomitantly in agriculture (Joo *et al.*, 2007a; Martinez *et al.*, 2018a; Scollon, Edward J. *et al.*, 2009; Yang, D. *et al.*, 2009). An *in vivo* animal study also showed that coexposure to organophosphate insecticides in a binary mixture impacted their rate of absorption and elimination (Timchalk *et al.*, 2005a).

Regarding pyrethroids more specifically, Bossou *et al.* (2020) experimentally assessed the influence of coexposure to the fungicide captan on the kinetic profiles of the main LCT metabolites . The *a priori* hypothesis was that captan could interfere with CYP450 metabolism pathway of pyrethroids or excretion mechanisms (Paolini *et al.*, 1999b; Wielgomas, B. et Krechniak, J., 2007) in a dose-dependent manner. In the study of Bossou *et al.* (2020), groups of male Sprague–Dawley rats were exposed orally by gavage to LCT alone (2.5 or 12.5 mg/kg bw) or to a binary mixture of LCT and captan (2.5/2.5 or 2.5/12.5 or 12.5/12.5 mg/kg bw) and results showed that coexposure led to a tendency to lower excretion levels of metabolites in the high-dose group, especially for 4-OH3PBA. These animal results cannot be directly extrapolated to humans for the interpretation of biomonitoring data because experimental exposure doses were much higher than those estimated in occupational exposure conditions in humans (< 1 µg/kg/d) (Chester *et al.*, 1992). Also, there could be interspecies differences in the toxicokinetics. Hence, the impact of coexposure in real-life contexts in workers remains to be verified.

The objective of the present study was to document the kinetic time courses of LCT metabolites in the urine of agricultural workers after spraying and working in strawberry fields treated with LCT, and to assess if coexposure with the fungicide captan had an apparent impact on the excretion profiles. Thus, in addition to providing novel excretion time course data for LCT metabolites valuable for the interpretation of biomonitoring data in workers, the current work also assessed mixture effect in the presence of captan, a largely used fungicide in crop fields.

MATERIELS AND METHODS

Study subjects

Workers were recruited using the Quebec Directory of horticultural producers obtained from the Quebec Produce Growers Association (QPGA), Canada. From this list of farms organized by city and crop types, strawberry growers located within 100 km from University of Montreal were contacted by phone – using a standardized script – to assess their willingness to solicit their employees performing spraying in the fields to participate in the study.

The eligibility criteria required that: i) the applicator planned to apply formulations containing LCT active ingredient (Matador®, Silencer®, Demand CS®, Warrior®) alone or in combination with captan fungicide (Captan®, Supra Captan 80 WDG®, Captan 80-WP®, Maestro®) during the summer as part of normal operations, and ii) he was willing to provide a 24-h urine collection prior to an application (-24 – 0 h prior to application) and all urine voided over a period of at least three days following spraying (0 – 72 h post-application). Inclusion criteria also included the absence of a medical condition and that the worker did not take (on a daily basis) medication that may interfere with the elimination of our biomarkers of interest. Eligible applicators interested in participating in the study received a recruitment letter by mail along with an information and consent form explaining the study steps and requirements, ethics considerations, the risks and benefits of the study, as well as a confirmation of meeting appointment. A few weeks after the documents were sent, a staff member from our lab met with the applicators to provide further details on the study, review the sampling protocol, answer questions and obtain their written informed consent to participate in the study.

Our recruitment objective was to enroll at least 5 to 7 applicators to obtain their detailed excretion profile of LCT exposure biomarkers following three pre-established exposure episodes. More specifically, applicators were monitored i) after spraying pesticide formulations containing LCT insecticide alone in a strawberry field (named E1), ii) after spraying pesticide formulations with LCT mixed with captan fungicide (named E2) or iii) after re-entering an area treated with both LCT and captan (named E3). Two other exposure scenarios are reported, one after applying LCT alone in a sweet corn field for one worker (E4) and one where captan was applied in a strawberry field previously treated the days before with a LCT containing formulation (E5).

The study was conducted during summer 2019 and 2020. After the first year of data collection, all the expected exposure scenarios for 5 to 7 participants were not covered to meet recruitment objective. Participants followed during the first year and planning to apply in next year were recruited once again during the second year. Efforts were also made to reach and recruit new farms during the second year of the study.

Fourteen application events of LCT alone (E1 and E4) and eleven application events of LCT mixed with captan (E2) were conducted. Two re-entry episodes (E3) were also monitored along with one scenario where captan was applied in a field treated three days before with LCT (E5).

Urine sampling and handling

Participants were requested to provide all urine voided over roughly a 24-h period before the onset of a spraying episode. They were then asked to collect all urine voided during the three days following spraying, using separate 500 mL polypropylene Nalgene® bottles for each complete micturition. Each

applicator provided urine samples over a three-day period after an application of a pesticide formulation containing LCT alone (E1) and after an application of formulations containing LCT mixed with captan (E2). Some applicators also performed tasks in the treated area in the days following spraying and therefore also provided urine samples over three days following entry in a treated field (E3). Participants were also asked not to change their work habits during the biomonitoring period.

Participants were asked to write the date and time of each micturition on the identification label affixed to the Nalgene collection bottles. The samples collected were kept either in the farmer's cold room or in coolers with ice packs provided by our team. Samples were picked up on daily basis at the workplace by a member of our research team and directly brought to the laboratory where urine volumes were measured using graduated cylinders. Two aliquots of 120 mL per collection bottle were prepared and placed in polypropylene Sarstedt tubes for storage at -20°C until analysis.

Urine analysis and metabolites quantification for data analysis

Concentrations of LCT metabolites were measured in each urine sample, namely CFMP, 3-PBA and 4-OH3PBA. CFMP is specific to LCT and cyfluthrin while 3-PBA and 4-OH3PBA are common to several pyrethroids including LCT, permethrin, cypermethrin. The metabolites *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acids (*cis*- and *trans*-DCCA), which are metabolites formed by permethrin and cypermethrin, were also measured to assess if workers were concomitantly exposed to other pyrethroids largely used on agricultural crops to avoid misinterpretation of results.

All metabolites were extracted from urine samples and quantified according to the method adapted from Khemiri *et al.* (2018). In short, 5 mL of urine were hydrolyzed with β-glucuronidase/arylsulfatase enzyme to obtain the sum of free and glucurono- and sulfo-conjugated metabolites. Samples were then subjected to a solid-phase extraction and the metabolites were eluted in methanol and analyzed by high performance liquid chromatography coupled to triple quadripole (QQQ) mass spectrometry (UHPLC-MS/MS). An Agilent Model 1260 HPLC system with an autosampler and thermostated column compartment coupled to a Model 6490 QQQ (Mississauga, Canada) with a jetstream electro-spray ionization (AJS ESI) source operating in negative mode was used. The analytes were separated using an Agilent Zorbax Eclipse plus C18 column (2.1 x 50 mm; 1.8 µm) from Agilent maintained at a temperature of 40°C. The mobile phase consisted of: eluent A composed of water and 0.01% acetic acid, and eluent B of acetonitrile and 0.01% acetic acid. Elution was performed using a solvent gradient, at a flow rate of 0.400 mL/min, with a total run time of 15 min. The following solvent program was used: (*i)* 2% eluent B for 2 min, (*ii)* linear gradient to 25% eluent B from 2 to 2.5min, *(iii)* maintained at 25% eluent B from 2.5 to 3.5 min, (*iv)* increased to 50% eluent B from 3.5 to 4 min and then *(v)* to 55% eluent B from 4 to 7.5 min, (*vi)* linear gradient to 98% eluent B from 7.5 to 8 min, (*vii*) maintained at 98% eluent B from 8 to 9.5 min, (*viii*) and returned to initial conditions of 2% eluent B in 0.5 min. Samples were kept at 5°C on the injection tray and the injection volume was set at 5 µL. Retention times of the analytes were: 8.3 min for CFMP, 7.6 min for 3PBA, 6.6 min for 4-OH3PBA, 7.6 min for *trans*-DCCA and 7.8 min for *cis*-DCCA.

Separated analytes were determined using a triple quadrupole MS operated in multiple reaction monitoring (MRM) mode using the following experimental conditions: sheath gas (N2) temperature at 365°C and sheath gas flow rate of 10 L/min, nebulizer gas pressure of 50 psi; drying temperature (N2) at 200°C and flow rate of 12 L/min; capillary voltage (Vcap) at 3000 V; nozzle voltage at 1000V; fragmentor at 380 V,

collision energy (CE) of 9V for CFMP, 21V for 4-OH3BPA, 9 and 33V for 3-PBA, 10V for *trans*- and *cis*-DCCA, 25V for the internal standard $^{13}C_6$ 3-PBA and 10V for the other internal standard $^{13}C_2$ 1D *trans*-DCCA. Data acquisition was performed across three different time segments. From 0 to 5 min, the chromatographic flow was diverted to waste in order to reduce instrument contamination from early eluting hydrophilic species. During the second time segment, from 5 to 13 min, the chromatographic flow was directed toward the mass spectrometer and the following MRM transitions were recorded: *m/z* 241.02>204.90, 35.00 for CFMP, *m/z* 213.05>169.00, 92.80 for 3-PBA, *m/z* 229.05>108.70, 99.00 for 4-OH3BPA and *m/z* 207.00>34.90 for *trans*- and *cis*-DCCA. The transitions for the internal standards were *m/z* 212.00>34.90 for $^{13}C_2$ 1D *trans*-DCCA and *m/z* 219.07>99.00 for $^{13}C_6$ 3-PBA. In the third time segment, from 13 to 15 min, the polarity of the mass spectrometer was switched to positive mode in order to diminish the instrument contamination from the very complex matrix. The limit of detection was 50 to 125 fmol of urinary extract injected in the column depending on the analyte, which corresponds to 2 to 5 pmol/ml of urine.

Creatinine concentrations were also measured in urine using the Jaffé method, an alkaline picric acid method with deproteinization (enzymatic colorimetric test PAP from Boehringer Mannheim, Germany). The time courses of creatinine-corrected concentrations of metabolites (µmol/mol creatinine) and cumulative excretion over the 3-day collection period post-application (pmol/kg bw) were then established for each participant and each exposure scenario (E1, E2, E3).

Questionnaire

At the time of enrolment, participants were asked to complete a first questionnaire to document demographics (age, sex, body weight (bw), and address of residence) as well as known health conditions, farm work experience and intention to use the pesticides of interest during the summer. On each day of urine collection post-application (0 – 24, 24 – 48 and 48 – 72 h) for each exposure scenario (application of LCT alone (E1) or application of LCT mixed with captan (E2) or work in a treated field post-application (E3)), participants were also invited to complete a self-administered questionnaire to document information on the studied pesticide application (date, hours, duration of mixing/ loading/spraying of pesticides or time spent in the treated area), personal protective equipment, residential use of pyrethroids at home during the 24 h preceding urine sampling, daily consumption of medication, alcohol, tobacco, portions of fruits, vegetables and cereals. Data from these questionnaires were used to verify potential factors that may influence urinary biomarkers excretion levels. Pre-exposure questionnaires (-24 – 0 h prior to application) were further filled to gather exposure information and relevant activities during the 24 h prior the onset of spraying. It was verified with this pre-application questionnaire that the workers monitored in the current work did not apply LCT-based formulations either in their work setting or for residential use in the 24-h prior to the onset of LCT or LCT plus captan spraying.

RESULTS

Study subjects and exposure characteristics during the three days of biomonitoring

After a telephone screening interview during early 2019 and 2020 to identify potentially eligible applicators and visit to the farms to recruit the farmers, 18 pesticide operators signed an informed consent to participate in the study. However, approximately 20% subsequently dropped out of the study, mostly citing the weather or the absence of insect threat requiring LCT spraying. Overall, fourteen pesticide operators from three regions of the Province of Quebec (Montérégie, Laurentides, Lanoraie) who applied pesticide formulations containing LCT alone or as a mixture with captan took part in the study. Those operators were professionally exposed during different tasks including mixing, loading and spraying of pesticides, cleaning of equipment in contact with the pesticides, maintenance/adjustment of the sprayer or inspection of the treated area.

The main characteristics of the study subjects are reported in Table 1. All participants were men; their age ranged from 23 to 64 years old, their height from 156 to 198 cm and their weight from 66 to 127 kg bw. According to questionnaire responses, 92.3% of applicators were French Canadians and 7.7% spoke Spanish and were from Mexico. The majority of participants reported to be in good health except one applicator with Crohn's disease. At the time of recruitment, 61.5% of participants mentioned that they intended to use LCT and captan pesticides under study while 38.5% were not sure to use them during the summer.

Table 1 Personal information on each worker, documented by self administered questionnaire

Worker	Age	Weight (kg)	Height (cm)	Highest educational level	Known health condition
Worker T101	64	80	182	University	No
Worker T102	32	109.1	189	DVS[a]	No
Worker T103	48	80	165	Primary school	No
Worker T105	55	77.3	177	College	No
Worker T106	34	95.5	182	DVS[a]	No
Worker T108	53	79.5	177	DVS[a]	No
Worker T109	34	76.4	156	DVS[a]	No
Worker T110	28	66	159	DVS[a]	No
Worker T111	48	66	155.5	College	No
Worker T112	44	84	180	University	No
Worker T113	56	127	155.5	High school	Crohn's disease
Worker T115	40	125	198	College	No
Worker T118	44	92	183	High school	No
Worker T119	23	75	156	High school	No

[a] Diploma of Vocational Studies (DVS): Qualification issued by the Quebec government after successful completion of programs lasting from one to two years offered at the end of the third, fourth, or fifth year of secondary school. DVS prepares students to train in a profession to directly enter the job market.

*All applicators were residing on their workplace at the time of the study, except T119 who was living on a farm different from his workplace.

**Applicators had more than 1-year experience in agriculture except for T119, who was hired for four months.

Of the 14 applicators recruited, it was possible to establish the urinary excretion profiles of metabolites for 25 application episodes. For seven applicators (T101; T103; T105; T106; T108; T109; T112), the time profiles were obtained following an exposure episode to formulations containing LCT alone (E1) on the one hand and in combination with captan on the other hand (E2). For the other six applicators, it was possible to determine the time profiles for only one of the two exposure scenarios, *i.e*. either after an exposure episode to formulations containing LCT alone (E1 for T110; T115; T119) or after applications of formulations containing LCT mixed with captan (E2 for T102; T111; T113). The individual time profiles and exposure conditions are shown for five of the applicators (T101; T103; T106; T106b; T108; see Figs. 1 to 5 and Table 2). While CFMP was not measured as part of large biomonitoring studies such as the Canadian Health Measure Survey (CHMS), Figures 1 to 5 (except Fig. 5B) show that all the applicators exhibited concentration values at some time points exceeding the median value reported for 3-PBA in cycle 6 of the CHMS (equivalent to 0.232 and 0.285 µmol/mol creat. for 20-39- and 40-59-year-old groups, respectively) and the 90e centile of the CHMS (1.954 and 1.373 µmol/mol creat. for 20-39- and 40-59-year-old groups, respectively) (HealthCanada, 2021).

Fig. 1. Concentration-time courses of CFMP (—■—), 3-PBA (—●—) and 4-OH3BPA (--○--), *trans*-DCCA (—▲—), and *cis*-DCCA (--Δ--) in strawberry field worker T101 following the onset of spraying of Matador® formulation (LCT alone - E1) **(A)** or in combination with Captan® (E2) **(B)** or work in a LCT-treated area (E3) **(C)**. Main graph compares the time courses of CMFP, 3-PBA and 4-OH3BPA; secondary graph on left compares the times courses of 3-PBA, *trans*-DCCA and *cis*-DCCA; secondary graph on the right highlights the time course of CFMP on a different scale. The red arrow represents spraying period. The grey marks below the x axis represent either tasks in the treated area, or handling and applying pesticides other than LCT.

Fig.1A

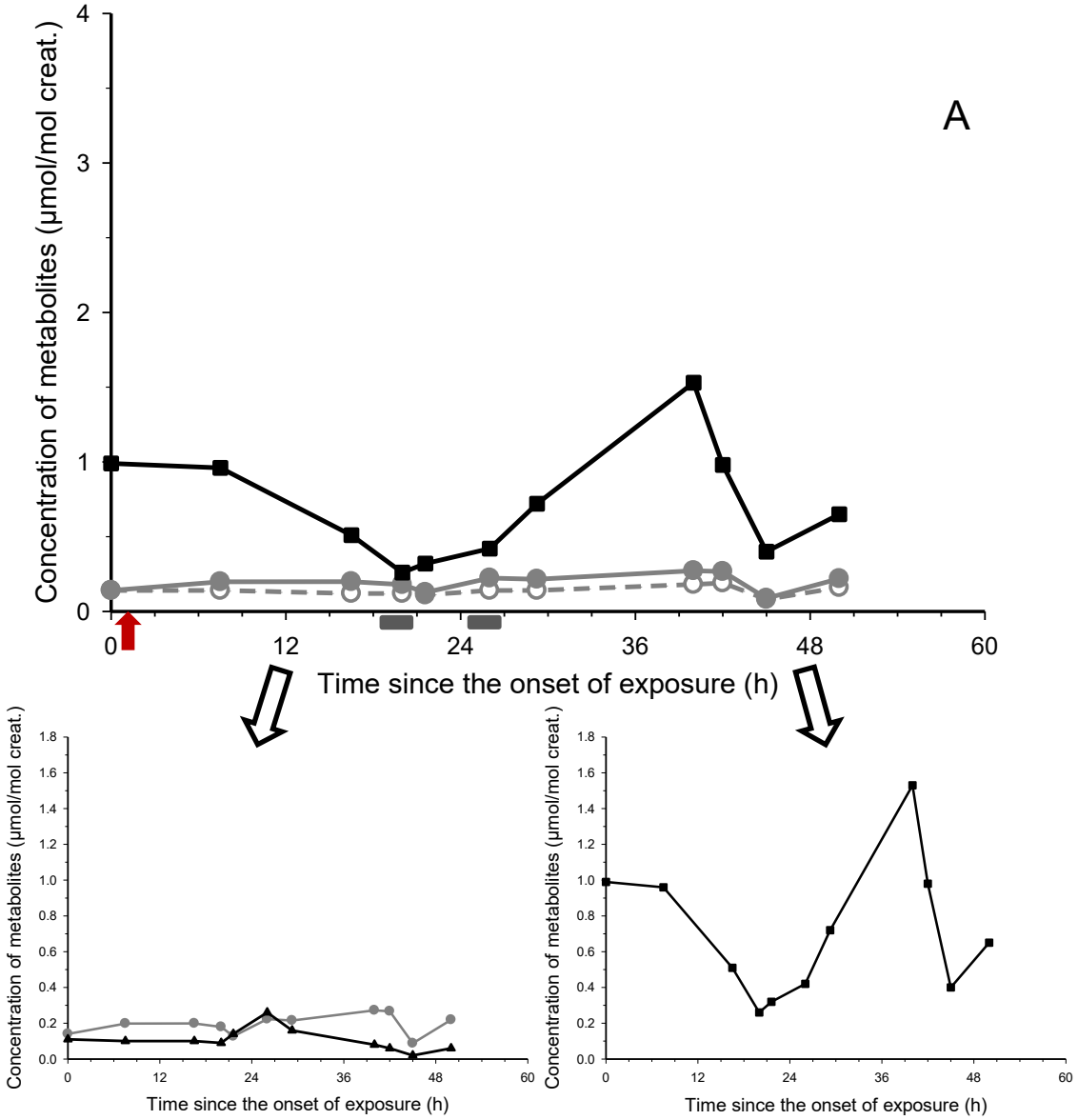

A
Concentration of metabolites (μmol/mol creat.)
Time since the onset of exposure (h)
0
1
2
3
4
0
12
24
36
48
60
Concentration of metabolites (μmol/mol creat.)
Time since the onset of exposure (h)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
0
12
24
36
48
60
Concentration of metabolites (μmol/mol creat.)
Time since the onset of exposure (h)

Fig.1B

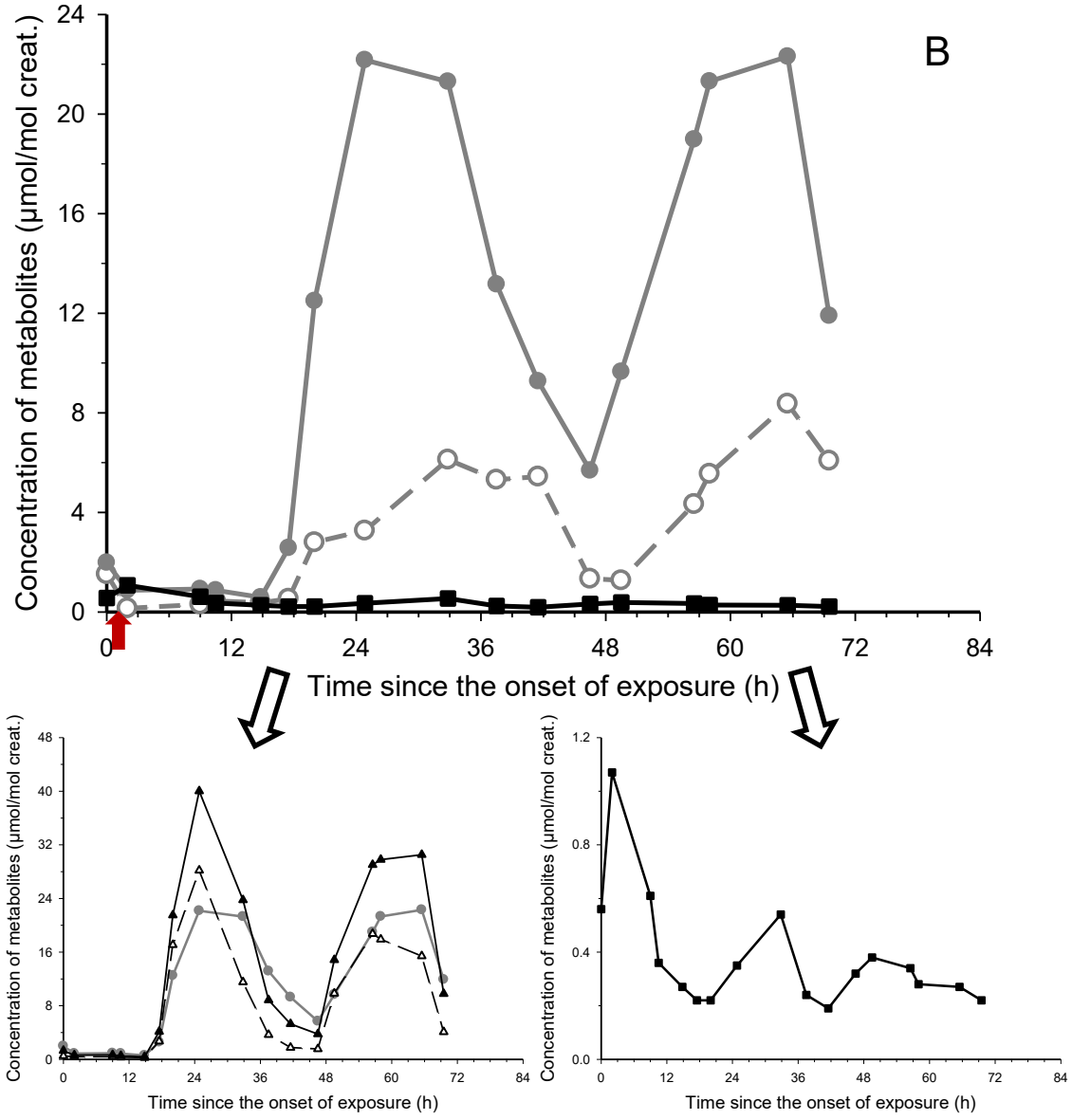

B
Concentration of metabolites (μmol/mol creat.)
Time since the onset of exposure (h)
Concentration of metabolites (μmol/mol creat.)
Time since the onset of exposure (h)
Concentration of metabolites (μmol/mol creat.)
Time since the onset of exposure (h)

Fig.1C

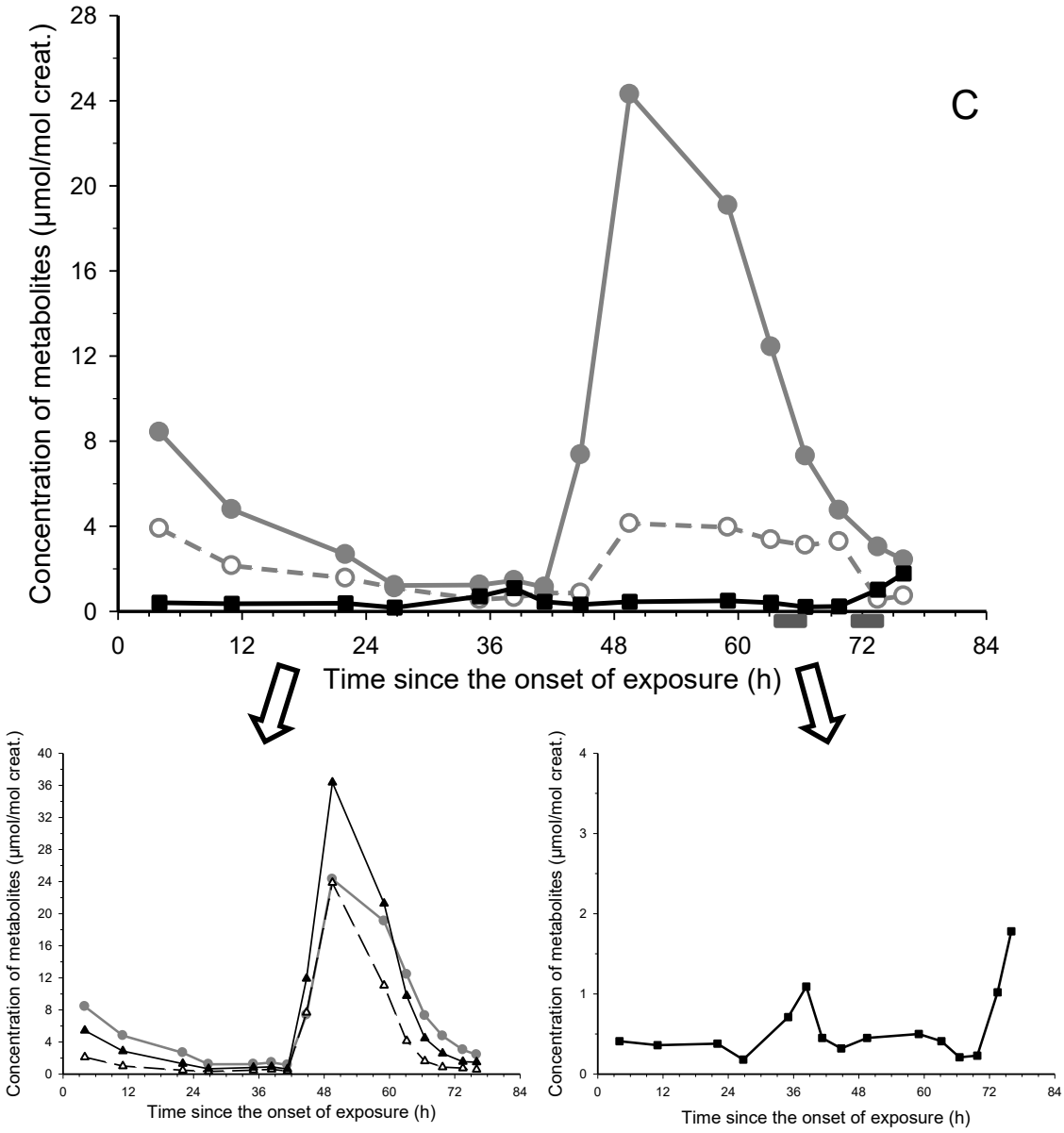

C
Concentration of metabolites (μmol/mol creat.)
Time since the onset of exposure (h)
0
4
8
12
16
20
24
28
0
12
24
36
48
60
72
84

Fig. 2. Concentration-time courses of CFMP (—■—), 3-PBA (—●—) and 4-OH3BPA (--○--), *trans*-DCCA (—▲—), and *cis*-DCCA (--Δ--) in strawberry field worker T103 following the onset of spraying of Silencer® formulation (LCT alone - E1) **(A)** or in combination with Captan® (E2) **(B)**. Main graph compares the time courses of CMFP, 3-PBA and 4-OH3BPA; secondary graph on left compares the times courses of 3-PBA, *trans*-DCCA and *cis*-DCCA; secondary graph on the right highlights the time course of CFMP on a different scale. The red arrow represents spraying period. The grey marks below the x axis represent either maintenance of equipment or inspection tasks in the treated area.

Fig. 2A.

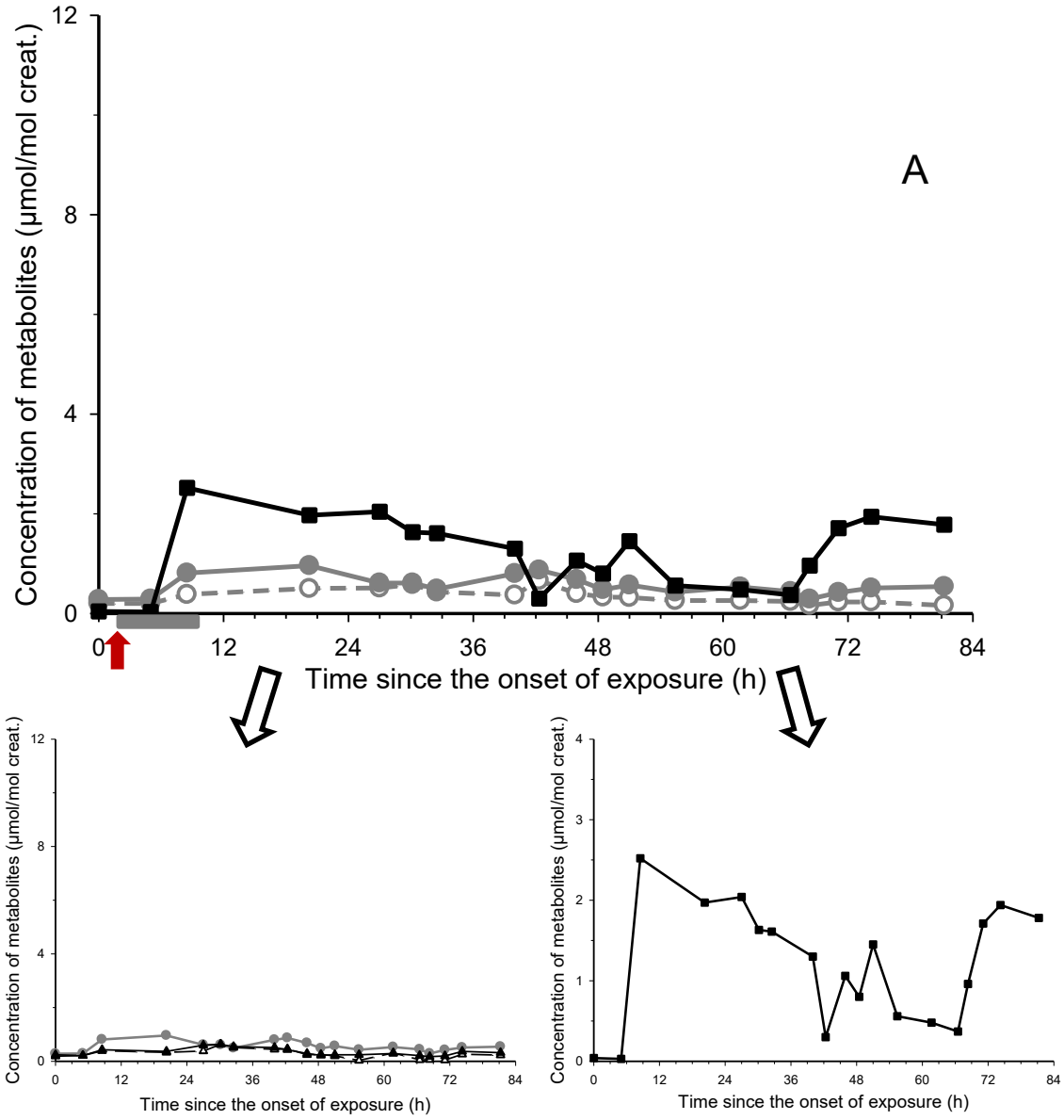

A
Concentration of metabolites (μmol/mol creat.)
Time since the onset of exposure (h)
0
4
8
12
24
36
48
60
72
84

Fig. 2B.

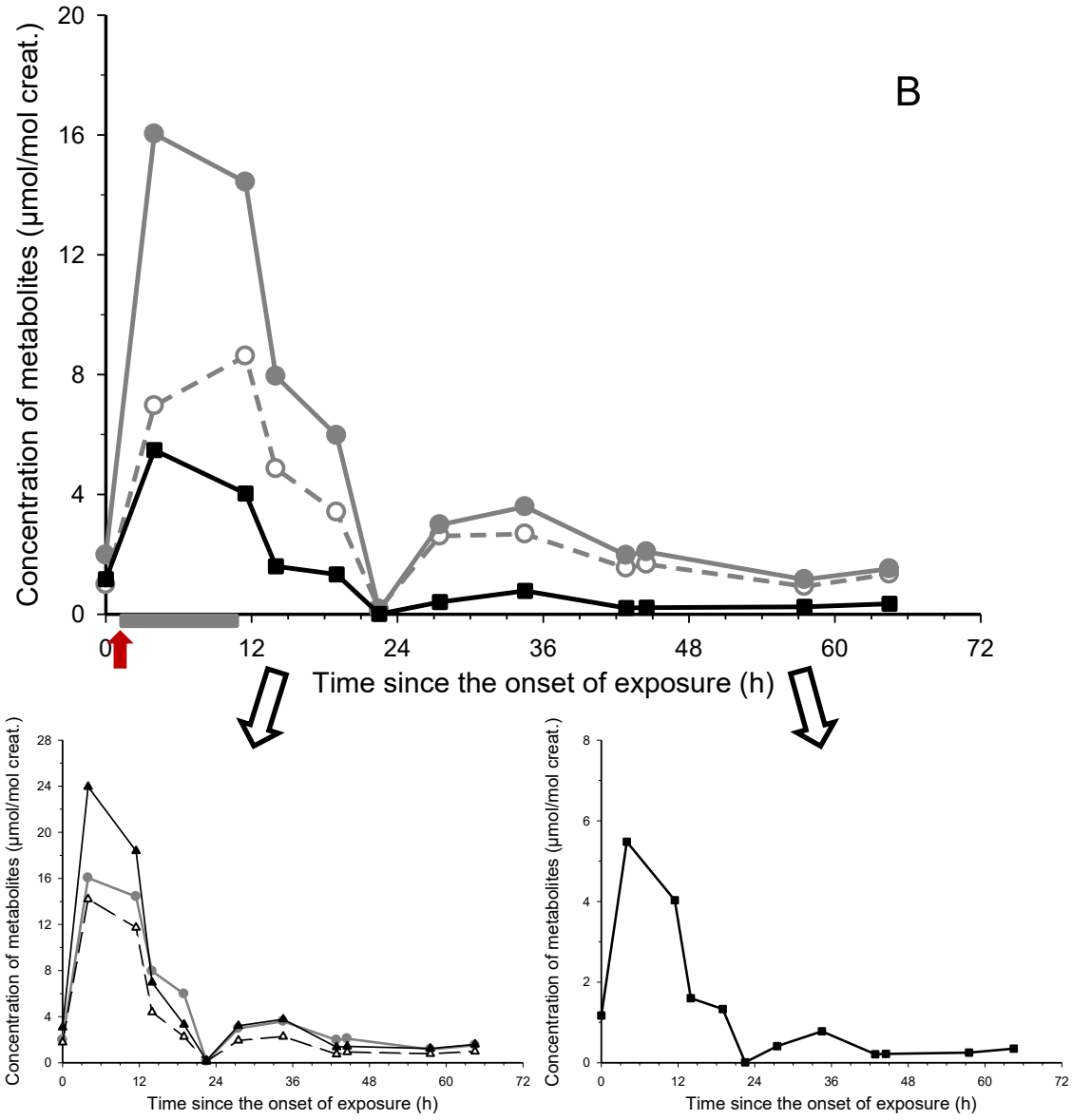

B
Concentration of metabolites (μmol/mol creat.)
Time since the onset of exposure (h)
0
4
8
12
16
20
0
12
24
36
48
60
72
Concentration of metabolites (μmol/mol creat.)
Time since the onset of exposure (h)
Concentration of metabolites (μmol/mol creat.)
Time since the onset of exposure (h)

Fig. 3. Concentration-time courses of CFMP (—■—), 3-PBA (—●—) and 4-OH3BPA (--○--), *trans*-DCCA (—▲—), and *cis*-DCCA (--Δ--) in strawberry field worker T106 following the onset of spraying of Matador® (LCT alone - E1) **(A)** or in combination with Captan® (E2) **(B)** or work in a LCT-treated area (E3) **(C)** and in the same workers after spraying Matador® in a sweet corn field (LCT alone – scenario E4 equivalent exposure scenario E1 but in a different field) **(D)**. Main graph compares the time courses of CMFP, 3-PBA and 4-OH3BPA; secondary graph on left compares the times courses of 3-PBA, *trans*-DCCA and *cis*-DCCA; secondary graph on the right highlights the time course of CFMP on a different scale. The red arrow represents spraying period. The grey marks below the x axis represent either tasks in the treated area, maintenance of equipment, or handling and applying pesticides other than LCT.

Fig3A

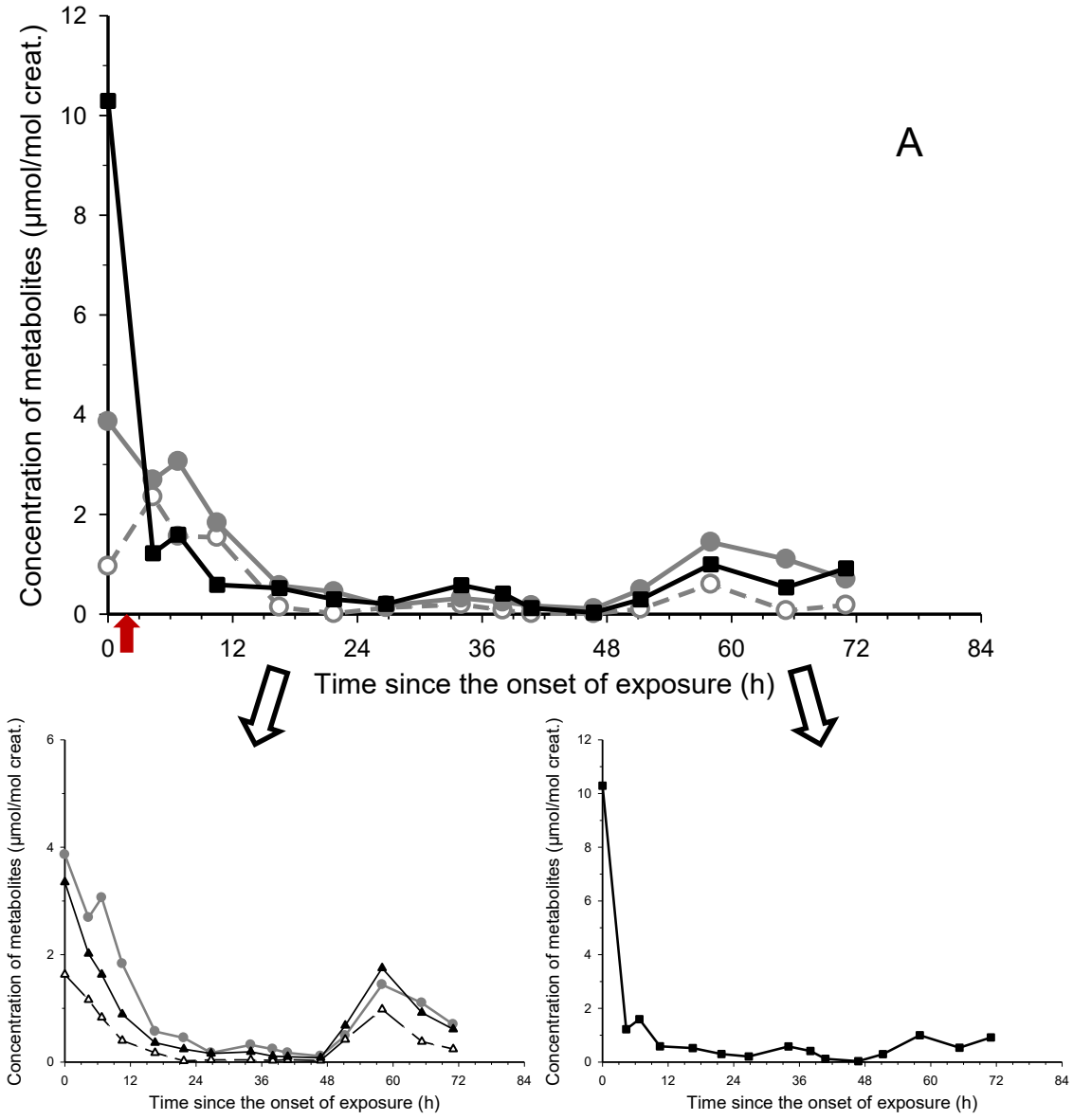

A
Concentration of metabolites (μmol/mol creat.)
Time since the onset of exposure (h)
0
12
24
36
48
60
72
84
0
2
4
6
8
10
12

Fig3B

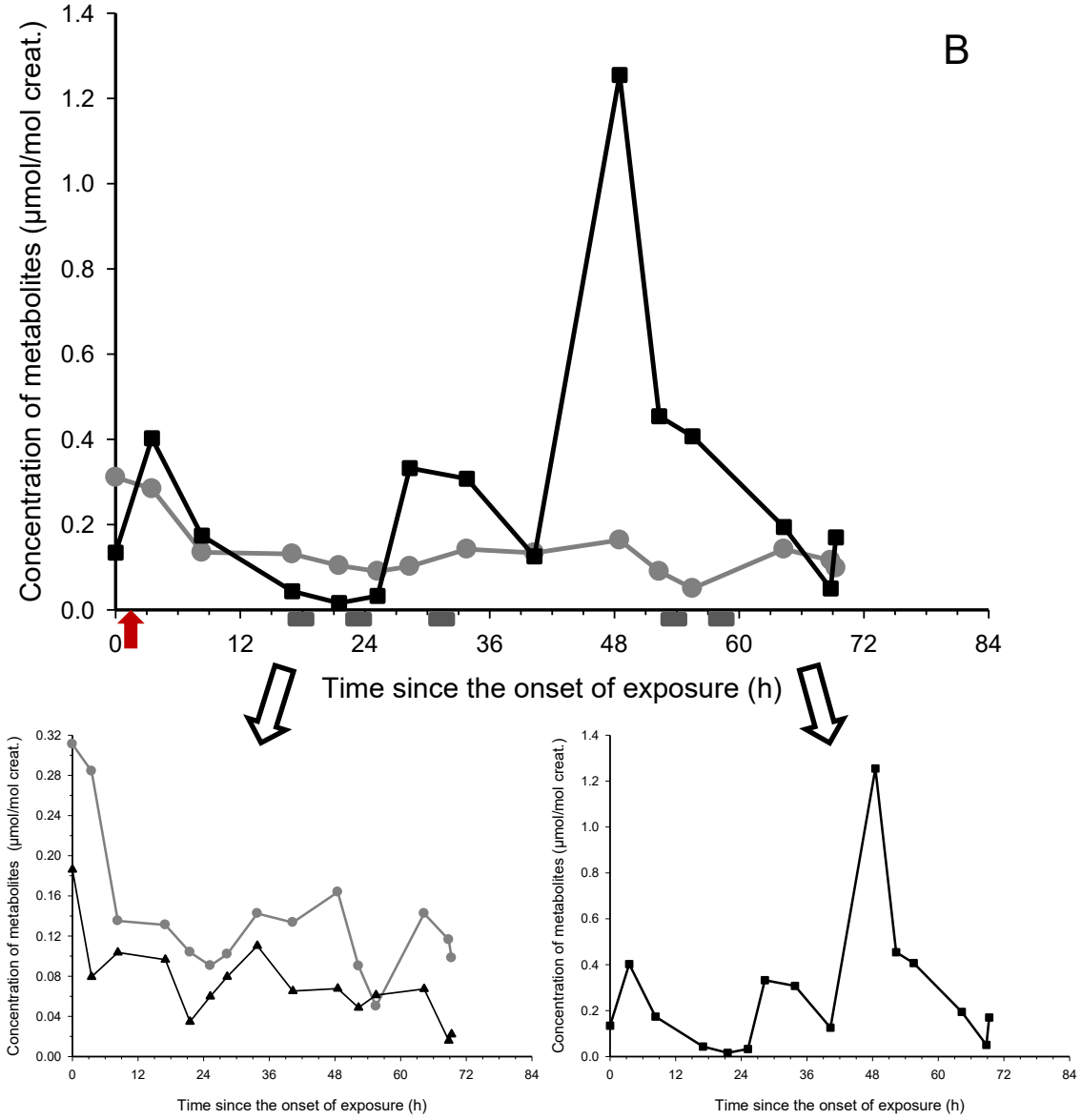

B
Concentration of metabolites (μmol/mol creat.)
Time since the onset of exposure (h)
Concentration of metabolites (μmol/mol creat.)
Time since the onset of exposure (h)
Concentration of metabolites (μmol/mol creat.)
Time since the onset of exposure (h)

Fig3C

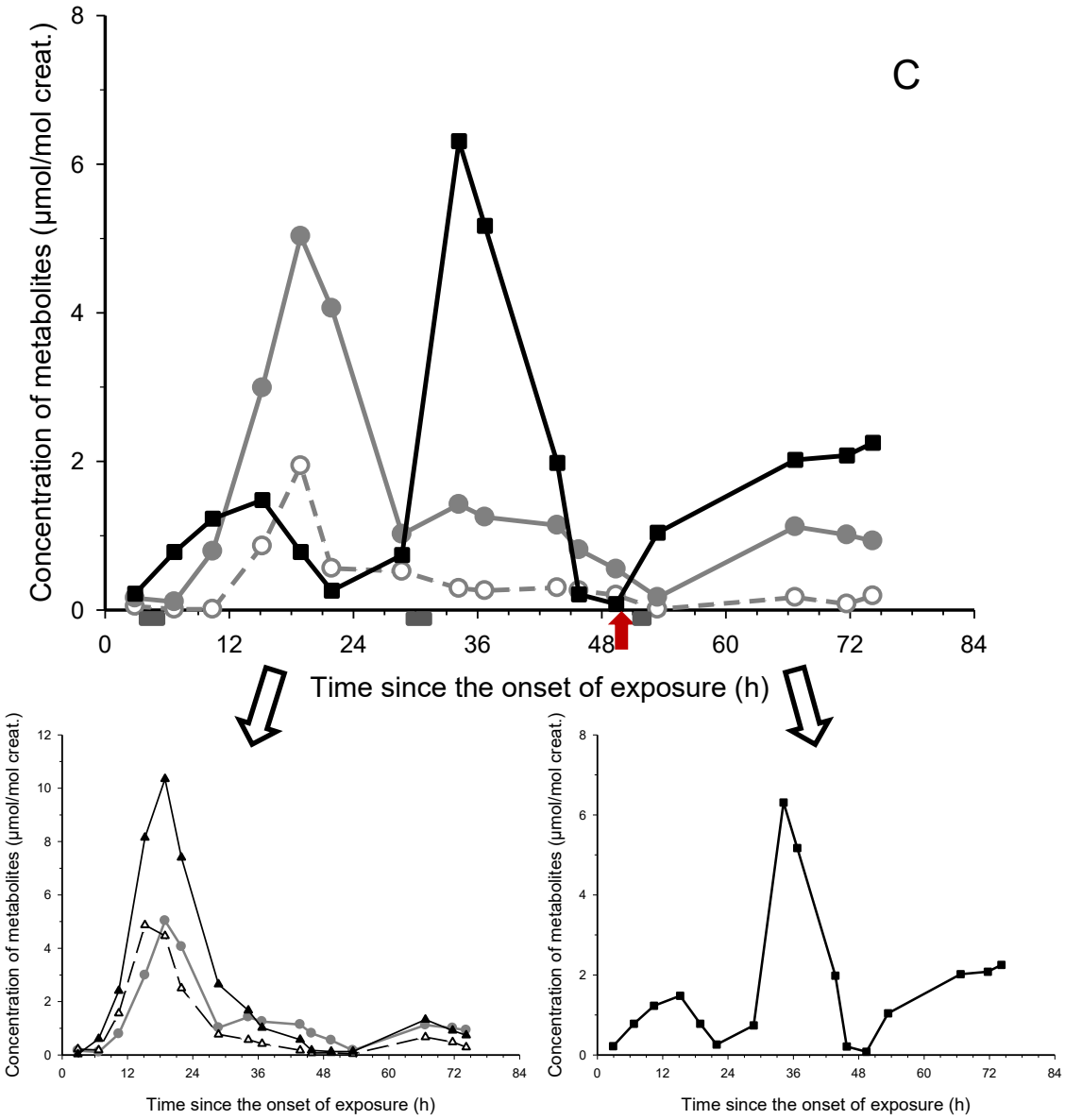

C
Concentration of metabolites (μmol/mol creat.)
Time since the onset of exposure (h)
0
2
4
6
8
0
12
24
36
48
60
72
84
Concentration of metabolites (μmol/mol creat.)
Time since the onset of exposure (h)
0
2
4
6
8
10
12
0
12
24
36
48
60
72
84
Concentration of metabolites (μmol/mol creat.)
Time since the onset of exposure (h)

Fig3D

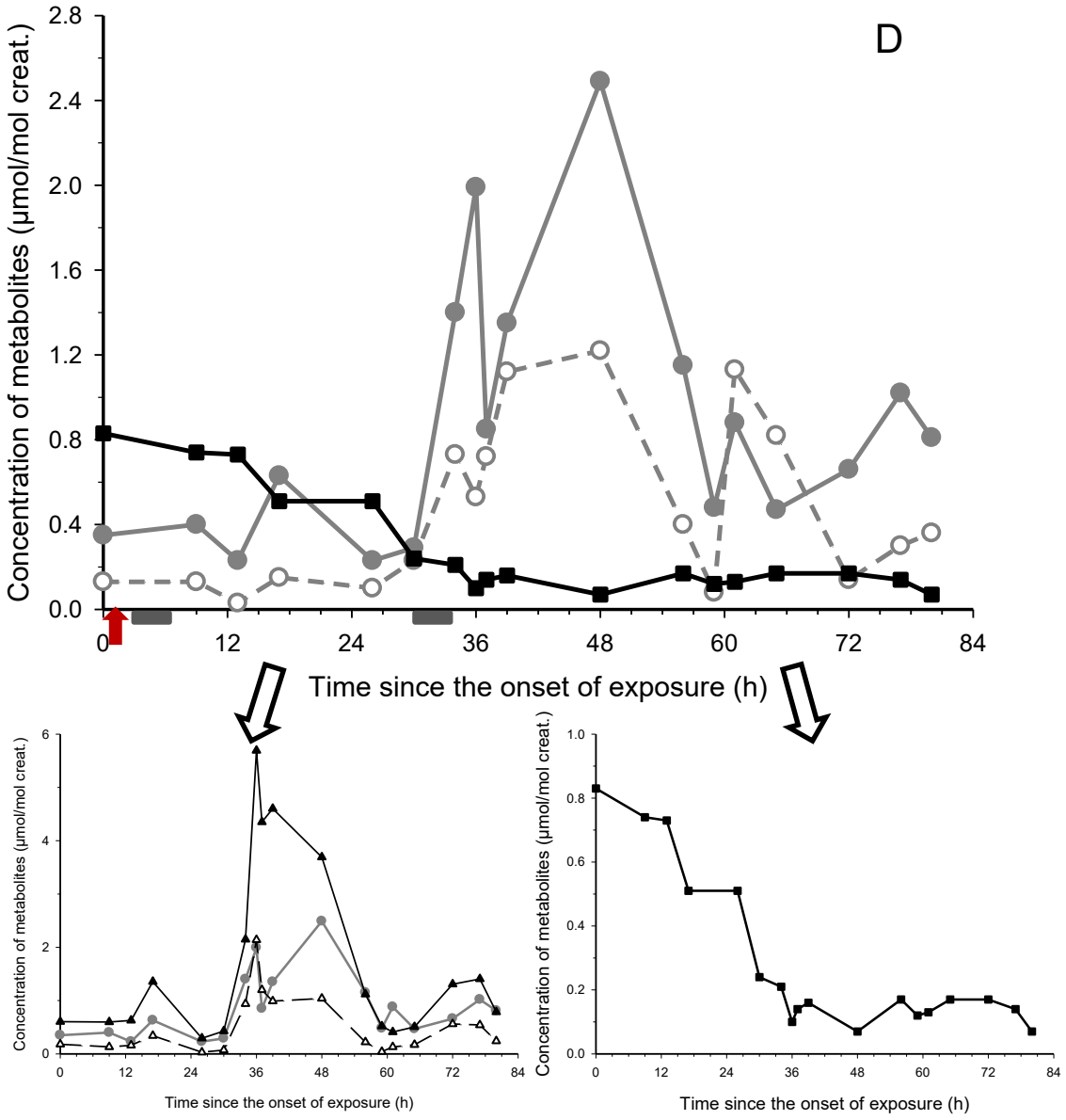

D
Concentration of metabolites (μmol/mol creat.)
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
0
12
24
36
48
60
72
84
Time since the onset of exposure (h)
Concentration of metabolites (μmol/mol creat.)
6
4
2
0
Time since the onset of exposure (h)
Concentration of metabolites (μmol/mol creat.)
1.0
0.8
0.6
0.4
0.2
0.0
Time since the onset of exposure (h)

Fig. 4. Concentration-time courses of CFMP (—■—), 3-PBA (—●—) and 4-OH3BPA (--○--), *trans*-DCCA (—▲—), and *cis*-DCCA (--Δ--) in strawberry field worker T106b following the onset of spraying of Silencer® (LCT alone - E1) **(A),** or Silencer® in combination with Captan® and Switch® fungicides (E5) **(B),** or Silencer® in combination with the Captan® (E2) **(C)**. Main graph compares the time courses of CMFP, 3-PBA and 4-OH3BPA; secondary graph on left compares the times courses of 3-PBA, *trans*-DCCA and *cis*-DCCA; secondary graph on the right highlights the time course of CFMP on a different scale. The red arrow represents spraying period. The grey mark below the x axis represents either maintenance of equipment or inspection tasks in the treated area.

FIG 4A

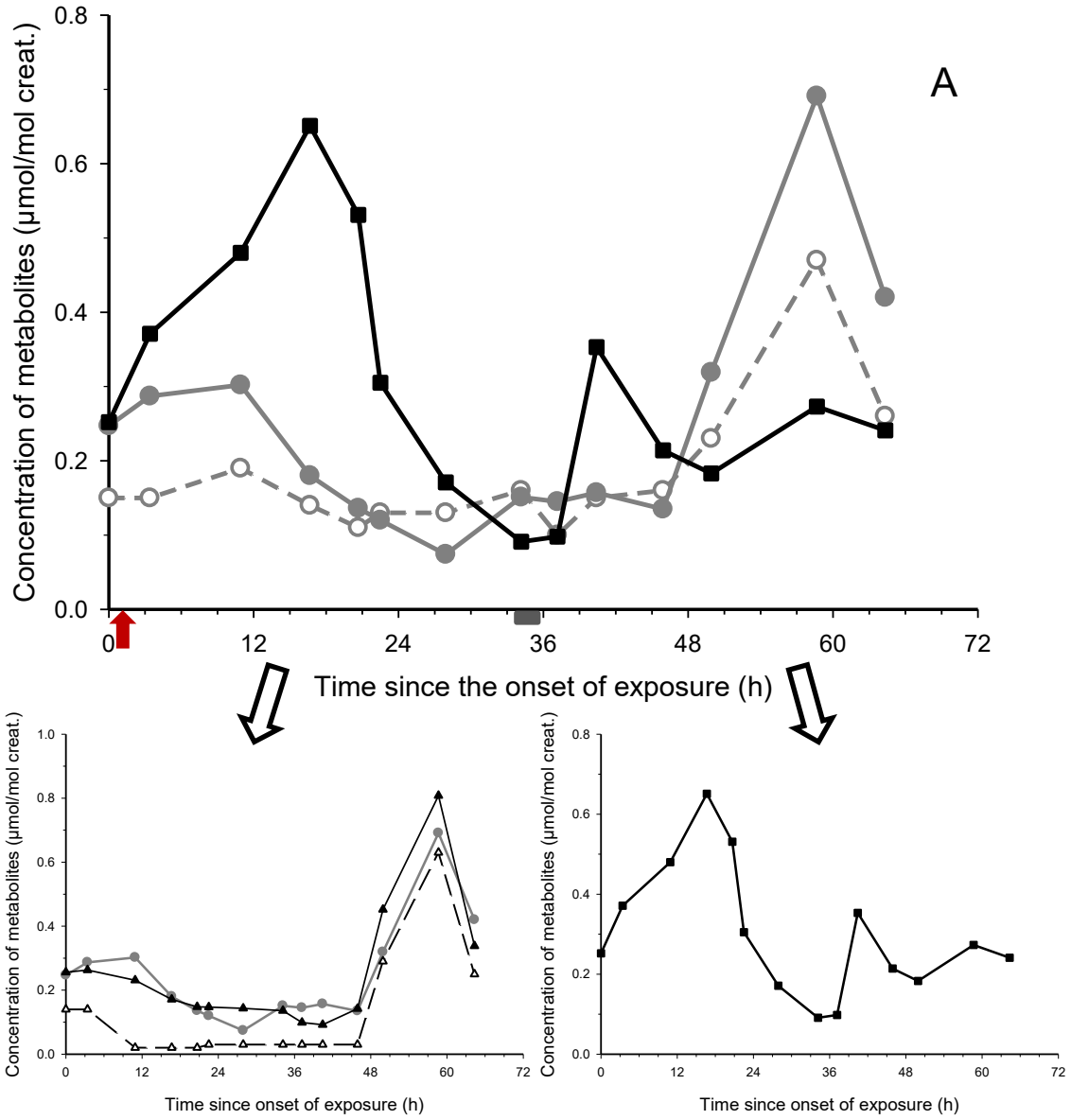

A
Concentration of metabolites (µmol/mol creat.)
0.0
0.2
0.4
0.6
0.8
0
12
24
36
48
60
72
Time since the onset of exposure (h)
Concentration of metabolites (µmol/mol creat.)
1.0
0.8
0.6
0.4
0.2
0.0
0
12
24
36
48
60
72
Time since onset of exposure (h)
Concentration of metabolites (µmol/mol creat.)
0.8
0.6
0.4
0.2
0.0
0
12
24
36
48
60
72
Time since onset of exposure (h)

FIG 4B

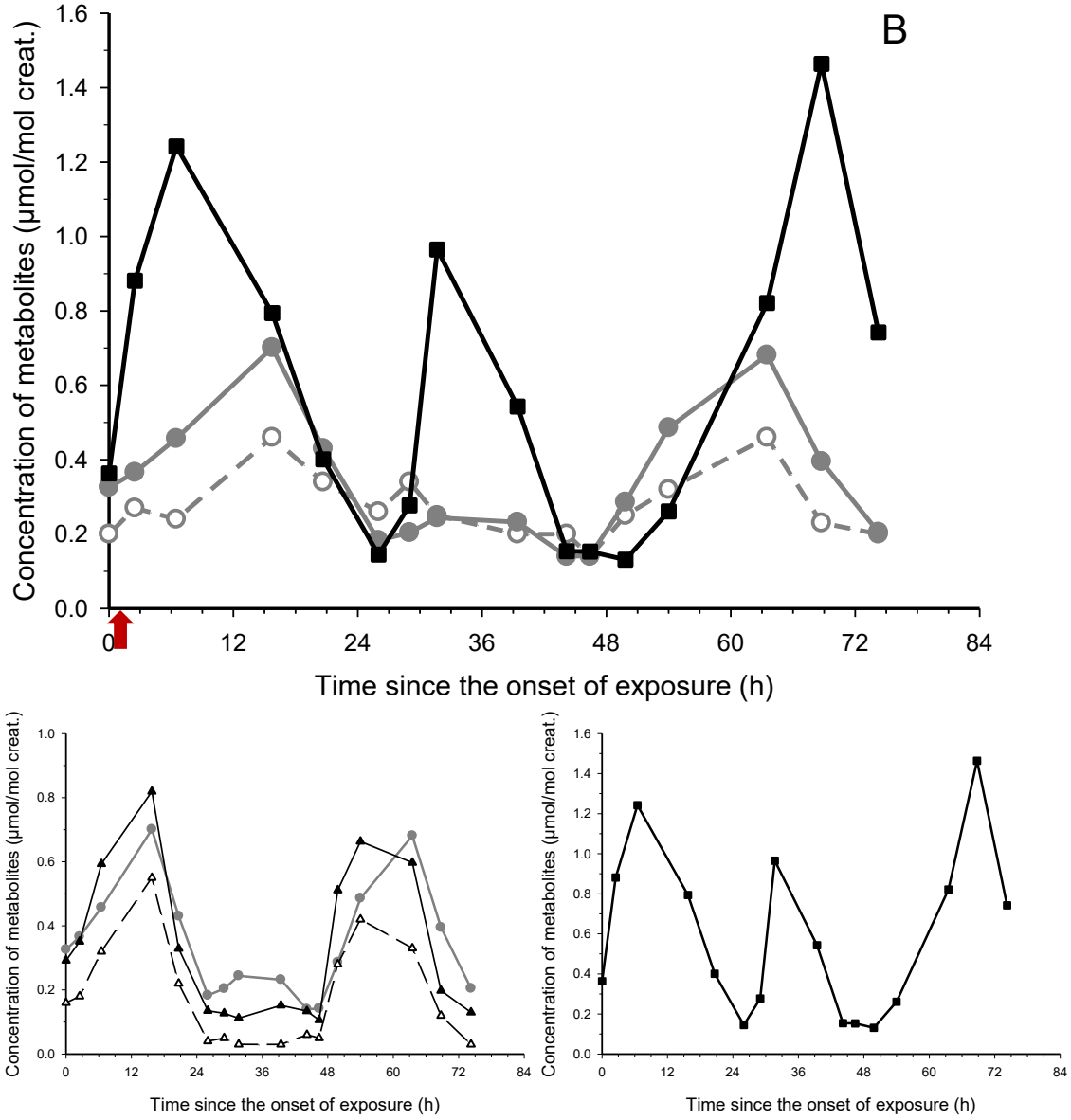

B
Concentration of metabolites (μmol/mol creat.)
Time since the onset of exposure (h)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
0
12
24
36
48
60
72
84

FIG 4C

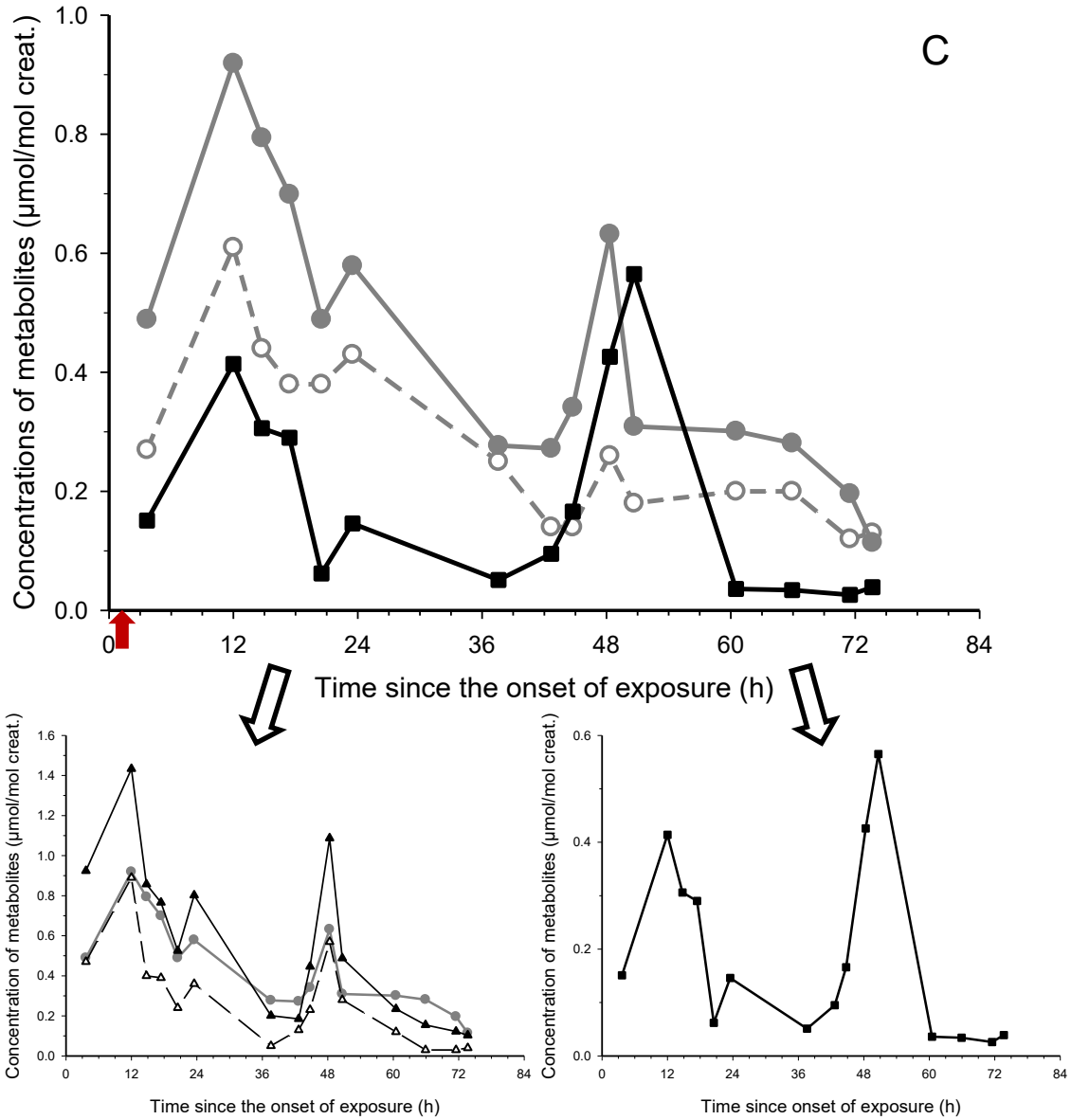

C
Concentrations of metabolites (μmol/mol creat.)
1.0
0.8
0.6
0.4
0.2
0.0
0
12
24
36
48
60
72
84
Time since the onset of exposure (h)
Concentration of metabolites (μmol/mol creat.)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Time since the onset of exposure (h)
Concentration of metabolites (μmol/mol creat.)
0.6
0.4
0.2
0.0
Time since onset of exposure (h)

Fig. 5. Concentration-time courses of CFMP (—■—), 3-PBA (—●—) and 4-OH3BPA (--○--), *trans*-DCCA (—▲—), in strawberry field worker T108 following the onset of spraying of Silencer® (LCT alone - E1) **(A)** or in combination with Captan® (E2) **(B)**. Main graph compares the time courses of CMFP, 3-PBA and 4-OH3BPA; secondary graph on left compares the times courses of 3-PBA and *trans*-DCCA; secondary graph on the right highlights the time course of CFMP. The red arrow represents spraying period. The grey mark below the x axis represents handling and applying pesticides other than LCT.

Fig5A

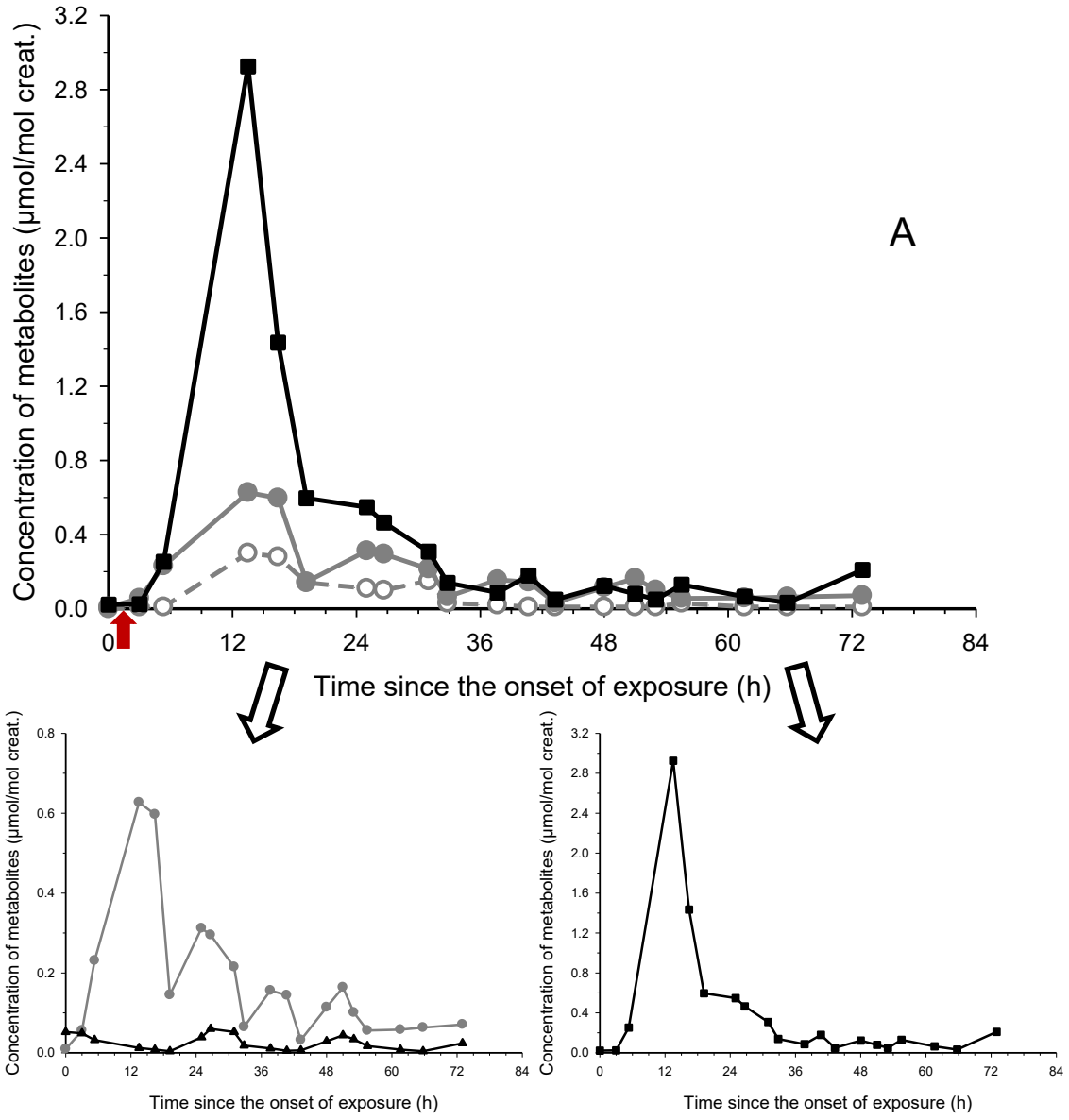
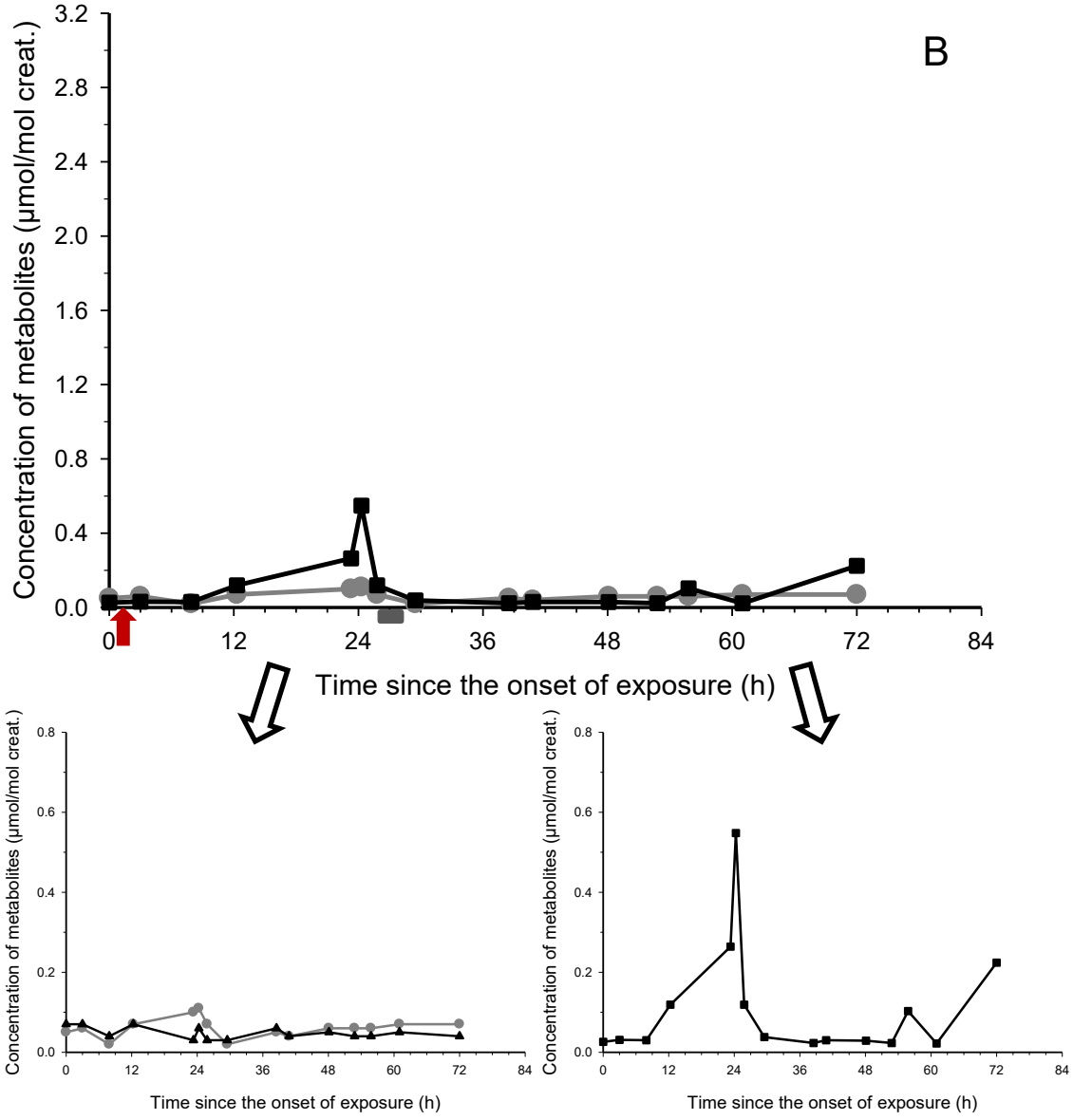

Concentration of metabolites (μmol/mol creat.)
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
0
12
24
36
48
60
72
84
A
Time since the onset of exposure (h)
Concentration of metabolites (μmol/mol creat.)
0.8
0.6
0.4
0.2
0.0
0
12
24
36
48
60
72
84
Time since the onset of exposure (h)
Concentration of metabolites (μmol/mol creat.)
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
0
12
24
36
48
60
72
84
Time since the onset of exposure (h)

Fig5B


B
Concentration of metabolites (μmol/mol creat.)
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
0
12
24
36
48
60
72
84
Time since the onset of exposure (h)
Concentration of metabolites (μmol/mol creat.)
0.8
0.6
0.4
0.2
0.0
0
12
24
36
48
60
72
84
Time since the onset of exposure (h)
Concentration of metabolites (μmol/mol creat.)
0.8
0.6
0.4
0.2
0.0
0
12
24
36
48
60
72
84
Time since the onset of exposure (h)

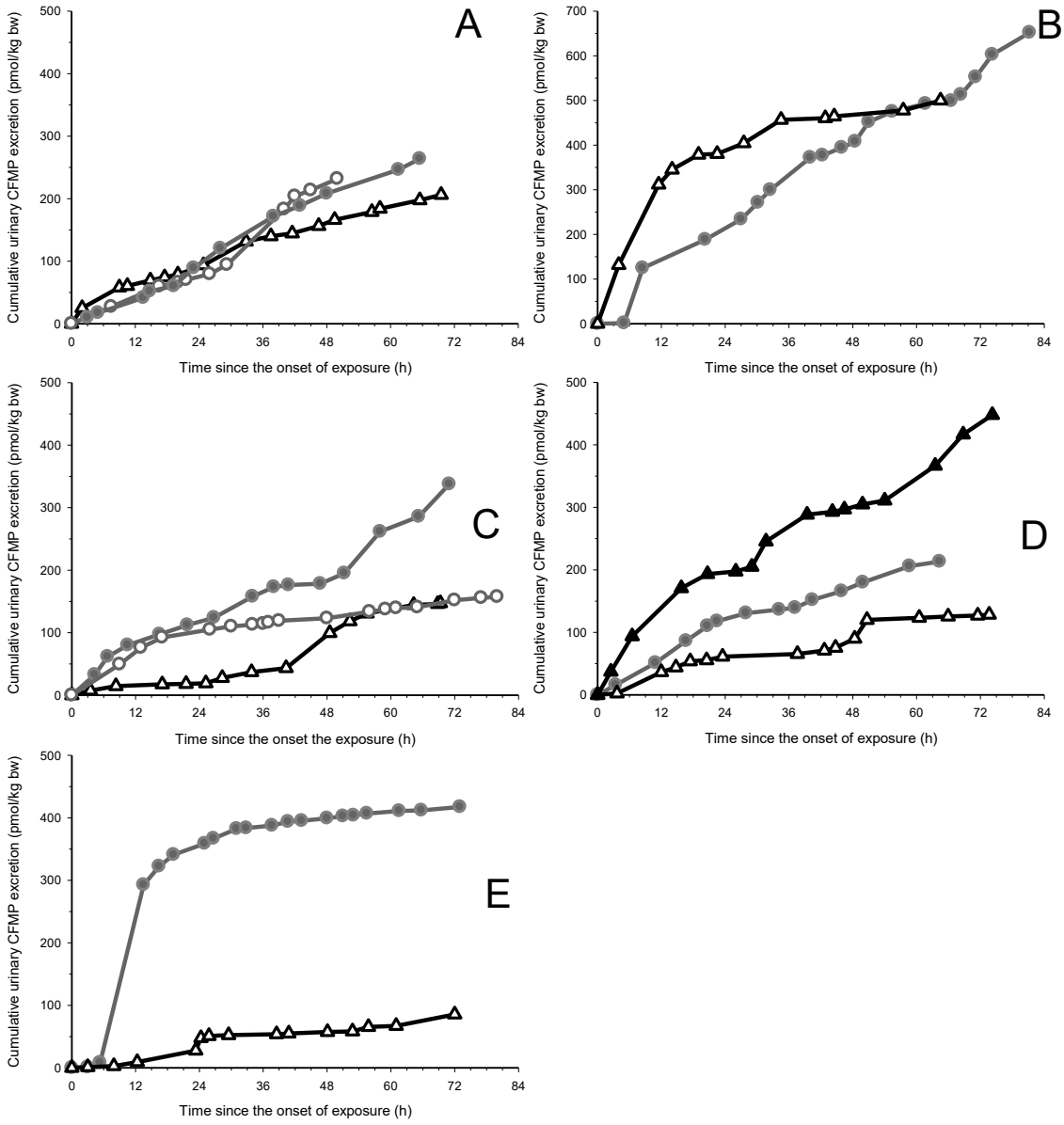


Fig. 6. Cumulative urinary excretion time courses of CFMP (pmol/kg bw) in agricultural workers T101 **(A)**, T103 **(B)**; T106 **(C)**; T106b **(D)** and T108 **(E)** following the onset of exposure to Matador® or Silencer® formulations containing LCT alone (E1 = —●—; E1b second application = —○—) or in combination with Captan® (E2 = ─Δ─) or Captan® and Switch® fungicides (E5 = ─▲─).

Table 2 Exposure conditions for each applicator during the 3-day biomonitoring period, as documented by self administered questionnaire.

Worker	Figure	Exposure scenario - Applied pesticide formulation	Tasks and time interval following the onset of biomonitoring		
			Day 1	Day 2	Day 3
T101	**Fig. 1A**	E1 - Matador®	3-4.5 h: Mixing/ loading outside/Spraying Matador® in tractor with cabin (duration: 60 min)	20-21.5 h: Mixing/ loading/ Spraying pesticides other than LCT (Bravo® and Switch®) in the field treated with LCT (duration: 90 min) 28.5-30 h: Handling other pesticides and application of these pesticides in a field not treated with LCT (Envidor® +Exirel® +Maxcel® +Ceyva® +Calcium) (duration: 90 min)	
	Fig. 1B	E2 - Matador® + Captan®	0- 1.5 h: Mixing/Loading outside/Spraying Matador®+captan® in tractor with cabin (duration: 90 min)		
	Fig. 1C	E3 (without re-entry in LCT treated field)			63-66 h: Handling other pesticides and application of these pesticides in a field not treated with LCT (Pixarro®) (duration: 180 min) 68-75 h: Handling other pesticides and application of these pesticides in a field not treated with LCT (Authority®) (duration: 7 h)
T103	**Fig. 2A**	E1 - Silencer®	0-3.5 h: Mixing/Loading outside/ Spraying Silencer® in tractor with cabin (duration: 210 min) 3.5-8.5 h: Maintenance of equipment in contact with pesticides/Inspection of LCT-treated field (duration: 300 min)		
	Fig. 2B	E2 - Silencer® + Captan®	0-9.5 h: Mixing/Loading outside/Spraying Silencer® + Captan® in tractor with cabin/Cleaning and maintenance of equipment in contact with pesticides/Inspection of treated		

Worker	Figure	Exposure scenario - Applied pesticide formulation	Tasks and time interval following the onset of biomonitoring		
			Day 1	Day 2	Day 3
			field (duration: 570 min - no reported time distinction between tasks)		
T106	**Fig. 3A**	E1 - Matador®	0.25-1 h: Mixing/Loading outside/Spraying Matador® in tractor with cabin/Cleaning equipment in contact with pesticides (duration: 60 min) 0.25-0.33 h: Handling other pesticides and application in a field not treated with LCT (duration: 5 min)		
	Fig. 3B	E2 - Matador® +Captan®	1.75-3.25 h: Mixing/Loading ouside/Spraying Matador®+Captan® in tractor with cabin/Cleaning equipment in contact with pesticides (duration: 95 min) 18.25-18.5 h: Inspection of LCT-treated field (duration: 15 min) 18.5-20.75 h: Handling other pesticides and application in a field not treated with LCT (Round-up® and Lumax®) (duration: 135 min)	23.5-25.5 h: Inspection of LCT-treated field (duration: 120 min) 29.5-32.5 h: Handling other pesticides and application in a field not treated with LCT (Round-up®) (duration: 120 min)	52.5-53.25 h: First daily inspection of LCT-treated field (duration: 15 min) 65.5-66 h: Second daily inspection of LCT-treated field (duration: 30 min)
	Fig. 3C	E3 - Work in field treated with Matador® +Captan®	7.2-7.4 h: First inspection of LCT-treated field (duration: 15 min) 28.2-28.4 h: Second inspection of LCT-treated field 21 h later (duration: 15 min)		50.2-51.2 h: Mixing/Loading outside/Spraying Matador® in tractor with cabin, and Luna Tranquility® handling and application in a field not treated with LCT (duration for both applications: 60 min); 57.2-59.2 h: Handling other pesticides and application in a field not treated with LCT (Round-up® and Malex®) (duration: 180 min)
	Fig. 3D	E4 - Matador® - in a sweet corn field	4.75-6.1 h: Mixing/loading outside/Spraying Matador® in tractor with cabin in a sweet corn	27.75- 27.8 h: Inspection of LCT-treated area (duration: 5 min)	

Worker	Figure	Exposure scenario - Applied pesticide formulation	Tasks and time interval following the onset of biomonitoring		
			Day 1	Day 2	Day 3
			field/maintenance and handling of the pulverizer and tractor (duration: 80 min) 7.0-7.7 h: Handling other pesticides and application in a field not treated with LCT (duration: 40 min)		
T106b	**Fig. 4A**	E1 - Silencer®	0.2-1.3 h: Mixing/loading outside/Spraying Silencer® in tractor with cabin (duration: 60 min)	34.4-34.9 h: Inspection of LCT-treated area (duration: 30 min)	
	Fig. 4B	E5 - Captan® in field treated days before with Silencer®	0.25-1.5 h: Captan® and Switch® fungicide handling and application in a field treated 3-days before with LCT (Silencer®) (duration: 60 min)		
	Fig. 4C	E2 - Silencer® + Captan®	2.5-3.5 h: Mixing/Loading outside/Spraying Silencer® + Captan® in tractor with cabin/Cleaning equipment in contact with pesticides (duration: 55 min)		
T108	**Fig. 5A**	E1 - Silencer®	1-2.5 h: Mixing/Loading/Spraying Silencer® in tractor with cabin (duration: 90 min)		
	Fig. 5B	E2 - Silencer® + Captan®	2-3.5 h: Mixing/ Loading outside/Spraying Silencer® + Captan® in tractor with cabin/Cleaning equipment in contact with pesticides (duration: 75 min)	26-27.5 h: Handling other pesticides and application in a field not treated with LCT (Intercept®) (duration: 150 min)	

Comparison of excretion profiles following exposure to LCT alone or in combination with captan

Figures 1 to 5 show that there were no obvious differences in the individual profiles of metabolites (CFMP, 3-PBA, 4-OH3BPA) after exposure to LCT alone or LCT in combination with captan on the basis of the time courses of creatinine concentrations during the 3-day period following spraying. The time courses of metabolites in urine expressed as creatinine adjusted concentrations (µmol/mol creat.) were also similar to those of excretion rates (pmol/kg bw/h) (data not shown). Figure 6 shows that the cumulative excretion time courses of CFMP – the more specific metabolite of LCT – during the 3-day period following the onset of a spraying episode of formulations containing LCT alone or mixed with captan also failed to show a different trend in excretion for these two exposure conditions.

Times courses of the different metabolites for a same individual

For most of the workers and exposure scenarios, CFMP was the main metabolite excreted (T101-E1 – Fig. 1A; T103-E1 – Fig. 2A; T106-E1 – Fig. 3A, T106-E2 – Fig. 3B and T106-E3 – Fig. 3C; T106b-E1 – Fig. 4A and T106b-E5 – Fig. 4B; T108-E1 – Fig. 5A and T108-E2 – Fig. 5B). However, data revealed that the time course of CFMP in the urine of some operators did not always follow that of 3-PBA and 4-OH3BPA, which are metabolites common to pyrethroids other than LCT (Figs. 1A, 1B, 1C, 2A, 3B, 3D, 4A). This was the case for workers T101, T103, T106 and T106b when exposed to formulations containing LCT alone (T101-E1, T103-E1, T106-E4 and T106b-E1) and T101 and T106 when coexposed to LCT and captan (T101-E2 and T106-E2). In several cases, the time courses of 3-PBA and 4-OH3BPA in urine followed that of *trans*-DCCA and *cis*-DCCA, which are metabolites of permethrin and cypermethrin (Fig.

1B, 1C, 2B, 3A, 3B, 3C, 3D, 4A, 4B, 4C). This was observed for workers T101 and T103 exposed to formulations containing LCT in combination with captan (E2) and for worker T106 and T106b exposed to both formulations containing LCT alone (E1) or in combination with captan (E2) (T101-E2 and E3 – Fig. 1B and 1C; T103-E2 – Fig. 2B; T106-E1, E2, E3, E4 – Fig. 3A, 3B, 3C and 3D; T106b-E1, E5 and E2 – Fig. 4A, 4B and 4C); in some cases, 3-PBA and 4-OH3BPA levels were also higher than that of CFMP (T101-E2 and E3 – Fig. 1B and 1C; T103-E2 – Fig. 2B; T106-E4 – Fig. 3D; T106b-E2 – Fig. 4C), suggesting a coexposure to pyrethroids other than LCT.

Times courses of metabolites with regards to spraying and other tasks

Furthermore, for several workers and exposure scenarios E1 and E2, values of CFMP increased in the hours following spraying compatible with an exposure due to this task (Figs. 1A, 1B, 2A, 2B, 3A, 3B, 4A, 4B, 4C, 5A). Also, regardless of the spraying exposure scenario (E1 or E2) and in the absence of subsequent pesticide exposure during other tasks, results show that most of the CFMP was eliminated from the body (hence urinary levels returned to baseline levels) during the first 24 h following the end of the exposure episode (Figs. 1A, 1B, 2B, 3A, 3B, 3C, 4A, 4B, 4C, 5A). However, for many pesticide operators, other peaks of CFMP were observed at later times (Fig. 1A, Fig. 1B, Fig. 1C, Fig. 2A, Fig. 2B, Fig. 3B, Fig. 3C, Fig. 4A, 4B, 4C), thus indicating that tasks other than spraying of LCT-containing formulations contributed to this increased exposure. For 3-PBA and 4-OH3BPA in some workers and some exposure scenarios, peak excretion levels of metabolites were not observed (or not only observed) in the hours following spraying but rather (also) on subsequent days (Fig. 1B, Fig. 1C, Fig. 3D, Fig. 4A, Fig. 4B, Fig. 4C), again confirming an exposure to LCT due to tasks other than spraying of LCT-containing formulations and/or an exposure to other pyrethroids than LCT.

Linking increased excretion to response to the self-administered questionnaire

In some cases, questionnaire responses (Table 2) allowed to link the increased excretions of metabolites in urine – at time points other than the hours following spraying of formulations containing LCT – to tasks such as handling/cleaning of equipment used for spraying (tractor or sprayer) (Figs. 2A, 2B) or work/inspection in the LCT-treated field (Figs. 2A, 2B, 3B, 3C, 4A). For two workers, it was also related to the application of another pesticide in the LCT-treated field during the 3-day biomonitoring period (Figs. 1A) or in an adjacent field not treated with LCT but using the same equipment (tractor, sprayer) (Fig. 3B, 3C). More specifically, for worker T101-E1 (Fig. 1A), this implied a re-entry in the treated area to apply the fungicide Bravo® in the strawberry field along with a contact with the equipment used to spray LCT, which was used on day two of biomonitoring to spray non-pyrethroid and non-captan pesticides (Endivor® + Exirel® + Maxcel® + Ceyva® + calcium) in an apple orchard that was not previously treated with pyrethroids (Table 2). For worker T103 and scenarios E1 and E2 (Fig. 2A and 2B), the increase in levels of CFMP observed is compatible with reported LCT spraying period but also handling/maintenance of the equipment used for spraying and work/inspection in LCT-treated field, as indicated in the questionnaire (Table 2). For worker T106 and scenarios E2 and E3 (Figs. 3B and 3C), varying levels of CFMP during the biomonitoring period and in particular during the 36- to 72-h period following application for scenario E2 was compatible with reported inspection of LCT-treated field and handling and application of a non-pyrethroid pesticide (Round-up®) in a field not treated with LCT, which implied a contact with possibly LCT-contaminated equipment used for application (tractor or sprayer…) (Table 2). Again, for worker T106b and scenario E1 (Fig. 4A), peak levels of metabolites observed between 45 and 72 h after application are consistent with the reported inspection task performed in the LCT-treated area (Table 2).

On the other hand, in some cases, increased metabolite levels in urine were observed whereas the applicator did not self-report a specific task in the field (T101-E2 – Fig. 1B; T106b-E5 – Fig. 4B and T106b-E2 – Fig. 4C). For worker T101 and scenario E2 (Fig. 1B), a significant increase in *trans*- and *cis*-DCCA levels was observed at the same time points as 3-PBA and 4-OH3BPA, hence showing similar time courses for all four metabolites; this confirmed that this excretion was not related to LCT exposure, but rather to other pyrethroids such as permethrin or cypermethrin. However, the elevated levels of 3-PBA, 4-OH3PBA, and *trans*- and *cis*-DCCA observed were not related to any tasks performed in LCT treated fields, as reported in Table 2. For worker T106b and scenarios E5 and E2 (Figs. 4B and 4C), although he did not state any task in the treated area after spraying period (Table 2), the metabolite excretion pattern was consistent with a repeated daily exposure to LCT and at least one pyrethroid other than LCT. Supplementary file S1 details results of individual kinetic profiles of metabolites for the different workers after exposure to LCT alone or coexposure with LCT and captan, and relation with questionnaire responses.

Similar to Table 2, Table 3 reports protective equipment used by workers represented in Figs.1 to 5. All workers wore long or short-sleeved shirt and long pants. Workers T103, T108, and T106b [for E1 (day 2) and E5 episodes] wore leather boots, while workers T106, T101 (only for E3 episode) and T106b [for E1 (day1) and E2 episode] wore closed shoes. Worker T101 also wore rubber boots (for E1 and E2). Only workers T101 and T106, and T106b wore rubber gloves during mixing and loading. Only T101 (for E3 episode), T106 (for E1 episode) and T108 [E1; E2 (days2)] wore glasses where handling and applying pesticide. Workers T106, T106b and T108 mentioned wearing a half-mask with filter during mixing and loading LCT; T108 mentioned wearing a full helmet with filter when mixing and loading the mix of LCT and captan or pesticides other than LCT in a field not treated with LCT.

Table 3 Personal protective equipment for each applicator during the 3-day biomonitoring period, as documented by self administered questionnaire

Worker	Figure	Exposure scenario - Applied pesticide formulation	Protective equipment and clothing		
			Day 1	Day 2	Day 3
T101	**Fig. 1A**	E1 - Matador®	3-4.5 h: Long-sleeved shirt, long pants, rubber gloves, rubber boots	20-30 h: Long-sleeved shirt, long pants, rubber gloves, rubber boots	
	Fig. 1B	E2 - Matador® + Captan®	0-1.5 h: Long-sleeved shirt, long pants, rubber gloves, rubber boots		
	Fig. 1C	E3 (without re-entry in LCT treated field)			63-75 h: Glasses, long-sleeved shirt, rubber gloves, closed shoes
T103	**Fig. 2A**	E1 - Silencer®	0-8.5 h: Long-sleeved shirt, long pants, leather boots		
	Fig. 2B	E2 - Silencer® + Captan®	0-9.5 h: Long-sleeved shirt, long pants, leather boots		
T106	**Fig. 3A**	E1 - Matador®	0.25-1h: Half-mask with filter during mixing and loading only, fabric hat, glasses or visor, shirt/long-sleeved shirt, long pants, rubber gloves, closed shoes 0.25-0.33 h: Fabrics hat, shirt/long-sleeved shirt, long pants, closed shoes		
	Fig. 3B	E2 - Matador® +Captan®	1.75-3.25 h: Half-mask with filter during mixing and loading, fabric hat, shirt/long-sleeved shirt, long pants, rubber gloves, closed shoes 18.25-18.5 h: Fabric hat, shirt/short-sleeved shirt, long pants, closed shoes 18.5-20.75 h: Half-mask with filter during mixing and loading, fabric hat, shirt/long-sleeved shirt, long pants, rubber gloves, closed shoes	23.5-25.5 h: Waterproof hat, short-sleeved shirt, long pants, closed shoes 29.5-32.5 h: Half-mask with filter, long-sleeved shirt/shirt, long pants, robber gloves, closed shoes	52.5-66 h: NR
	Fig. 3C	E3 - Work in field treated with Matador® + Captan®	7.2-28.4 h: Waterproof hat, long-sleeved shirt, long pants, closed shoes		50.2-59.2 h: NR

Worker	Figure	Exposure scenario - Applied pesticide formulation	Protective equipment and clothing		
			Day 1	Day 2	Day 3
	Fig. 3D	E4 - Matador® - in a sweet corn field	4.75-6.1 h: Fabric hat, shirt/short-sleeved shirt, long pants, rubber gloves, closed shoes 7.0-7.7 h: Fabric hat, shirt/short-sleeved shirt, long pants, closed shoes	27.75-27.8 h: Fabric hat, shirt/short-sleeved shirt, long pants, closed shoes	
T106b	**Fig. 4A**	E1 - Silencer®	0.2-1.3 h: Half-mask with filter during mixing and loading only, fabric hat, shirt/long-sleeved shirt, long pants, rubber gloves, closed shoes	34.4-34.9 h: Fabric hat, shirt/short-sleeved shirt, long pants, leather boots	
	Fig. 4B	E5 - Captan® in field treated days before with Silencer®	0.25-1.5 h: Half-mask with filter, fabric hat, shirt/short-sleeved shirt, rubber gloves, leather boots		
	Fig. 4C	E2 - Silencer® + Captan®	2.5-3.5 h: Half-mask with filter during mixing and loading only, fabric hat, shirt/long-sleeved shirt, long pants, rubber gloves, closed shoes		
T108	**Fig. 5A**	E1 - Silencer®	1-2.5 h: Half mask with filter during mixing and loading only, glasses otherwise, shirt/short-sleeved shirt, long pants, leather boots		
	Fig. 5B	E2 - Silencer® + Captan®	2-3.5 h: Full helmet with filter for mixing and loading only, long-sleeved shirt/ shirt, long pants, leather boots	26-27.5 h: Full helmet with filter during mixing and loading, glasses otherwise, shirt/long-sleeved shirt, long pants, leather boots	

DISCUSSION

Captan coexposure effect on the concentration-time course of LCT metabolites

This study is the first to assess the effect of mixed fungicide-pyrethroid exposure in an occupational environment on biomarkers of exposure in workers. Such coexposure effect on the toxicokinetics (CYP 450 metabolism or excretion) of pesticide compounds was identified in animals exposed to high doses (Bossou *et al.*, 2020; Hirosawa *et al.*, 2011; Wielgomas, B. et Krechniak, J., 2007). *In vitro* studies also showed that pyrethroids and tetrahydrophthalimide metabolites of captan share common CYP450 hepatic metabolism pathways (such as CYP3A4 in humans (Scollon, Edward J. *et al.*, 2009)) and that captan can impair CYP450 metabolism of lipophilic compounds (Guengerich, 2008; Paolini *et al.*, 1999). Therefore, coexposure effect should also be considered in the context of biomonitoring of exposure in workers using metabolite levels in urine, as excretion levels may be biased if such effects were to occur at exposure levels observed in occupational settings. Assessment of the concentration-time courses of LCT metabolites (CFMP, 3-PBA, 4-OH3BPA) in agricultural workers performing spraying tasks revealed the absence of captan coexposure effect when comparing profiles during a 3-day period following spraying of LCT alone or in combination with captan. A lack of trend was also observed when comparing excretion rates of the three metabolites or 3-day cumulative excretion of the more specific CFMP metabolite of LCT post-application. The only available comparable kinetic study was performed in rats following oral gavage of LCT alone (2.5 or 12.5 mg/kg bw) or as a binary mixture with captan (2.5/2.5 or 2.5/12.5 or 12.5/12.5 mg/kg bw) (Bossou *et al.*, 2020) and pointed to the absence of interaction at

the no-observed adverse effect level (NOAEL) dose of LCT of 2.5 mg/kg bw reported by the U.S. EPA (2004). However, an effect of coexposure on the benzyl metabolite pathway was apparent at that high dose of 12.5 mg/kg bw corresponding to the lowest-observed adverse effect level (LOAEL) reported by the U.S. EPA (2004). More specifically, this experimental study in rats showed the absence of coexposure effect on the kinetic profiles of CFMP and 3-PBA metabolites in plasma when comparing results after administration of 2.5 mg LCT alone/kg bw or in combination with 2.5 and 12.5 mg captan/kg bw. On the other hand, plasma levels of 3-PBA in the first 24 h post-dosing were lower in rats administered a binary mixture of 12.5 mg LCT and captan/kg bw compared to those exposed to the 12.5 mg LCT alone/kg bw. This experimental study in rats also revealed that increasing the LCT dose had an effect on the temporal profiles of metabolites in plasma and in excreta. Nevertheless, both the current study in workers and the experiment in rats by Bossou *et al.* (2020) concur to indicate that measurements of CFMP and 3-PBA metabolites are not influenced by coexposure when pesticide exposure levels remain below the toxicological reference values.

Only a few studies have looked at the impact of coexposure on levels of metabolites used for the biomonitoring of exposure to pesticides in individuals. These studies were mostly performed in rats at relatively high doses. In particular, Timchalk *et al.* (2005) showed that coexposure to organophosphate (OP) insecticides in binary mixture had an impact on their rate of absorption and elimination. In the case of coexposure with pyrethroids more specifically, Hirosawa *et al.* (2011) reported a significantly lower excretion of 3-PBA in rats pretreated intraperitoneally with the OP insecticide dichlorvos at 0.3 or 1.5 mg/kg bw and then intravenously injected with a single high dose of cis-permethrin (20 mg/kg bw). Wielgomas et Krechniak (2007) further reported a decrease in the excretion of urinary metabolites of the pyrethroid α-cypermethrin in rats after coexposure with

the OP insecticide chlorpyrifos by gavage at 5 mg/kg bw of each of the compounds, seven days/week for 28 days as compared to 10 mg/kg bw of α-cypermethrin alone.

Similar to our results, in one of the only published study on the impact of coexposure on the kinetics of pyrethroids and their metabolites in humans, there was no apparent effect of coexposure to the pyrethroid deltamethrin in combination with the OP chlorpyrifos-methyl at the acceptable daily intake (ADI) dose of 0.01 mg/kg bw on the excretion profiles of metabolites (3-(2,2-dibromovinyl)-2,2-dimethyl-(1-cyclopropane)carboxylic acid (DVBA) and 3-PBA) in urine (Sams et Jones, 2011b).

Comparison of the time courses of the different pyrethroid metabolites

Furthermore, in the current study, CFMP was found in highest concentrations in urine of most of the assessed workers following the different exposure scenarios, but the concentration-time profile of CFMP did not always follow that of the phenoxybenzoic acid metabolites (3-PBA and 4-OH3BPA) common to other pyrethroids. In some cases where excretion of 3-BPA and 4-OH3BPA in urine was observed to be higher than that of CFMP, their time courses followed that of *trans*-DCCA and *cis*-DCCA, which points to a concomitant exposure to pyrethroids generating these metabolites such as permethrin and cypermethrin. These results underline the importance of assessing the specific metabolites for the biomonitoring of exposure and impact of coexposure with other pesticides such as captan. The importance of monitoring specific metabolites to assess occupational exposure was also highlighted in the biomonitoring study by Berthet *et al.* (2012) in workers exposed to captan and folpet.

In addition, the study of Khemiri *et al.* (2017) depicted similar time profiles of CFMP and 3-PBA in both plasma and urine of volunteers orally exposed to the Reference dose (RfD) level of 2.5 µg/kg bw established by the U.S. EPA (2004) under controlled conditions (peak plasma concentrations around 3-4 h following ingestion and mean elimination half-life of 5-6 h). On the other hand, the experimental study of Bossou *et al.* (2020) in rats revealed kinetic differences between metabolites with a faster elimination of 3-PBA and 4-OH3BPA compared to CFMP (with a shorter residence time of the phenoxybenzoic acids in plasma). This can be either attributed to species specific differences in the kinetics or a high-dose effect in the rat study, not likely to be observed at lower exposure levels such as those observed in workers.

Times courses of metabolites with regards to spraying and other tasks

For several workers, results of the current study also allowed to link a LCT spraying episode to an increase in urinary levels of CFMP metabolite during the following hours regardless of the spraying exposure scenario (*i.e.*, exposure to LCT alone (scenario E1) or in combination with captan (scenario E2)). This confirmed that the spraying task contributed to an increase in exposure to LCT with urinary concentration values that exceeded at some time points the baseline values observed in the general population, which are exposed mainly through diet (HealthCanada, 2021). The spraying task was also associated with the highest biomarker peak excretion in a study on a related pyrethroid, cypermethrin (Ratelle, M. *et al.*, 2016) and in a study with captan (Berthet *et al.*, 2012) compared to weeding, harvest, and inspection tasks.

Following peak excretion of CFMP, the observed rapid decline in metabolite concentrations in urine in the applicators who did not handle or perform tasks in the LCT-treated field during the days following spraying confirms previous results in volunteers where an elimination half-life of 5-6 h was reported after oral exposure (Khemiri *et al.*, 2017).

Linking increased excretion to response to the self-administered questionnaire

As observed in Ratelle *et al.* (2016), for several of the assessed workers, increased CFMP or 3-PBA/4-OH3BPA concentrations were not always compatible with the LCT-spraying period but rather with later tasks performed. These tasks include reported inspection of LCT-treated field or spraying other pesticides in the LCT-treated field after the re-entry delay or simply handling

equipment to apply pesticides other than LCT in a field not treated with LCT in some instances. Ferland *et al.* (2015) also showed that inspection and corn picking tasks contributed to increased concentrations of permethrin metabolites in the urine of corn field workers. More specifically in the current study, the questionnaire responses were useful to link increased excretions of metabolites in urine – at time points other than the hours following spraying of formulations containing LCT – to tasks such as handling/maintenance of equipment used for spraying (tractor or sprayer) or work/inspection in the LCT-treated area. For two workers, it was also related to the application of another pesticide in the LCT-treated field during the 3-day biomonitoring period or in an adjacent field not treated with LCT but using the same equipment (tractor, sprayer), which implied a contact with possibly LCT-contaminated equipment used for application.

On the other hand, in some cases, increased metabolite levels in urine were observed whereas the applicator did not self-report performing a specific task in the field, similar to what was observed in the study of Hardt et Angerer (2003). In particular, for exposure scenarios E5 and E3 where tasks were supposed to be performed in the LCT-treated field, worker T106b did not self-report (by questionnaire) any task in the LCT-treated area after the spraying period but the metabolite excretion pattern was consistent with a repeated daily exposure to LCT and at least one pyrethroid other than LCT (Figs. 4B and 4C). The concomitant assessment of *trans*- and *cis*-DCCA levels in urine showing time courses evolving in parallel with 3-PBA and 4-OH3BPA was useful in confirming that phenoxybenzoic acid excretion was partly related exposure to pyrethroids other than LCT, such as permethrin or cypermethrin. Link between excretion levels and personal protective equipment remains to be better assessed.

CONCLUSION

In conclusion, comparison of excretion profiles following LCT exposure alone or in combination with captan did not show a general elimination pattern of CFMP that could be attributed to the impact of coexposure to captan. Meanwhile, factors other than coexposure to captan (contact with contaminated devices or plants) appeared to have more impact on the cumulative time courses and amounts of CFMP excreted in urine over approximately 72 hours following the onset of a spraying episode. The self-administered questionnaire results were also useful to interpret biomonitoring data, that is the rise and decline in metabolite concentrations. However, our analysis pointed out that sometimes this is not enough, and metabolite profiles from other pesticides should be measured in combination. This is most obvious when biomonitoring was based on a non-specific biomarker of exposure as in the case of 3-PBA.

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Author Contributions

Yélian Marc Bossou: Methodology, Formal analysis, Investigation, Writing – Original draft preparation. **Jonathan Côté:** Formal analysis, Investigation, Writing – Reviewing and Editing. **Louiza Mahrouche:** Methodology, Formal analysis, Writing – Reviewing and Editing. **Marc Mantha:** Formal analysis, Investigation, Writing – Reviewing and Editing. **Naïma El Majidi:** Methodology, Writing – Reviewing and Editing. Alexandra Furtos**:** Methodology, Formal analysis, Writing – Reviewing and Editing. **Michèle Bouchard:** Conceptualization, Methodology, Formal analysis, Supervision, Project Management, Writing – Original draft preparation, Writing – Reviewing and Editing, Funding acquisition.

Data Availability

All data generated during this study are included in this article or are available on reasonable request from the corresponding author.

Ethics approval

The study protocol, the information and consent form, and other relevant documents were approved by Research Ethics Committee of the University of Montreal (*Comité d'éthique de la recherche Clinique de Université de Montréal* #CERC-19-007-D).

Consent to participate

The study was based on a voluntary participation. Participants received a slight financial compensation for their involvement and time. Subjects wishing to participate in the study signed an informed consent form, after receiving all necessary information about the project. Each participant was free to withdraw from the study at any time, without any prejudice.

Consent for publication

This manuscript has not been published or presented elsewhere and is not under consideration by another journal. All authors read and approved the final manuscript and consent for publication in Environmental Health.

Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

REFERENCES

Abass K, Pelkonen O (2013) The inhibition of major human hepatic cytochrome P450 enzymes by 18 pesticides: Comparison of the N-in-one and single substrate approaches. Toxicol In Vitro 27(5):1584-1588. https://doi.org/10.1016/j.tiv.2012.05.003

Barr DB, Olsson AO, Wong LY, et al. (2010) Urinary concentrations of metabolites of pyrethroid insecticides in the general U.S. population: National Health and Nutrition Examination Survey 1999-2002. Environ Health Perspect 118(6):742-8. https://doi.org/10.1289/ehp.0901275

Berthet A, Heredia-Ortiz R, Vernez D, Danuser B, Bouchard M (2012) A detailed urinary excretion time course study of captan and folpet biomarkers in workers for the estimation of dose, main route-of-entry and most appropriate sampling and analysis strategies. Ann Occup Hyg 56(7):815-28. https://doi.org/10.1093/annhyg/mes011

Bossou YM, Cote J, Mantha M, Haddad S, Achard S, Bouchard M (2020) Impact of pesticide coexposure: an experimental study with binary mixtures of lambda-cyhalothrin (LCT) and captan and its impact on the toxicokinetics of LCT biomarkers of exposure. Arch Toxicol 94(9):3045-3058. https://doi.org/10.1007/s00204-020-02810-6

Bouchard M, Côté J, Khemiri R (2019) Lambda-Cyhalothrin Used as an Insecticide in Agriculture: Study of Biomarker Toxicokinetics to Monitor Worker Exposure. Institut de recherche Robert-Sauvé en santé et en sécurité du travail (IRSST). https://www.irsst.qc.ca/en/publications-tools/publication/i/101029/n/lambda-cyhalothrine

Buchholz BA, Ahn KC, Huang H, et al. (2021) Pharmacokinetics, Metabolite Measurement, and Biomarker Identification of Dermal Exposure to Permethrin Using Accelerator Mass Spectrometry. Toxicol Sci 183(1):49-59. https://doi.org/10.1093/toxsci/kfab082

Burns CJ, Pastoor TP (2018) Pyrethroid epidemiology: a quality-based review. Crit Rev Toxicol:1-15. https://doi.org/10.1080/10408444.2017.1423463

Chester G, Sabapathy NN, Woollen BH (1992) Exposure and health assessment during application of lambda-cyhalothrin for malaria vector control in Pakistan. Bull World Health Organ 70(5):615-9.

Coronado GD, Thompson B, Strong L, Griffith WC, Islas I (2004) Agricultural task and exposure to organophosphate pesticides among farmworkers. Environ Health Perspect 112(2):142-7. https://doi.org/10.1289/ehp.6412

EPA US (2004) Lambda-cyhalothrin and an isomer gamma-cyhalothrin; Tolerances for residues. Environmental Protection Agency. https://www.federalregister.gov/d/04-7979

Ferland S, Cote J, Ratelle M, Thuot R, Bouchard M (2015) Detailed Urinary Excretion Time Courses of Biomarkers of Exposure to Permethrin and Estimated Exposure in Workers of a Corn Production Farm in Quebec, Canada. Ann Occup Hyg 59(9):1152-67. https://doi.org/10.1093/annhyg/mev059

Fortin MC, Bouchard M, Carrier G, Dumas P (2008a) Biological monitoring of exposure to pyrethrins and pyrethroids in a metropolitan population of the Province of Quebec, Canada. Environ Res 107(3):343-50. https://doi.org/10.1016/j.envres.2008.03.002

Fortin MC, Carrier G, Bouchard M (2008b) Concentrations versus amounts of biomarkers in urine: a comparison of approaches to assess pyrethroid exposure. Environmental health : a global access science source 7:55. https://doi.org/10.1186/1476-069X-7-55

Grover R, Cessna A, Muir N, Riedel D, Franklin C, Yoshida K (1986) Factors affecting the exposure of ground-rig applicators to 2,4-D dimethylamine salt. Arch Environ Contam Toxicol 15:677-686. https://doi.org/10.1007/BF01054915

Guengerich FP (2008) Cytochrome p450 and chemical toxicology. Chem Res Toxicol 21(1):70-83. https://doi.org/10.1021/tx700079z

Hardt J, Angerer J (2003) Biological monitoring of workers after the application of insecticidal pyrethroids. Int Arch Occup Environ Health 76(7):492-8. https://doi.org/10.1007/s00420-003-0451-8

HealthCanada (2021) Sixth report on human biomonitoring of environmental chemicals in Canada. Minister of Health, Ottawa, ON. Available at: https://www.canada.ca/en/health-canada/services/environmental-workplace-health/reports-publications/environmental-contaminants/sixth-report-human-biomonitoring.html

Hirosawa N, Ueyama J, Kondo T, et al. (2011) Effect of DDVP on urinary excretion levels of pyrethroid metabolite 3-phenoxybenzoic acid in rats. Toxicol Lett 203(1):28-32. https://doi.org/10.1016/j.toxlet.2011.02.016

Jones K (2020) Human Biomonitoring in occupational health for exposure assessment. Port J Public Health 38(1):2-5. https://doi.org/10.1159/000509480

Joo H, Choi K, Rose RL, Hodgson E (2007) Inhibition of fipronil and nonane metabolism in human liver microsomes and human cytochrome P450 isoforms by chlorpyrifos. J Biochem Mol Toxicol 21(2):76-80. https://doi.org/10.1002/jbt.20161

Khemiri R, Cote J, Fetoui H, Bouchard M (2017) Documenting the kinetic time course of lambda-cyhalothrin metabolites in orally exposed volunteers for the interpretation of biomonitoring data. Toxicol Lett 276:115-121. https://doi.org/10.1016/j.toxlet.2017.05.022

Khemiri R, Cote J, Fetoui H, Bouchard M (2018) Kinetic time courses of lambda-cyhalothrin metabolites after dermal application of Matador EC 120 in volunteers. Toxicol Lett 296:132-138. https://doi.org/10.1016/j.toxlet.2018.08.008

Krieger RI, Dinoff TM (2000) Captan fungicide exposures of strawberry harvesters using THPI as a urinary biomarker. Arch Environ Contam Toxicol 38(3):398-403. https://doi.org/10.1007/s002449910053

Martinez MA, Ares I, Rodriguez JL, et al. (2018) Pyrethroid insecticide lambda-cyhalothrin induces hepatic cytochrome P450 enzymes, oxidative stress and apoptosis in rats. Sci Total Environ 631-632:1371-1382. https://doi.org/10.1016/j.scitotenv.2018.03.030

Maule AL, Scarpaci MM, Proctor SP (2019) Urinary concentrations of permethrin metabolites in US Army personnel in comparison with the US adult population, occupationally exposed cohorts, and other general populations. Int J Hyg Environ Health 222(3):355-363. https://doi.org/10.1016/j.ijheh.2019.02.005

Paolini M, Barillari J, Trespidi S, Valgimigli L, Pedulli GF, Cantelli-Forti G (1999) Captan impairs CYP-catalyzed drug metabolism in the mouse. Chem-Biol Interact 123(2):149-70. https://doi.org/10.1016/s0009-2797(99)00134-9

Ratelle M, Cote J, Bouchard M (2016) Time courses and variability of pyrethroid biomarkers of exposure in a group of agricultural workers in Quebec, Canada. Int Arch Occup Environ Health 89(5):767-83. https://doi.org/10.1007/s00420-016-1114-x

Ravula AR, Yenugu S (2021) Pyrethroid based pesticides - chemical and biological aspects. Crit Rev Toxicol 51(2):117-140. https://doi.org/10.1080/10408444.2021.1879007

Saillenfait AM, Ndiaye D, Sabate JP (2015) Pyrethroids: exposure and health effects--an update. Int J Hyg Environ Health 218(3):281-92. https://doi.org/10.1016/j.ijheh.2015.01.002

Sams C, Jones K (2011) Human volunteer studies investigating the potential for toxicokinetic interactions between the pesticides deltamethrin; Pirimicarb and chlorpyrifos-methyl following oral exposure at the acceptable daily intake. Toxicol Lett 200(1-2):41-45. https://doi.org/10.1016/j.toxlet.2010.10.012

Scollon EJ, Starr JM, Godin SJ, DeVito MJ, Hughes MF (2009) In vitro metabolism of pyrethroid pesticides by rat and human hepatic microsomes and cytochrome p450 isoforms. Drug Metab Dispos 37(1):221-228. https://doi.org/10.1124/dmd.108.022343

Tang J, Cao Y, Rose RL, Hodgson E (2002) In vitro metabolism of carbaryl by human cytochrome P450 and its inhibition by chlorpyrifos. Chem-Biol Interact 141(3):229-41. https://doi.org/10.1016/s0009-2797(02)00074-1

Thatheyus AJ, Selvam AG (2013) Synthetic pyrethroids: toxicity and biodegradation. Appl Ecol Environ Sci 1(3):33-36. https://doi.org/10.12691/aees-1-3-2

Timchalk C, Poet TS, Hinman MN, Busby AL, Kousba AA (2005) Pharmacokinetic and pharmacodynamic interaction for a binary mixture of chlorpyrifos and diazinon in the rat. Toxicol Appl Pharmacol 205(1):31-42. https://doi.org/10.1016/j.taap.2004.09.004

Wielgomas B, Krechniak J (2007) Toxicokinetic interactions of alpha-cypermethrin and chlorpyrifos in rats. Pol J Environ Stud 16(2):267. https://doi.org/10.1007/s00204-019-02546-y

Yang D, Wang X, Chen YT, Deng R, Yan B (2009) Pyrethroid insecticides: isoform-dependent hydrolysis, induction of cytochrome P450 3A4 and evidence on the involvement of the pregnane X receptor. Toxicol Appl Pharmacol 237(1):49-58. https://doi.org/10.1016/j.taap.2009.02.012

Supplementary file S1:
Individual kinetic profiles of metabolites after exposure to LCT alone or coexposure with captan

Worker T101 – E1

Worker T101 was first followed after Matador® application in strawberry fields (E1). Figure 1A (T101-E1) shows a high concentration of the specific metabolite CFMP in the control urine prior to Matador® spraying and in the urines collected shortly after application with a return close to baseline levels within 18 h after the onset of application. During the 24- to 42-h period following application, CFMP excretion values increased and this was compatible with a re-entry in the LCT-treated area to apply the fungicide Bravo®, as indicated in the questionnaire (Table 2). Variations in CFMP excretions during that time period are also compatible with a contact with LCT-contaminated equipment, which was used on day two of biomonitoring to spray non-pyrethroid and non-captan pesticides (Endivor® + Exirel® + Maxcel® + Ceyva® + calcium) in an apple orchard that was not previously treated with pyrethroids. Throughout the follow-up period for T101-E1, CFMP was the main metabolite excreted (CFMP > 3-PBA > 4-OH3PBA > *trans*-DCCA) and 3-PBA and 4-OH3BPA did not evolve in parallel with CFMP. *trans*-DCCA metabolite levels remained low throughout the biomonitoring period.

Worker T101 – E2

Worker T101 was also followed after application of formulations containing LCT in combination with captan (E2) as depicted in Fig. 1B (T101-E2). Peak excretion of CFMP was observed in the hours following spraying, hence compatible with an exposure due to this task and levels then rapidly returned to baseline levels with the next 24 h. However, contrary to the prior exposure scenario for this worker (Fig. 1A – E1), CFMP was the lowest LCT metabolite detected and showed a time course pattern that differed from that of 3-PBA and 4-OH3PBA (CFMP < 4-OH3PBA < 3-PBA). The urinary excretion time courses of 3-PBA and 4-OH3PBA were similar to those of *trans*-DCCA and *cis*-DCCA, indicating a concomitant exposure with other pyrethroid insecticides (such as permethrin and cypermethrin). Two peaks were observed at approximatively 24 and 57 h after the end of the spraying episode; these peaks were not compatible with any potential exposures reported by questionnaire and the applicator did not report any application of other pyrethroids during the biomonitoring period (Table 2).

Worker T101 - E3

Worker T101 not only provided urine samples during the three days following spraying of formulations containing LCT in combination with captan but also for an additional three days, during which some tasks in fields were performed (E3) (T101-E3). As shown in Fig. 1C, CFMP levels remained quite low during this biomonitoring period, while a significant rise in 3-PBA and 4-OH3PBA levels was observed between 42 and 51 h followed by a progressive decline up to 72 h. A significant increase in *trans*- and *cis*-DCCA levels was also observed at the same time points as 3-PBA and 4-OH3BPA, hence showing similar time courses for all four metabolites; this

confirmed that this excretion was not related to LCT exposure, but rather to other pyrethroids such as permethrin or cypermethrin. Furthermore, the elevated levels of 3-PBA, 4-OH3PBA, and *trans*- and *cis*-DCCA observed between 42 and 72 h were not related with any task performed in LCT-treated fields, as reported in Table 2. This worker only applied pesticides in a field not treated with LCT (Pixarro® and Authority®) between 63 h and 72 h.

Worker T103 – E1

Following application of Silencer® in strawberry fields (E1), worker T103 exhibited an increase in excretion of CFMP during the first 12 h following application, which remained elevated during the next 24 h followed by a progressive decrease (Fig. 2A - T103-E1). As observed for T101-E1, throughout the follow-up period, CFMP was the main metabolite excreted (CFMP > 3-PBA ≈ 4-OH3BPA ≈ *trans*-DCCA ≈ *cis*-DCCA) and 3-PBA and 4-OH3BPA did not evolve in parallel with CFMP. *trans*-DCCA and *cis*-DCCA metabolite levels also remained low throughout the biomonitoring period. The increase in levels of CFMP observed is compatible with reported LCT spraying period and subsequent maintenance of the equipment used for spraying and inspection of LCT-treated field, as indicated in the questionnaire (Table 2).

Worker T103 – E2

Worker T103 was also followed after application of formulations containing LCT in combination with captan (E2), as depicted in Fig. 2B (T103-E2). Peak excretion of CFMP was observed in the hours following spraying, hence compatible with an exposure due to this task and levels then rapidly returned to baseline levels within the next 24 h. CFMP also showed a time course pattern similar to that of 3-PBA and 4-OH3BPA, but was excreted in lower concentrations (*trans*-DCCA > 3-PBA ≥ *cis*-DCCA > 4-OH3PBA ≥ CFMP). Interestingly, the urinary excretion time courses of *trans*-DCCA followed the time courses of the other assessed metabolites (CFMP, 3-PBA and 4-OH3BPA); however, the high levels of *trans*-DCCA observed indicated a concomitant exposure to other pyrethroid insecticides (such as permethrin or cypermethrin), although a coexposure with LCT due to LCT spraying was evident from CFMP profiles. A second moderate rise in levels of all metabolites was also observed between 30 and 36 h following application, indicating a potential exposure from the same source. Nevertheless, this worker failed to report any time spent handling pesticides or performing tasks in the field in the days following this exposure scenario (E2) (Table 2).

Worker T106 – E1

During summer 2019, worker T106 first provided urine samples during a 3-day period following Matador® application in strawberry fields (E1). Figure 3A shows that CFMP excretion peaked at the onset of sampling period with a return to baseline levels within the next 12 h. This offset suggests that urine collection started after application; this worker only reported the spraying period

at the onset of exposure and no other tasks before or after, according to the questionnaire (Table 2). CFMP also showed a time course pattern relatively similar to that of 3-PBA and 4-OH3BPA, and showed higher peak concentrations (CFMP > 3-PBA > 4-OH3PBA > *trans*-DCCA ≥ *cis*-DCCA). Again, as observed for T103-E2, the urinary excretion time courses of *trans*-DCCA and *cis*-DCCA followed the time courses of the other assessed metabolites (CFMP, 3-PBA and 4-OH3BPA), although in slightly lower concentrations; the presence of *trans*- and *cis*-DCCA indicated a concomitant exposure to other pyrethroid insecticides (such as permethrin or cypermethrin), although a coexposure with LCT at early time points was evident from CFMP profiles.

Worker T106 – E2

Later during the summer, worker T106 was also followed after application of formulations containing LCT (Matador®) in combination with captan (E2). Figure 3B (T106-E2) shows varying levels of CFMP during the three-day period following application. In particular, during the 36- to 72-h period following application, CFMP excretion values increased followed by a progressive decline. This was compatible with questionnaire responses indicating inspection of field treated with LCT-containing formulation, and also handling and application of non-pyrethroid pesticides (Round-up®) in a field not treated with LCT suggesting a possible contact with contaminated equipment used for application (tractor or sprayer) (Table 2). Throughout the follow-up period for T106-E2, CFMP was the main metabolite excreted (CFMP > 3-PBA > *trans*-DCCA). 3-PBA did not evolve in parallel with CFMP but rather followed the time course of *trans*-DCCA; for these latter two metabolites, highest levels were observed in pre-exposure samples, but the applicator

stated in the questionnaire that he applied LCT in an adjacent field one day before the control urine sampling (detail not reported in Table 2).

Worker T106-E3

As for worker T101, worker T106 provided urine samples for an additional 3 days following an initial spraying of formulations containing LCT in combination with captan (6-consecutive biomonitoring days in total), during which some tasks in LCT-treated fields were performed (E3) (T106-E3). Figure 3C shows that CFMP levels peaked about 6 to 9 h after each of the three reported tasks in the LCT-treated fields (see Table 2), hence inspection for the first two field tasks and mixing/loading and application in relation to the third peak. First peak excretion of CFMP was accompanied by an increase in 3-PBA and 4-OH3BPA but also of *trans*- and *cis*-DCCA concentrations – to levels exceeding those of CFMP – confirming a coexposure to other pyrethroids such as permethrin or cypermethrin during this initial follow-up period. Second and third peak levels of CFMP were higher than those of the other assessed metabolites and accompanied by a concomitant (although less pronounced) rise in 3-PBA and 4-OH3BPA concentrations but not of *trans*- and *cis*-DCCA; this suggests in these later time-periods an exposure to LCT mainly, respectively during inspection of LCT-treated fields and subsequent spraying of LCT-containing formulation, as reported by questionnaire (Table 2).

Worker T106-E4

Worker T106 was sampled a fourth time period during 3 days following Matador® application in a sweet corn crop (E4) (equivalent exposure scenario E1 but in a different field). Figure 3D shows that CFMP excretion peaked at the onset of sampling period with a progressive return to baseline levels within the next 36 h. Elevated levels of CFMP were observed in the control sample provided prior to application of Matador®; according to questionnaire responses (Table 2), this worker applied Coragen® insecticide and Round-up® just before the control urine collection. The time course of CFMP did not evolve in parallel with that of 3-PBA and 4-OH3BPA and the excretion profiles of these latter two metabolites were more similar to that of *trans*-DCCA, the metabolite showing highest peak levels in urine (*trans*-DCCA > 3-PBA ≥ *cis*-DCCA ≥ 4-OH3PBA > CFMP). Increased levels of 3-PBA, 4-OH3BPA and *trans*-DCCA observed between 30 and 60 h following the onset of the biomonitoring period, but not accompanied by an increase in CFMP concentration, could be linked to the reported inspection of treated field (Table 2).

Worker T106b-E1

During summer 2020, worker T106 was again followed during a 3-day period after Silencer® application in strawberry fields (T106b-E1). Figure 4A depicts that during the first 36 h following spraying, CFMP was the main metabolite excreted and showed a time course pattern relatively similar to that of 3-PBA and 4-OH3BPA with a progressive increase in concentrations during the first 12 h post-application followed by a decline up to 36 h (CFMP ≥ 3-PBA > 4-OH3PBA ≈ *trans*-DCCA ≥ *cis*-DCCA). Between 45 and 72 h after spraying, a second peak was observed for 3-PBA and 4-OH3BPA (not clearly for CFMP) but this was accompanied by a parallel increase in *trans*-

DCCA and *cis*-DCCA levels (*trans*-DCCA ≈ 3-PBA ≥ *cis*-DCCA ≥ 4-OH3PBA > CFMP), indicating a concomitant exposure to other pyrethroid insecticides (such as permethrin or cypermethrin) during that period. The second peak is consistent with the reported inspection task performed in the LCT-treated area (Table 2).

Workers T106b-E5

At the end of the 3-day biomonitoring period following Silencer® application in strawberry fields, worker T106b was monitored for another 3 days following re-entry in the previously LCT-treated area to apply captan and Switch® fungicides (T106b-E5). Figure 4B shows three peaks in the excretion of CFMP, the most abundant metabolite excreted, with an initial increase in the hours following application of captan in the fields up to 6 h post-application followed by a progressive decrease with a return to baseline levels at 24 h. Two other peaks were observed at around 30 h and 70 h. The time course pattern was relatively similar for CFMP, 3-PBA, 4-OH3BPA, *trans*- and *cis*-DCCA for the first and last peaks in particular (CFMP > 3-PBA ≈ *trans*-DCCA > 4-OH3PBA ≥ *cis*-DCCA). Although the applicator did not state any task in the treated area (Table 2), the metabolite excretion pattern was consistent with a repeated daily exposure to LCT and at least one pyrethroid other than LCT.

Worker T106b-E2

Worker T106b was also sampled during summer 2020 following application of formulations containing LCT (Silencer®) in combination with captan (E2) (Fig. 4C). The time courses of CFMP, 3-PBA and 4-OH3BPA roughly evolved in parallel but so did those of *trans*- and *cis*-DCCA. It was also observed that *trans*-DCCA > 3-PBA > *cis*-DCCA ≥ 4-OH3BPA > CFMP during the first 36 h with a peak at 12 h for all metabolites; this suggests a concomitant exposure to pyrethroid pesticides other than LCT during application event.

Worker T108-E1

Following application of Silencer® in strawberry fields (E1), worker T108 exhibited an increase in the excretion of CFMP during the first 12 h, where a peak was reached followed by a progressive decrease up to 24 h post-application (Fig 5A - T108-E1). Throughout the follow-up period, CFMP was the main metabolite excreted, and 3-PBA and 4-OH3BPA evolved in parallel with CFMP (CFMP > 3-PBA > 4-OH3BPA > *trans*-DCCA; *cis*-DCCA was not detectable). *trans*-DCCA metabolite levels remained low throughout the biomonitoring period. The increase in levels of CFMP observed is compatible with reported LCT spraying period, as indicated in the questionnaire (Table 2).

Worker T108-E2

Worker T108 was also followed after application of formulations containing LCT in combination with captan (E2) as depicted in Fig. 5B (T106-E2). While CFMP was the main metabolite found in urine with a peak about 24 h following application (Fig.5B), concentration values of all metabolites remained close to the LOD at most time points throughout the biomonitoring period. There was no peak associated with the reported period of preparation and application of a non-pyrethroid pesticide (Intercept®) on day two (Table 2).

ARTICLE 3

ARTICLE 3

Assessing the impact of coexposure on the measurement of biomarkers of exposure to the pyrethroid lambda-cyhalothrin in agricultural workers

Yélian Marc Bossou[a], Jonathan Côté[a], Éloïse Morin, Étienne Dumais, Clara Bianchi, Michèle Bouchard[a,*]

[a] Department of Environmental and Occupational Health, Chair in Toxicological Risk Assessment and Management, and Public Health Research Center (CReSP), University of Montreal, Roger-Gaudry Building, U436, P.O. Box 6128, Main Station, Montreal, Quebec, Canada, H3C 3J7

* Correspondence to:
Michèle Bouchard, Department of Environmental and Occupational Health University of Montreal, P.O. Box 6128, Main Station, Montreal, Quebec, Canada, H3C 3J7.
E-mail: michele.bouchard@umontreal.ca
Telephone number: (514) 343-6111 ext 1640
Fax number: (514) 343-2200

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ASTRACT

There are few published data on the impact of combined exposure to multiple pesticides (coexposure) on levels of biomarkers of exposure in workers, which may alter their toxicokinetics and thus the interpretation of biomonitoring data. This study aimed to assess the impact of coexposure to two pesticides with shared metabolism pathways on levels of biomarkers of exposure to pyrethroid pesticides in agricultural workers. The pyrethroid lambda-cyhalothrin (LCT) and the fungicide captan were used as sentinel pesticides, since they are widely sprayed concomitantly in agricultural crops. Eighty-seven (87) workers assigned to different tasks (application, weeding, picking) were recruited. The recruited workers provided two-consecutive 24-h urine collections following an episode of lambda-cyhalothrin application alone or in combination with captan or following tasks in the treated fields, as well as a control collection. Concentrations of lambda-cyhalothrin metabolites – 3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethyl-cyclopropanecarboxylic acid (CFMP) and 3-phenoxybenzoic acid (3-PBA) – were measured in the samples. Potential determinants of exposure established in a previous study, including the task performed and personal factors were documented by questionnaire. Multivariate analyses showed that coexposure did not have a statistically significant effect on the observed urinary levels of 3-PBA (Exp(β) (95% confidence interval (95% CI)): 0.94 (0.78 - 1.13)) and CFMP (1.10 (0.93 - 1.30). The repeated biological measurements ("time variable") – defined as the within-subjects variable – was a significant predictor of observed biological levels of 3-PBA and CFMP; the within-subjects variance (Exp(β) (95% (95% CI)) for 3-PBA and CFMP was 1.11 (1.09 - 3.49) and 1.25 (1.20 - 1.31). Only the main occupational task was associated with urinary levels of 3-PBA and CFMP. Compared to the weeding or picking task, the pesticide application task was associated with higher urinary 3-PBA and CFMP concentrations. In sum, coexposure to agricultural pesticides

in the strawberry fields did not increase pyrethroid biomarker concentrations at the exposure levels observed in the studied workers. The study also confirmed previous data suggesting that applicators were more exposed than workers assigned to field tasks such as weeding and picking.

Keywords: Biomonitoring; toxicokinetics; co-exposure; pyrethroids; lambda-cyhalothrin; captan

Résumé

Il existe peu de données publiées sur l'impact d'une exposition combinée à plusieurs pesticides (coexposition) sur les niveaux de biomarqueurs d'exposition chez les travailleurs, ce qui peut modifier leur toxicocinétique et donc l'interprétation des données de biosurveillance. Cette étude visait à évaluer l'impact de la coexposition à deux pesticides ayant des voies métaboliques communes sur les niveaux de biomarqueurs d'exposition aux pesticides pyréthrinoïdes chez les travailleurs agricoles. Le pyréthrinoïde lambda-cyhalothrine (LCT) et le fongicide captane ont été utilisés comme pesticides sentinelles, car ils sont largement pulvérisés de manière concomitante dans les cultures agricoles. Quatre-vingt-sept (87) travailleurs affectés à différentes tâches (application, désherbage, cueillette) ont été recrutés. Les travailleurs recrutés ont fourni deux prélèvements d'urine consécutifs sur 24 heures après un épisode d'application de lambda-cyhalothrine seule ou en combinaison avec du captane ou après des tâches dans les champs traités, ainsi qu'un prélèvement de contrôle. Les concentrations de métabolites de la lambda-cyhalothrine - l'acide 3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)- 2,2-diméthyl-cyclopropanecarboxylique (CFMP) et l'acide 3-phénoxybenzoïque (3-PBA) - ont été mesurées dans les échantillons. Les déterminants potentiels de l'exposition établis dans une étude précédente, y compris la tâche effectuée et les facteurs personnels, ont été documentés au moyen d'un questionnaire. Les analyses multivariées ont montré que la coexposition n'avait pas d'impact statistiquement significatif sur les concentrations urinaires observés de 3-PBA (Exp(β) (intervalle de confiance à 95 % (IC 95 %)) : 0,94 (0,78 - 1,13)) et de CFMP (1,10 (0,93 - 1,30)). Les mesures biologiques répétées ("variable temporelle") - définies comme la variable intra-sujet - étaient un prédicteur significatif des niveaux biologiques observés de 3-PBA et de CFMP ; la variance intra-sujet (Exp(β) (95% (95% CI)) pour le 3-PBA et le CFMP était de 1,11 (1,09 - 3,49) et de 1,25 (1,20 - 1,31). Seule la tâche

professionnelle principale était associée à des niveaux urinaires de 3-PBA et de CFMP. Comparée à la tâche de désherbage ou de cueillette, la tâche d'application de pesticides était associée à des concentrations urinaires plus élevées de 3-PBA et de CFMP. En résumé, la coexposition aux pesticides agricoles dans les champs de fraises n'a pas augmenté les concentrations de biomarqueurs pyréthrinoïdes aux niveaux d'exposition observés chez les travailleurs étudiés. L'étude a également confirmé des données antérieures suggérant que les applicateurs étaient plus exposés que les travailleurs affectés à des tâches sur le terrain telles que le désherbage et la cueillette.

Mots clés : Biosurveillance, toxicocinétique, coexposition, pyréthrinoïdes, lambda-cyhalothrine, captane.

INTRODUCTION

The assessment of risks associated with pyrethroid exposure is among the priorities of major government agencies such as Health Canada, the United States Environmental Protection Agency (U.S. EPA), and the French Agency for Food, Environmental and Occupational Health & Safety (ANSES), as several *in vitro* cellular and *in vivo* animal studies have shown that exposure to repeated high doses of these chemicals induces early biological alterations, such as oxidative stress, immune alterations, and endocrine disruption (Barrón Cuenca *et al.*, 2019; Costa *et al.*, 2013; El Okda *et al.*, 2017; Lee *et al.*, 2020; Ravula et Yenugu, 2021; Shearer *et al.*, 2019; Wang, X. *et al.*, 2016; Zepeda-Arce *et al.*, 2017). Cases of acute intoxication or incidents in workers exposed to pyrethroids have also been reported, including respiratory and neurological symptoms (Amoatey *et al.*, 2020; Curl *et al.*, 2020; de Graaf *et al.*, 2022; Ismail *et al.*, 2017; Lucero et Muñoz-Quezada, 2021; Mattila *et al.*, 2021; Ratanachina *et al.*, 2020). The U.S. EPA has classified some pyrethroids, including permethrin, as possibly carcinogenic to humans, based on observations of (benign) lung and liver tumors in mice exposed to high doses, although these findings are not supported by available epidemiological studies (Burns et Juberg, 2021; De Roos *et al.*, 2021; U.S. EPA 2018). It is therefore important to develop and apply tools to properly evaluate and control exposure to these contaminants, which are still insufficiently evaluated in some agricultural workplaces. To assess internal exposure to pesticides such as pyrethroids, urinary measurements of metabolites is considered a preferred tool (Arcury *et al.*, 2018; Buchholz *et al.*, 2021; Curl *et al.*, 2021; Maule *et al.*, 2019).

However, our latest work has raised the issue of multiple exposures to several pesticides and the impact that this coexposure (concomitant or combined exposure on the same day or on sequential

days (OECD, 2018)) could have on the interpretation of biomonitoring data used to assess exposure to pyrethroids (Bossou *et al.*, 2020; Bouchard *et al.*, 2016; Khemiri *et al.*, 2017; Ratelle *et al.*, 2015a, 2015b). Currently, there is a lack of data on the impact of multiple pesticide coexposure on levels of exposure biomarkers to commonly used pyrethroids in agricultural settings.

Overall, the data published in the scientific literature on the impact of coexposure on the biological behavior of pyrethroids and their metabolites used as biomarkers of exposure are very limited in real-life context such as agricultural settings. Experimentally or in controlled studies, some studies reported decreased urinary excretion of pyrethroid exposure biomarkers in animals or volunteers coexposed with organophosphate insecticides (Hirosawa *et al.*, 2011; Sams et Jones, 2011; Wielgomas et Krechniak 2007). However, the doses administered were relatively high in relation to worker exposure levels and the exposure scenarios (pyrethroid/organophosphate coexposure) were not very representative of the exposure context of workers. Recently, our team experimentally evaluated the influence of coexposure to the fungicide captan on the kinetic profiles of the main metabolites of the pyrethroid lambda-cyhalothrin in a rat study, 3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethyl-cyclopropanecarboxylic acid (CFMP), 3-phenoxybenzoic acid (3-PBA) and 4-hydroxy-3-phenoxybenzoic acid (4-OH3PBA) (Bossou *et al.*, 2020). The *a priori* hypothesis was that captan could interfere with the CYP450 metabolism pathway of pyrethroids or the excretion mechanisms (Paolini *et al.*, 1999) in a dose-dependent manner. More specifically, lambda-cyhalothrin metabolism to its main metabolites used as biomarkers of exposure (CFMP and 3-PBA) is catalyzed by CYP450 enzymes also implicated in the metabolism of captan (Kaneko, 2011; Paolini *et al.*, 1999; Scollon *et al.*, 2009). The results of the animal study of Bossou *et al.* (2020) showed that captan and lambda-cyhalothrin coexposure resulted in a trend toward lower levels of metabolite excretion, in particular on the benzyl metabolite pathway leading to 3-PBA

formation. This was observed in the higher dose group exposed to the "Lowest-Observed Adverse Effect Level" (LOAEL) of 12.5 mg/kg bw/day reported by the U.S. EPA (2004) but not in the lower exposure group exposed to the "No-Observed Adverse Effect Level" (NOAEL) of 2.5 mg lambda-cyhalothrin/kg bw/day. Again, these animal results cannot be directly extrapolated to humans for interpretation of biomonitoring data because the experimental exposure doses were much higher than those estimated under human occupational exposure conditions (< 1 µg/kg bw/day) (Chester *et al.*, 1992). In addition, there may be interspecies differences in toxicokinetics.

In real-life exposure situations in workers, the impact of coexposure to different pesticides in workers on variability in levels of biomarkers of exposure, relative to other factors, remains to be verified. In order to properly interpret the significance of a measurement of these exposure biomarkers, it becomes necessary to fully understand the influence of parameters such as coexposure to other pesticides. We hypothesize that, at a certain exposure level, combined exposure to multiple pesticides may alter biomarker concentrations in urine used to assess internal exposure in agricultural workers and hence may have an impact on the interpretation of biomonitoring data. This research thus specifically aimed to evaluate the impact of coexposure on biomarkers of exposure to pyrethroid pesticides in agricultural workers and to identify the contribution of this factor to the variability in biological monitoring data. The pyrethroid lambda-cyhalothrin (LCT) and the fungicide captan were used as sentinel pesticides, since they are widely sprayed concomitantly on agricultural crops and share common metabolism pathways (Paolini *et al.*, 1999b).

METHODS

Study population, crops and targeted pesticide active ingredients

A biomonitoring study was conducted in agricultural workers exposed to lambda-cyhalothrin alone or in combination with captan. Strawberry workers were targeted because this crop represents an important production in Quebec; it involves a large number of workers and pyrethroids and fungicides are widely used in these fields (MAPAQ 2020, 2021). The workers were recruited using the Quebec Directory of Horticultural Producers obtained from the Quebec Fruit and Vegetable Growers' Association. From this list of farms organized by city and crop type, strawberry farm owners within a 100 km radius from the University of Montreal were contacted by telephone (using a standard text) to assess their willingness to solicit their field workers to participate in the study.

A total of 87 workers assigned to different tasks (application, weeding, strawberry picking) were recruited, and evaluated under their usual working conditions. The target workers were exposed to lambda-cyhalothrin alone, or alternatively, in combination with captan. In the case of combined exposure in applicators, lambda-cyhalothrin and captan were mixed and spayed at the same time; in the case of field workers, they entered an area previously treated with both chemicals. This sample size was based on the number of workers used in a previous study, which assessed the impact of various personal factors and exposure determinants on the kinetics of biomarkers of exposure to cypermethrin in vegetable crop workers by statistical multivariate analysis (Ratelle *et al.*, 2016).

The recruitment strategy used for this study was the same as that described in Bossou *et al.* (2022). Eligibility criteria were: i) the worker anticipated being exposed to formulations containing the active ingredient lambda-cyhalothrin (Matador®, Silencer®, Demand CS®, Warrior®) alone or in combination with captan fungicide (Captan®, Supra Captan 80 WDG®, Captan 80-WP®, Maestro®) during the summer as part of their normal activities; ii) they were willing to provide a 24-h urine collection prior to application (-24 - 0 h prior to application) and two consecutive 24-h urine collections (0-24 h and 24-48 h) following the onset of an exposure episode (after spraying the pesticide or working in a treated field).

Subjects who participated in the study signed a free and informed consent form after receiving all necessary information about the project. Each participant was free to withdraw at any time. The study protocol, consent form, and other relevant documents were approved by the Clinical Research Ethics Committee (CERC) of the Université de Montréal. The anonymity of the subjects was also respected by coding the samples.

Urine collections and measurement of exposure biomarkers

Recruited workers were asked to provide a first full 24-h urine collection prior to an exposure episode to establish baseline exposure levels as well as two consecutive 24-h urine collections following an episode of lambda-cyhalothrin spaying alone or in combination with captan or tasks in treated fields (weeding and strawberry picking). Each 24-h samples were collected in 1.5 L polypropylene Nalgene® bottles. Workers were asked to write down the date and time of each micturition on the identification label affixed to the Nalgene collection bottles. The samples

collected were kept in coolers with ice packs provided by our team. Samples were picked up on a daily basis at the workplace by a member of our research team and directly brought to the laboratory where urine volumes were measured using graduated cylinders. On days when the team were not able to arrive before the end of the workday, workers transferred samples into the farm cold room until our team members arrived. Two aliquots of 120 mL per collection bottle were prepared and placed in polypropylene Sarstedt tubes for storage at – 20°C until analysis.

All participants were well-informed of the importance of complete urine collections without omissions. They were also compensated for their time and efforts to ensure compliance to the protocol and limit the proportion of incomplete urine collection. However, one participant failed to collect all his 24 hour-urine samples and was excluded in analyses.

The urine samples (5 mL) were subjected to an enzyme hydrolysis with β-glucuronidase/arylsulfatase enzyme to obtain the sum of free and glucurono- and sulfo-conjugated metabolites followed by solid-phase extraction where the metabolites were recovered in methanol (1 mL). The concentrations of lambda-cyhalothrin metabolites used as biomarkers of exposure, 3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethyl-cyclopropanecarboxylic acid (CFMP; otherwise known as ClF_3CA) and 3-phenoxybenzoic acid (3-PBA), were then analyzed in the methanolic extracts using a high performance liquid chromatography coupled to triple quadripole (QQQ) mass spectrometry (UHPLC-MS/MS) method validated in our laboratory and published elsewhere (Bossou *et al.*, 2022; Khemiri *et al.*, 2018).

The limit of detection (LOD) was calculated to be 0.7-2.5 pmol/mL of methanolic extract for 3-PBA and 3.9 to 6 pmol/mL for CFMP. The limit of quantification (LOQ), which represents the

lowest level of the calibration curve that is quantified with a less than 20% error, was 6 pmol/mL and 12 pmol/mL of methanolic extract for 3-PBA and CFMP, respectively. Corresponding LOQ values in urine are 1-2 pmol/mL of urine considering that 5 mL of urine were analyzed and the residue obtained after solid-phase extraction and evaporation was redissolved in 1 mL of methanol. This method allowed quantifying 3-PBA and CFMP metabolites in 88% and 43% of the analyzed samples, respectively.

Creatinine concentrations were also measured in urine using the Jaffé method, an alkaline picric acid method with deproteinization (PAP enzymatic colorimetric assay from Boehringer Mannheim, Germany). Concentrations of lambda-cyhalothrin metabolites corrected for creatinine (µmol/mol creatinine) were then established for each sample. For values below the LOQ, they were treated using a robust method called "regression on order statistics" (ROS) (Helsel, 2005). This method was used because it is less sensitive to small sample sizes, low censoring percentages and is more resistant to non-normality in the data (Huston et Juarez-Colunga, 2009).

Questionnaires and field observations

Potential determinants of metabolite levels used as biomarkers of exposure or potential confounders were assessed by questionnaire in addition to coexposure to lambda-cyhalothrin and captan. These included the main work tasks performed, work practices and hygiene, personal protective equipment as well as personal factors and lifestyle habits (see supplementary file S1). Main questions were used in a previous work on cypermethrin exposure in workers (Ratelle *et al.*, 2016). Members of the team also conducted field observations during the first day of exposure. On

the third day post-exposure, research team members also checked the daily questionnaire responses, and participants were asked to record any missing urine collection.

Data analysis

The impact of coexposure on variations in urinary levels of exposure biomarkers was established using *linear mixed effects models* (MIXM). The focus was on 3-PBA which is the most measured metabolite of pyrethroids and CFMP which is more specific. The potential determinants of exposure established in a previous study (Ratelle *et al.*, 2016), including the task performed and personal factors, were considered in the models. Specifically, the subject variable was set as a *random effect* and a *compound symmetry* covariance *structure among repeated measurements* was considered. The levels of CFMP and 3-PBA metabolites expressed as concentrations (µmol/mol creat.) were considered as dependent variables in the models. Since the biomarker levels showed a log-normal distribution and not normal after analysis by the Kolmogorov-Smirnov test, the exposure biomarker levels (CFMP and 3-PBA) were expressed as log-transformed values to obtain a normal distribution with constant variance.

Potential determinants of biological levels of 3-PBA and CFMP were considered, including: 1) coexposure, *i.e.* exposure to lambda-cyhalothrin alone or lambda-cyhalothrin in combination with captan; 2) main occupational task performed (application including mix preparation and equipment cleaning, weeding, or picking); 3) time since the onset of lambda-cyhalothrin spraying alone or in combination with captan (≤7 days and >7 days); and 4) farm size (≤ 10 workers or >10 workers). Potential confounding variables considered in the models included age (log-transformed

continuous variable in years), body mass index (log-transformed continuous BMI), ethnicity (Caucasian or Latino American), education (primary/high school or college/university), alcohol use (yes/no during the study period), cigarette smoking (yes/no), ibuprofen or acetaminophen use, other medication use.

Associations between biomarker levels (3-PBA or CFMP metabolites) in urine of farmworkers and potential determinants of exposure including the influence of coexposure or potential confounding factors were initially assessed in univariate models (explanatory variables considered one by one in the models). Multivariate models were then constructed by first inserting all variables and then sequentially subtracting those that did not contribute to the model according to the Akaike's information criterion (AIC) following the approach proposed by Zuur *et al.* (2009). Only predictors and confounders contributing to the fit of the multivariate models to the data were retained in the final models. Statistical analyses were performed using SPSS plus (SPSS Inc, Chicago). The level of statistical significance for the final multivariate models was set at $p \leq 0.05$.

RESULTS

Study of the impact of coexposure versus other factors on the measurement of biomarkers of exposure in workers

After a screening telephone interview in early 2019 and 2020 to identify potentially eligible workers followed by a farm visit to recruit farmers, a total of 87 workers of strawberry fields – where lambda-cyhalothrin alone or in combination with captan was sprayed –signed informed consent to participate in the study. They provided urine samples during summer 2019 and 2020. Workers were recruited from 13 farms in three regions of the Province of Quebec (Montérégie, Laurentides, Lanaudière) and the main professionals tasks performed were spraying of pesticides (lambda-cyhalothrin or lambda-cyhalothrin combined with captan), weeding or strawberry picking in a treated area. In total, 70 of the recruited workers had an exposure episode to lambda-cyhalothrin alone and 49 had an exposure episode to lambda-cyhalothrin in combination with captan over the two-year study period. Some workers were thus monitored more than once since they performed different tasks in the fields (weeding versus picking) or were evaluated for more than one exposure scenario (exposure to lambda-cyhalothrin alone or in combination with captan) at different periods.

Table 1 shows the main personal characteristics of the 87 workers included in the study who provided serial urine collections following an exposure episode to lambda-cyhalothrin alone (70 workers) or in combination with captan (49 workers). A participant may have been evaluated for more than one task or more than one exposure scenario. Only 1% of all study participants were

women and 82% of them were of Latin origin (from Guatemala, Honduras and Mexico); the median age was 33 years and their education level was low, with 84% reporting a high school level or less as the highest education level. In addition, during the biological sampling period, 13% of the workers (11 workers out of 87) reported smoking tobacco; 16% (14 workers out of 87) reported consuming alcohol during at least one of their biological follow-ups; 13% (11 workers out of 87) mentioned taking ibuprofen or acetaminophen; and 9% (8 workers out of 87) reported taking other types of medication.

Table 1. Characteristics of participants

	All participants (n=87[a])	**LCT exposed** (n=70[b])	**LCT + Captan exposed** (n=49[c])
Sex: n (%)			
Women	1 (1.1%)	0 (0%)	1 (2.0%)
Men	86 (98.9%)	70 (100%)	48 (98.0%)
Age: years			
Average (SD)	34.8 (10.2)	34.1 (9.91)	36.3 (10.2)
Median [Min, Max]	33.0 [20.0, 64.0]	31.5 [21.0, 64.0]	34.0 [20.0, 64.0]
Age categories: years (%)			
20-30	35 (40.2%)	31 (44.3%)	15 (30.6%)
31-40	30 (34.5%)	22 (31.4%)	22 (44.9%)
≥ 41	22 (25.3%)	17 (24.3%)	12 (24.5%)
Body weight: kg			
Average (SD)	72.3 (14.0)	71.3 (12.4)	74.3 (14.2)
Median [Min, Max]	70.0 [50.0, 127]	70.0 [50.0, 125]	71.2 [54.5, 127]
Height: cm			
Average (SD)	165 (9.38)	165 (9.34)	167 (9.19)
Median [Min, Max]	162 [150, 198]	162 [150, 198]	165 [155, 189]
BMI: kg/m^2			

	All participants (n=87[a])	**LCT exposed** (n=70[b])	**LCT + Captan exposed** (n=49[c])
Average (SD)	26.4 (4.11)	26.1 (2.97)	26.6 (4.60)
Median [Min, Max]	26.3 [17.9, 52.5]	26.2 [17.9, 34.1]	26.3 [21.2, 52.5]
BMI categories: kg/m^2 (%)			
< 24.9	30 (34.5%)	25 (35.7%)	17 (34.7%)
25-29.9	46 (52.9%)	38 (54.3%)	26 (53.1%)
≥ 30	11 (12.6%)	7 (10.0%)	6 (12.2%)
Country of birth: n (%)			
Bosnia	1 (1.1%)	0 (0%)	1 (2.0%)
Canada	15 (17.2%)	11 (15.7%)	10 (20.4%)
Guatemala	35 (40.2%)	33 (47.1%)	13 (26.5%)
Honduras	24 (27.6%)	16 (22.9%)	20 (40.8%)
Mexico	12 (13.8%)	10 (14.3%)	5 (10.2%)
Ethnicity: n (%)			
Caucasian	16 (18.4%)	11 (15.7%)	11 (22.4%)
Latino American	71 (81.6%)	59 (84.3%)	38 (77.6%)
Language: n (%)			
French	16 (18.4%)	11 (15.7%)	11 (22.4%)
Spanish	71 (81.6%)	59 (84.3%)	38 (77.6%)
Education: n (%)			
High school or less	73 (83.9%)	59 (84.3%)	39 (79.6%)
College and University	14 (16.1%)	11 (15.7%)	10 (20.4%)

[a] Number of participants included in the study.

[b] Number of participants who provided biological samples following application of lambda-cyhalothrin alone. A participant may have been evaluated for more than one task or more than one exposure scenario. [c] Number of participants who provided biological samples following application of lambda-cyhalothrin in combination with captan. A participant may have been evaluated for more than one task or more than one exposure scenario.

In terms of biological follow-up, considering that some workers provided more than one set of urine collections, tobacco use was reported for 10% of the biological follow-ups (i.e., for 14 of the 139 biological follow-ups); alcohol use was indicated for 12% of the biological follow-ups (16 out of 139 biological follow-ups); ibuprofen or acetaminophen use was reported in 10% of the follow-ups (14 out of 139 follow-ups) and other types of medication were reported in 7% of the follow-ups (10 out of 139 follow-ups).

The consumption of fruits, vegetables and cereals (number of servings according to Canada's Food Guide (2011)) was documented by questionnaire, but this variable was not considered in the results, as workers did not appear to be able to adequately answer these questions. Only one person reported the use of lice treatment and no participants reported the use of animal treatment or the use of pesticides for residential purposes. None of the participants reported signs or symptoms (as mentioned in supplementary file S1) that, although not specific, could be associated with exposure to this type of pesticide.

While most workers wore long pants and long-sleeved shirts and boots, only a proportion of workers wore gloves, goggles, and a hat (Table 2). Although only 45% of workers wore gloves, no significant effect of glove wearing on urinary levels of CFMP and 3-PBA was observed ($p>0.05$). Wearing goggles and hats was also not significantly associated with urinary levels of CFMP and 3-PBA. Other personal protective clothing or equipment (mask, raincoat, scarf) were worn by only a small number of workers such that the association between wearing the latter and urinary levels of CFMP and 3-PBA was not tested.

Table 2. Protective equipment (PPE) for all participants as well as for participants stratified by exposure group (exposure to lambda-cyhalothrin alone or combination with captan)

Type of PPE	All[a]	Population Exposure to the LCT[b]	Exposure to LCT+Captan[b]	P-value
Long pants: n (%)				
Yes	125 (89)	78 (90)	47 (89)	0.857
No	15 (11)	9 (10)	6 (11)	
Long sleeve shirt: n (%)				
Yes	121 (86)	72 (83)	49 (92)	0.106
No	19 (14)	15 (17)	4 (8)	
Hat: n (%)				
Yes	94 (67)	60 (69)	34 (64)	0.558
No	46 (33)	27 (31)	19 (36)	
Goggles: n (%)				
Yes	22 (16)	8 (9)	14 (26)	0.007
No	118 (84)	79 (91)	39 (74)	
Scarf: n (%)				
Yes	2 (1)	1 (1)	1 (2)	NA
No	138 (99)	86 (99)	52 (98)	
Raincoat: n (%)				
Yes	15 (11)	10 (11)	5 (9)	0.703
No	125 (89)	77 (89)	48 (91)	
Gloves: n (%)				
Yes	63 (45)	27 (31)	36 (68)	0.00002
No	77 (55)	60 (69)	17 (32)	
Boots: n (%)				
Yes	111 (79)	70 (80)	41 (77)	0.662
No	29 (21)	17 (20)	12 (23)	

[a] The n is the number of biomonitoring for all participants across all tasks (application, weeding, and harvesting) and all exposure scenarios (lambda-cyhalothrin alone versus lambda-cyhalothrin in combination with captan). A participant may have been evaluated for more than one task or more than one exposure scenario. The percentage represents the proportion of workers who reported wearing the equipment over the entire biological monitoring.

[b] The n is the number of biomonitoring for all participants across all tasks (application, weeding and harvesting) but for a given exposure scenario (lambda-cyhalothrin alone or in combination with captan). A participant may have been assessed for more than one task. The percentage

represents the reported proportion of workers who reported wearing the equipment for all biological monitoring but for a given exposure scenario.

Table 3 presents the distribution of urinary concentrations of CFMP, the more specific metabolite of lambda-cyhalothrin, for all participants and for participants stratified by exposure group, either exposure to lambda-cyhalothrin alone or in combination with captan. The results show that the distribution of CFMP values was similar for all groups in control urine as well as in 24-48 h urine collections post-exposure (for all participants, participants exposed to lambda-cyhalothrin alone and participants exposed to lambda-cyhalothrin plus captan). In 0-24 h collections post-exposure, CFMP metabolite values for the upper extremes of the distribution (75^{th} and 95^{th} percentiles in the table) were higher than in control urine or in urine collected 24-48 h after the onset of an exposure period (application or working in a field treated with lambda-cyhalothrin alone or in combination with captan). For the extremes of the distribution (75^{th} and 95^{th} percentiles), CFMP values in urine collected after the onset of an exposure episode to lambda-cyhalothrin combined with captan were also higher than in urine collected after exposure to lambda-cyhalothrin alone. Table 4 shows that the trend was the same for 3-PBA metabolite, which is not specific to lambda-cyhalothrin as it is a common metabolite of several pyrethroids. However, exceptionally, a high 3-PBA value was obtained for the 95^{th} percentile of the distribution in control urine of workers to be later assessed for exposure to lambda-cyhalothrin plus captan.

Table 3. Distribution of CFMP concentrations in urine for all participants as well as for participants stratified by exposure group (exposure to lambda-cyhalothrin alone or in combination with captan)

Time since the onset of an exposure episode (h)	Exposure group	N of samples[a]	CFMP concentration (µmol/mol creat.)						
			Geometric mean	Percentile					
				5th	10th	25th	50th	75th	95th
-24-0[b]	All	134	0.132	0.011	0.018	0.067	0.180	0.290	0.776
	LCT	85	0.141	0.012	0.025	0.076	0.182	0.309	0.799
	LCT+Captan	49	0.117	0.010	0.015	0.053	0.173	0.259	0.691
0-24	All population	138	0.146	0.010	0.023	0.056	0.147	0.389	1.598
	LCT	85	0.132	0.012	0.024	0.061	0.129	0.295	1.417
	LCT+Captan	53	0.173	0.008	0.018	0.039	0.180	0.780	2.829
24-48	All	135	0.122	0.019	0.031	0.063	0.133	0.281	0.582
	LCT	84	0.115	0.013	0.028	0.061	0.139	0.267	0.554
	LCT+Captan	53	0.136	0.028	0.035	0.064	0.131	0.309	0.620

[a] Some workers performed biological monitoring for more than one exposure scenario. This number represents the number of biological samples per exposure period and exposure scenario.

[b] Control urine collection in workers to be assessed for exposure to LCT or LCT+captan.

Table 4. Distribution of 3-PBA concentrations in urine for all participants as well as for participants stratified by exposure group (exposure to lambda-cyhalothrin alone or in combination with captan)

Time since the beginning of the exposure (h)	Exposure group	N of samples[a]	3-PBA concentration (µmol/mol creat.)						
			Geometric mean	Percentile					
				5th	10th	25th	50th	75th	95th
-24-0 [b]	All	134	0.125	0.016	0.034	0.059	0.129	0.231	0.670
	LCT	85	0.136	0.015	0.029	0.082	0.151	0.263	0.589
	LCT+Captan	49	0.107	0.018	0.034	0.044	0.101	0.175	1.979
0-24	All population	138	0.151	0.021	0.032	0.081	0.160	0.260	1.418
	LCT	85	0.146	0.022	0.040	0.084	0.161	0.247	0.998
	LCT+Captan	53	0.159	0.015	0.031	0.067	0.148	0.322	3.020
24-48	All	135	0.129	0.014	0.029	0.057	0.133	0.305	0.729
	LCT	84	0.122	0.015	0.025	0.058	0.138	0.263	0.647
	LCT+Captan	51	0.140	0.010	0.030	0.057	0.104	0.408	2.064

[a] Some workers performed biological monitoring for more than one exposure scenario. This number represents the number of biological samples per exposure period and exposure scenario.

[b] Control urine collection in workers to be assessed for exposure to LCT or LCT+captan.

Tables 5 and 6 present the potential determinants of urinary 3-PBA and CFMP levels in exposed strawberry workers, in particular the variable "exposure group" or so called "coexposure" (exposure to lambda-cyhalothrin alone or in combination with captan) but also potential confounding factors documented by questionnaire. Urinary metabolite levels showed a log-normal distribution as did age and body mass index (BMI); univariate and multivariate statistical analyses were therefore performed on the log-transformed values for these variables. When considered individually in the univariate model, age, ethnicity, education, farm size, main occupational task, and time since pesticide application showed a statistically significant association ($p<0.05$) with 3-PBA levels and, in the case of CFMP, ibuprofen or acetaminophen use. For the 3-PBA, using the linear mixed effects model (MIXM), farm size, main occupational task and time since pesticide application as well as alcohol consumption, ibuprofen or acetaminophen use, and other medication use were retained for adjustment of the multivariate model, according to the Akaike's information criterion (AIC). These variables were considered as variables contributing to the final multivariate model. The variable "time," representing repeated measurements and defined as a within-subjects variable, was a significant predictor of observed biological levels of 3-PBA; the within-subjects variance (95% confidence interval (95% CI)) was 1.11 (1.09 - 3.49), $p<0.001$. However, coexposure did not have any statistically significant effect on observed urinary 3-PBA levels (0.94 (0.78 - 1.13); $p=0.48$). Table 5 shows that the main occupational task (pesticide application, weed control, or picking), time since exposure, and farm size were the main three predictors of observed biological levels in the final model. Compared to the picking task, the weeding and pesticide spraying tasks were overall associated with higher urinary 3-PBA concentrations ($p<0.05$). However, the pesticide application task had a greater effect than the weeding task. No statistically significant associations ($p<0.05$) were detected with the other factors assessed in the multivariate model (alcohol consumption, ibuprofen or acetaminophen use, or other medications).

For CFMP, farm size, main occupational task, and time since pesticide application as well as alcohol consumption, ibuprofen or acetaminophen use, and other medication use were also included in the multivariate MIXM model, using Akaike's information criterion (AIC). The variable "time", representing repeated measurements and defined as a within-subject variable, was a significant predictor of the observed urinary CFMP levels; the within-subject variance (95% CI) was 1.25 (1.20 - 1.31), $p<0.001$. However, coexposure had no significant effect on observed urinary CFMP levels (1.10 (0.93 - 1.30); $p=0.26$) (Table 6). In the final model, only the main occupational task (pesticide application, weeding or picking), was significantly associated with observed urinary CFMP levels. Compared to the weeding or picking task, the pesticide application task was overall associated with higher urinary CFMP concentrations. No statistically significant associations ($p<0.05$) were detected with the other factors assessed in the multivariate model (farm size, time since pesticide application, alcohol consumption, ibuprofen or acetaminophen use, or other medications).

Table 5. Predictors of 3-PBA levels in workers' urine using a linear mixed effects model (MIXM)

Predictors		3-PBA concentration (µmol/mol creat.) (n= 139)[a]		
		Univariate Analysis[b.c]	Multivariate analysis[c.d.e]	
		Exp(β) (CI95%)	Exp(β) (CI95%)	P-value
Coexposure	LCT	1.02 (0.87 - 1.18)	0.94 (0.78 - 1.13)	0.48
	LCT+captan	Reference	Reference	
Age	Years (log)	1.93 (1.03 - 3.61)		
BMI	(kg/m^2) (log)	0.49 (0.13 - 1.89)		
Ethnicity	Caucasian	1.36 (1.12 - 1.65)		
	Latino American	Reference		
Education	Primary or High school	0.74 (0.62 - 0.90)		
	College or University	Reference		
Alcohol consumption	No	0.97 (0.76 - 1.23)	1.15 (0.90 - 1.45)	0.26
	Yes	Reference	Reference	
Cigarette consumption	No	0.81 (0.63 - 1.05)		
	Yes	Reference		
Ibuprofen or acetaminophen	No	1.18 (0.92 - 1.53)	1.14 (0.82 - 1.60)	0.43
	Yes	Reference	Reference	
Other medications	No	0.84 (0.62 - 1.13)	0.82 (0.60 - 1.12)	0.21
	Yes	Reference	Reference	
Size of the farm	≤ 10 workers	1.23 (1.01 - 1.51)	0.69 (0.50 - 0.95)	0.02
	>10 workers	Reference	Reference	
Main professional task	Pesticide application	1.38 (1.13 - 1.67)	2.49 (1.74 - 3.56)	<0.001
	Weeding	0.89 (0.76 - 1.04)	1.29 (1.00 - 1.67)	0.053
	Picking	Reference	Reference	
Time since pesticide application	≤ 7 days	0.87 (0.75 - 1.01)	0.56 (0.44 - 0.71)	<0.001
	> 7 days	Reference	Reference	

[a] This number represents the number of biological monitoring for all participants across all tasks (application, weeding, and harvesting) and all exposure scenarios (lambda-cyhalothrin alone or in combination with captan). A participant may have been evaluated for more than one task or more than one exposure scenario.

[b] All variables were tested individually in the model. The variable "time" (urine collections at -24-0, 0-24, and 24-48 h following an exposure episode) was considered a repeated measure.

[c] The β estimates and 95% CIs were exponentially transformed from log values.

[d] The variable "time" was considered a repeated measure and a within-subject variable in the multivariate model. The within-subject variance (95% CI) was: 1.11 (1.09 – 3.49); $p<0.001$.

[e] Intra-class correlation coefficient (ICC) was 0.54.

Table 6. Predictors of CFMP levels in workers' urine using a linear mixed effects model (MIXM)

Predictors		CFMP concentration (µmol/mol creat.) (n= 139)[a]		
		Univariate Analysis[b.c]	Multivariate analysis[c. d. e]	
		Exp(β) (CI95%)	Exp(β) (CI95%)	P-value
Coexposure	LCT	0.966 (0.84 - 1.10)	1.10 (0.93 - 1.30)	0.26
	LCT+captan	Reference	Reference	
Age	Years (log)	0.964 (0.55 - 1.69)		
BMI	(kg/m^2) (log)	1.837 (0.55 - 6.10)		
Ethnicity	Caucasian	1.050 (0.88 - 1.26)		
	Latino American	Reference		
Education	Primary or high school	0.875 (0.88 - 1.26)		
	College or University	Reference		
Alcohol consumption	No	1.038 (0.85 - 1.27)	1.13 (0.91 - 1.40)	0.26
	Yes	Reference	Reference	
Cigarette consumption	No	1.133 (0.91 - 1.41)		
	Yes	Reference		
Ibuprofen or acetaminophen	No	0.771 (0.62 - 0.96)	0.80 (0.59 - 1.08)	0.14
	Yes	Reference	Reference	
Other medications	No	1.018 (0.79 - 1.30)	1.22 (0.92 - 1.63)	0.16
	Yes	Reference	Reference	
Size of the farm	≤ 10 workers	1.041 (0.87 - 1.25)	0.94 (0.70 - 1.27)	0.69
	>10 workers	Reference	Reference	
Main professional task	Pesticide application	1.17 (0.98 - 1.40)	1.41 (1.01 - 1.96)	0.04
	Weeding	1.04 (0.91 - 1.21)	1.13 (0.89 - 1.43)	0.30
	Picking	Reference	Reference	
Time since pesticide application	<7 days	1.09 (0.95 - 1.24)	1.04 (0.84 - 1.30)	0.70
	> 7 days	Reference	Reference	

[a] This number represents the number of biological monitoring for all participants across all tasks (application, weeding and harvesting) and all exposure scenarios (lambda-cyhalothrin alone or in combination with captan). A participant may have been evaluated for more than one task or more than one exposure scenario.

[b] All variables were tested individually in the model. The variable "time" (urine collections at -24-0, 0-24 and 24-48 h) was considered a repeated measure.

[c] The β estimates and 95% CIs were exponentially transformed from log values.

[d] The variable "time" was considered a repeated measure and a within-subject variable in the multivariate model. The within-subject variance (95% CI) was: 1.25 (1.20 - 1.31); $p<0.001$.

[e] Intra-class correlation coefficient (ICC) was 0.20.

DISCUSSION

Impact of coexposure and other factors on urinary 3-PBA and CFMP levels

This study is the first to assess the impact of pyrethroid-fungicide (lambda-cyhalothrin-captan) coexposure on levels of urinary biomarkers of exposure in workers, while accounting for other determinants. It is also the first to assess the impact of the work tasks on levels of biomarkers of exposure to lambda-cyhalothrin. Multivariate statistical analyses showed that coexposure to lambda-cyhalothrin and captan was not a significant contributor to the variability in CFMP or 3-PBA concentrations in urine, used as biomarkers of exposure. Only the main task was consistently associated with variations in urinary levels of CFMP and 3-PBA; the pesticide application task was associated with higher levels of metabolites in urine compared to the weeding or picking tasks. These results are very similar to those reported in a previous study on exposure to another pyrethroid, cypermethrin, which is metabolized to 3-PBA, as is lambda-cyhalothrin (Bouchard *et al.*, 2019; Ratelle *et al.*, 2016). In the latter study, some individually assessed personal or occupational characteristics were associated with the excretion of the metabolite 3-PBA after exposure to cypermethrin, but only the main occupational task was associated with the excretion of exposure biomarkers in the multivariate MIXM model. Similar to the present study, cypermethrin pesticide applicators had higher overall urinary levels of 3-PBA than workers performing tasks such as weeding, harvesting, or inspecting fields in an area treated with this pesticide.

In a German study, Hardt et Angerer (2003) also reported higher levels of the pyrethroid metabolites DCCA and 3-PBA in the urine of pesticide sprayers compared to the overall workers assessed (farmers, greenhouse workers or building exterminators). In a Japanese biological monitoring study of pyrethroid exposure in pesticide sprayers, geometric mean concentrations of 3-PBA in the urine of operators who sprayed in the two days preceding the survey were significantly higher than those who did not perform any spraying (5.4 µg/g creat. vs. 0.9 µg/g creat.) (Wang *et al.*, 2007). However, a significant association between urinary 3-PBA levels and pyrethroid spraying was observed only in winter and not in summer. Hence, the fact that all biomonitoring data were collected during summer in our study excludes to some extent the seasonal variability.

In the present study, time since pesticide application (lambda-cyhalothrin alone or mixed with captan) as well as farm size were also associated with urinary 3-PBA levels in the multivariate MIXM model (but the result did not come out significant for CFMP). In the study by Ratelle *et al.* (2016), farm size – when considered individually in the MIXM model – showed an association with urinary 3-PBA excretion, but this association was not significant in the multivariate model (*i.e.* in combination with other factors).

Also, in the present study, when considered individually in the MIXM model, ethnicity, age, and education were associated with urinary 3-PBA levels, but these variables did not contribute significantly to the fit of the final multivariate model. On the other hand, alcohol consumption and the use of ibuprofen, acetaminophen or other medications contributed to the fit of the multivariate model (according to the AIC criterion), but did not show a statistically significant association with urinary 3-PBA or CFMP levels in the multivariate model.

In an exposure biomonitoring study conducted by our group in the general population of Montreal, Québec, Canada, prescription and over-the-counter drug use was associated with higher urinary excretion of pyrethroid metabolites, including 3-PBA (Fortin *et al.*, 2008). In addition, Barr *et al.* (2010) reported that urinary excretion of 3-PBA in individuals from the general U.S. population was significantly associated with ethnicity and age in a multivariate model. In our study, the sample size was relatively small, and the group evaluated was composed mainly of healthy Latino workers.

Lopez-Galvez *et al.* (2018) evaluated pesticide exposure in 20 migrant farmers in a study in Sonora, Mexico and the impact of different factors, based on urine metabolite measurements including 3-PBA. Farmworkers age, language, personal protective equipment, time spent on the farm and season were important determinants of exposure. In a biomonitoring study of 50 textile workers in eastern China, Lu *et al.* (2013) also reported an effect of age and task on biological levels of pyrethroid metabolites (*trans*-DCCA, *cis*-DCCA and 3-PBA). In addition, in a study among farmers and their families, Trunnelle *et al.* (2014) reported a positive association between poor housing conditions and levels of urinary 3-PBA metabolites. They reported that poor housing conditions was a contributing factor to the higher levels of 3-PBA observed in the urine of these farmworker families.

Furthermore, in the present study, information on clothing worn was fairly consistent among workers (long pants (89%) and long-sleeved shirt or sweater (86%) and boots (79%) for the majority of workers); the small number of workers who reported wearing half-masks or helmets with filters (only nine pesticide sprayers) did not allow for a specific assessment of the impact of this protective equipment on biological levels of CFMP and 3-PBA. Only 45% of workers wore gloves, but there was no effect of glove wearing on urinary levels of CFMP and 3-PBA. Wearing

goggles and hats was also not significantly associated with urinary levels of CFMP and 3-PBA. In contrast, in a recent biomonitoring study in which the metabolites 3-PBA and 4F-3PBA were measured in the urine of 45 farmers in northwestern Catalonia, Spain (Bravo *et al.*, 2022), the use of specific personal protective equipment among farm workers, such as the use of gloves and masks during mixing, was associated with lower biological levels, although the differences were not statistically significant. However, a positive association was found between the use of a cap during mixing and during application. The authors reported that these caps were primarily used for sun protection, and when not cleaned after handling pesticides, they could represent a continuous source of exposure through dermal contact. In the same study, farm workers using tractors with cabin also had statistically lower concentrations of the metabolite of another pesticide, 2-diethylamino-6-methyl pyrimidin-4-ol, than those using tractors without cabin. In the present study, most applicators used a tractor for pesticide spraying, with cabin in eight cases and without cabin in four cases; only one applicator used a backpack sprayer. Because there was little difference in application conditions in our study, it was not possible to associate spraying equipment with biological levels.

Furthermore, in our study, there was no significant difference between the wearing of personal protective equipment (PPE) for the group exposed to lambda-cyhalothrin alone or in combination with captan, except for wearing gloves and goggles, which was more worn in the group coexposed to lambda-cyhalothrin and captan. Occupational hygiene practices in each exposure group (lambda-cyhalothrin alone or in combination with captan) were therefore relatively homogeneous, and the variability that could be associated with PPE between exposure groups was low.

Comparison of 3-PBA and CFMP levels with reference values in the general population or in exposed volunteers

The study also provided an overall indication of the magnitude of exposure of strawberry workers relative to the general population. In order to assess the importance of this exposure of all workers in the study, concentrations of 3-PBA metabolites in urine (24-hour) from workers in our study were compared with those reported (in spot urine collections) in the general Canadian population and collected in the CHMS Cycle 6 (Health Canada, 2021). The 95th percentiles of the distribution of 3-PBA concentrations in the urine of our study workers reached values (3.02 µmol/mol creat. in urine collections performed 0-24 h after spraying lambda-cyhalothrin in combination with captan; see Table 4) similar to the 95th percentile (CI) of 3.1 (1.06-5.02) µmol/mol creat. reported in the CHMS (Health Canada, 2021). The CHMS sample may have included workers exposed to pesticides. CFMP was not measured in the CHMS study but lower 95th percentile values (0.145 and 0.261 µmol/mol creat.) were reported in spot urine samples collected from Sweden adolescents (Norén *et al.*, 2020) and German individuals from the general population (Schettgen *et al.*, 2016). Conversely, the 95th percentile concentration value of CFMP reported in the urine of the UK general population (0.839 µmol/mol creat.) (Bevan *et al.*, 2013) was close to the values reported in our study (0.554-0.799 µmol/mol creat.) at -24-0 h prior to exposure and 24-48 h post-exposure.

Urinary levels of 3-PBA in the present study were comparable to those of Ratelle *et al.* (2016) where 34 patterns of 3-PBA excretion were observed in vegetable crop workers. Depending on the profile, median urinary 3-PBA values for a given individual ranged from 0.073 to 1.28 µmol/mol creat. and the 95^{e} percentiles were as high as 9.07 µmol/mol creat. In the latter study, 16 profiles had geometric mean urinary 3-PBA concentrations above those reported in the CHMS at that time

(Health Canada, 2013), and five profiles had 95th percentile values of 3-PBA higher than those reported in that survey. The observed levels of 3-PBA in our study were also comparable to those reported in German and Japanese studies where spot urine measurements (Panuwet *et al.*, 2008; Wang *et al.*, 2007) or 24-hour collections were made (Hardt et Angerer, 2003). Pesticide exposure and uptake, and thus observed biological levels, may vary depending on several factors, including work habits, protective equipment, climate (heat and humidity) (Havenith, 1999).

The levels of 3-PBA and CFMP observed in the urine of workers can also be compared to the maximum urine values obtained from eight volunteers for whom the temporal profiles of CFMP and 3-PBA in urine were determined over a period of 84 hours following the administration of an acute oral dose of 0.0025 mg lambda-cyhalothrin/kg bw, which is the EFSA acceptable daily intake (ADI) value (EFSA 2014) or 0.025 mg lambda-cyhalothrin/kg bw (Khemiri *et al.*, 2017). In the study of Khemiri *et al.* (2017), the maximum concentration of 3-PBA in urine was 10 µmol/mol creat. in the volunteer exposed to the ADI and ranged from 60 to 211 µmol/mol creat. in the other seven volunteers exposed to 0.025 mg/kg bw. The corresponding values for CFMP were 41 and 63-431 µmol/mol creat. In the present study, the 95th percentiles of the distribution of 3-PBA and CFMP concentrations in urine reached 2.8 and 3.0 µmol/mol creat. respectively; these values are 3.6 and 14 times lower than the urinary levels observed in the ADI-exposed volunteer.

Limitations and interest of the current biomonitoring Study

In multivariate analyses, the levels of CFMP and 3-PBA between the group exposed to lambda-cyhalothrin alone and that coexposed to lambda-cyhalothrin and captan were compared while

controlling for other factors that may influence urinary levels. The contribution of personal factors (age, ethnicity, BMI) and lifestyle habits (smoking and alcohol consumption or use of medication), as well as occupational exposure conditions (task, time since application, size of the farm in terms of number of employees) were therefore tested. One limitation was that some factors documented by questionnaire were not reported much so that their impact on exposure biomarker levels could not be tested (such as the impact of the "sex" variable since the farmers were mostly men, or the impact of domestic treatments or residential pesticide use since it was reported only in one case). In addition, although the questionnaires were translated in Spanish and a Spanish-speaking member of our team was available to assist and verify the participants' answers, the questions on the consumption of foods that may contain pesticide residues (consumption of cereals, fruits and vegetables, and number of servings) were not answered adequately or were poorly answered, so that they were not considered in the analyses. Also, the clothing and PPE worn by the workers were not considered in the multivariate analyses because, as mentioned above, the information on the clothing worn was fairly uniform among the workers (mostly long pants, long-sleeved shirts and boots), and the wearing of specific PPE (wearing half-masks or helmets with filters) was rarely reported. In addition, wearing gloves, goggles and a hat did not show a significant association with urinary levels of CFMP and 3-PBA.

Another limitation related to biological exposure monitoring concerns the fact that it involves the measurement of 3-PBA, the metabolite common to several pyrethroids, in addition to the more specific CFMP metabolite. As noted in a previous study on cypermethrin (Bouchard *et al.*, 2016), the measurement of specific metabolites is important to confirm the source of exposure. It is only recently that the measurement of CFMP metabolites was conducted in large surveys (*e.g.*, Apel *et al.* (2023)). Furthermore, although it was confirmed that both captan and lambda-cyhalothrin were

sprayed concomitantly by the applicators in our study and that field workers entered areas previously treated with both compounds, the metabolites of captan were not specifically measured in the urine of the workers under study to confirm internal exposure to both pyrethroids and captan.

Despite these limitations, this study showed that coexposure did not significantly impact biological levels of lambda-cyhalothrin metabolites, while controlling for other factors such as the main task performed, time since application, age, ethnicity, education level, medication, which may contribute to biological variability. In the study of Khemiri *et al.* (2017, 2018) in volunteers exposed to lambda-cyhalothrin orally and dermally under controlled conditions, variability in urine levels of CFMP and 3-PBA was significant despite identical exposure doses and the absence of coexposure. This indicates that physiological factors, related to absorption, distribution, metabolism and excretion, contribute significantly to inter-individual variability in the biological levels of metabolites used as biomarkers of exposure. The results of the present study thus suggest that at the levels of pesticide exposure observed in the targeted workers, coexposure does not contribute significantly to increasing this variability in the biological levels of CFMP and 3-PBA. At these exposure levels, coexposure therefore had no significant impact on the kinetics of lambda-cyhalothrin and its assessed metabolites used as biomarkers of exposure.

Furthermore, while the measurement of 3-PBA is not specific to lambda-cyhalothrin, the analyses carried out on the basis of this metabolite allow *a contrario* to assume that the results on the impact of coexposure to lambda-cyhalothrin and captan can be extrapolated and therefore generalized to other pyrethroids. Considering that the workers in the study were also exposed to other pesticides in their workplace (e.g. application of Roundup reported in some cases or other fungicides), the observed results indirectly point to a lack of impact of exposure to other pesticides used in the

workplace on the levels of biomarkers of exposure to pyrethroids at the observed levels, although this remains to be confirmed

Overall, this study provided novel data confirming that at the levels observed in the agricultural workers under study, biomarker concentrations in urine used to assess exposure to pyrethroids should not be influenced by coexposure to captan. As concluded from the animal results of Bossou et al. (2020), the present study thus suggests that the pyrethroid metabolites CFMP and 3-PBA, mostly measured in biomonitoring studies, remain useful as biomarkers of exposure in mixtures, when pesticide exposure levels are in the range of the sampled workers or at the general populational levels. Future perspectives include the use of a toxicokinetic model specific to lambda-cyhalothrin and Monte Carlo simulations to obtain the reconstructed absorbed dose possibilities for each worker, based on the amounts of the more specific metabolite CFMP measured in urine, throughout the biological monitoring period. The reconstructed daily dose results for applicators and other agricultural weed control and harvest workers can then be compared to limit values such as the *Acceptable Operator Exposure Level* (AOEL) reference value established by the European Commission (EFSA, 2014).

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Author Contributions

Yélian Marc Bossou: Methodology, Formal analysis, Investigation, Writing – Original draft preparation. **Jonathan Côté:** Formal analysis, Investigation, Writing – Reviewing and Editing. **Étienne Dumais:** Formal analysis, Investigation, Writing – Reviewing and Editing. **Éloïse Morin:** Formal analysis, Investigation, Writing – Reviewing and Editing. **Clara Bianci:** Formal analysis, Investigation, Writing – Reviewing and Editing. **Michèle Bouchard:** Conceptualization, Methodology, Formal analysis, Supervision, Project Management, Writing – Original draft preparation, Writing – Reviewing and Editing, Funding acquisition.

Data Availability

All data generated during this study are included in this article or are available on reasonable request from the corresponding author.

Ethics approval

The study protocol, the information and consent form, and other relevant documents were approved by Research Ethics Committee of the University of Montreal (*Comité d'éthique de la recherche Clinique de Université de Montréal* #CERC-19-007-D).

Consent to participate

The study was based on a voluntary participation. Participants received a slight financial compensation for their involvement and time. Subjects wishing to participate in the study signed an informed consent form, after receiving all necessary information about the project. Each participant was free to withdraw from the study at any time, without any prejudice.

Consent for publication

This manuscript has not been published or presented elsewhere and is not under consideration by another journal. All authors read and approved the final manuscript and consent for publication.

Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

REFERENCES

Amoatey, P., Al-Mayahi, A., Omidvarborna, H., Baawain, M. S. et Sulaiman, H. (2020). Occupational exposure to pesticides and associated health effects among greenhouse farm workers. *Environ Sci Pollut Res Int, 27*(18), 22251-22270. 10.1007/s11356-020-08754-9

Apel, P., Lamkarkach, F., Lange, R., Sissoko, F., David, M., Rousselle, C., . . . Health, E. (2023). Human biomonitoring guidance values (HBM-GVs) for priority substances under the HBM4EU Initiative–New values derivation for deltamethrin and cyfluthrin and overall results. *248*, 114097.

Arcury, T. A., Laurienti, P. J., Talton, J. W., Chen, H., Howard, T. D., Barr, D. B., . . . Quandt, S. A. (2018). Pesticide urinary metabolites among latina farmworkers and non-farmworkers in North Carolina. *Journal of occupational environmental medicine, 60*(1), e63.

Barr, D. B., Olsson, A. O., Wong, L. Y., Udunka, S., Baker, S. E., Whitehead, R. D., . . . Needham, L. L. (2010a). Urinary concentrations of metabolites of pyrethroid insecticides in the general U.S. population: National Health and Nutrition Examination Survey 1999-2002. *Environ. Health Perspect, 118*(6), 742-748. 10.1289/ehp.0901275

Barrón Cuenca, J., Tirado, N., Barral, J., Ali, I., Levi, M., Stenius, U., . . . Dreij, K. (2019). Increased levels of genotoxic damage in a Bolivian agricultural population exposed to mixtures of pesticides. *Sci Total Environ, 695*, 133942. 10.1016/j.scitotenv.2019.133942

Bevan, R., Jones, K., Cocker, J., Assem, F. L. et Levy, L. S. (2013). Reference ranges for key biomarkers of chemical exposure within the UK population. *International Journal of Hygiene & Environmental Health, 216*(2), 170-174. 10.1016/j.ijheh.2012.03.005

Bossou, Y. M., Côté, J., Mahrouche, L., Mantha, M., El Majidi, N., Furtos, A. et Bouchard, M. (2022). Excretion time courses of lambda-cyhalothrin metabolites in the urine of strawberry farmworkers and effect of coexposure with captan. *Archives of Toxicology*, 1-22.

Bossou, Y. M., Cote, J., Mantha, M., Haddad, S., Achard, S. et Bouchard, M. (2020). Impact of pesticide coexposure: an experimental study with binary mixtures of lambda-cyhalothrin (LCT) and captan and its impact on the toxicokinetics of LCT biomarkers of exposure. *Arch. Toxicol., 94*(9), 3045-3058. 10.1007/s00204-020-02810-6

Bouchard, M., Côté, J. et Khemiri, R. (2019). *La lambda-cyhalothrine comme insecticide en milieu agricole : Étude de la toxicocinétique de biomarqueurs pour le suivi de l'exposition des travailleurs* (Publication nº R-1043). Institut de recherche Robert-Sauvé en santé et en sécurité du travail (IRSST). https://www.irsst.qc.ca/en/publications-tools/publication/i/101029/n/lambda-cyhalothrine

Bouchard, M., Ratelle, M. et Côté, J. (2016). *Développement et application d'une approche toxicocinétique pour l'évaluation de l'exposition des travailleurs agricoles aux pyréthrinoïdes* nº R-936). https://www.irsst.qc.ca/media/documents/PubIRSST/R-936.pdf?v=2019-04-17

Bravo, N., Gari, M. et Grimalt, J. O. (2022). Occupational and residential exposures to organophosphate and pyrethroid pesticides in a rural setting. *Environ Res, 214*(Pt 4), 114186. 10.1016/j.envres.2022.114186

Buchholz, B. A., Ahn, K. C., Huang, H., Gee, S. J., Stewart, B. J., Ognibene, T. J. et Hammock, B. D. (2021). Pharmacokinetics, metabolite measurement, and biomarker identification of dermal exposure to permethrin using accelerator mass spectrometry. *Toxicol. Sci, 183*(1), 49-59. 10.1093/toxsci/kfab082

Burns, C. J. et Juberg, D. R. (2021). Cancer and occupational exposure to pesticides: an umbrella review. *International Archives of Occupational & Environmental Health, 94*(5), 945-957. 10.1007/s00420-020-01638-y

Chester, G., Sabapathy, N. N. et Woollen, B. H. (1992). Exposure and health assessment during application of lambda-cyhalothrin for malaria vector control in Pakistan. *Bull. World Health Organ., 70*(5), 615-619. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2393370/

Costa, C., Rapisarda, V., Catania, S., Di Nola, C., Ledda, C. et Fenga, C. (2013). Cytokine patterns in greenhouse workers occupationally exposed to alpha-cypermethrin: an observational study. *Environ Toxicol Pharmacol, 36*(3), 796-800. 10.1016/j.etap.2013.07.004

Curl, C. L., Meierotto, L., Castellano, R. L., Spivak, M. R. et Kannan, K. (2021). Measurement of urinary pesticide biomarkers among Latina farmworkers in southwestern Idaho. *Journal of exposure science environmental epidemiology, 31*(3), 538-548.

Curl, C. L., Spivak, M., Phinney, R. et Montrose, L. (2020). Synthetic Pesticides and Health in Vulnerable Populations: Agricultural Workers. *Curr Environ Health Rep, 7*(1), 13-29. 10.1007/s40572-020-00266-5

de Graaf, L., Boulanger, M., Bureau, M., Bouvier, G., Meryet-Figuiere, M., Tual, S., . . . Baldi, I. (2022). Occupational pesticide exposure, cancer and chronic neurological disorders: A systematic review of epidemiological studies in greenspace workers. *Environ Res, 203*, 111822. 10.1016/j.envres.2021.111822

De Roos, A. J., Schinasi, L. H., Miligi, L., Cerhan, J. R., Bhatti, P., 't Mannetje, A., . . . Cocco, P. (2021). Occupational insecticide exposure and risk of non-Hodgkin lymphoma: A pooled case-control study from the InterLymph Consortium. *Int J Cancer, 149*(10), 1768-1786. 10.1002/ijc.33740

El Okda, E. S., Abdel-Hamid, M. A. et Hamdy, A. M. (2017). Immunological and genotoxic effects of occupational exposure to α-cypermethrin pesticide. *Int J Occup Med Environ Health, 30*(4), 603-615. 10.13075/ijomeh.1896.00810

EPA. (2004). *Lambda-cyhalothrin and an isomer gamma-cyhalothrin; Tolerances for residues*. U.S. Environmental Protection Agency

European Food Safety Authority. (2014). Conclusion on the peer review of the pesticide risk assessment of the active substance lambda-cyhalothrin. *EFSA Journal, 12*(5), 170. doi:10.2903/j.efsa.2014.3677

Fortin, M. C., Bouchard, M., Carrier, G. et Dumas, P. (2008). Biological monitoring of exposure to pyrethrins and pyrethroids in a metropolitan population of the Province of Quebec, Canada. *Environ. Res, 107*(3), 343-350. 10.1016/j.envres.2008.03.002

Hardt, J. et Angerer, J. (2003). Biological monitoring of workers after the application of insecticidal pyrethroids. *Int. Arch. Occup. Environ. Health, 76*(7), 492-498. 10.1007/s00420-003-0451-8

Havenith, G. (1999). Heat balance when wearing protective clothing. *Ann Occup Hyg, 43*(5), 289-296. https://www.ncbi.nlm.nih.gov/pubmed/10481628

Health Canada. (2013). Second report on human biomonitoring of environmental chemicals in Canada. In: Minister of Health, O., ON (Eds.), Health Canada.

Health Canada. (2021). *Sixth report on human biomonitoring of environmental chemicals in Canada*. Minister of Health, Ottawa, ON. Available at: https://www.canada.ca/en/health-canada/services/environmental-workplace-health/reports-publications/environmental-contaminants/sixth-report-human-biomonitoring.html

Helsel, D. R. (2005). *Nondetects and data analysis : statistics for censored environmental data*. Wiley-Interscience, Hoboken, N.J.

Hirosawa, N., Ueyama, J., Kondo, T., Kamijima, M., Takagi, K., Fujinaka, S., . . . Wakusawa, S. (2011). Effect of DDVP on urinary excretion levels of pyrethroid metabolite 3-phenoxybenzoic acid in rats. *Toxicol. Lett, 203*(1), 28-32. 10.1016/j.toxlet.2011.02.016

Huston, C. et Juarez-Colunga, E. (2009). Guidelines for computing summary statistics for data-sets containing non-detects. *Bulkley Valley Research Center*.

Ismail, A. A., Bonner, M. R., Hendy, O., Abdel Rasoul, G., Wang, K., Olson, J. R. et Rohlman, D. S. (2017). Comparison of neurological health outcomes between two adolescent cohorts exposed to pesticides in Egypt. *PLoS One, 12*(2), e0172696. 10.1371/journal.pone.0172696

Kaneko, H. (2011). Pyrethroids: mammalian metabolism and toxicity. *J Agric Food Chem, 59*(7), 2786-2791. 10.1021/jf102567z

Khemiri, R., Cote, J., Fetoui, H. et Bouchard, M. (2017). Documenting the kinetic time course of lambda-cyhalothrin metabolites in orally exposed volunteers for the interpretation of biomonitoring data. *Toxicol Lett, 276*, 115-121. 10.1016/j.toxlet.2017.05.022

Khemiri, R., Cote, J., Fetoui, H. et Bouchard, M. (2018). Kinetic time courses of lambda-cyhalothrin metabolites after dermal application of Matador EC 120 in volunteers. *Toxicol Lett, 296*, 132-138. 10.1016/j.toxlet.2018.08.008

Lee, G.-H., Choi, K.-C. J. C. B., Toxicology, P. P. C. et Pharmacology. (2020). Adverse effects of pesticides on the functions of immune system. *235*, 108789.

Lopez-Galvez, N., Wagoner, R., Beamer, P., de Zapien, J. et Rosales, C. (2018). Migrant Farmworkers' Exposure to Pesticides in Sonora, Mexico. *Int J Environ Res Public Health, 15*(12). 10.3390/ijerph15122651

Lu, D., Wang, D., Feng, C., Jin, Y., Zhou, Z., Wu, C., . . . Wang, G. (2013). Urinary concentrations of metabolites of pyrethroid insecticides in textile workers, Eastern China. *Environ Int, 60*, 137-144. 10.1016/j.envint.2013.08.004

Lucero, B. et Muñoz-Quezada, M. T. (2021). Neurobehavioral, Neuromotor, and Neurocognitive Effects in Agricultural Workers and Their Children Exposed to Pyrethroid Pesticides: A Review. *Front Hum Neurosci, 15*, 648171. 10.3389/fnhum.2021.648171

Ministère de l'Agriculture des Pêcheries et de l'Alimentation du Québec. (2020). *Agir, pour une agriculture durable plan 2020-2030*. https://cdn-contenu.quebec.ca/cdn-contenu/adm/min/agriculture-pecheries-alimentation/publications-adm/dossier/plan_agriculture_durable/PL_agriculture_durable_MAPAQ.pdf?1661972140

Ministère de l'Agriculture des Pêcheries et de l'Alimentation du Québec. (2021). Profil régional de l'industrie bioalimentaire au Québec https://cdn-contenu.quebec.ca/cdn-contenu/adm/min/agriculture-pecheries-alimentation/agriculture/industrie-agricole/regions/FS_profilregionalbioalimentaire_complet_MAPAQ.pdf

Mattila, T., Santonen, T., Andersen, H.R., Katsonouri, A., Szigeti, T., Uhl, M.,Wąsowicz, W., Lange, R., Bocca, B., Ruggieri, F., Kolossa-Gehring, M., Sarigiannis, D. A., Tolonen, H., 2021. Scoping review-the association between asthma and environmental chemicals. Int. J. Environ. Res. Publ. Health 18.

Maule, A.L., Scarpaci, M.M., Proctor, S.P. (2019). Urinary concentrations of permethrin metabolites in US Army personnel in comparison with the US adult population, occupationally exposed cohorts, and other general populations. Int. J. Hyg Environ. Health 222, 355–363.

Norén, E., Lindh, C., Rylander, L., Glynn, A., Axelsson, J., Littorin, M., . . . Nielsen, C. (2020). Concentrations and temporal trends in pesticide biomarkers in urine of Swedish adolescents, 2000-2017. *Expo Sci Environ Epidemiol, 30*(4), 756-767. 10.1038/s41370-020-0212-8

OECD. (2018). *Considerations for assessing the risks of combined exposure to multiple chemicals, Series on Testing and Assessment No. 296* (Publication nº 9264952977). https://www.oecd.org/chemicalsafety/risk-assessment/considerations-for-assessing-the-risks-of-combined-exposure-to-multiple-chemicals.pdf

Panuwet, P., Prapamontol, T., Chantara, S., Thavornyuthikarn, P., Montesano, M. A., Whitehead Jr, R. D. et Barr, D. B. (2008). Concentrations of urinary pesticide metabolites in small-scale farmers in Chiang Mai Province, Thailand. *Science of the Total Environment, 407*(1), 655-668.

Paolini, M., Barillari, J., Trespidi, S., Valgimigli, L., Pedulli, G. F. et Cantelli-Forti, G. (1999). Captan impairs CYP-catalyzed drug metabolism in the mouse. *Chem Biol Interact, 123*(2), 149-170. https://www.ncbi.nlm.nih.gov/pubmed/10597907

Perkins, A., Walters, F., Sievert, J., Rhodes, B., Morrissey, B. et Karr, C. J. (2016). Home Use of a Pyrethroid-Containing Pesticide and Facial Paresthesia in a Toddler: A Case Report. *International Journal of Environmental Research and Public Health, 13*(8), 829. 10.3390/ijerph13080829

Ratanachina, J., De Matteis, S., Cullinan, P. et Burney, P. (2020). Pesticide exposure and lung function: a systematic review and meta-analysis. *Occup Med (Lond), 70*(1), 14-23. 10.1093/occmed/kqz161

Ratelle, M., Côté, J. et Bouchard, M. (2015a). Time profiles and toxicokinetic parameters of key biomarkers of exposure to cypermethrin in orally exposed volunteers compared with previously available kinetic data following permethrin exposure. *Appl Toxicol, 35*(12), 1586-1593. 10.1002/jat.3124

Ratelle, M., Côté, J. et Bouchard, M. (2015b). Toxicokinetics of permethrin biomarkers of exposure in orally exposed volunteers. *Toxicol Lett, 232*(2), 369-375. 10.1016/j.toxlet.2014.12.003

Ratelle, M., Côté, J. et Bouchard, M. (2016). Time courses and variability of pyrethroid biomarkers of exposure in a group of agricultural workers in Quebec, Canada. *Int Arch Occup Environ Health, 89*(5), 767-783. 10.1007/s00420-016-1114-x

Ravula, A. R. et Yenugu, S. (2021). Pyrethroid based pesticides - chemical and biological aspects. *Crit. Rev. Toxicol, 51*(2), 117-140. 10.1080/10408444.2021.1879007

Sams, C. et Jones, K. (2011). Human volunteer studies investigating the potential for toxicokinetic interactions between the pesticides deltamethrin; Pirimicarb and chlorpyrifos-methyl following oral exposure at the acceptable daily intake. *Toxicology Letters, 200*(1-2), 41-45. 10.1016/j.toxlet.2010.10.012

Schettgen, T., Dewes, P. et Kraus, T. (2016). A method for the simultaneous quantification of eight metabolites of synthetic pyrethroids in urine of the general population using gas chromatography-tandem mass spectrometry. *Anal Bioanal Chem, 408*(20), 5467-5478. 10.1007/s00216-016-9645-2

Scollon, E. J., Starr, J. M., Godin, S. J., DeVito, M. J. et Hughes, M. F. (2009). In vitro metabolism of pyrethroid pesticides by rat and human hepatic microsomes and cytochrome p450 isoforms. *Drug Metab. Dispos, 37*(1), 221-228. 10.1124/dmd.108.022343

Shearer, J. J., Beane Freeman, L. E., Liu, D., Andreotti, G., Hamilton, J., Happel, J., . . . Hofmann, J. N. (2019). Longitudinal investigation of haematological alterations among permethrin-exposed pesticide applicators in the Biomarkers of Exposure and Effect in Agriculture study. *Occup Environ Med, 76*(7), 467-470. 10.1136/oemed-2018-105559

Trunnelle, K. J., Bennett, D. H., Ahn, K. C., Schenker, M. B., Tancredi, D. J., Gee, S. J., . . . Hammock, B. D. (2014). Concentrations of the urinary pyrethroid metabolite 3-

phenoxybenzoic acid in farm worker families in the MICASA study. *Environmental research, 131*, 153-159.

United States Environmental Protection Agency. (2018). *Chemicals Evaluated for Carcinogenic Potential (Annual Cancer Report 2018)*. US Environmental Protection Agency, Office of Pesticide Programs. https://apublica.org/wp-content/uploads/2020/05/chemicals-evaluated.pdf

Wang, D., Kamijima, M., Imai, R., Suzuki, T., Kameda, Y., Asai, K., . . . Wakusawa, S. (2007). Biological monitoring of pyrethroid exposure of pest control workers in Japan. *Occup Health, 49*(6), 509-514. 10.1539/joh.49.509

Wang, X., Martínez, M. A., Dai, M., Chen, D., Ares, I., Romero, A., . . . Yuan, Z. (2016). Permethrin-induced oxidative stress and toxicity and metabolism. A review. *Environ Res, 149*, 86-104. 10.1016/j.envres.2016.05.003

Wielgomas, B. et Krechniak, J. (2007). Toxicokinetic interactions of alpha-cypermethrin and chlorpyrifos in rats. *Pol. J. Environ. Stud, 16*(2), 267. 10.1007/s00204-019-02546-y

Zepeda-Arce, R., Rojas-García, A. E., Benitez-Trinidad, A., Herrera-Moreno, J. F., Medina-Díaz, I. M., Barrón-Vivanco, B. S., . . . Bernal-Hernández, Y. Y. (2017). Oxidative stress and genetic damage among workers exposed primarily to organophosphate and pyrethroid pesticides. *Environ Toxicol, 32*(6), 1754-1764. 10.1002/tox.22398

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A. et Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R* (vol. 574). Springer.

SUPPLEMENTARY FILES

Table S1. Variables documented by questionnaire

	Variable	Description
Physiological information	Age	Expressed in years
	Type	Male/Female
	Body weight	Expressed in kg (or pounds)
	Size	Expressed in m (or feet)
	Body mass index (BMI)	Calculated as follows: body weight/height^2 and processed as a continuous variable
	Symptoms	Nausea, headache, stomach cramps, diarrhea, loss of appetite, dizziness, sweating, tearing, skin irritation, eye irritation, dry skin, excessive fatigue
Sociodemographic data	Education	Primary and High school or CEGEP and University treated as a dichotomous variable
	Ethnicity	Ethnicity of the participant and his/her parents, treated as a dichotomous variable: Caucasian or Latin o-American
Lifestyle habits	Smoking status	Treated in dichotomous: yes or no
	Alcohol consumption	Treated in dichotomous: yes or no
	Medication	Dichotomous drug intake: yes or no. Type of medication and time of administration for all medications or natural products
Household exposure	Household or animal treatments	Treatment in the house (extermination or other), treatment against lice, use of insect repellent, treatment of pets against fleas and ticks. Treated in dichotomous variable: yes or no
	Consumption of cereals, fruits and vegetables (organic and non-organic)	Number of daily servings of each food category and type (frozen, whole, fresh) averaged over the three days of biological monitoring. Expressed according to the serving size found in Canada's *Food Guide* (Santé Canada, 2011)
Occupational exposure	Duration of exposure	Preparation/mixing/application period of lambda-cyhalothrin alone or in combination with captan or working in the treated area
	Personal protective equipment (PPE)	PPE category: clothing, eye protection, respiratory protection, feet, hands and head protection
	Work tasks	Treated as a categorical variable: preparation/mixing/application of pesticide and decontamination of equipment, weeding and picking

CHAPITRE QUATRIÈME :

DISCUSSION GÉNÉRALE

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Contribution au domaine de la santé au travail

Afin de mieux évaluer et prévenir les risques pour la santé liés à l'utilisation de pesticides sur le lieu de travail, la surveillance biologique de l'exposition est désormais utilisée. Cette approche consiste à mesurer les composés parents ou les métabolites (produits de biotransformation) de ces pesticides dans les urines (Angerer *et al.*, 2007; Hardt et Angerer, 2003; Linda, 2022). Comme mentionné plus tôt, les niveaux urinaires de ces biomarqueurs d'exposition sont susceptibles de varier considérablement d'une personne à l'autre, ainsi que chez la même personne au fil du temps, et peuvent être influencés par plusieurs facteurs. Certaines études menées auprès des travailleurs agricoles avaient souligné la nécessité de mieux comprendre ces facteurs afin d'interpréter de manière plus précise les données de biosurveillance. La plupart des recherches scientifiques se sont concentrées sur des facteurs liés aux tâches, tels que les expositions résultant des différentes méthodes de manipulation et d'application des pesticides (Coronado *et al.*, 2004; Grover *et al.*, 1986), ainsi que l'utilisation d'équipements de protection individuelle (Krieger et Dinoff, 2000).

Cette présente recherche est originale et novatrice, car il s'agit, à notre connaissance, de la première à évaluer l'impact de la coexposition sur les biomarqueurs d'exposition aux pesticides pyréthrinoïdes chez les travailleurs agricoles. L'hypothèse, a priori, était que le captane pouvait interférer avec la voie métabolique des pyréthrinoïdes catalysée par les CYP450 ou avec les mécanismes d'excrétion (Paolini *et al.*, 1999; Wielgomas et Krechniak, 2007). En effet, des études *in vitro* ont montré que les pyréthrinoïdes et les métabolites tétrahydrophtalimides du captane

partagent des voies métaboliques communes catalysées par les CYP450 au niveau du foie (comme le CYP3A4 chez l'humain (Scollon *et al.*, 2009)). Elles ont aussi montré que le captane peut altérer le métabolisme de composés lipophiles par les CYP450s (Guengerich, 2008; Paolini *et al.*, 1999). Il était donc important de comprendre comment de tels biomarqueurs d'exposition se comportent dans l'organisme lors de coexposition afin de mieux interpréter les données de biosurveillance chez les travailleurs agricoles fréquemment exposés à des mélanges de pesticides en milieu de travail.

Impact de la coexposition sur les biomarqueurs d'exposition aux pyréthrinoïdes (lambda-cyhalothrine)

Globalement, les résultats obtenus dans cette recherche ont révélé l'existence d'un impact de la coexposition LCT-captan sur la voie de formation des métabolites phénoxybenzoïques chez des rats exposés à fortes doses, mais n'a pas démontrer d'impact de la coexposition à faibles doses, et ce sur la base de l'étude cinétique menée auprès des applicateurs de pesticides et de l'étude transversale menée chez des travailleurs agriculteurs.

Le premier volet de cette recherche, basé sur une étude expérimentale *in vivo* (Bossou *et al.*, 2020) a contribué à documenter l'impact de la coexposition sur le devenir des biomarqueurs d'exposition aux pyréthrinoïdes dans des conditions expérimentales contrôlées. Nous avons pu montrer que la coexposition aux faibles doses de LCT n'a pas eu d'impact observé sur la toxicocinétique des métabolites CFMP et 3-PBA, d'après l'évaluation combinée des profils temporels de ces métabolites dans le plasma, l'urine et les fèces. Le volet de la recherche menée sur le terrain en

milieu agricole (Bossou *et al.*, 2022) a analysé les profils temporels d'excrétion urinaires des biomarqueurs d'exposition au pyréthrinoïde LCT (CFMP, 3-PBA, 4-OH3BPA) chez les travailleurs agricoles effectuant des tâches de pulvérisation de la LCT seule ou en combinaison avec le captane dans des champs de fraises. L'analyse comparative a montré qu'il n'y avait pas de différences évidentes dans les profils temporels individuels des concentrations de ces trois métabolites, ni dans l'excrétion cumulative après une exposition à la LCT seule ou en combinaison avec le captane. Enfin, dans le troisième volet de la recherche (Bossou *et al.*, 2023), une étude transversale a été réalisée pour évaluer l'impact de la coexposition en relation avec plusieurs d'autres facteurs pouvant influencer les niveaux urinaires des biomarqueurs mesurés chez les travailleurs. Des analyses statistiques multivariées ont été utilisées pour évaluer la contribution de la coexposition à la variation dans les niveaux de biomarqueurs d'exposition. Contrairement au deuxième volet de la recherche (Bossou *et al.*, 2022) qui s'était concentré principalement sur un petit nombre de pulvérisateurs mais un grand nombre de collectes urinaires par travailleur, cette troisième étude a inclus 87 travailleurs exerçant différentes tâches (application, désherbage, cueillette) et exposés à la lambda-cyhalothrine seule ou coexposés avec le captane. Les participants ont fourni deux collectes urinaires de 24 h consécutives après exposition à la lambda-cyhalothrine ou en coexposés avec le captane. Une collecte témoin avait également été envisagée. Les résultats des analyses multivariées ont indiqué que la coexposition n'avait pas eu d'influence statistiquement significative sur les concentrations urinaires de 3-PBA et de CFMP.

La littérature disponible à ce jour concernant des études examinant l'impact de la coexposition sur les niveaux de métabolites utilisés pour la biosurveillance de l'exposition aux pesticides chez les individus est relativement pauvre. Néanmoins, les données rapportées dans cette recherche sont

conformes aux rares études publiées. Plus spécifiquement, l'équipe de Chata *et al.* (2019) a évalué la toxicocinétique de 17 pesticides, largement mesurés dans des études de biosurveillance, et de leurs principaux métabolites dans le plasma de rats Long-Evans femelles après une dose orale unique du mélange complexe. Dans leur étude, huit familles de pesticides ont été mesurées, notamment les anilines, les carbamates, les carboxamides, les organochlorés, les organophosphates, les oxadiazines, les phénylpyrazoles et les pyréthroïdes (LCT, perméthrine, cyperméthrine) ; le captane n'était pas inclus dans le mélange. Les métabolites évalués étaient le CFMP et le 3-PBA. La demi-vie d'élimination terminale moyenne du CFMP, estimée dans le plasma par ces auteurs et le TRM étaient similaires à ceux obtenus dans la présente étude (t1/2β moyen : 11,0 versus 9,2-12,1 h et de 19,0 versus 21,6-24,1 h respectivement pour le CFMP et TRM). Dans l'étude de Chata *et al.* (2019), le mélange comprenait à la fois de la cyperméthrine et de la perméthrine, en plus de la LCT. Ces substances forment toutes deux des métabolites du 3-PBA. Cependant, la demi-vie moyenne d'élimination terminale du 3-BPA dans le plasma et le TRM étaient similaires à ceux rapportés dans notre étude, que ce soit dans les groupes de rats exposés uniquement au LCT ou en mélange avec le captane (t1/2β moyen respectif de 7,0 contre 7,4-7,9 h et TRM de 10,0 contre 11,3-12,5 h). Ces résultats suggèrent que nos conclusions pourraient être généralisées aux mélanges complexes à faibles doses. De plus, (Chata *et al.*, 2019) ont également conclu que les enzymes métaboliques n'étaient pas saturées à la dose du mélange testé.

De même, une des rares études, menée chez l'humain en conditions contrôlées, sur l'impact de la coexposition aux pyréthrinoïdes et à leurs métabolites a rapporté des conclusions similaires (Sams et Jones, 2011). Cette étude a examiné l'impact de la coexposition au pyréthrinoïde deltaméthrine en combinaison avec l'organophosphoré chlorpyrifos-méthyle, à une dose journalière admissible

(DJA) de 0,01 mg/kg p.c., sur les profils d'excrétion des métabolites (notamment l'acide 3-(2,2-dibromovinyl) -2,2-diméthyl-(1-cyclopropane) carboxylique (DVBA) et le 3-PBA) dans l'urine. Les résultats de cette étude ont indiqué qu'il n'y avait pas d'impact apparent de la coexposition sur les profils d'excrétion des métabolites (Sams et Jones, 2011).

Par ailleurs, nos résultats ont également montré qu'aux doses élevées de LCT ($LOAEL_{LCT}$:12,5 mg/kg pc) et de captane ($NOAEL_{captane}$: 12,5 mg/kw pc), un potentiel impact de la coexposition sur la voie de formation des métabolites phénoxybenzoïques conduisant à la formation de 3-PBA mais pas sur la voie du CFMP était observable. Plus particulièrement, les niveaux plasmatiques de 3-PBA étaient plus faibles dans le groupe coexposé à la dose élevée de 12,5 mg/kg de p.c., correspondant à la dose minimale avec effet nocif observé (LOAEL) de LCT rapportée par l'EFSA (2014a). Un impact significatif de la coexposition sur l'excrétion urinaire du 4-OH3PBA (augmentation de l'excrétion du 4-OH3PBA) a également été observé, tandis que l'excrétion fécale n'a pas été affectée. Ces données sont comparables aux études animales disponibles, réalisées chez le rat à des doses relativement élevées. En particulier, Timchalk *et al.* (2005) ont montré que la coexposition à des insecticides organophosphorés (OP) en mélange binaire avait un impact sur leur vitesse d'absorption et d'élimination. Dans le cas de la coexposition avec des pyréthrinoïdes plus spécifiquement, Hirosawa *et al.* (2011) ont rapporté une excrétion significativement plus faible de 3-PBA chez des rats prétraités par voie intrapéritonéale avec l'insecticide OP dichlorvos à 0,3 ou 1,5 mg/kg pc, puis injectés par voie intraveineuse avec une dose unique élevée de cis-perméthrine (20 mg/kg pc). Wielgomas et Krechniak (2007) ont également signalé une réduction de 30 % de l'excrétion d'un métabolite urinaire du pyréthrinoïde α-cyperméthrine (soit le 4-OH3PBA) chez les rats après une coexposition avec l'insecticide OP chlorpyrifos par gavage à 5 mg/kg pc de chacun

des composés, sept jours/semaine pendant 28 jours, par rapport à 10 mg/kg pc de α-cyperméthrine seule.

Bien que notre étude signale un impact de la coexposition chez les animaux exposés à la forte dose de LCT (LOAEL :12,5 mg/kg pc), l'extrapolation d'un tel résultat ne peut être directement appliquée aux travailleurs agricoles car une telle dose administrée aux rats équivaudrait à 2,01 mg/kg pc chez l'humain. Ce niveau de dose a été obtenue à partir d'une équation d'ajustement allométrique [DEH = NOAELanimal [mg/kg] × (Poids animal [kg] / Poids humain [kg])(1-0.67)] (Nair et Jacob, 2016; Saadh *et al.*, 2020) , et dépasse 3000 fois la valeur limite AOEL de 0,63 µg/kg pc/jour pour la LCT (EFSA, 2014a) et 800 fois les doses absorbées reconstruites chez certains travailleurs agricoles exposés aux pyréthrinoïdes (Ferland *et al.*, 2015; Ratelle *et al.*, 2016). Toutefois, les résultats obtenus dans l'étude cinétique menée chez les applicateurs de pesticides (Bossou *et al.*, 2022) ainsi que dans l'étude expérimentale sur des rats exposés à de faibles doses (Bossou *et al.*, 2020) concourent à indiquer que les mesures des métabolites du CFMP et du 3-PBA ne sont pas influencées par la coexposition lorsque les niveaux d'expositions aux pesticides restent inférieurs aux valeurs toxicologiques de référence.

Importance des biomarqueurs spécifiques

En plus de fournir des données sur l'impact de la coexposition sur la cinétique des biomarqueurs d'expositions, cette recherche a permis de mettre en évidence l'intérêt des métabolites spécifiques pour évaluer l'exposition des travailleurs. Dans l'étude cinétique menée auprès des applicateurs de

pesticides, le CFMP s'est avéré être le métabolite retrouvé en concentrations urinaires les plus élevées chez la plupart des applicateurs évalués, quel que soit le scénario d'exposition. Cependant, le profil temporel des concentrations de CFMP ne correspondait pas toujours à celui des métabolites des dérivés phénoxybenzoïques (3-PBA et 4-OH3BPA) communs à d'autres pyréthrinoïdes. Dans certains cas où l'excrétion urinaire de 3-BPA et de 4-OH3BPA étaient supérieure à celle du CFMP, l'évolution temporelle de ces métabolites suivait celle du trans-DCCA et du cis-DCCA, suggérant une exposition concomitante à des pyréthrinoïdes telles que la perméthrine et la cyperméthrine, et ceci a pu être confirmé par l'analyse des réponses aux questionnaires. Des travaux antérieurs de biosurveillance menée par Berthet *et al.* (2012) chez des travailleurs exposés au captane et au folpet ont souligné l'intérêt de mesurer les métabolites spécifiques pour évaluer l'exposition professionnelle. Ces résultats renforcent donc l'idée de l'importance de mesurer et d'analyser les métabolites spécifiques pour une meilleure évaluation de l'exposition professionnelle aux pesticides.

Concernant l'évaluation de l'exposition à la LCT spécifiquement, notre étude a confirmé que le métabolite CFMP restait plus longtemps dans l'organisme que le 3-PBA après une exposition à la LCT (21,6 ± 1,2 h versus 11,8±0,6 h, TRM respectif de CFMP et 3PBA). De plus, le volet terrain et la précédente étude menée chez les volontaires (Khemiri *et al.*, 2017) a confirmé que la LCT était majoritairement excrétée dans l'urine sous forme de CFMP après avoir été absorbée dans l'organisme humain. De tels résultats sont d'un grand intérêt pour la communauté scientifique et ont des implications pour l'étude des schémas d'excrétion de LCT. Ces résultats peuvent également fournir une base pour l'élucidation des processus métaboliques de la LCT et d'autres pyréthrinoïdes. De plus, il n'y a pas de composés métabolisés en CFMP urinaire parmi les produits

d'usage courant homologués au Canada tels que les insecticides ménagers ou agricoles. Par conséquent, le CFMP devrait être considéré comme l'un des biomarqueurs optimaux et appropriés pour évaluer l'absorption de la LCT dans l'organisme des travailleurs.

Portée et Limites

Limites et portée de l'étude expérimentale chez l'animal

L'étude expérimentale menée chez des rats en laboratoire (Bossou *et al.*, 2020), présente l'avantage de pouvoir contrôler précisément les conditions d'exposition. Cet environnement contrôlé a réduit les facteurs de confusion et a renforcé la validité interne des résultats rapportés. De plus, l'étude toxicocinétique a fourni des informations précieuses sur l'absorption, la distribution, le métabolisme et l'excrétion des biomarqueurs d'exposition à la LCT. Ainsi, en examinant le mélange binaire spécifique de la LCT et du captane, les effets potentiels de la coexposition sur la toxicocinétique des biomarqueurs d'expositions aux pesticides pyréthrinoïdes, en absence de potentiels facteurs de confusion, ont pu être élucidés dans ce volet.

Une des limites principales de ce volet expérimental était le nombre réduit de doses expérimentales de LCT envisagées. Les doses choisies et testées correspondaient aux NOAEL et LOAEL de la LCT. Les animaux exposés aux fortes doses ont manifesté des signes cliniques de toxicité très rapidement et ont dû être euthanasiés précocement afin de limiter leur souffrance (24 h à 30 h après exposition). En conséquence, il n'était pas possible d'observer clairement l'élimination des biomarqueurs et d'estimer les paramètres toxicocinétiques appropriés dans aucun des deux

scénarios testés. Une perspective future serait d'expérimenter une dose intermédiaire de la LCT afin de documenter les paramètres toxicocinétiques et surtout de vérifier si les effets potentiels de la coexposition seraient observés sur le métabolite 3-PBA.

Limites et portée de l'étude de suivi biologique chez les travailleurs

Concernant le volet terrain (Bossou *et al.*, 2022; 2023), les principales limites résidaient dans la difficulté à effectuer des observations sur le terrain auprès des applicateurs, car ces derniers ne prévenaient pas à l'avance du moment de la pulvérisation. De plus, il était également difficile d'organiser systématiquement des observations sur le terrain tout au long de la journée pour les autres personnels agricoles travaillant dans les champs, compte tenu des différentes fermes impliquées.

En outre, bien que le projet ait cherché à déterminer l'impact de la coexposition à la lambda-cyhalothrine et au captane sur les niveaux de biomarqueurs d'exposition tels que les métabolites CFMP et 3-PBA, d'autres facteurs susceptibles d'influencer les niveaux biologiques mesurés ont été pris en compte grâce à un questionnaire individuel auto-administré lors du suivi biologique. Ces facteurs comprennent des informations personnelles (âge, sexe, ethnie, poids et taille pour le calcul de l'IMC) et socioéconomiques (niveau d'éducation) renseignés lors de l'inclusion au moment du recrutement. D'autres informations concernaient des habitudes de vie (tabagisme, consommation d'alcool, prise de médicaments), des expositions domestiques (utilisation de traitements personnels contre les poux, traitement des animaux domestiques contre les puces ou les

tiques, utilisation de pesticides à des fins résidentielles) et la consommation d'aliments susceptibles de contenir des résidus de pesticides (notamment les céréales, les fruits et les légumes), ont été documentés.

Les facteurs professionnels tels que les tâches effectuées et les équipements de protection individuelle (EPI) portés lors des activités, susceptibles d'influencer les niveaux de CFMP et de 3-PBA lors de l'exposition, ont également été documentés grâce à un questionnaire rempli à chaque journée de suivi biologique. Une attention particulière a été portée à la partie concernant les EPI, car les travailleurs ne portaient pas nécessairement les mêmes vêtements ou EPI pour chaque journée de suivi biologique ou pendant la période évaluée, comme par exemple le port d'un chapeau ou de gants pour les travailleurs dans les champs ou encore le port de masques et de gants pour les applicateurs.

Lors des analyses statistiques multivariées, les niveaux de CFMP et de 3-PBA ont été comparés entre le groupe exposé à la LCT seule et le groupe coexposé à la LCT et au captane, tout en tenant compte d'autres facteurs pouvant influencer les niveaux urinaires. Les contributions des facteurs personnels tels que l'âge, l'ethnie et l'IMC, ainsi que des habitudes de vie telles que la consommation de tabac et d'alcool ou la prise de médicaments, ainsi que des conditions d'exposition professionnelle telles que la tâche effectuée, le temps écoulé depuis l'application et la taille de la ferme en termes d'effectifs ont été testées. Cependant, certains facteurs documentés par questionnaire ont été peu rapportés, ce qui ne nous a pas permis de tester leur impact sur les niveaux de biomarqueurs d'exposition (exemple de la variable "sexe"). De plus, certaines questions sur les

traitements domestiques ou l'utilisation de pesticides à des fins résidentielles n'ont été rapportées que dans un seul cas, limitant ainsi leur inclusion dans les analyses.

Concernant les habits et les EPI portés par les travailleurs, ils n'ont pas été inclus dans les analyses multivariées car, comme mentionné précédemment, les informations sur les vêtements portés étaient assez uniformes (principalement des pantalons longs, des chandails à manches longues et des bottes) et le port d'EPI spécifiques (comme les demi-masques ou les casques avec filtres) était peu rapporté. De plus, les réponses concernant le port de gants, de lunettes et de chapeau n'ont pas permis d'établir une association significative avec les niveaux urinaires de CFMP et de 3-PBA.

Il convient de noter que les questionnaires utilisés lors de l'étude terrain ont été traduits en espagnol et qu'un membre hispanophone de notre équipe était disponible pour aider et vérifier les réponses des participants. Malgré cela, les questions sur la consommation d'aliments pouvant contenir des résidus de pesticides, comme les céréales, les fruits et les légumes, ont été peu ou mal documentées ou ont reçu peu de réponses, ce qui a empêché leur inclusion dans les analyses.

Une autre limite, liée au suivi biologique de l'exposition concerne la mesure du métabolite spécifique CFMP, ainsi que du métabolite commun à plusieurs pyréthrinoïdes, le 3-PBA. La mesure simultanée des métabolites spécifiques trans-DCCA et cis-DCCA a permis de confirmer, dans certains cas, que les travailleurs étaient également exposés à la perméthrine ou à la cyperméthrine, qui génèrent également du 3-PBA. Comme cela a été souligné dans une étude

antérieure portant sur la cyperméthrine (Bouchard *et al.*, 2016), la mesure de métabolites spécifiques est importante pour confirmer la source d'exposition.

Malgré ces contraintes, cette recherche a réussi à démontrer que la coexposition n'avait pas d'impact significatif sur les niveaux biologiques des métabolites de la lambda-cyhalothrine, en tenant compte d'autres facteurs tels que la tâche effectuée, le temps écoulé depuis l'application, l'âge, l'ethnie, le niveau d'éducation et la médication, qui sont des facteurs pouvant contribuer à la variabilité biologique selon la littérature. Dans les études menées par Khemiri *et al.* (2017, 2018) sur des volontaires exposés à la LCT par voie orale et cutanée dans des conditions contrôlées, une variabilité importante dans les niveaux urinaires de CFMP et de 3-PBA a été observée malgré des doses d'expositions identiques et l'absence de coexposition pour tous les volontaires. Cette observation suggère que des facteurs physiologiques liés à l'absorption, à la distribution, au métabolisme et à l'excrétion contribuent largement à la variabilité interindividuelle dans les niveaux biologiques des métabolites utilisés comme biomarqueurs d'exposition. Les résultats de cette étude suggèrent donc qu'à ces niveaux d'exposition aux pesticides observés chez les travailleurs ciblés, la coexposition ne contribue pas de manière significative à accroître cette variabilité dans les niveaux biologiques de CFMP et de 3-PBA. Ainsi, à ces niveaux d'exposition, la coexposition n'a pas d'impact significatif sur la cinétique de la LCT et de ses métabolites évalués en tant que biomarqueurs d'exposition.

Par ailleurs, il convient de rappeler que la mesure du 3-PBA n'est pas spécifique à la LCT. Malgré cela, les analyses basées sur ce métabolite suggèrent que les résultats sur l'impact de la coexposition

à la lambda-cyhalothrine et au captane peuvent être extrapolés et généralisés à d'autres pyréthrinoïdes. Étant donné que les travailleurs de l'étude ont également été exposés à d'autres pesticides sur leur lieu de travail (par exemple, l'application de Roundup®, de Coragen® ou d'autres fongicides rapportés dans certains cas), les résultats observés indiquent indirectement une absence d'impact de l'exposition à d'autres pesticides utilisés en milieu de travail sur les niveaux de biomarqueurs d'exposition aux pyréthrinoïdes.

Toutefois, il est important de noter que les résultats présentés dans ce travail de recherche ne peuvent être généralisés à l'ensemble des coexpositions observées dans les fermes québécoises. De plus, les facteurs de variabilité identifiés ne peuvent être considérés comme représentatifs de tous les facteurs d'exposition dans ce secteur car il existe un grand nombre de facteurs susceptibles d'influencer le niveau d'exposition et ils pourraient ne pas être présents dans l'échantillon restreint des exploitations agricoles ayant participé à notre étude.

Références bibliographiques

Anadon, A., Martinez-Larranaga, M. R., Diaz, M. J. et Bringas, P. (1991). Toxicokinetics of permethrin in the rat. *Toxicology & Applied Pharmacology, 110*(1), 1-8. 10.1016/0041-008x(91)90284-l

Anadon, A., Martınez, M., Martınez, M. A., Dıaz, M. J. et Martınez-Larranaga, M. R. (2006). Toxicokinetics of lambda-cyhalothrin in rats. *Toxicology Letters, 165*, 47-56.

Angerer, J., Ewers, U. et Wilhelm, M. (2007). Human biomonitoring: state of the art. *International Journal of Hygiene & Environmental Health, 210*(3-4), 201-228. 10.1016/j.ijheh.2007.01.024

Ansari, R. W., Shukla, R. K., Yadav, R. S., Seth, K., Pant, A. B., Singh, D., . . . Khanna, V. K. (2012). Involvement of dopaminergic and serotonergic systems in the neurobehavioral toxicity of lambda-cyhalothrin in developing rats. *Toxicol Lett, 211*(1), 1-9. 10.1016/j.toxlet.2012.02.012

Aouey, B., Derbali, M., Chtourou, Y., Bouchard, M., Khabir, A. et Fetoui, H. (2017). Pyrethroid insecticide lambda-cyhalothrin and its metabolites induce liver injury through the activation of oxidative stress and proinflammatory gene expression in rats following acute and subchronic exposure. *Environ Sci Pollut Res Int, 24*(6), 5841-5856. 10.1007/s11356-016-8323-4

Berthet, A., Heredia-Ortiz, R., Vernez, D., Danuser, B. et Bouchard, M. (2012). A detailed urinary excretion time course study of captan and folpet biomarkers in workers for the estimation of dose, main route-of-entry and most appropriate sampling and analysis strategies. *Ann Occup Hyg, 56*(7), 815-828. 10.1093/annhyg/mes011

Bossou, Y. M., Côté, J., Mahrouche, L., Mantha, M., El Majidi, N., Furtos, A. et Bouchard, M. (2022). Excretion time courses of lambda-cyhalothrin metabolites in the urine of strawberry farmworkers and effect of coexposure with captan. *Archives of Toxicology*, 1-22.

Bossou, Y. M., Cote, J., Mantha, M., Haddad, S., Achard, S. et Bouchard, M. (2020). Impact of pesticide coexposure: an experimental study with binary mixtures of lambda-cyhalothrin (LCT) and captan and its impact on the toxicokinetics of LCT biomarkers of exposure. *Arch. Toxicol., 94*(9), 3045-3058. 10.1007/s00204-020-02810-6

Bossou, Y. M., Côté, J., Morin, É., Dumais, É., Bianchi, C. et Bouchard, M. (2023). Assessing the impact of coexposure on the measurement of biomarkers of exposure to the pyrethroid lambda-cyhalothrin in agricultural workers. *International Journal of Hygiene and Environmental Health, 251*, 114194. https://doi.org/10.1016/j.ijheh.2023.114194

Bouchard, M., Côté, J. et Khemiri, R. (2019). *La lambda-cyhalothrine comme insecticide en milieu agricole : Étude de la toxicocinétique de biomarqueurs pour le suivi de l'exposition des travailleurs* (Publication n° R-1043). Institut de recherche Robert-Sauvé en santé et en

sécurité du travail (IRSST). https://www.irsst.qc.ca/en/publications-tools/publication/i/101029/n/lambda-cyhalothrine

Bouchard, M., Ratelle, M. et Côté, J. (2016). *Développement et application d'une approche toxicocinétique pour l'évaluation de l'exposition des travailleurs agricoles aux pyréthrinoïdes* nº R-936). https://www.irsst.qc.ca/media/documents/PubIRSST/R-936.pdf?v=2019-04-17

Burns, C. J. et Pastoor, T. P. (2018). Pyrethroid epidemiology: a quality-based review. *Crit. Rev. Toxicol*, 1-15. 10.1080/10408444.2017.1423463

Chata, C., Palazzi, P., Grova, N., Haan, S., Emond, C., Vaillant, M. et Appenzeller, B. M. R. (2019). Blood pharmacokinetic of 17 common pesticides in mixture following a single oral exposure in rats: implications for human biomonitoring and exposure assessment. *Arch Toxicol, 93*(10), 2849-2862. 10.1007/s00204-019-02546-y

Chester, G., Sabapathy, N. N. et Woollen, B. H. (1992). Exposure and health assessment during application of lambda-cyhalothrin for malaria vector control in Pakistan. *Bull. World Health Organ., 70*(5), 615-619. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2393370/

Coronado, G. D., Thompson, B., Strong, L., Griffith, W. C. et Islas, I. (2004). Agricultural task and exposure to organophosphate pesticides among farmworkers. *Environ. Health Perspect., 112*(2), 142-147. 10.1289/ehp.6412

Côté, J. et Bouchard, M. (2018). Dose reconstruction in workers exposed to two major pyrethroid pesticides and determination of biological reference values using a toxicokinetic model. *Expo Sci Environ Epidemiol, 28*(6), 599-614. 10.1038/s41370-017-0004-y

European Food Safety Authority. (2014a). Conclusion on the peer review of the pesticide risk assessment of the active substance lambda-cyhalothrin. *EFSA Journal, 12*(5), 170. doi:10.2903/j.efsa.2014.3677

European Food Safety Authority. (2014b). *Final addendum to the Renewal Assessment Report*).

Ferland, S., Côté, J., Ratelle, M., Thuot, R. et Bouchard, M. (2015). Detailed Urinary Excretion Time Courses of Biomarkers of Exposure to Permethrin and Estimated Exposure in Workers of a Corn Production Farm in Quebec, Canada. *Ann Occup Hyg, 59*(9), 1152-1167. 10.1093/annhyg/mev059

Fortin, M. C., Bouchard, M., Carrier, G. et Dumas, P. (2008). Biological monitoring of exposure to pyrethrins and pyrethroids in a metropolitan population of the Province of Quebec, Canada. *Environ. Res, 107*(3), 343-350. 10.1016/j.envres.2008.03.002

Gorse, I. et Balg, C. J. (2013). Bilan des ventes de pesticides au Québec pour l'année 2010, Québec.Ministère du Développement durable, de l'Environnement, de la Faune et des Parcs, Gouvernement du Québec, Québec, Canada.

Gouvernement du Québec. (2023). *Bilan des ventes de pesticides au Québec*. https://cdn-contenu.quebec.ca/cdn-contenu/adm/min/environnement/pesticides/bilan-ventes-pesticides-quebec.pdf

Grover, R., Cessna, A., Muir, N., Riedel, D., Franklin, C. et Yoshida, K. (1986). Factors affecting the exposure of ground-rig applicators to 2,4-D dimethylamine salt. *Arch. Environ. Contam. Toxicol., 15*, 677-686.

Guengerich, F. P. (2008). Cytochrome p450 and chemical toxicology. *Chem. Res. Toxicol, 21*(1), 70-83. 10.1021/tx700079z

Hardt, J. et Angerer, J. (2003). Biological monitoring of workers after the application of insecticidal pyrethroids. *International Archives of Occupational & Environmental Health, 76*(7), 492-498. 10.1007/s00420-003-0451-8

Hirosawa, N., Ueyama, J., Kondo, T., Kamijima, M., Takagi, K., Fujinaka, S., . . . Wakusawa, S. (2011). Effect of DDVP on urinary excretion levels of pyrethroid metabolite 3-phenoxybenzoic acid in rats. *Toxicol. Lett, 203*(1), 28-32. 10.1016/j.toxlet.2011.02.016

Hudson, N. L., Kasner, E. J., Beckman, J., Mehler, L., Schwartz, A., Higgins, S., . . . Calvert, G. M. (2014). Characteristics and magnitude of acute pesticide-related illnesses and injuries associated with pyrethrin and pyrethroid exposures--11 states, 2000-2008. *Am J Ind Med, 57*(1), 15-30. 10.1002/ajim.22216

Kaneko, H. (2011). Pyrethroids: mammalian metabolism and toxicity. *J Agric Food Chem, 59*(7), 2786-2791. 10.1021/jf102567z

Khemiri, R., Cote, J., Fetoui, H. et Bouchard, M. (2017). Documenting the kinetic time course of lambda-cyhalothrin metabolites in orally exposed volunteers for the interpretation of biomonitoring data. *Toxicol. Lett, 276*, 115-121. 10.1016/j.toxlet.2017.05.022

Khemiri, R., Cote, J., Fetoui, H. et Bouchard, M. (2018). Kinetic time courses of lambda-cyhalothrin metabolites after dermal application of Matador EC 120 in volunteers. *Toxicol. Lett, 296*, 132-138. 10.1016/j.toxlet.2018.08.008

Krieger, R. I. et Dinoff, T. M. (2000). Captan fungicide exposures of strawberry harvesters using THPI as a urinary biomarker. *Archives of Environmental Contamination & Toxicology, 38*(3), 398-403. https://link.springer.com/content/pdf/10.1007/s002449910053.pdf

Linda, R. (2022). Occupational Biomonitoring Guidance Document-Series on Testing and Assessment No. 370.

Mamane, A., Baldi, I., Tessier, J. F., Raherison, C. et Bouvier, G. (2015). Occupational exposure to pesticides and respiratory health. *Eur Respir Rev, 24*(136), 306-319. 10.1183/16000617.00006014

Ministère de l'Agriculture des Pêcheries et de l'Alimentation du Québec. (2020). *Agir, pour une agriculture durable plan 2020-2030*. https://cdn-contenu.quebec.ca/cdn-

contenu/adm/min/agriculture-pecheries-alimentation/publications-adm/dossier/plan_agriculture_durable/PL_agriculture_durable_MAPAQ.pdf?1661972140

Nair, A. B. et Jacob, S. (2016). A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm, 7*(2), 27-31. 10.4103/0976-0105.177703

Panuwet, P., Prapamontol, T., Chantara, S., Thavornyuthikarn, P., Montesano, M. A., Whitehead Jr, R. D. et Barr, D. B. (2008). Concentrations of urinary pesticide metabolites in small-scale farmers in Chiang Mai Province, Thailand. *Science of the Total Environment, 407*(1), 655-668.

Paolini, M., Barillari, J., Trespidi, S., Valgimigli, L., Pedulli, G. F. et Cantelli-Forti, G. (1999). Captan impairs CYP-catalyzed drug metabolism in the mouse. *Chem.-Biol. Interact, 123*(2), 149-170. 10.1016/s0009-2797(99)00134-9

Ratanachina, J., De Matteis, S., Cullinan, P. et Burney, P. (2020). Pesticide exposure and lung function: a systematic review and meta-analysis. *Occup Med (Lond), 70*(1), 14-23. 10.1093/occmed/kqz161

Ratelle, M., Côté, J. et Bouchard, M. (2015a). Time profiles and toxicokinetic parameters of key biomarkers of exposure to cypermethrin in orally exposed volunteers compared with previously available kinetic data following permethrin exposure. *Appl Toxicol, 35*(12), 1586-1593. 10.1002/jat.3124

Ratelle, M., Côté, J. et Bouchard, M. (2015b). Toxicokinetics of permethrin biomarkers of exposure in orally exposed volunteers. *Toxicol Lett, 232*(2), 369-375. 10.1016/j.toxlet.2014.12.003

Ratelle, M., Côté, J. et Bouchard, M. (2016). Time courses and variability of pyrethroid biomarkers of exposure in a group of agricultural workers in Quebec, Canada. *Int. Arch. Occup. Environ. Health, 89*(5), 767-783. 10.1007/s00420-016-1114-x

Righi, D. A., Xavier, F. G. et Palermo-Neto, J. (2009). Effects of type II pyrethroid cyhalothrin on rat innate immunity: a flow cytometric study. *Int Immunopharmacol, 9*(1), 148-152. 10.1016/j.intimp.2008.10.009

Saadh, M. J., Haddad, M., Dababneh, M. F., Bayan, M. F. et Al-Jaidi, B. A. J. S. R. P. (2020). A guide for estimating the maximum safe starting dose and conversion it between animals and humans. *11*, 98-101.

Saillenfait, A. M., Ndiaye, D. et Sabate, J. P. (2015). Pyrethroids: exposure and health effects--an update. *Int. J. Hyg. Environ. Health, 218*(3), 281-292. 10.1016/j.ijheh.2015.01.002

Sams, C. et Jones, K. (2011). Human volunteer studies investigating the potential for toxicokinetic interactions between the pesticides deltamethrin; Pirimicarb and chlorpyrifos-methyl following oral exposure at the acceptable daily intake. *Toxicol. Lett, 200*(1-2), 41-45. 10.1016/j.toxlet.2010.10.012

Santé Canada. (2021). *Lambda-cyhalothrine et préparations commerciales connexes* (Publication nº RVD2021-04). Agence de réglementation de la lutte antiparasitaire, Santé Canada

Sarazin, P., Lavoué, J., Tardif, R., Lévesque, M. (2022). Guide de surveillance biologique de l'exposition Stratégie de prélèvement et interprétation des résultats, 8e édition corrigée 3. Institut de recherche Robert-Sauvé en santé et en sécurité du travail (IRSST). https://www.irsst.qc.ca/media/documents/PubIRSST/T-03.pdf?v=2023-11-06

Scollon, E. J., Starr, J. M., Godin, S. J., DeVito, M. J. et Hughes, M. F. (2009). In vitro metabolism of pyrethroid pesticides by rat and human hepatic microsomes and cytochrome p450 isoforms. *Drug Metab. Dispos, 37*(1), 221-228. 10.1124/dmd.108.022343

Statistique Canada. (2022). *Statistiques sur la main-d'œuvre agricole et agroalimentaire*. Le Quotidien. https://www150.statcan.gc.ca/n1/fr/daily-quotidien/220613/dq220613d-fra.pdf?st=pnv9pacg

Syngenta. (2022). Approved Matador 120EC 24984 2022-04-26. https://assets.syngenta.ca/pdf/ca/labels/MATADOR_120EC_24984_en_pamphlet.pdf

Timchalk, C., Poet, T. S., Hinman, M. N., Busby, A. L. et Kousba, A. A. (2005). Pharmacokinetic and pharmacodynamic interaction for a binary mixture of chlorpyrifos and diazinon in the rat. *Toxicology & Applied Pharmacology, 205*(1), 31-42. 10.1016/j.taap.2004.09.004

Wielgomas, B. et Krechniak, J. (2007). Toxicokinetic interactions of alpha-cypermethrin and chlorpyrifos in rats. *Polish Journal of Environmental Studies, 16*(2), 267.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A. et Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R* (vol. 574). Springer.