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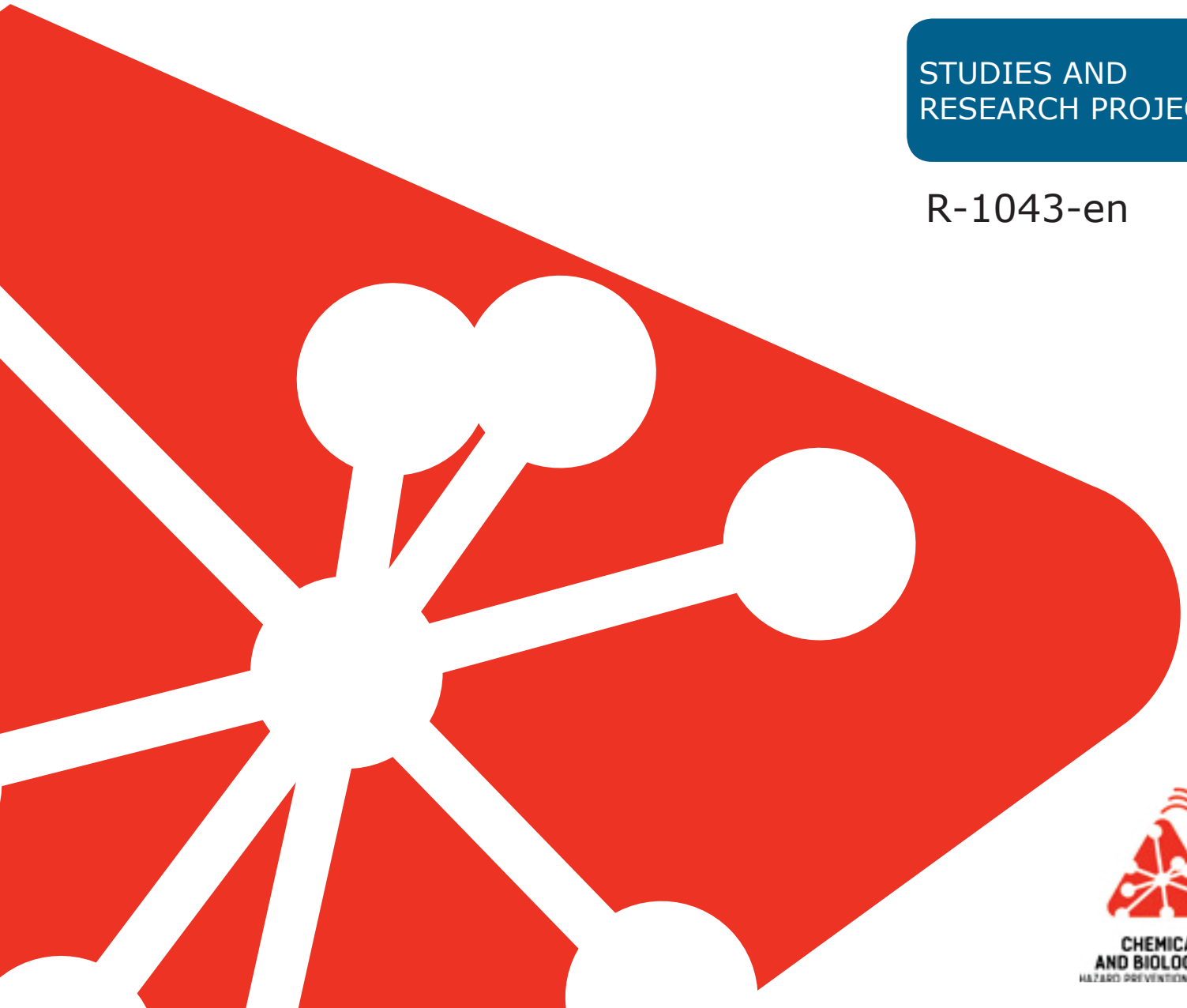
Lambda-Cyhalothrin Used as an Insecticide in Agriculture

Study of Biomarker Toxicokinetics to Monitor Worker Exposure

Michèle Bouchard
Jonathan Côté
Rania Khemiri

STUDIES AND RESEARCH PROJECTS

R-1043-en



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IRSST – Communications, Strategic Watch and Knowledge Mobilization Division
505 De Maisonneuve Blvd. West
Montréal, Québec
H3A 3C2
Phone: 514 288-1551
publications@irsst.qc.ca
www.irsst.qc.ca

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Department of Environmental Health and Occupational Health, Université de Montréal

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SUMMARY

Pyrethroid insecticides are a family of pesticides widely used in Quebec to control insect pests in market garden crops. One such insecticide is lambda-cyhalothrin. Despite its extensive use, there is very little data on this pyrethroid's biological behaviour in the human body. It has therefore become essential to develop tools to accurately assess worker exposure to these pesticides during spraying or work in already treated areas. Biological monitoring, by measuring products (called metabolites) excreted in the urine, is considered to be an appropriate method of assessing the doses actually absorbed of this type of product in the workplace. Measuring these exposure biomarkers in farmers provides an indication of combined respiratory, dermal and inadvertent oral exposure and allows varied exposure conditions to be taken into account. Interpretation of biological monitoring data requires a good knowledge of the fate (toxicokinetic behaviour) of the substance of interest in the human body, so that biomarker levels in workers can be related to doses actually absorbed. That relationship was recently established by this research team for permethrin and cypermethrin.

The overall objective of this research project was to address the lack of knowledge about the toxicokinetics of biomarkers of exposure to lambda-cyhalothrin in humans, and so to enable better interpretation of biomonitoring data for workers exposed to this pesticide and better assessment of the associated risks.

The project was divided into two parts. In the first part, a controlled kinetic study was conducted on volunteers exposed acutely to low oral (oral reference dose) and dermal (lambda-cyhalothrin-based formulation) doses of lambda-cyhalothrin, in order to analyze the time profiles of the exposure biomarkers (CFMP and 3-PBA) in their plasma and urine. In the second part, a toxicokinetic model was developed based on data from study volunteers to simulate the kinetics of biomarkers of exposure to lambda-cyhalothrin for various exposure scenarios and to provide a tool for recreating the doses absorbed by exposed workers.

The kinetic study of the volunteers and the data modelling showed that lambda-cyhalothrin enters the body quickly, but that it is also quickly eliminated following exposure through ingestion or skin contact. Measurement of metabolites in plasma or urine therefore reflects recent exposure. The research also revealed differences in the rate of absorption and elimination of lambda-cyhalothrin depending on whether the exposure is oral or dermal. The differences can be explained by the fact that the skin can cause the biotransformation of lambda-cyhalothrin into its metabolites (CFMP and 3-PBA), which serve as exposure biomarkers. They can also be explained by the fact that the parent product and the resulting metabolites are retained in the skin. The modelling did show, however, that the skin penetration of the molecule was limited, so that a worker's exposure doses by that route must be very high to make a significant contribution to the total amounts absorbed by the different routes (combination of oral, dermal and respiratory).

The results also showed that the behaviour of lambda-cyhalothrin exposure biomarkers (measured in plasma and urine) is similar to that of the metabolites of other pyrethroids already studied: permethrin and cypermethrin. The different metabolites of those pyrethroids in accessible biological fluids like urine can therefore be measured to assess overall exposure to pyrethroids. The modelling was also essential to proposing a urinary level of metabolites that can serve as a biological reference value that should not be exceeded, to reduce the risks of health effects.

Exceeding this threshold would be an indicator that workplace practices and hygiene need to be adjusted to limit the risks of harmful effects.

The new kinetic data obtained through the study, as well as the modelling performed, can be used directly to interpret biological monitoring data on worker exposure to lambda-cyhalothrin or other pyrethroids, and to reconstruct the doses absorbed under different exposure scenarios. Furthermore, comparing a farmworker's urinary biomarker levels with the biological reference value proposed as a benchmark can serve to assess the risks associated with exposure to lambda-cyhalothrin and, more broadly, to pyrethroids in general. This study has helped advance current knowledge, which will lead to better assessment of exposure and the risks associated with the use of pesticides in agriculture, from a prevention perspective.

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3-PBA:	3-phenoxybenzoic acid
4-OH-3-PBA:	3-(4'-hydroxyphenoxy) benzoic acid
ADI:	Acceptable daily intake
AJS ESI:	Jet Stream Technology Ion Source (Agilent Jet Stream electrospray ionization)
AOEL:	Acceptable operator exposure level
AUC:	Area under the curve
AUMC:	Area under the first moment curve
BRV:	Biological reference value
CAS:	Chemical Abstracts Service
CFMP:	*Cis*-3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethylcyclopropane carboxylic acid
CHMS:	Canadian Health Measures Survey
Cis-DCCA:	*Cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid
CL:	Plasma clearance
EFSA:	European Food Safety Authority
EIC:	Extracted Ion Chromatogram
FAO:	Food and Agriculture Organization
HPLC:	High-performance liquid chromatography
IPCS:	International Programme on Chemical Safety
IRSST:	Institut de recherche Robert-Sauvé en santé et en sécurité du travail
LOD:	Limit of detection
MAPAQ	Ministère de l’Agriculture, des Pêcheries et de l’Alimentation du Québec
MDDELCC:	Ministère du Développement durable, de l’Environnement et de la Lutte contre les changements climatiques du Québec

MRT:	Mean residence time
MS:	Mass spectrometry
n:	Number
NHANES:	National Health and Nutrition Examination Survey (US)
NOEL:	No-observed-effect level
PMRA:	Pest Management Regulatory Agency of Canada
Q-ToF:	Quadrupole time-of-flight
RfD:	Reference dose per ingestion established by the US Environmental Protection Agency
$t_{1/2:}$	Half life, or time needed to eliminate 50% of the substance absorbed by the body
Trans-DCCA:	*Trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid
UHPLC/TOFMS:	Ultra-high-performance liquid chromatography coupled with time-of-flight mass spectrometry
UPA:	Union des producteurs agricoles du Québec
US EPA:	United States Environmental Protection Agency
Vcap:	Capillary voltage
V_d:	Volume of distribution
WHO:	World Health Organization

1. INTRODUCTION

1.1 Occupational health and safety problem

Pyrethroid insecticides are a family of pesticides widely used in Quebec and elsewhere in the world to control insect pests in agricultural environments. Thus, farmworkers use these products widely while spraying farm fields. To properly assess exposure to this kind of chemical, we now use biological monitoring, which involves measuring the products excreted in the urine (parent products or metabolites) to obtain an indication of the doses actually absorbed. In farmers, the measurement of these products in the urine provides biomarkers of exposure and gives one an indication of combined respiratory, dermal and inadvertent oral exposure. Interpretation of biological monitoring data requires a good knowledge of the fate (toxicokinetic behaviour) of the substance of interest in the human body, so that biomarker levels in workers can be related to doses actually absorbed.

Recently, the biological behaviour and thus the toxicokinetics of two widely used pyrethroids – permethrin and cypermethrin – were established by our research team. In market gardening, other pyrethroids are also commonly used, but there are few data on their biological behaviour in humans. One of these pyrethroids is lambda-cyhalothrin. Thus, there is a need to better understand the kinetic behaviour of biomarkers of exposure to these other pyrethroids such as lambda-cyhalothrin in human beings. A controlled study of the temporal profiles of these biomarkers in accessible biological matrices such as blood and urine in volunteers could fill this gap. These data could then be used in a toxicokinetic model, which simulates the absorption, distribution, metabolism and excretion of the compound and its metabolites in the human body. When it is validated, this biomathematical model could be an excellent tool for better understanding the essential biological determinants of the profiles observed. Based on urinary biomarker measurements, it could be used to reconstruct the doses absorbed by workers who are exposed to formulations containing lambda-cyhalothrin and predict the main routes of exposure. The acquisition of this kind of data and the development of such a tool will also make it possible to better predict, and thus prevent, the risks associated with exposure to this kind of pesticide in the workplace.

2. STATE OF KNOWLEDGE

2.1 Agriculture: An important industry in Quebec

Agriculture is an industry that employs a large number of Quebec workers. According to the latest census by the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ, 2016a), this sector comprised approximately 28,150 farms and 54,500 workers in 2015. These businesses may or may not be family farms. Agriculture has a high proportion of employees hired on a seasonal basis and temporary foreign workers, of whom there were 7,780 in 2014 (MAPAQ, 2016b).

2.2 Use of pesticides in agriculture

In agriculture, pesticides are used regularly during the spring and summer to limit the damage caused by insects, fungi and weeds. Pyrethroid insecticides are among the most widely used (Gorse & Balg, 2013; MDDELCC, 2016). They are sprayed heavily, especially in market gardening. Thus, their use represents a risk factor for workers' health.

Permethrin and cypermethrin have long been among the most commonly applied pyrethroids in market gardening. Today, lambda-cyhalothrin (Figure 1), another pyrethroid, is used even more heavily on these crops. According to the latest report on sales of pesticides, annual sales of lambda-cyhalothrin and permethrin ranged from 1,001 to 10,000 kg (class C) while sales of cypermethrin ranged from 0.1 to 1,000 kg (class B) (MDDELCC, 2016). Lambda-cyhalothrin is a fast-acting ingestion and contact pyrethroid and is effective at protecting crops from a wide range of pests. It is also widely used to control pests and parasites inside buildings and around their perimeters (Syngenta, 2014). In Quebec, lambda-cyhalothrin is sold as various commercial formulations, mainly Matador 120EC® and Warrior®. The formulation is diluted in water before being applied with sprayers operated by cab tractors in vegetable farms or with manual or electric sprayers (in the form of droplets) in and around buildings. However, lambda-cyhalothrin is not very soluble in water; it adsorbs strongly to particles in the soil and its degradation rate in soil is slow (Pest Management Regulatory Agency [PMRA], 2004, 2017).

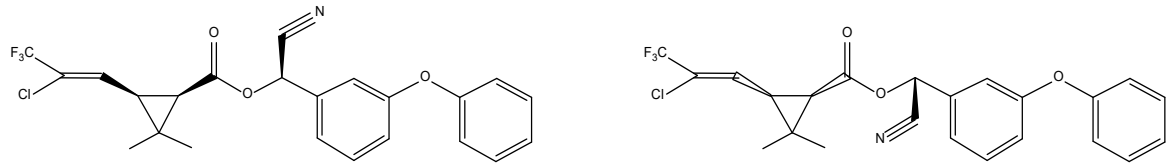


Figure 1. Lambda-cyhalothrin (composed of two pairs of diastereoisomeric enantiomers) (S)-α-cyano-3-phenoxybenzyl (Z)-(1R,3R)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate; (R)-α-cyano-3-phenoxybenzyl (Z)-(1S,3S)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate; CAS Number 91465-08-6).

2.3 Toxic effects of pyrethroids

Pyrethroids exert their toxic action on insects' neurological system by acting on voltage-gated ion channels (mainly sodium), which underlie nerve activity. They bind to these channels and prevent their transition from an activated state (which conducts ions) to an inactive state (which no longer conducts ions). Thus, the neuron cell membranes remain depolarized, which leads to the paralysis and death of the insect (Field, Davies, O'Reilly, Williamson, & Wallace, 2017). These insecticides also produce the same neurotoxic reaction in rats and humans, although to a much lesser degree (over 2,000 times less toxic) given the differences in their sodium channels (Choi & Soderlund, 2006; Clark & Symington, 2007; He et al., 1989; Le Quesne, Maxwell, & Butterworth, 1981). Cases of poisoning or incidents related to occupational exposure to pyrethroids, including respiratory and neurological disorders, have been described in reports from local and national monitoring programs and in case studies (Saillenfait, Ndiaye, & Sabate, 2015). Walters et al. (2009) point out that such incidents are very probably considerably under-reported to health care institutions. Moreover, very few epidemiological studies have examined the associations between exposure to pyrethroids and neurological effects, particularly in workers. Nevertheless, some authors have observed significant associations between exposure to pyrethroids and neurodevelopmental disorders in children (Oulhote & Bouchard, 2013; Shelton et al., 2014; Viel et al., 2015; Watkins et al., 2016).

In addition, some animal studies have shown endocrine and immune changes following the administration of high doses of pyrethroids, such as lambda-cyhalothrin (Ansari et al., 2012; Righi, Xavier, & Palermo-Neto, 2009). Oxidative stress has also been observed in rats after the administration of high doses of lambda-cyhalothrin (Aouey et al., 2017; Fetoui & Gdoura, 2012). However, the applicability of these results to the assessment of risk in humans exposed to doses that are much lower but repeated over time has not been established.

2.4 Established reference doses

To prevent the harmful effects of acute and chronic ingestion of certain pyrethroids in the general population, the United States Environmental Protection Agency has established reference doses (RfD) for ingestion on the basis of animal studies. Acute RfDs of 0.0025 mg/kg bw and chronic ones of 0.001 mg/kg bw/day have been established for lambda-cyhalothrin on the basis of a study of chronic neurotoxicity in dogs exposed by the oral route (no-observed-effect level [NOEL] of 0.25 mg/kg bw and 0.1 mg/kg bw/day, respectively, divided by an uncertainty factor of 100) (United States Environmental Protection Agency [US EPA], 2004). The corresponding values for cypermethrin are 0.1 mg/kg bw and 0.06 mg/kg bw/day, whereas the chronic RfD for permethrin is 0.25 mg/kg bw/day (US EPA, 2008, 2009).

The European Food Safety Authority (EFSA, 2014) has published an acceptable daily intake (ADI) for lambda-cyhalothrin of 0.0025 mg/kg bw/day, based on a NOEL of 0.5 mg/kg bw/day in a multigenerational study of rats exposed to cyhalothrin, to which an uncertainty factor of 200 was applied (the standard factor of 100 for a chronic animal study and an additional factor of 2 for the conversion of cyhalothrin to lambda-cyhalothrin). A systemic acceptable operator exposure level (AOEL) of 0.00063 mg/kg bw/day was established on the basis of this same NOEL and considering a limited absorption fraction of 0.25 (EFSA, 2014). This AOEL corresponds to a maximum internal dose (absorbed) that must not be exceeded to prevent harmful long-term effects in operator workers, who are repeatedly exposed and involved in activities related to

pesticide application (mixing and loading of the product in machinery, operation of machinery, including repairs and cleaning after use) (EFSA, 2006). According to our previous study done with workers exposed to cypermethrin and permethrin, but potentially also to lambda-cyhalothrin (Bouchard, Ratelle, & Côté, 2016; Ferland, Côté, Ratelle, Thuot, & Bouchard, 2015; Ratelle, Côté, & Bouchard, 2016), the reconstructed absorbed doses reached 2.4 µg/kg bw/day. This maximum value exceeds the AOEL of 0.63 µg/kg bw/day for lambda-cyhalothrin. It is therefore possible that workers may be exposed to levels of lambda-cyhalothrin that exceed this limit.

2.5 Multiroute exposure to pyrethroids in farmworkers

Farmworkers may be exposed to pyrethroids, such as lambda-cyhalothrin, during application in fields or due to a worker's presence in a treated area, following the prescribed re-entry interval of 24 hours for the insecticide formulations Matador® and Warrior® (Syngenta, 2014).

According to several studies carried out in agricultural settings, respiratory exposure may occur during spraying, when protective equipment is not properly used. Exposure due to skin contact with treated crops or contaminated equipment has also been reported (Vermeulen, Stewart, & Kromhout, 2002). However, recent data provided by our research team on biological monitoring of workers' exposure to permethrin and cypermethrin suggest that inadvertent ingestion during the workday could explain the majority of the temporal urinary metabolite profiles observed after an application episode (Bouchard et al., 2016; Côté & Bouchard, 2018).

2.6 Measurement of biomarkers of exposure and knowledge of toxicokinetics for assessment of absorbed doses

The measurement of metabolites of pyrethroids in the urine is considered to be a gold-standard approach for assessing exposure to these pesticides (Bouchard et al., 2016; Canadian Health Measures Survey [CHMS], 2013; National Health and Nutrition Examination Survey [NHANES], 2017). Nevertheless, in order to properly interpret the significance of a measurement of these biomarkers of exposure and establish the best sampling strategies to track exposure, it is necessary to clearly understand their toxicokinetic behaviour in the body, that is to say, the speed with which they are absorbed, distributed, metabolized and excreted. This step is crucial in order to be able to determine the link between levels of biomarkers of exposure measured in the urine of exposed workers and the doses they have actually absorbed.

To help determine this link, biomathematical models have been developed to simulate the fate of the compounds in question and their biomarkers in the body. In these models, the body is represented in the form of compartments; each compartment represents a tissue or set of tissues. The variation in the amounts of the product and its metabolites in each compartment is then determined by the difference between the incoming and outgoing amounts per unit of time. These models simulate different exposure scenarios and different absorption routes for workers. Thus, they can be used to reconstruct the doses of a product absorbed on the basis of the biomarkers measured in matrices such as urine, for different exposure scenarios. To develop a robust toxicokinetic model, it is important to obtain reliable kinetic data under controlled conditions in which the exposure doses are known and the critical biological determinants of the biological levels observed (including intra- and inter-individual variability) can be accurately defined.

Recently the toxicokinetics of two pyrethroid pesticides, permethrin and cypermethrin, was analyzed in workers in controlled and actual exposure conditions (Bouchard et al., 2016; Ferland et al., 2015; Ratelle, Côté, & Bouchard 2015a, 2015b; Ratelle et al., 2016). More specifically, in that study, the temporal profiles of the biomarkers of exposure to permethrin and cypermethrin were established in the plasma and urine of volunteers exposed orally and in the urine of workers exposed in vegetable and sweet corn farming. The data acquired from the volunteers were then used to refine a toxicokinetic model of permethrin and cypermethrin and their metabolites, in order to reconstruct the doses absorbed by workers based on the urine profiles of biomarkers of exposure. However, kinetic data on other pyrethroids, such as lambda-cyhalothrin, remain very incomplete. At present, this limits the use of biological monitoring to assess exposure to this extensively used pyrethroid.

The kinetic profiles of the metabolites of permethrin and cypermethrin studied in the plasma and urine of volunteers exposed in controlled conditions and the subsequent toxicokinetic modelling confirmed that the kinetic parameters were similar for permethrin and cypermethrin. Thus, a single model could be used to predict the temporal profiles of both permethrin and cypermethrin and their metabolites following different routes of exposure (oral, dermal or respiratory) and different temporal exposure scenarios, one-time or repeated, continuous or intermittent (Bouchard et al., 2016; Côté & Bouchard, 2018; Ratelle et al., 2015a, 2015b). *A priori,* it seems that the model could be adapted to simulate the kinetics of other pyrethroids and their metabolites, such as lambda-cyhalothrin, and thus act as a generic tool to reconstruct the doses absorbed and predict the main routes of exposure.

2.7 Available data on the kinetics of lambda-cyhalothrin and its metabolites

Before this study, data on the kinetic behaviour of lambda-cyhalothrin in humans were all but nonexistent. The available data were based essentially on animal experiments. Anadon, Martinez, Martinez, Diaz, and Martinez-Larranaga (2006) were among the few authors to publish data on the kinetic behaviour of lambda-cyhalothrin; they were limited to rats and the fate of the parent compound in the body after the administration of high doses intravenously and orally (3 and 20 mg/kg bw, respectively). It is well known that there are differences in the kinetic behaviour of pyrethroids in animals and humans. Moreover, several researchers have stated that toxicokinetic data should be gathered from human studies (Brzak, 2000; Krieger & Thongsinthusak, 1993; Woollen, Marsh, Laird, & Lesser, 1992).

Nevertheless, according to the available studies in rats and data from our laboratory, lambda-cyhalothrin is rapidly broken down in the body by carboxylesterases and cytochromes P450, generating several metabolites (Figure 2). The metabolites are excreted in the urine and feces a few days after exposure; the plasma elimination half-life of the parent compound has been established at 7.5 and 10 hours following intravenous injection and oral administration, respectively, in rats (Anadon et al., 2006; Food and Agriculture Organization [FAO], 2003; World Health Organization [WHO], 2013). Four metabolites were identified as major metabolites in the urine, in these animal studies (Table 1). One of these major metabolites, which is easily detected in urine, is 3-phenoxybenzoic acid (3-PBA) (CAS Number 3739-38-6). This metabolite is common to other pyrethroids, including permethrin, cypermethrin and deltamethrin. Our research team recently quantified *cis*-3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethylcyclopropanecarboxylic acid (CFMP) (CAS Number: 72748-35-7) as a major metabolite in the blood, tissues and excreta of rats exposed to lambda-cyhalothrin orally (Aouey et al., 2017).

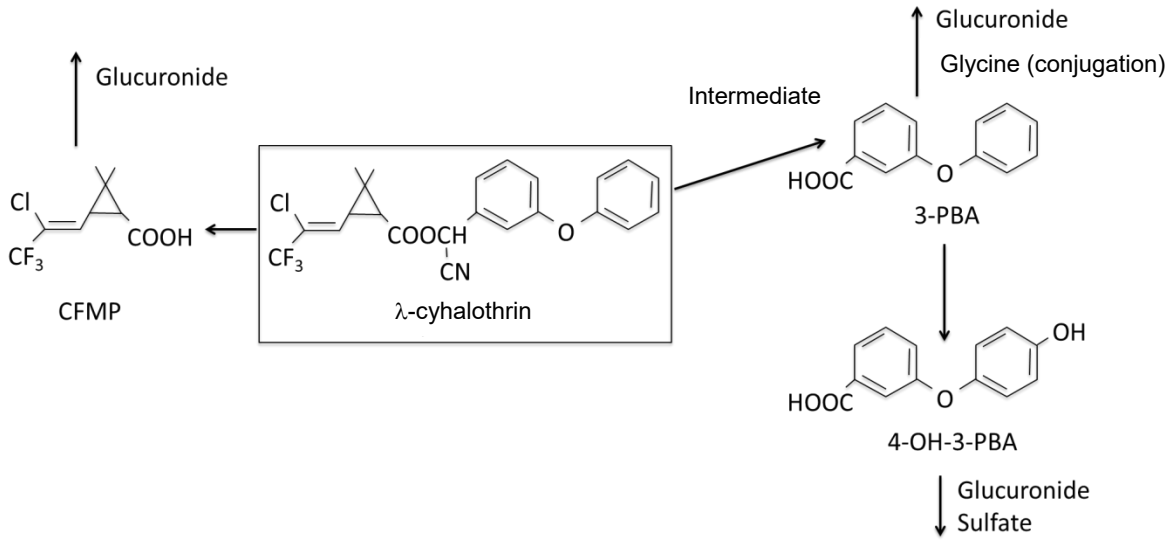
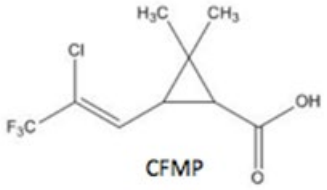
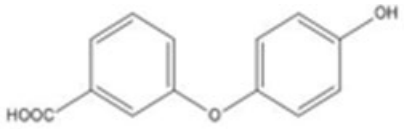
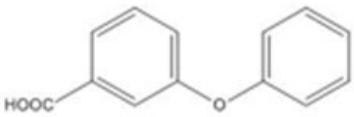


Figure 2. Metabolism of lambda-cyhalothrin.

Table 1. Major metabolites of lambda-cyhalothrin identified from rat studies

Cyclopropane carboxylic fragment	Phenoxybenzoic fragment
(Z)-3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropane carboxylic acid (CFMP or CF_3CA) (CAS Number 72748-35-7)  CFMP	3-(4'-hydroxy)-phenoxybenzoic acid (4-OH-3-PBA) (CAS Number 35065-12-4)  -4-OH-3-PBA 3-phenoxybenzoic acid (3-PBA) (CAS Number 3739-38-6)  3-PBA

3-PBA is easily measurable in workers' urine (Bouchard et al., 2016; CHMS, 2013; Couture et al., 2009; Fortin, Bouchard, Carrier, & Dumas, 2008; NHANES, 2009). However, to the best of our knowledge, there is no published study that measured CFMP in workers, although it appears to be a major and specific metabolite of lambda-cyhalothrin. 3-(4'-hydroxy)-phenoxybenzoic acid (4-OH-3-PBA) has been identified as a major metabolite in rats as well (Aouey et al., 2017), but it has not often been measured in human studies.

To sum up, in order to better assess exposure and the risks associated with the growing use of lambda-cyhalothrin as a pyrethroid insecticide in farming, it is clear that the kinetic behaviour of its biomarkers of exposure must be analyzed. The biomathematical tool developed by our research team can then be adapted to link measurements of biomarkers of exposure to the doses that individuals have actually absorbed.

3. RESEARCH OBJECTIVES

In the context of a previous study, biomonitoring tools for exposure to permethrin and cypermethrin were developed and validated by our team to assess exposure to pyrethroid pesticides (Bouchard et al., 2016). Field studies done in this context on farmworkers revealed that farmers were tending to make increasing use of another pyrethroid insecticide, lambda-cyhalothrin, which had not yet been evaluated much (CAS Number 91465-08-6). The data recently obtained by our team suggest that there are similarities in the kinetic behaviour of different pyrethroids. It has therefore become necessary to do an in-depth analysis of the toxicokinetic behaviour of this pyrethroid, lambda-cyhalothrin, to determine, among other things, whether the biological monitoring of metabolites common to several pyrethroids would provide an overall indication of exposure.

Based on the approach applied in the last project, the general objective of this research project was to fill the gap in knowledge of the toxicokinetics of the biomarkers of exposure to lambda-cyhalothrin in humans in order to better interpret biomonitoring data and better assess the risks for exposed workers.

The toxicokinetic approach developed in the previous project (Bouchard et al., 2016) was therefore applied to this new pyrethroid as follows:

1) assessment of the toxicokinetics of lambda-cyhalothrin in controlled conditions in volunteers;
2) use of toxicokinetic modelling to:
    i) establish the essential determinants of the biological behaviour of lambda-cyhalothrin and its metabolites;
    ii) provide a tool to reconstruct doses absorbed on the basis of biomarker measurements.

4. METHODOLOGY

The project was subdivided into two complementary parts:

1) A controlled kinetic study (under medical supervision) in volunteers subjected to acute exposure to a low dose of lambda-cyhalothrin.

   This first phase of the project consisted in characterizing the kinetics of the biomarkers of exposure to lambda-cyhalothrin in volunteers exposed in controlled conditions.

2) The establishment of a toxicokinetic model for lambda-cyhalothrin that can be used as a tool to reconstruct the doses absorbed by workers on the basis of measurements of biomarkers in the accessible biological matrices.

   This second phase of the project consisted in using the kinetic data obtained with volunteers in the first part to develop a toxicokinetic model making it possible to relate the doses of lambda-cyhalothrin absorbed in humans with the biomarkers of exposure observed in accessible biological matrices such as urine.

4.1 Controlled kinetic study

4.1.1 Design of the study

As in our study of permethrin and cypermethrin (Bouchard et al., 2016; Ratelle et al., 2015a, 2015b), a controlled study in volunteers was done to determine the kinetic profile of the biomarkers of exposure to lambda-cyhalothrin in humans. These data were then used to develop a biomathematical model of the toxicokinetics of the multiple metabolites of lambda-cyhalothrin.

4.1.2 Participants

The participants in the controlled kinetic study were recruited on a volunteer basis from students at Université de Montréal and their families and friends. Five men and three women were recruited. The volunteers were aged between 20 and 41 years, weighed between 53 and 93 kg (mean of 70 kg) and were between 154 and 186 cm tall. All were non-smokers in good health and were not taking any medications. A check was done to make sure that the participants had not been exposed to pyrethroids during the month before the first experimental administration, except perhaps in food. In addition, during the three days before each administration, the volunteers were not allowed to eat fruit, vegetables or nuts; they were given organic bread, cereals, milk and jam to limit the ingestion of contaminated food, since it is known that food is generally the main route for pesticide absorption in the general population (Schettgen, Heudorf, Drexler, & Angerer, 2002). During the biological monitoring of exposure, meals and snacks prepared with certified organic ingredients were also given to each participant. They were also asked to avoid consuming tea, tisane, alcohol or medications during this period, as the latter two items can affect the metabolism of certain chemical compounds by interfering with enzyme activity (Choi, Rose, & Hodgson, 2002; Gueguen et al., 2006).

4.1.3 Administration and biological sampling

The volunteers were exposed to both a single oral dose of lambda-cyhalothrin (purity <100%, purchased from Supelco, Sigma-Aldrich, Oakville, ON, Canada) and a dermal dose of the formulation of Matador® containing lambda-cyhalothrin (purity of 120 g/l, purchased from Synagri-Syngenta, Saint-Hyacinthe, QC, Canada). A three-week period was respected between each series of exposures in order to ensure the complete elimination of the product from the body between two exposures.

For oral exposure, a pre-test was initially done on a volunteer who received a dose of lambda-cyhalothrin equal to the acute oral reference dose (RfD) of 0.0025 mg/kg bw, established by the US EPA (US EPA, 2004) (corresponds to 0.175 mg for an individual weighing 70 kg). According to a recent evaluation by the US EPA, this dose is considered to have no harmful effects in acute and chronic exposure scenarios in the general population, including children (US EPA, 2004). This pre-test was intended to verify whether the levels of metabolites were sufficiently high to establish a kinetic profile in plasma and urine. Since the levels were almost non-quantifiable most of the time in plasma, this dosage could not clearly establish the plasma kinetics. The dose was therefore increased to 0.025 mg/kg bw for the main oral kinetic study. Seven other volunteers took part in this main oral kinetic study. The compound administered orally was dissolved in organic olive oil (1 mg of product in 2 ml of oil for the dose of 0.0025 mg/kg bw and 10 mg of product in 2 ml for oil for the dose of 0.025 mg/kg bw). The volunteers were then asked to drink 100 ml of water. The tip used for administration was rinsed with pure oil and the oil was then administered to participants, with another 100 ml of water.

For the dermal exposure, six of the seven volunteers exposed to 0.025 mg/kg bw orally were then exposed to the formulation of Matador®, at a dose corresponding to 0.25 mg/kg bw of lambda-cyhalothrin. The product was applied on 40 cm^2 on one forearm and left for 6 hours. A cardboard frame 20 x 2 cm was attached to each volunteer's forearm to delimit the application area. The dose was applied with a plastic pipette. The treated area was left uncovered and washed with soap and water 6 hours after application. Thus, this kind of application is similar to that of the exposed workers.

As was done for permethrin and cypermethrin, a series of 30 ml (3 tubes x 10 ml) blood samples were taken by venipuncture from the arm before exposure (t = 0, 30 minutes before administration of the pesticide) and at set times over a 72-hour period after administration, namely 0.5, 1, 1.5, 2, 4, 6, 8, 10, 24, 48, and 72 hours after administration (n = 12 samples per individual per exposure) to study the kinetics of metabolites in blood. To facilitate blood sampling, a catheter was inserted for the whole day after exposure, then sampling was done with venipuncture over the following days. Immediately after each sample was taken, the blood samples were centrifuged to precipitate the red cells and isolate the plasma. The plasma samples were then divided into aliquots, carefully labelled and stored at -20 °C until the analysis was done.

To study the urinary kinetics of the metabolites, complete urine samples were also collected in separate, clearly identified pots at set periods, namely -3–0 hours before exposure and 0–3, 3–6, 6–9, 9–12, 12–24, 24–36, 36–48, 48–60, 60–72, and 72–84 hours post-administration (n = 11 collected per individual per exposure). All the urine produced during a given period was collected in the same container, namely a 500 ml Nalgene® polypropylene bottle, already identified beforehand. The specimens were then coded to respect participants' anonymity, and the date and time of sampling were noted on the containers. Once the urine samples were collected, they

were kept in the refrigerator before the total urine volume was measured. To avoid repeated freezing and thawing, each sample was then divided into aliquots, in 3 labelled 15 ml tubes and one 120 ml container, before storage at -20 °C until the analysis was done.

During the first 12 hours following the administration of the dose, the volunteers were confined to a room in the Department of Environmental and Occupational Health at the School of Public Health at Université de Montréal. During the following four days of sampling, participants had to visit the department each morning to undergo blood sampling and bring their urine samples. The volunteers were also asked to complete a questionnaire to provide: (1) personal information (weight, height); (2) lifestyle information, such as their (i) physical activity; (ii) occupational or accidental exposure while handling products (use of shampoo, conditioner, lotions, or creams that might have contained pesticides to deal with pests or the use of products containing pesticides for pets to protect them against fleas, mange, etc.) during the 3 days preceding the start of the study; (iii) medications taken; (iv) alcohol consumption; (v) tobacco consumption; (vi) consumption of non-organic food during this period; and (3) any symptoms potentially related to the pesticide administration.

4.1.4 Processing of samples and laboratory analyses

The samples of plasma and urine were processed with a method previously developed in the research team's laboratory to measure the metabolites of permethrin and cypermethrin, but with certain adjustments to measure the metabolites of lambda-cyhalothrin and to take account of the fact that the dose administered was lower than in the previous study (Ratelle et al., 2015a, 2015b).

To analyze the metabolites in the various samples from the exposed volunteers, 2 ml of plasma was processed. Each sample was fortified by adding 5 µl of a mix of labelled internal standards, $^{13}C_2$ ^{1}D *trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid ($^{13}C_2$ 1D *trans*-DCCA) and $^{13}C_6$-3-PBA, at a concentration of 20 nmol/ml (Cambridge Isotope Laboratories, Inc., Andover, MA, USA). There is no labelled standard of CFMP since it is a rarely measured molecule; $^{13}C_2$ 1D *trans*-DCCA was therefore used as an internal standard for CFMP. Two millilitres of sodium acetate buffer at 0.1 M, pH 5, was added and the samples were incubated for 16 hours with 20 µl of β-glucuronidase/arylsulfatase (100,000 U/ml of Fishman and 800 U/ml of *Helix pomatia*; Roche Diagnostics, Laval, QC, Canada) at 37 °C in a shaking water bath to obtain the sum of free and conjugated metabolites. Liquid-liquid extraction was then done twice with 4 ml of ethyl acetate (Fisher Scientific, Ottawa, ON, Canada) saturated in water (shaking for 20 minutes and centrifuging at 3,400 rpm for 15 minutes at 4 °C). The upper organic phase recovered after each extraction was combined in a 10 ml glass tube and the solvent was evaporated to dryness under a light nitrogen stream in a bath at 35 °C. The residues were resuspended in 500 µl of MS grade methanol from Honeywell, and the samples were centrifuged for 60 seconds at 3,000 rpm, then transferred into HPLC vials for analysis.

To analyze the metabolites of pyrethroids in the various samples from the exposed volunteers, 5 ml of urine was processed. Each sample was fortified by adding 10 µl of a mix of internal standards of $^{13}C_2$ ^{1}D *trans*-DCCA and $^{13}C_6$-3-PBA, at a concentration of 20 nmol/ml. Five millilitres of sodium acetate buffer (0.1 M, pH 5) was added and enzymatic hydrolysis was done with 20 µl of β-glucuronidase/arylsulfatase for 16 hours at 37 °C. The analytes were then extracted in solid phase with Sep-Pak C18 cartridges (Waters, Milford, MA, USA). The cartridges were first conditioned with 4 ml of methanol followed by 8 ml of water. The urine was then passed through the column. The cartridges were washed with 8 ml of water. The analytes were then eluted in the

column with 8 ml of methanol, in 10 ml glass tubes. The solvent was evaporated to dryness under a nitrogen stream in a bath at 35 °C. The residues were resuspended in 1,000 µl of methanol, and the samples were centrifuged for 60 seconds at 3,000 rpm, then transferred into HPLC vials for analysis.

The analysis of CFMP and 3-PBA in the plasma and urine was done using an ultra-high-performance liquid chromatography (UHPLC) system from Agilent, Model LC-1290, equipped with a binary UHPLC gradient pump (Agilent, Mississauga, ON, Canada), an autosampler and a thermostatted column compartment (Agilent, Mississauga, ON, Canada), and coupled with a quadrupole time-of-flight (Q-ToF) mass spectrometer (MS), model 6530 (Agilent, Mississauga, ON, Canada), with a jet stream technology ion source (AJS ESI). The ionization was done in negative ion mode and all the specific ESI-MS parameters were manually optimized. The compounds were separated using a C18 ZORBAX Eclipse Plus column (2.1 x 50 mm; 1.8 µm, HD) from Agilent (Mississauga, ON, Canada). The column temperature was maintained at 40 °C. The mobile phase consisted in an eluent A composed of water and 0.01% acetic acid and an eluent B of methanol with 0.01% acetic acid. The elution was done in 11 minutes using a solvent gradient, at a flow of 0.4 ml/minute. The following program was used: (i) 2% of eluent B for 2 minutes, (ii) linear gradient up to 30% of eluent B from 2 to 2.5 minutes, (iii) maintained at 30% of eluent B from 2.5 to 3.5 minutes, (iv) increased to 55% of eluent B from 3.5 to 4 minutes, (v) to 60% of eluent B from 4 to 7.5 minutes, (vi) linear gradient to 98% of eluent B from 7.5 to 8 minutes, (vii) maintained at 98% of eluent B from 8 to 9.5 minutes, (viii) return to the initial conditions of 2% of eluent B in 1.5 minutes. The retention time (elution) of the different analytical compounds ranged from 7.2 to 8.1 minutes. The solvent was removed to waste before 4 minutes, to eliminate salts and prevent ion suppression in the mass spectrometer by the salts contained in the matrices; it was then directed to the mass spectrometer after 4 minutes. The samples were kept at 5 °C on the injection tray and 5 µl was injected.

The exact masses of the analytes were determined in the following optimized MS conditions (parameters as defined by the instrument): sheath gas (N_2) temperature: 200 °C; sheath gas flow rate: 10 l/minute; nebulizer gas pressure: 50 psi; drying gas (N_2) temperature: 365 °C; drying gas flow rate: 12 l/minute; capillary voltage (Vcap): 3,000 V; nozzle voltage: 0 V; fragmentor: 75 V; skimmer: 65 V; octopole: 750 V. The precursor ions $[M-H]^-$ analyzed were m/z 241.02487 for CFMP, m/z 213.05572 for 3-PBA, m/z 212.00955 for $^{13}C_2$ 1D *trans*-DCCA and m/z 219.07585 for $^{13}C_6$-3-PBA. Identification and quantification were done using Extracted Ion Chromatogram (EIC) mode. Quantification was based on calibration curves in the urine or the plasma. Response signals were established based on the surface ratio for the precursor ions of each analyte over that of the internal standard.

Quality control was ensured by inserting positive and negative controls into the analysis sequence at regular intervals. Analytical repeatability was also evaluated by determining the variation in the results of the analysis of replicates of the urine and plasma samples in the same calibration and setting conditions (blanks fortified at two levels of concentration with the reference standards). The limit of detection (LOD) in plasma and urine was approximately 10 fmol injected into the UHPLC system (equal to 3 standard deviations of the response ratio of the repeated analysis of a blank divided by the mean slope of the standard curves). The repeatability of the analysis of samples in the same calibration and setting conditions (blanks fortified by authentic reference standards at two levels or positive controls) was lower than 5%. The reproducibility between the analysis series (variability from one day to another of the results of the analysis of blank samples

fortified with authentic reference standards at two levels and one positive control) on five different days was lower than 15%.

4.1.5 Determination of biological levels

Plasma levels were expressed as a concentration (pmol/ml/kg bw). Urine levels were expressed as an excretion rate (pmol/h/kg bw) and cumulative excretion (% dose). For purposes of comparison, urine levels were also expressed as a concentration, adjusted for creatinine (µmol/mol creatinine), as recommended by Viau, Lafontaine, and Payan (2004). Creatinine in the urine was measured with Jaffe's method, which uses alkaline picrate with deproteinization (enzymatic colorimetric Creatinine PAP test from Boehringer Mannheim, Germany).

4.1.6 Determination of toxicokinetic parameters

As the first step in the analysis, the baseline toxicokinetic parameters were determined using the kinetic profiles of the metabolites of pyrethroids in plasma and urine after oral exposure. To do this, it was considered that, following administration, a dynamic equilibrium was quickly reached between tissue and plasma levels of metabolites (the plasma and tissue levels soon started evolving in parallel) and thus that the body could be represented using a one-compartment model with first-order elimination (Renwick, 2008; Tornero-Velez et al., 2012). Based on the kinetics of other pyrethroids, it was also considered that the metabolism of lambda-cyhalothrin into CFMP and 3-PBA is almost instantaneous (on the order of minutes compared with hours for elimination). The baseline kinetic parameters were therefore derived by using the least squares method to fit the following general equation to the observed experimental data (using MATLAB software and a program developed in Visual Basic for Applications in Microsoft Excel): $C(t) = Ae^{-kabs.t} + Be^{-kelim.t}$, where C (t) is the plasma concentration as a function of time (or of urinary excretion rate), A and B are pre-exponential coefficients, and k_{abs} and k_{elim} are hybrid coefficients of speed for the absorption and elimination phases, respectively. The time needed to achieve the maximum concentration (T_{max}) and the apparent absorption ($\ln(2)/k_{abs}$) and elimination ($\ln(2)/k_{elim}$) half-lives were also determined.

Based on the temporal profiles of plasma concentrations (C), the area under the curve (AUC), area under the first moment curve (AUMC), mean residence time (MRT), plasma clearance (CL) and apparent volume of distribution (V_d) were also calculated. The equations used to calculate these parameters are as follows:

$$AUC = \frac{1}{2}\sum_{\forall i}(t_i - t_{i+1})\left[C(t_i) + C(t_{i+1})\right] \quad (1)$$

$$AUMC = \frac{1}{2}\sum_{\forall i}(t_i - t_{i+1})\left[t_i C(t_i) + t_{i+1} C(t_{i+1})\right] \quad (2)$$

$$MRT = \frac{AUMC}{AUC} \quad (3)$$

$$CL = \frac{\text{Dose absorbed (fraction of dose administered)}}{AUC} \quad (4)$$

$$Vd = \frac{CL}{k_{elim}} \quad (5)$$

4.1.7 Ethical considerations

The experimental protocol and the consent forms were approved by the Université de Montréal's research ethics committee (certificate 15-085-CERES-P). All participants gave their consent in writing and were informed of the risks related to participation and their right to withdraw from the study at any time, without prejudice. Subjects' anonymity was ensured by coding the samples. Participants received financial compensation for their time and any inconvenience caused.

In addition, the proposed exposure doses were selected to be safe and not to induce harmful systemic effects in volunteers. Clinical signs and symptoms were monitored by certified medical staff throughout the study. None of the volunteers reported any symptoms during the oral study period. On the other hand, two volunteers felt stinging sensations and noticed the appearance of redness after dermal application; these volunteers were men, the hair on whose forearms had been shaved the morning of exposure. No systemic symptoms (headache, numbness, abdominal pain) were reported.

4.2 Toxicokinetic modelling

The experimental data obtained from volunteers exposed to lambda-cyhalothrin during this study contributed greatly to our understanding of the kinetics of lambda-cyhalothrin in humans. Indeed, in the context of this project, we noted that there is very little information on the kinetics of lambda-cyhalothrin in the literature. This information is limited to the results of one animal study (Anadon et al., 2006) and one study in workers (Chester, Sabapathy, & Woollen, 1992). The International Programme on Chemical Safety (IPCS, 1990) also summarizes some animal studies, but those studies could not be consulted. Since only very limited data have been published on the kinetics of lambda-cyhalothrin, the available information on pyrethroids of the same type was also consulted. Thus, considering the kinetics of similar pyrethroids and the experimental data obtained during this study, it was possible to develop a conceptual model of the kinetics of lambda-cyhalothrin and the major metabolites that could be used as biomarkers of exposure.

Mathematically, the model specific to lambda-cyhalothrin reflects the essential determinants of the temporal evolution of the parent product and its metabolites in the human body and in the excreta. The critical biological processes taking place at key points in time were used to determine the overall dynamics of the system. The model makes it possible to link the absorbed dose, the blood concentration and the urinary measurement and thus to reconstruct the doses absorbed by workers based on the measurement of metabolites in accessible biological matrices, such as urine.

The structure of the model we developed is similar to that already used to describe the kinetics of permethrin and cypermethrin in humans (Bouchard et al., 2016; Côté & Bouchard, 2018). This kind of model has compartments to which biological significance was attributed and the emphasis was on monitoring mass balance, which means that the doses may be related to the total of the amounts in the body and in the excreta at all times. It has the purpose of predicting the temporal evolution of urinary biomarkers. Thus, only the key processes determining the kinetics of biomarker excretion need to be established. Moreover, since the model is used to interpret biological monitoring data on exposure in humans, the values of the model's parameters are established directly based on human data. This toxicokinetic modelling approach was also applied in the past to the following organophosphate and carbamate insecticides: malathion, parathion, chlorpyrifos and carbaryl (Bouchard, Carrier, & Brunet, 2008; Bouchard, Carrier, Brunet, Bonvalot, & Gosselin, 2005; Bouchard, Carrier, Brunet, Dumas, & Noisel, 2006; Bouchard et al., 2003; Gosselin, Bouchard, Brunet, Dumoulin, & Carrier, 2004).

4.2.1 Functional representation of the model

The representation of the model (description of the compartments and intercompartmental transfers) is illustrated in Figure 3. The parameters of the model are described in Table 2. The loads in the body and the excreta (accumulation in the urine of the parent product and its metabolites) are represented by compartments. The changes in these loads were described mathematically by a system of differential equations that ensures the conservation of mass at all time (Appendix A). Thus, the distribution of a substance and its biotransformation were represented by transfers from one compartment to another at rates proportional to the load in the source compartment. The rate of change in the amounts in each compartment (dXi(t)/dt) (on a molar basis) therefore corresponds to the difference between incoming and outgoing amounts per unit of time.

Specific compartments, D(t), GI(t), RT(t), were used to describe the amounts of lambda-cyhalothrin on the surface of the skin, in the lumen of the gastrointestinal tract and in the respiratory tract, respectively. The tissue loads of lambda-cyhalothrin, which quickly achieved equilibrium with the blood loads, were combined in a single compartment, B(t), given that the amounts evolved in parallel. A compartment for lambda-cyhalothrin storage, S(t), was also introduced into the model to describe the accumulation in lipids or tissue protein binding. The compartment S(t) was added to the model to take into consideration the biphasic elimination of metabolites of lambda-cyhalothrin in volunteers exposed orally. The compartment M(t) was used to represent the body burdens of the metabolites CFMP and 3-PBA. A compartment $M_{not\_observed}(t)$ was used to represent the body burden of metabolites not measured in the blood and urine. The compartment U(t) represented the cumulative excretion of CFMP and 3-PBA in the urine, and F(t) the cumulative fecal excretion of CFMP and 3-PBA. To take account of differences in the kinetics of the metabolites CFMP and 3-PBA in the matrices of volunteers with dermal exposure, a compartment $D_{in}(t)$ was added to represent the load of lambda-cyhalothrin absorbed by the skin structures. A compartment MD(t) was also added to represent the load of lambda-cyhalothrin metabolized into CFMP and 3-PBA inside the skin structures.

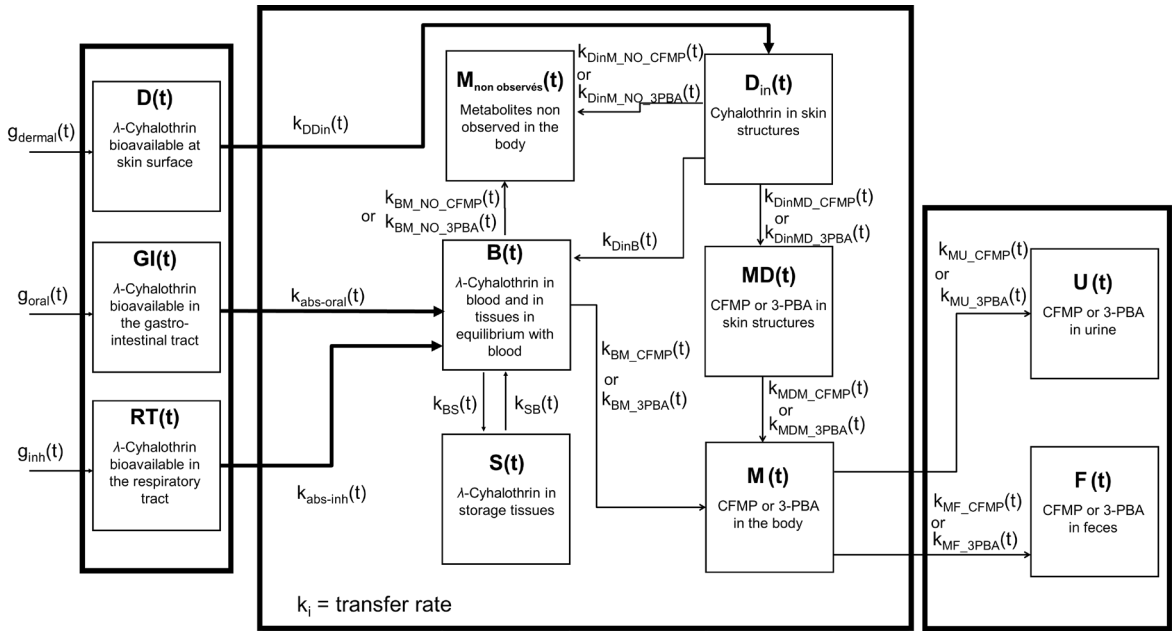


Figure 3. Conceptual model of the kinetics of lambda-cyhalothrin and its metabolites that can be used as biomarkers of exposure in humans.

This model also supposes that there is no saturation in the processes of metabolism and clearance. At the exposure doses modelled in the volunteers (Khemiri, Côté, Fetoui, & Bouchard, 2017, 2018), the kinetic profiles of CFMP and 3-PBA were accurately predicted without having to introduce saturation. In animals exposed to a very high dose of lambda-cyhalothrin intravenously (3 mg/kg bw) or orally (20 mg/kg bw), no saturation was apparent based on the temporal profile of the parent compound (Anadon et al., 2006). However, the model cannot be used to predict the kinetics at saturating exposure doses. The solution of the differential equations simulating the kinetics of the parent product and its metabolites in the body made it possible to generate mathematical functions ($X_i(t)$) describing the temporal profiles of these models in the different compartments.

Table 2. Description of the symbols used for the conceptual and functional representations of the kinetic model of lambda-cyhalothrin and its metabolites

Parameter	Definition
$g_{oral}(t)$	Available oral dose (mol) per unit of time that is able to describe temporal variations in inputs
$g_{dermal}(t)$	Available dermal dose (mol) per unit of time that is able to describe temporal variations in inputs
$g_{inh}(t)$	Available inhalation dose (mol) per unit of time that is able to describe temporal variations in inputs
D(t)	Amounts of lambda-cyhalothrin (mol) available on the surface of the skin as a function of time
$D_{in}(t)$	Amounts of lambda-cyhalothrin (mol) inside the skin structures as a function of time
GI(t)	Amounts of lambda-cyhalothrin (mol) available in the gastrointestinal tract as a function of time
RT(t)	Amounts of lambda-cyhalothrin (mol) available in the respiratory tract as a function of time
B(t)	Amounts of lambda-cyhalothrin (mol) in the blood and the tissues in dynamic equilibrium with the blood as a function of time
S(t)	Amounts of lambda-cyhalothrin (mol) retained in the tissues (mol) as a function of time
M(t)	Body burden of CFMP or 3-PBA (mol) as a function of time
MD(t)	Amounts of CFMP or 3-PBA (mol) inside the skin structures as a function of time
$M_{not\_observed}(t)$	Body burden of non-observed metabolites (mol) as a function of time
U(t)	Cumulative amounts of CFMP or 3-PBA in the urine (mol) as a function of time
QU(t)	Rate of excretion of CFMP or 3-PBA in the urine (mol) as a function of time = $M(t) \times k_{MU}$
F(t)	Cumulative amounts of CFMP or 3-PBA in the feces (mol) as a function of time
f_{abs_oral}	Oral absorption fraction of lambda-cyhalothrin
f_{abs_dermal}	Dermal absorption fraction of lambda-cyhalothrin
k_{abs_oral}	Oral absorption rate of lambda-cyhalothrin (h^{-1})
k_{DDin}	Dermal absorption rate of lambda-cyhalothrin by the internal skin structures (h^{-1})
k_{abs_inh}	Respiratory absorption rate of lambda-cyhalothrin (h^{-1})
k_{DinB}	Rate of transfer of lambda-cyhalothrin from the internal skin structures to the blood (h^{-1})
k_{BS}	Rate of transfer of lambda-cyhalothrin from the blood to the storage tissues (h^{-1})

Parameter	Definition
k_{SB}	Rate of transfer of lambda-cyhalothrin from the storage tissues to the blood (h^{-1})
k_{BM_CFMP}	Rate of biotransformation of lambda-cyhalothrin into CFMP (h^{-1})
k_{BM_3PBA}	Rate of biotransformation of lambda-cyhalothrin into 3-PBA (h^{-1})
k_{DinMD_CFMP}	Rate of biotransformation of lambda-cyhalothrin into CFMP in the internal skin structures (h^{-1})
k_{DinMD_3PBA}	Rate of biotransformation of lambda-cyhalothrin into 3-PBA in the internal skin structures (h^{-1})
k_{MDM_CFMP}	Rate of transfer of CFMP from the internal skin structures to the body (h^{-1})
k_{MDM_3PBA}	Rate of transfer of 3-PBA from the internal skin structures to the body (h^{-1})
$k_{BM\_NO\_CFMP}$ and $k_{DinM\_NO\_CFMP}$	Rate of biotransformation of lambda-cyhalothrin into non-observed metabolites derived from the CFMP form (h^{-1})
$k_{BM\_NO\_3PBA}$ and $k_{DinM\_NO\_3PBA}$	Rate of biotransformation of lambda-cyhalothrin into non-observed metabolites derived from the phenoxybenzoic form (h^{-1})
k_{MU_CFMP}	Rate of transfer of CFMP from the body to urine (h^{-1})
k_{MU_3PBA}	Rate of transfer of 3-PBA from the body to urine (h^{-1})
k_{MF_CFMP}	Rate of transfer of CFMP from the body to feces (h^{-1})
k_{MF_3PBA}	Rate of transfer of 3-PBA from the body to feces (h^{-1})

4.2.2 Determination of the model's parameters

A mathematical program was created to determine the parameters of the model specific to lambda-cyhalothrin and its metabolites, using MATLAB (version R2017a). A value determination sequence was programmed in successive iterations, fitting the mathematical functions (by minimization with the least squares method) to the temporal profiles established in this project with volunteers exposed to lambda-cyhalothrin. The approach to determining parameters is similar to that described in Côté et al. (2014) and Côté and Bouchard (2018) to establish the parameters specific to permethrin and cypermethrin. However, since lambda-cyhalothrin does not have a stereoisomer, the computer programming used to determine the parameters proved to be less complex, which reduced the computation time.

In view of this simplification, other parameters could be verified. Indeed, in Côté et al. (2014) and Côté and Bouchard (2018), the metabolism rate constant for permethrin or cypermethrin into 3-PBA in the blood and tissues in equilibrium with the blood was considered identical to the metabolism rate constant for permethrin or cypermethrin into *trans*- and *cis*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid (*trans*- and *cis*-DCCA). In fact, in that model, it was impossible to determine a metabolism rate constant other than the one for permethrin or cypermethrin into *cis*- or *trans*-DCCA. In the model specific to lambda-cyhalothrin, it was possible to determine a different metabolism rate constant for the parent product metabolized into each of its metabolites, CFMP and 3-PBA. This differentiation is plausible since the metabolite CFMP is produced immediately following the scission of the parent product while the formation of 3-PBA

requires an additional step following the scission of the parent molecule (Kaneko & Miyamoto, 2001). In addition, in the previous studies conducted by our team, the value of the oral absorption fraction was set at 0.8. In this project, the value of the oral absorption fraction could be investigated.

More specifically, to determine the parameters of the model specific to lambda-cyhalothrin and its metabolites, the computerized routine developed in the studies by Côté et al. (2014) and Côté and Bouchard (2018) was adapted. The analytical equations representing the evolution of the metabolites CFMP and 3-PBA in blood and urine as a function of time were used, and then the computerized routine derived the best set of parametric values by fitting the simulation points to the experimental data, using minimization with the least square fit method. The parameters were determined by executing a series of inner iterations, which made it possible to systematically test all the possible sets of parametric values. For each parameter, all the possible values within a given interval were tested (unlike a Monte Carlo simulation, where the values are selected randomly within an interval according to a distribution). For some parameters, an interval of values was set at between 1 and 10,000 minutes of half-life (the highest value was a possible upper limit). The routine then tested each minute of half-life in this interval. The value for the parameter sought was therefore located within this interval. However, it was impossible to set this interval for every parameter, since the computation time would have been too long. For other parameters, the intervals of parametric values were set taking physiological restrictions (related to the conceptual model or the metabolite profiles) into consideration. For example, a first estimate of the value of the parameter k_{SB} (transfer of the parent compound from the storage compartment to the blood) was made based on the slope of the last time points of the urinary profile of metabolites on a log-linear graph. However, since all the parameters can have a general impact on the simulation profiles, lower and upper limits around the value were then estimated in order to include all the possibilities associated with this parameter.

For the oral absorption fraction specifically, the interval of values was determined logically. The maximum value of the interval was set at 100%, but the minimum value could not be lower than the smallest value (as a % of the exposure dose) of the metabolite found in the urine. For example, if there was 20% of CFMP in the urine, the interval could range from 0.2 to 1. For reasons of computation time, the oral absorption fraction was increased in increments of 0.05. The dermal absorption fraction was determined in a different way. The compartment D(t) corresponds to lambda-cyhalothrin available on the surface of the skin. It penetrates to the interior of the skin at a speed associated with the constant k_{DDin}. The surface of the skin was cleaned 6 hours after application in volunteers. Thus, the amounts that penetrated the skin could be determined by reducing the model to two compartments: one compartment associated with the surface of the skin and one compartment associated with the interior of the skin. By solving the differential equations, it is possible to find out how much has penetrated in 6 hours. This amount corresponds to the dermal absorption fraction.

For the respiratory route, no study had been published on the kinetics of lambda-cyhalothrin and its metabolites. However, Leng et al. (1997) did a study of volunteers exposed to cyfluthrin by inhalation. That study revealed a rapid apparent elimination half-life (Leng et al., 1997), on the same order of magnitude as was observed in studies of volunteers exposed orally to cypermethrin, permethrin and lambda-cyhalothrin (Khemiri et al., 2017, 2018; Ratelle et al., 2015a, 2015b; Woollen et al., 1992). The kinetics of the respiratory route was therefore considered to be similar to the oral route. Thus, to simulate exposure by the respiratory route, the

mean pulmonary ventilation rate is used and it is considered that absorption is very fast (minutes of half-life) with a high absorption fraction (≥ 80%).

With the mathematical routine used, several sets of parametric values were determined by fitting the data of each of the volunteers exposed to lambda-cyhalothrin and to the mean profiles. These sets of values all provide good fits for the experimental curves. However, ultimately, a single set of parametric values was retained to describe each of the available profiles (one for each volunteer), along with a profile representing the mean for volunteers. The set of parametric values retained corresponds to the one that showed the least variance between the values for the different volunteers' parameters and that gave the best fit.

More specifically, the parameters were first determined based on data from the seven volunteers exposed orally and 8 sets of parametric values were retained to simulate the available data for this exposure route (seven sets of values from the fitting to the individual profiles and an eighth from the fitting to the mean profile). The parameters related to dermal absorption were then estimated using data from four of the six volunteers exposed to Matador® dermally. The values of the data for the other two volunteers were too low to allow modelling. Similarly, for the four volunteers used to determine the specific parameters for dermal exposure, the data obtained for the initial times were also eliminated. To determine the specific parameters for the dermal route, the sets of parametric values obtained by fitting to the individual profiles and the mean profiles in volunteers exposed orally were first set in the model and only the parameters specific to dermal absorption were then determined. As with the determination of parameters for the oral data, a set of specific parametric values for the dermal route that provided the best fit was retained to describe each individual profile (one for each volunteer) and the mean profile for the dermal route.

4.2.3 Simulations

4.2.3.1 Simulation of data with volunteers

Once the parameters of the model were established based on data observed in volunteers exposed to lambda-cyhalothrin orally and dermally, the system of differential equations describing the complete functional model of the kinetics of the parent product and its metabolites was solved in MATLAB with the final parameters retained, to reproduce the temporal profiles of the metabolites CFMP and 3-PBA in the blood and urine for a simulation corresponding to the volunteers' oral and dermal exposure. The simulated profiles were then represented graphically and compared to the data observed in the volunteers.

4.2.3.2 Prediction of temporal profiles following different exposure scenarios

The model allows us to predict the temporal profile of lambda-cyhalothrin and its metabolites CFMP and 3-PBA in the body and the excreta at any point in time and for different exposure scenarios. Simulations of one-time or repeated, continuous or intermittent exposure scenarios can be done by introducing a term g(t) to describe exposure doses over time.

Several exposure scenarios, for a single exposure route (oral, dermal or respiratory) or for concomitant exposure by different routes and for different periods, can be simulated. For example, three different simulations were done to reproduce a plausible exposure scenario for a farmworker doing a weeding task in the fields for a typical workweek of five days followed by two days off. For each workday, the simulation reproduced a scenario of seven hours of work in the fields, followed

by a one-hour break to eat, then another seven-hour work period. The simulation considered that the task was repeated for five consecutive days, followed by two days off.

The first simulation reproduced a worker's exposure (i) dermally for the first seven hours of work in the fields, (ii) by extemporaneous ingestion, inadvertently, during the meal break, reflecting "hand-to-mouth behaviour" (contact between contaminated hands and the mouth), and (iii) dermally again for several hours after the return to the fields. Thus, this simulation considered continuous dermal exposure over two periods within a workday, plus extemporaneous oral exposure once during a workday. For the lunch break, the reconstruction presupposed that the worker washed the exposed skin area, which removed almost all of the product on the skin (approximately 99.5%); thus, for oral exposure, the scenario considered a hypothesis whereby the worker ingested a dose corresponding to 0.5% of the amounts accumulated on the skin during the 7-hour period following the start of the shift. This simulation also considered that the exposed skin area was washed after work, removing all the remaining product from the skin. Moreover, the first simulation considered a total daily dose of 1 µmol/kg bw/day; dermal exposure corresponding to 0.9975 µmol/kg bw/day (0.49875 µmol/kg bw for each seven-hour exposure period) and oral exposure to 0.0025 µmol/kg bw/day. The dermal dose was divided equally over the two seven-hour periods.

The second simulation presupposed only dermal exposure, spread over an exposure period of seven consecutive hours, a one-hour period without exposure during the lunch break, considering that the worker washed his/her hands, followed by a new seven-hour exposure period. The third simulation assumed extemporaneous exposure by ingestion only, due to "hand-to-mouth behaviour," during the lunch break. For the second simulation, the dermal dose was 0.9975 µmol/kg bw/day and, for the third, the oral dose was 0.0025 µmol/kg bw/day, which allowed for direct comparison of the results with those of the first simulation.

For purposes of these simulations, the set of parametric values associated with the kinetics of the mean profile was used. All these parameters were introduced into the computer routine created with MATLAB. Thus, the temporal profiles of the hourly excretion rates (pmol/kg bw/h) of metabolites of lambda-cyhalothrin in the urine were simulated for a period of seven days following the start of the exposure scenarios described above. For the first scenario, the total amounts excreted in the urine for periods of 12 consecutive hours were also simulated in addition to the hourly excretion rates.

4.2.3.3 Proposed biological reference value for CFMP in urine

With modelling, it was also possible to propose a biological reference value (BRV) for acute and chronic exposures, considering a simulation of a plausible exposure scenario in a farmworker. This BRV was established for the specific metabolite of lambda-cyhalothrin, CFMP. The exposure scenario retained to derive this BRV corresponded to continuous daily dermal exposure over an entire typical 15-hour shift. The simulation was conducted such that the value of the absorbed dose corresponded both to the US EPA's acute oral RfD (0.0025 mg/kg bw/day) and to its chronic oral RfD (0.001 mg/kg bw/day), multiplied by the oral absorption fraction (fabs) determined by the modelling of data in volunteers exposed orally. This value for the absorbed dose was divided uniformly over the 15-hour exposure period to simulate continuous dermal exposure throughout the shift. The total amounts of CFMP excreted in the urine over a period of 24 hours following the start of this exposure scenario was then predicted by the model. This simulated total daily amounts of CFMP in the urine was proposed as a BRV. These simulations were done using each

of the sets of parametric values established for dermal exposure, that is, those for volunteers 2, 3, 5 and 6 and the one for the mean profile. The smallest value for the simulated daily amounts of CFMP in the urine – that is, the most protective – was retained as a BRV. It was expressed first in pmol/kg bw/day and converted into µmol/mol of creatinine. To establish the latter BRV unit, a value for daily excretion of creatinine of 25 mg/kg bw/day was considered, which corresponds to an amount of 221 µmol/kg bw/day of creatinine. The BRV in pmol/kg bw/day determined in advance was therefore divided by the daily amounts of creatinine expressed in µmol/kg bw/day to obtain a CFMP concentration in µmol/mol of creatinine.

5. RESULTS

5.1 Temporal profiles of biomarkers of exposure observed in the plasma and urine of volunteers, and toxicokinetic parameters

The temporal profiles of CFMP and 3-PBA in the plasma of the seven volunteers exposed to 0.025 mg/kg bw of lambda-cyhalothrin orally are presented in Figure 4. The profiles of the two metabolites were similar, indicating similar kinetic behaviour for both biomarkers of exposure. A rapid increase in blood levels of the metabolites was clearly visible in the hours following exposure, but elimination was also rapid and complete in less than 72 hours.

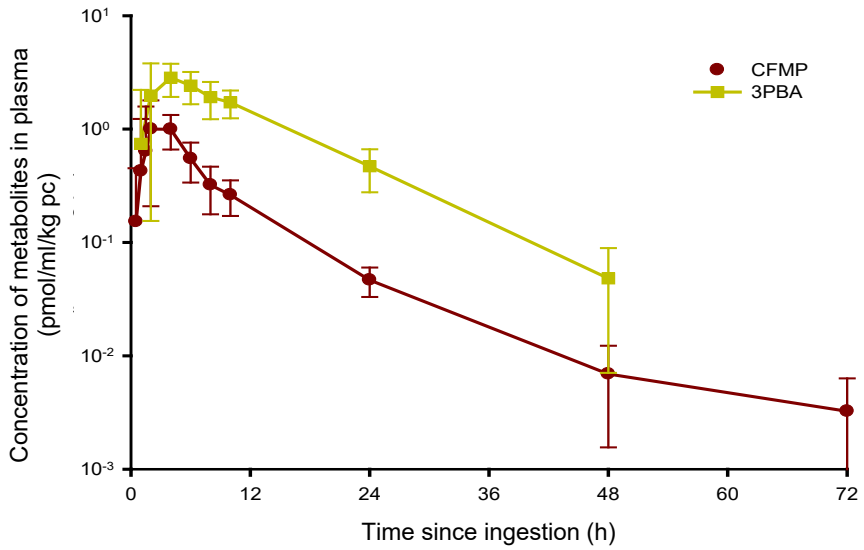


Figure 4. Temporal profiles of concentrations of CFMP (●) and 3-PBA (▽) observed in the plasma of volunteers following ingestion of 0.025 mg/kg bw of lambda-cyhalothrin. The symbols represent mean values and the vertical bars show standard deviations.

The baseline toxicokinetic parameters calculated on the basis of the plasma profiles of CFMP and 3-PBA (Table 3 and Table 4) confirm the rapid absorption of lambda-cyhalothrin, with a mean calculated rate of 0.3 and 0.2 h^{-1}, respectively, which corresponds to a calculated mean apparent absorption half-life ($t_{1/2}$) of 2.0 and 3,0 hours, respectively. The elimination rate (k_{elim}) calculated on the basis of the plasma profiles of CFMP and 3-PBA was also high, with a mean of ≈0.1 h^{-1} in both cases, which corresponds to a calculated mean apparent elimination $t_{1/2}$ of 5.3 and 6.4 hours, respectively. The mean residence time (MRT) was similar for both metabolites. The calculated apparent volume of distribution (V_d) also shows limited tissue distribution, with somewhat greater distribution for the cyclopropane portion (according to the profile of the metabolite CFMP).

The temporal profiles of CFMP and 3-PBA observed in the urine of the seven volunteers exposed to 0.025 mg/kg bw of lambda-cyhalothrin orally are presented in Figure 5. The urinary profiles of the metabolites again show similar kinetic behaviour for both biomarkers of exposure, with rapid and complete excretion in 72 hours.

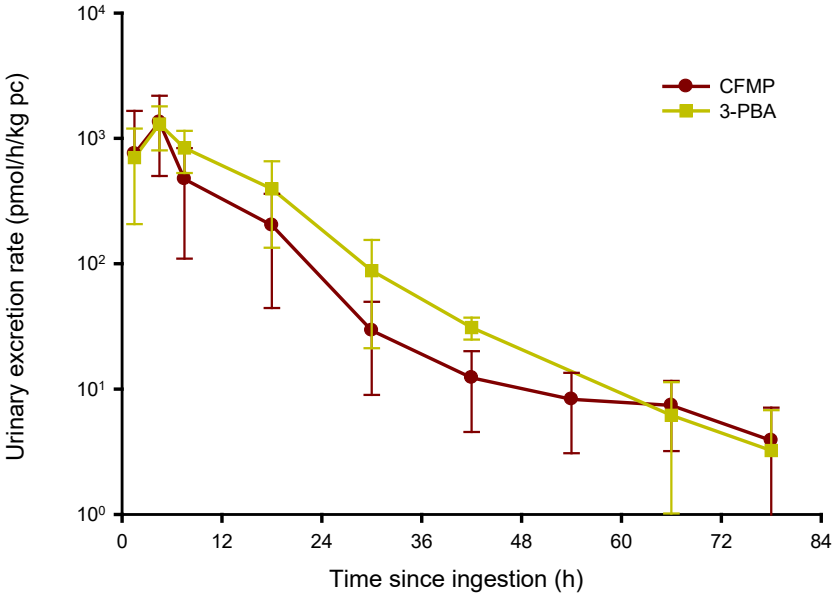


Figure 5. Temporal profiles of excretion of CFMP (●) and 3-PBA (▿) observed in the urine of volunteers following ingestion of 0.025 mg/kg bw of lambda-cyhalothrin. The symbols represent mean values and the vertical bars show standard deviations.

The toxicokinetic parameters established on the basis of these urinary profiles confirm the absorption rate and elimination rate values calculated based on the plasma profiles. The mean apparent absorption $t_{1/2}$ values calculated on the basis of the urinary profiles of CFMP and 3-PBA were 2.7 and 2.6 hours, and the elimination $t_{1/2}$ values were 4.2 and 5.9 hours, respectively (Table 3). However, for both CFMP and 3-PBA, the plasma levels (nmol) were 4 to 10 times higher than the urinary excretion rates (nmol/h), indicating that the rate of transfer of the metabolites from plasma to urine was 0.3 to 0.1 h^{-1}, corresponding to a half-life of ≈2.7 to 6.7 hours.

Moreover, the cumulative urinary excretion of metabolites observed during the 84-hour collection period after ingestion of 0.025 mg/kg bw of lambda-cyhalothrin in the seven volunteers shows that, on average, 21% and 30% of the lambda-cyhalothrin was excreted in the urine in the form of CFMP and 3-PBA, respectively (Figure 6). These data confirm that these molecules are major metabolites of lambda-cyhalothrin in humans. The data also show that urinary excretion is relatively variable from one individual to another.

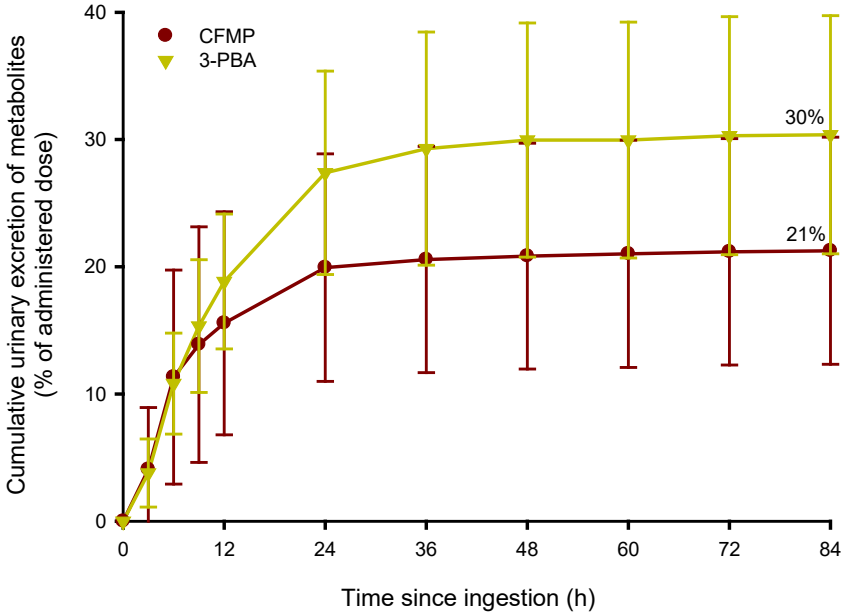


Figure 6. Cumulative excretion of CFMP (●) and 3-PBA (▼) in the urine observed as a function of time in volunteers following ingestion of 0.025 mg/kg bw of lambda-cyhalothrin. The symbols represent mean values and the vertical bars show standard deviations.

Table 3. Baseline toxicokinetic parameters (mean ± SD) determined on the basis of plasma and urine profiles of CFMP and 3-PBA in volunteers following ingestion of 0.025 mg/kg bw of lambda-cyhalothrin

Toxicokinetic parameter (mean ± SD;[a] n = 7)				
	CFMP		3-PBA	
	Plasma	Urine	Plasma	Urine
T_{max} (h)	3.1 ± 1.2[b]	5.6 ± 2.1	4.0 ± 1.15[b]	6.4 ± 2.7
Apparent absorption half-life (h)	2.0 ± 1.9	2.7 ± 2.1	3.0 ± 1.9	2.6 ± 2.2
Apparent elimination half-life (h)	5.3 ± 1.6	4.2 ± 1.5	6.4 ± 1.3	5.9 ± 1.4
Fraction excreted in the urine (molar % of dose)[c]		21.3 ± 8.9		30.4 ± 9.4

[a] Represents the mean ± SD of the toxicokinetic parameters derived from the temporal profiles of each volunteer.

[b] Mean time (± SD) to achieve the maximum levels, calculated based on the values observed for each volunteer. Figure 4 presents the mean temporal profiles of the metabolites and shows a peak of concentration at ≈ 3 hours for CFMP, and ≈ 4 hours for 3-PBA.

[c] Represents the molar fraction of the administered dose of lambda-cyhalothrin found in the urine in the form of CFMP or 3-PBA.

Table 4. Other toxicokinetic parameters calculated on the basis of the temporal profiles of CFMP and 3-PBA in the plasma of volunteers following ingestion of 0.025 mg/kg bw of lambda-cyhalothrin

Toxicokinetic parameter		Mean ± SD (n = 7)	
		CFMP	3-PBA
AUC [(nmol x h /l)/kg bw]		8.8 ± 2.6	42.0 ± 12.7
AUMC [(nmol x h^2 /l)/kg bw]		75.9 ± 24.0	511 ± 181
MRT (h)		8.9 ± 2.4	12.2 ± 2.3
CL (l/h)	Lower limit of estimate[a]	1.5 ± 0.9	0.4 ± 0.2
	Upper limit of estimate[b]	6.8 ± 2.1	1.5 ± 0.5
V_d (l)	Lower limit of estimate[a]	13.9 ± 8.6	5.3 ± 2.5
	Upper limit of estimate[b]	62.3 ± 30.1	17.7 ± 6.8

[a] The lower limit of the estimated clearance (CL) and volume of distribution (V_d) of the metabolites in the plasma was calculated considering that the absorbed dose corresponded to the total amounts excreted in the urine.

[b] The upper limit of the estimated clearance (CL) and volume of distribution (V_d) of the metabolites in the plasma was calculated considering that the absorbed dose was equal to the exposure dose (i.e., considering an absorption fraction of 1).

The temporal profiles of CFMP and 3-PBA in the plasma of four volunteers exposed to 0.25 mg/kg bw of lambda-cyhalothrin after dermal application of a formulation of Matador EC 120® for 6 hours are presented in Figure 7. As with the oral route, the profiles of the two metabolites were similar, indicating similar kinetic behaviour in both biomarkers of exposure. A rapid increase in blood levels of the metabolites was clearly visible in the hours following exposure, but the peak of excretion of the two metabolites was achieved later than with the oral route, namely 10 and 13.5 hours following the start of application, according to the profiles of CFMP and 3-PBA, respectively, which corresponds to 4 and 7.5 hours after cleaning of the treated area. Elimination was also rapid and complete in 84 hours.

The baseline toxicokinetic parameters calculated on the basis of the plasma profiles of CFMP and 3-PBA confirm the rapid absorption of lambda-cyhalothrin after dermal application; the calculated mean apparent absorption half-life ($t_{1/2}$) was 3 and 7.3 hours, respectively (Table 5). Differences were observed in the apparent absorption half-life calculated based on the profiles of each metabolite. The mean apparent elimination $t_{1/2}$ calculated on the basis of the plasma profiles of 3-PBA was similar to that obtained after ingestion, at 7.6 hours, whereas the mean apparent elimination half-life for CFMP was longer, at 11.2 hours. These differences suggest a certain metabolism at the site of introduction and storage of the metabolites with the dermal route. The calculated apparent volume of distribution (V_d) suggests greater tissue distribution after dermal application than after ingestion.

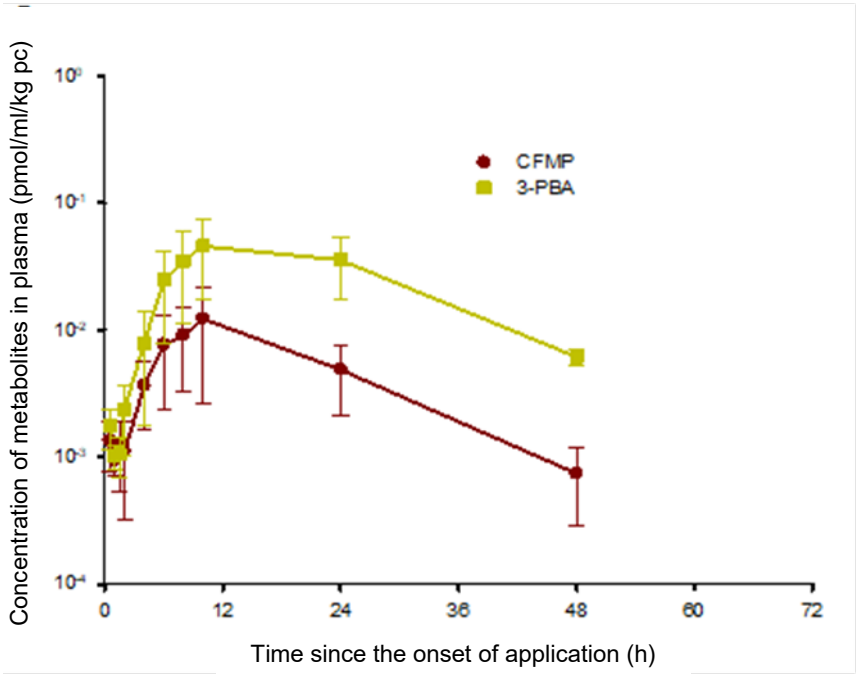


Figure 7. Temporal profiles of concentrations of CFMP (●) and 3-PBA (ν) observed in the plasma of volunteers following dermal application of Matador EC 120® for 6 hours (0.25 mg/kg bw of lambda-cyhalothrin). The symbols represent mean values and the vertical bars show standard deviations.

The temporal profiles of CFMP and 3-PBA observed in the urine of the four volunteers exposed to 0.25 mg/kg bw of lambda-cyhalothrin after dermal application of Matador EC 120® for 6 hours are presented in Figure 8. Again, the urinary profiles of the metabolites show similar kinetic behaviour by both biomarkers of exposure, with rapid and complete excretion in 84 hours. As with the oral route, the toxicokinetic parameters calculated on the basis of these urinary profiles confirm the values for the absorption and elimination rates calculated based on the plasma profiles. The mean apparent absorption $t_{1/2}$ values calculated based on the urinary profiles of CFMP and 3-PBA were 3.2 and 7.0 hours and the elimination $t_{1/2}$ were 15.4 and 7.4 hours, respectively (Table 5 and Table 6).

Moreover, the cumulative urinary excretion of metabolites observed during the 84-hour collection period after the dermal application of Matador EC 120® (0.25 mg/kg bw of lambda-cyhalothrin) for 6 hours in the four volunteers (Figure 9) shows that, on average, 0.12% and 0.08% of lambda-cyhalothrin was excreted in the urine in the form of CFMP and 3-PBA, respectively. This indicates that these metabolites are excreted in the urine in amounts 250 to 280 times lower after dermal administration than after oral administration. As with the oral route, the data also show that urinary excretion is relatively variable from one person to another.

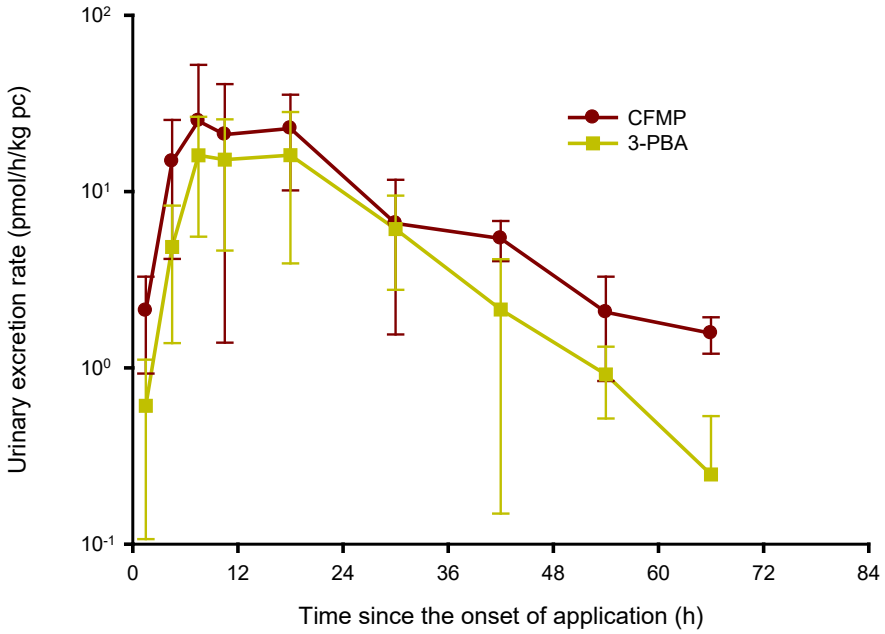


Figure 8. Temporal profiles of the urinary excretion rates of CFMP (●) and 3-PBA (ν) observed in volunteers following application of Matador EC 120® (0.25 mg/kg bw of lambda-cyhalothrin) for 6 hours. The symbols represent mean values and the vertical bars show standard deviations.

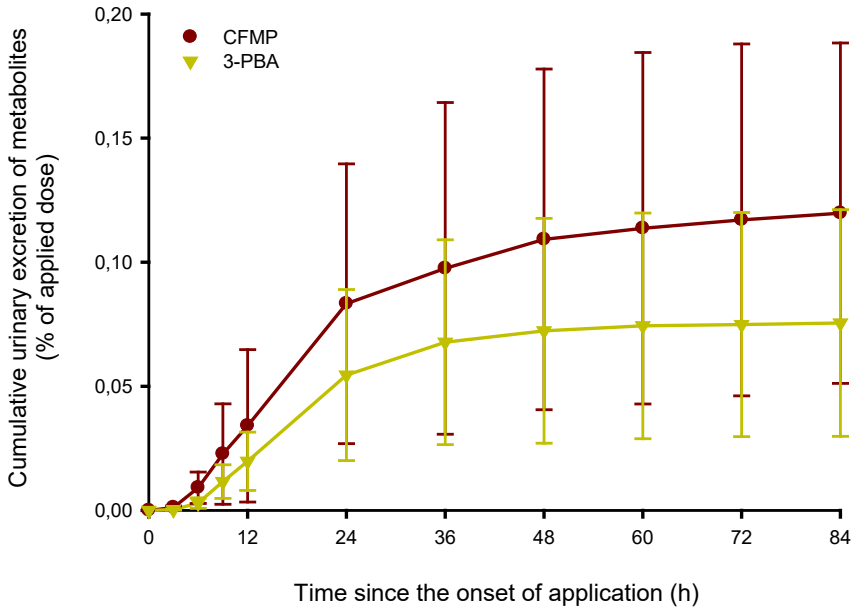


Figure 9. Cumulative urinary excretion of CFMP (●) and 3-PBA (▽) observed as a function of time in volunteers following application of Matador EC 120® (0.25 mg/kg bw of lambda-cyhalothrin) for 6 hours. The symbols represent mean values and the vertical bars show standard deviations.

Table 5. Baseline toxicokinetic parameters (mean ± SD) determined on the basis of plasma and urinary profiles of CFMP and 3-PBA in volunteers after dermal application of Matador 120 EC® (0.25 mg/kg bw of lambda-cyhalothrin) for 6 hours

Toxicokinetic parameter (mean ± SD;[a] n = 4)				
	CFMP		3-PBA	
	Plasma	Urine	Plasma	Urine
T_{max} (h)	10.0[b]	13.5 ± 5.3	13.5 ± 7.0[b]	16.1 ± 3.8
Apparent absorption half-life (h)	3.0 ± 1.7	3.2 ± 2.3	7.3 ± 1.7	7.0 ± 1.4
Apparent elimination half-life (h)	11.2 ± 7.9	15.4 ± 12.6	7.6 ± 1.7	7.4 ± 1.2
Fraction excreted in the urine (molar % of dose)		0.12 ± 0.07		0.08 ± 0.05

[a] Given that the concentrations were close to the limit of detection, it was not possible to establish the temporal profiles of metabolites for two of the volunteers.

[b] Given that the concentrations were close to the limit of detection, these values are estimates. The values were obtained based on statistical fitting of the following general equation to the experimental data observed: $C(t) = Ae^{-kabs.t} + Be^{-kelim.t}$, where C(t) is the plasma concentration as a function of time (or the urinary excretion rate).

Table 6. Other toxicokinetic parameters calculated on the basis of the temporal profiles of CFMP and 3-PBA in the plasma of volunteers following dermal application of Matador 120 EC® (0.25 mg/kg bw of lambda-cyhalothrin) for 6 hours

Toxicokinetic parameter		Mean ± SD (n = 4)	
		CFMP	3-PBA
AUC [(nmol x h /l)/kg bw]		0.25 ± 0.15	1.3 ± 0.67
AUMC [(nmol x h^2 /l)/kg bw]		4.3 ± 1.9	28 ± 12
MRT (h)		19 ± 3.9	22 ± 3.5
CL (l/h)	Lower limit of estimate[a]	2.8 ± 0.7	0.3 ± 0.1
V_d (l)	Lower limit of estimate[a]	55 ± 23	6.6 ± 0.9

[a] The lower limit of the estimated clearance (CL) and volume of distribution (V_d) of the metabolites in the plasma was calculated considering that the absorbed dose corresponded to the total amounts excreted in the urine.

5.2 Toxicokinetic modelling of lambda-cyhalothrin and its metabolites

5.2.1 Determination of the model's parameters and simulation of the data in volunteers

The kinetic data obtained in volunteers exposed to lambda-cyhalothrin enabled us to estimate the parametric values of the model. Table 7 presents the values of the constant for transfer of the metabolites from the blood to the urine (k_{MU}), a key parameter that affects the search for the values of all the other parameters retained in this model. Table 8 presents the values for the parameters in volunteers exposed orally and Table 9 shows the specific values for the dermal route. They allowed a very good fit to the experimental data on the blood and urinary profiles of CFMP and 3-PBA collected in this project from volunteers exposed orally to lambda-cyhalothrin (Figure 10 and Figure 11). These parametric values also allowed a very good fit to the blood and urinary profiles of the metabolites observed in volunteers exposed dermally (Figure 12 and Figure 13).

Table 7. Determination of parametric values (interval of possible values) of the constant for the transfer of metabolites from blood to urine (k_{MU}), established based on data obtained in volunteers exposed orally to lambda-cyhalothrin

Constant k_{MU} (h^{-1})				
	Based on CFMP profile		Based on 3-PBA profile	
Profile	**Min**	**Max**	**Min**	**Max**
Volunteer 1	0.06	0.18	0.04	0.13
Volunteer 2	0.18	0.54	0.07	0.20
Volunteer 3	0.06	0.17	0.05	0.14
Volunteer 4	0.08	0.25	0.06	0.19
Volunteer 5	0.22	0.66	0.07	0.21
Volunteer 6	0.22	0.67	0.05	0.14
Volunteer 7	0.07	0.22	0.02	0.07
Mean [a]	0.15	0.45	0.05	0.16

[a] Estimate of k_{MU} parameter based on mean profile of volunteers exposed to lambda-cyhalothrin.

Table 8. Values of the constants of the model determined on the basis of experimental data on the urinary profiles of the metabolites in volunteers exposed orally to lambda-cyhalothrin

		Lambda-cyhalothrin										
		CFMP							3-PBA			
Profile	**f_{abs_oral}**	**k_{abs} (h^{-1})**	**k_{BS} (h^{-1})**	**k_{SB} (h^{-1})**	**k_{BM_cfmp} (h^{-1})**	**$k_{BM\_NO\_cfmp}$ (h^{-1})**	**k_{MU_cfmp} (h^{-1})**	**k_{MF_cfmp} (h^{-1})**	**k_{BM_3PBA} (h^{-1})**	**$k_{BM\_NO\_3PBA}$ (h^{-1})**	**k_{MU_3PBA} (h^{-1})**	**k_{MF_3PBA} (h^{-1})**
Volunteer 1	0.90	1.54	0.53	0.19	0.62	0.08	0.16	0.72	0.62	0.00	0.07	0.11
Volunteer 2	0.80	0.51	21	0.23	13	1.18	0.31	0.73	13	8.15	0.08	0.06
Volunteer 3	0.90	0.55	42	0.16	32	8.85	0.15	0.72	32	10	0.06	0.11
Volunteer 4	0.85	0.35	42	0.10	42	0.48	0.25	0.59	35	6.03	0.08	0.04
Volunteer 5	0.70	0.58	0.21	0.05	0.80	0.59	0.63	0.04	0.66	0.004	0.14	0.21
Volunteer 6	0.75	0.32	0.09	0.04	0.73	0.50	0.33	0.27	0.71	0.47	0.07	0.04
Volunteer 7	0.80	0.44	21	0.16	19	2.00	0.15	0.66	17	3.8	0.04	0.08
Mean [a]	0.85	0.35	0.55	0.11	0.86	0.09	0.30	0.79	0.86	0.01	0.09	0.16

[a] Determination of parameters on the basis of the mean profile of volunteers exposed to lambda-cyhalothrin.

Table 9. Values of the constants of the model determined on the basis of experimental data on the urinary profiles of the metabolites in volunteers exposed dermally to lambda-cyhalothrin

		Lambda-cyhalothrin							
		CFMP					3-PBA		
Profile	**f_{abs_dermal}**	**k_{DDin} (h^{-1})**	**k_{DinB} (h^{-1})**	**k_{DinMD_CFMP} (h^{-1})**	**$k_{DinM\_NO\_CFMP}$ (h^{-1})**	**k_{MDM_cfmp} (h^{-1})**	**k_{DinMD_3PBA} (h^{-1})**	**$k_{DinM\_NO\_3PBA}$ (h^{-1})**	**k_{MDM_3PBA} (h^{-1})**
Volunteer 2	0.006	0.001	0.05	0.05	0.05	0.05	0.02	0.21	0.05
Volunteer 3	0.01	0.002	0.05	0.05	0.05	0.05	0.03	0.21	0.05
Volunteer 5	0.012	0.002	0.05	0.02	0.21	0.05	0.03	0.21	0.05
Volunteer 6	0.004	0.001	0.02	0.02	0.12	0.03	0.01	0.21	0.03
Mean [a]	0.006	0.001	0.03	0.05	0.04	0.05	0.03	0.10	0.05

[a] Determination of parameters on the basis of the mean profile of volunteers exposed to lambda-cyhalothrin.

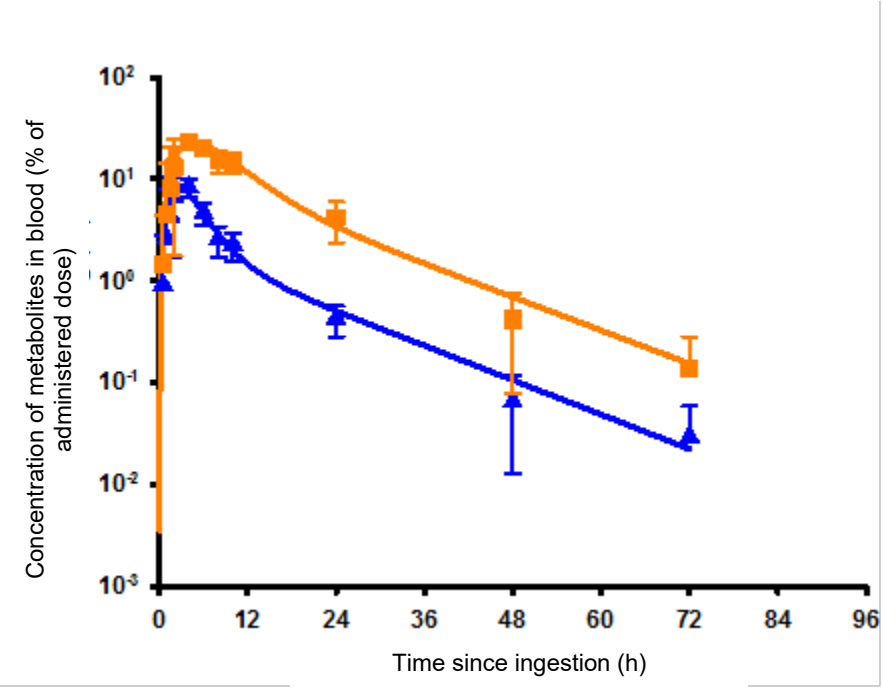


Figure 10. Comparison of simulations (lines) to experimental data (symbols) on the temporal profiles of CFMP (▲; —) and 3-PBA (ν;—) in the blood of volunteers following oral administration of 0.025 mg/kg bw of lambda-cyhalothrin.

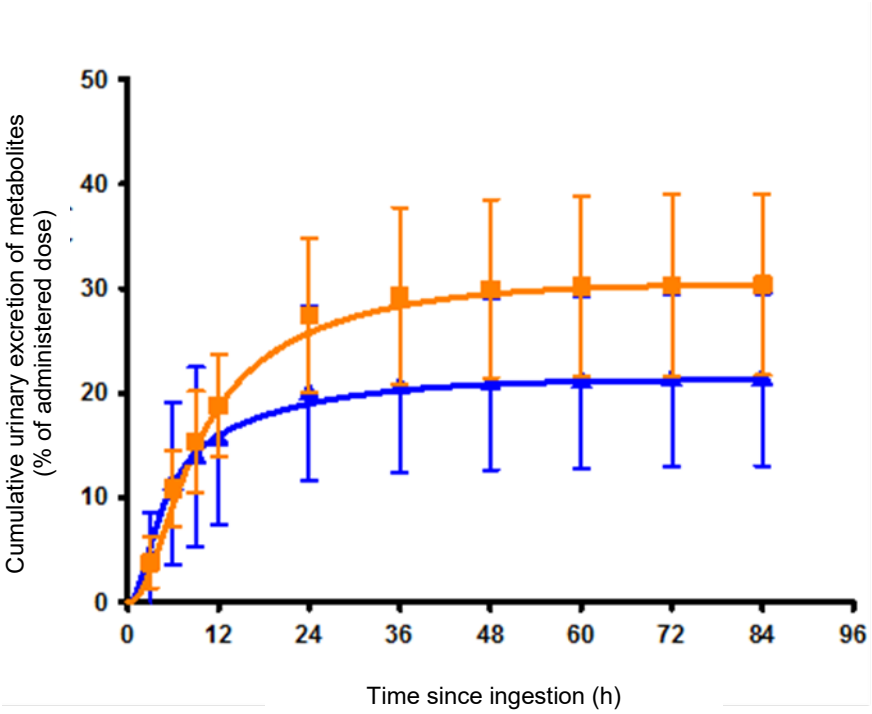


Figure 11. Comparison of simulations (lines) to experimental data (symbols) on the temporal profiles of cumulative urinary excretion of CFMP (▲; —) and 3-PBA (▽;—) in volunteers following oral administration of 0.025 mg/kg bw of lambda-cyhalothrin.

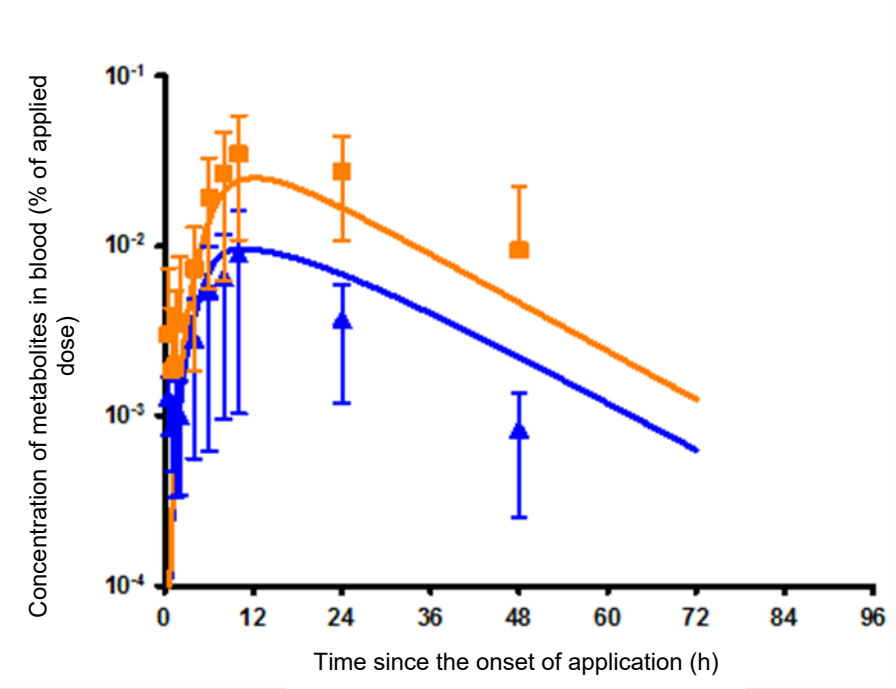


Figure 12. Comparison of simulations (lines) to experimental data (symbols) on the temporal profiles of CFMP (▲; —) and 3-PBA (ν;—) in the blood of volunteers following dermal application of 0.25 mg/kg bw of lambda-cyhalothrin for 6 hours.

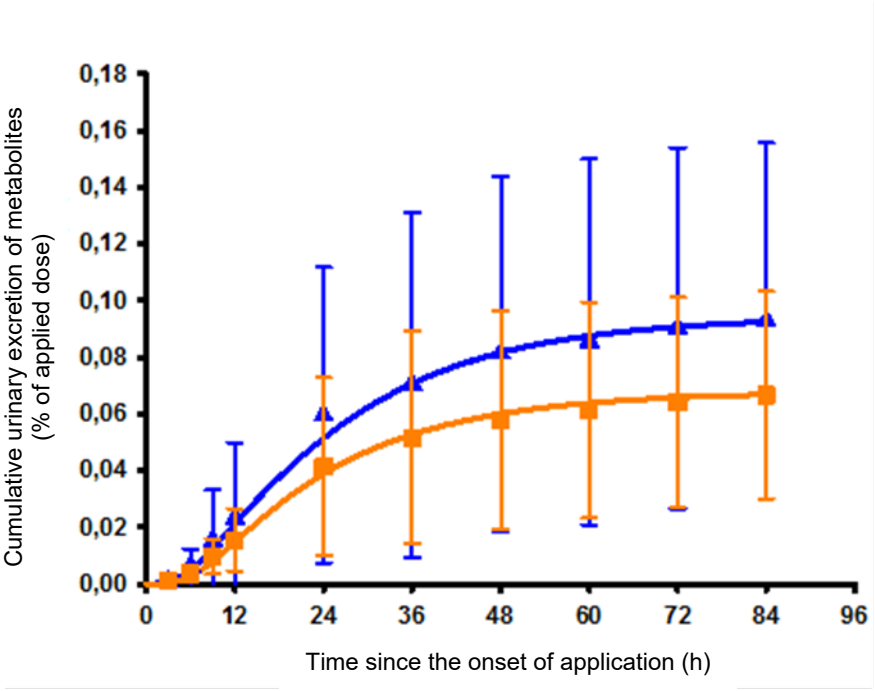


Figure 13. Comparison of simulations (lines) to experimental data (symbols) on the temporal profiles of cumulative urinary excretion of CFMP (▲; —) and 3-PBA (ν;—) in volunteers following dermal application of 0.25 mg/kg bw of lambda-cyhalothrin for 6 hours.

5.2.2 Prediction of the temporal profiles for different realistic exposure scenarios in workers

Figures 14 and 15 present the simulation of a temporal profile of the metabolite CFMP in the urine of a worker (expressed as the excretion rate per hour and the total amounts over periods of 12 consecutive hours, respectively), assuming daily exposure to lambda-cyhalothrin for five consecutive days according to the following sequence: (i) continuous dermal contact for 7 hours; (ii) one-hour break when the contaminated area is washed and thus there is no more dermal penetration of the product; (iii) ingestion as a bolus dose during this break via “hand-to-mouth behaviour”; and (iv) continuous dermal contact for 7 hours followed by complete washing of the contaminated area. The daily exposure dose corresponds to 1 µmol/kg bw/day (0.9975 µmol/kg bw/day dermally and 0.0025 µmol/kg bw/day orally). The profile is simulated for the five days of exposure and the two days following the end of this exposure (Table 10).

Table 10. Simulations of temporal profiles of the metabolite CFMP in the urine for different typical exposure scenarios in a worker

Figures	Type of representation	Oral exposure[a]	Dermal exposure[b]	Daily oral exposure dose (µmol/kg bw/day)	Daily dermal exposure dose[c] (µmol/kg bw/day)	Total daily exposure dose (µmol/kg bw/day)
14	Urinary excretion rate	Yes	Yes	0.0025	0.9975	1
15	12-hour urine collection	Yes	Yes	0.0025	0.9975	1
16	Urinary excretion rate	No	Yes	-----	0.9975	0.9975
17	Urinary excretion rate	Yes	No	0.0025	-----	0.0025

[a] Oral exposure 7.5 hours following the start of the shift and repeated for five consecutive days, followed by two days without exposure.

[b] Exposure scenario: (i) continuous dermal contact for a 7-hour period following the start of the shift; (ii) one-hour lunch break during which the contaminated area is washed (and dermal penetration ceases); (iii) continuous dermal contact for the period from 8 to 15 hours following the start of the shift; and (iv) washing of the contaminated area 15 hours after the start of the shift (dermal penetration ceases). This scenario was repeated for five consecutive days, followed by two days without exposure.

[c] Dose divided equally throughout the duration of daily exposure.

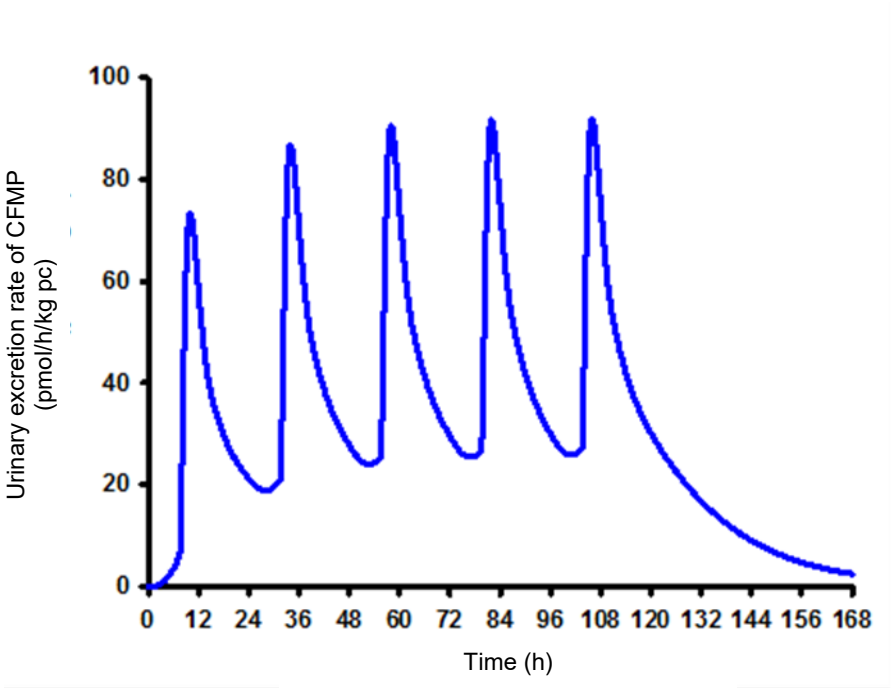


Figure 14. Simulation of the temporal profile of CFMP in the urine of a fictitious worker, expressed as excretion rate per hour, following a scenario involving concomitant dermal and oral exposure. The scenario considers daily exposure to lambda-cyhalothrin for five consecutive days followed by two days without exposure: (i) continuous dermal contact for a 7-hour period following the start of the shift; (ii) one-hour lunch break during which the contaminated area is washed (and dermal penetration ceases); (iii) ingestion as a bolus dose 7.5 hours following the start of the shift; (iv) continuous dermal contact for the period from 8 to 15 hours following the start of the shift; and (v) washing of the contaminated area 15 hours after the start of the shift (dermal penetration ceases). The daily exposure dose is 1 µmol/kg bw/day, with 0.9975 µmol/kg bw/day dermally and 0.0025 µmol/kg bw/day orally.

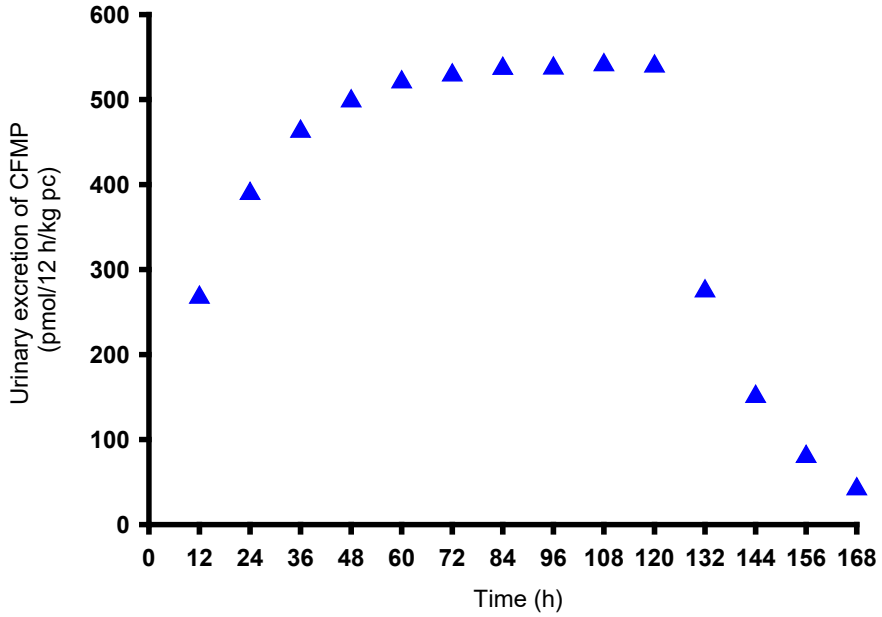


Figure 15. Simulation of the temporal profile of CFMP in the urine of a fictitious worker, expressed as total amounts over periods of 12 consecutive hours, following a scenario involving concomitant dermal and oral exposure. The exposure scenario is the same as in Figure 14.

This simulation of concomitant dermal and oral exposure shows an increase in the maximum and minimum values over the days of exposure and thus a certain accumulation during a typical workweek. This indicates that a certain portion of the daily dose did not have time to be eliminated before the next dose in this repeated exposure scenario. However, equilibrium is achieved after three consecutive days of exposure.

Figure 16 presents the simulation of a temporal profile of the metabolite CFMP in the urine of a worker (expressed as excretion rate per hour) assuming daily exposure to lambda-cyhalothrin only dermally, for five consecutive days, according to the following sequence (i) continuous dermal contact for 7 hours; (ii) one-hour break when the contaminated area is washed and thus there is no more dermal penetration of the product; and (iii) continuous dermal contact for 7 hours followed by complete washing of the contaminated area. The daily exposure dose corresponds to 0.9975 µmol/kg bw/day. The profile is simulated for the five days of exposure and the two days following the end of this exposure (Table 10). This simulation of dermal exposure shows an increase in the maximum and minimum values over the days of exposure and thus a certain accumulation during a typical workweek. This indicates that a certain portion of the daily dose did not have time to be eliminated before the next dose in this repeated exposure scenario. However, equilibrium is achieved after three consecutive days of exposure.

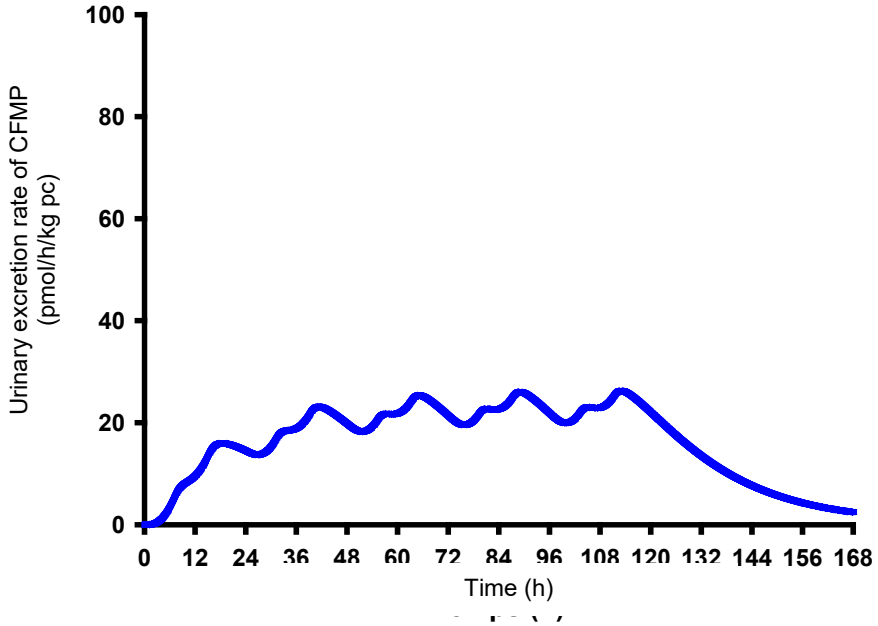


Figure 16. Simulation of the temporal profile of CFMP in the urine of a fictitious worker, expressed as excretion rate per hour, following a dermal exposure scenario. The scenario considers daily dermal exposure to lambda-cyhalothrin, at a dose of 0.9975 µmol/kg bw/day, for five consecutive days followed by two days without exposure: (i) continuous dermal contact for a 7-hour period following the start of the shift; (ii) one-hour lunch break during which the contaminated area is washed (and dermal penetration ceases); (iii) continuous dermal contact for the period from 8 to 15 hours following the start of the shift; and (iv) washing of the contaminated area 15 hours after the start of the shift (dermal penetration ceases).

Figure 17 presents the simulation of a temporal profile of the metabolite CFMP in the urine of a worker (expressed as excretion rate per hour), assuming daily exposure to lambda-cyhalothrin orally, 7.5 hours following the start of the shift, repeated for five consecutive days, followed by two days off. This scenario corresponds to inadvertent exposure by “hand-to-mouth behaviour” at mealtimes. The daily oral exposure dose corresponds to 0.0025 µmol/kg bw/day (Table 10). This oral exposure simulation shows that there is little daily variation in the maximum and minimum values and thus little accumulation during a typical workweek. This indicates that most of the dose is eliminated before the following dose in this repeated exposure scenario. Moreover, comparison of the daily excretion rates in the three simulated exposure scenarios, namely concomitant dermal and oral exposure, dermal exposure only and oral exposure only (Figure 15, Figure 16 and Figure 17, respectively), shows that the dermal route makes a limited contribution to the daily absorbed dose.

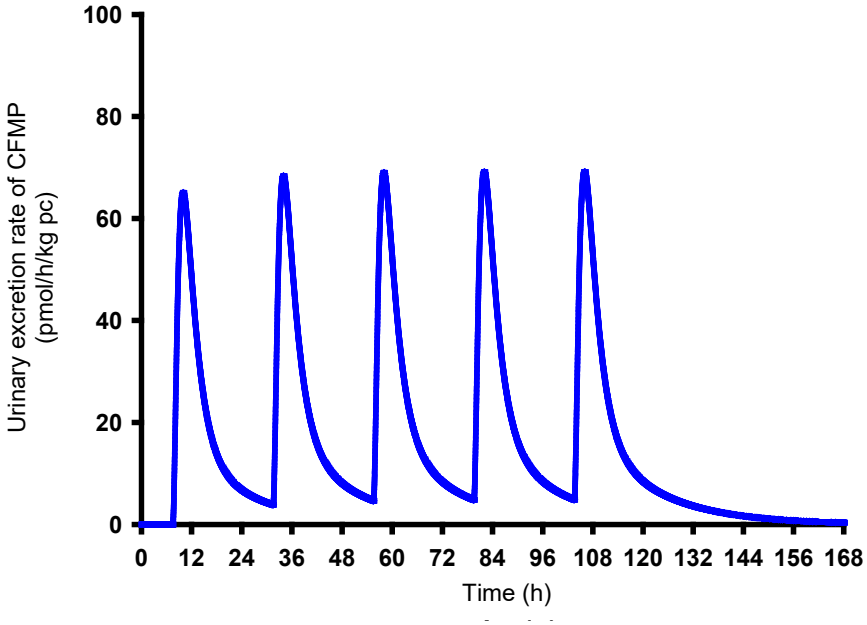


Figure 17. Simulation of the temporal profile of CFMP in the urine of a fictitious worker, expressed as excretion rate per hour, following an oral exposure scenario. The scenario considers daily oral exposure to lambda-cyhalothrin, at a dose of 0.0025 µmol/kg bw/day, 7.5 hours following the start of the shift, repeated for five consecutive days, followed by two days without exposure.

5.2.3 Proposal of a biological reference value for CFMP in urine

Biological reference values (BRV) for CFMP in urine for acute and chronic exposure were proposed using the set of parametric values that generated the most protective value, namely the set corresponding to volunteer 6 (Table 8 and Table 9), in the model. The BRV for CFMP derived for a 24-hour collection of urine was therefore 198.9 pmol/kg bw/day or 0.2 nmol/kg bw/day, which corresponds to 0.9 µmol/mol of creatinine for an acute exposure scenario. The BRV for a chronic exposure scenario is 0.08 nmol/kg bw/day or 0.3 µmol/mol of creatinine.

6. DISCUSSION

6.1 Temporal profiles of biomarkers of exposure to lambda-cyhalothrin in volunteers subject to controlled exposure

The controlled study in volunteers exposed by the oral and dermal routes advanced our knowledge of the toxicokinetics of the biomarkers of exposure to lambda-cyhalothrin. These results promote a better understanding of the behaviour of these biomarkers of exposure, for a better interpretation of biomonitoring data on individuals who have been exposed, such as workers who apply this pesticide or work in areas that have been treated with it. The temporal profiles of the biomarkers of exposure, CFMP and 3-PBA, established in the volunteers' plasma and urine, showed that lambda-cyhalothrin was quickly absorbed in systemic circulation but was also rapidly eliminated from the body following the administration of a single low dose (apparent absorption and elimination half-lives of a few hours), whether orally or dermally.

Furthermore, the kinetic behaviour of the two biomarkers of exposure assessed here was similar after oral administration. However, by the dermal route, differences were observed in the apparent absorption and elimination constants (a factor of approximately 1.5–2.5) and in the apparent volume of distribution calculated on the basis of the plasma profiles of CFMP and 3-PBA.

Nevertheless, absorption and elimination remained rapid after dermal application. The peaks of plasma concentration and the urinary excretion rate were reached about 10 to 16 hours after the start of dermal application (for a period of 6 hours), which represents 4 to 10 hours after the washing of the treated area, whereas it was observed 3 to 6 hours after ingestion. In fact, almost all of the dose was eliminated 84 hours after the start of dermal application, compared with 24 to 36 hours after ingestion. For the biological monitoring of workers' exposure, these data indicate that the time to reach the peak plasma concentration or urinary excretion should be similar following the end of an episode of exposure by these two routes. On the other hand, the percentage of the dose of lambda-cyhalothrin found in the urine in the form of biomarkers of exposure after dermal administration (approximately 0.1%) was considerably lower than that observed after ingestion (20%–30%), which indicates that dermal absorption is limited. Thus, dermal exposure doses must be very high to contribute significantly to the doses absorbed by multiple routes (combination of oral, dermal and respiratory routes).

The kinetic data obtained in volunteers exposed to lambda-cyhalothrin were also compared with the only kinetic animal data available for this pesticide, namely those from Anadon et al. (2006). Those authors determined the plasma and tissue profiles of the parent compound, unchanged lambda-cyhalothrin, after an intravenous injection and oral administration using feeding tubes in rats (3 and 20 mg/kg bw, respectively). The authors reported that the bioavailability (mean ± SD) of lambda-cyhalothrin after oral administration was 67 ± 9%. The plasma peaks were reached in the same time as those observed in the volunteers (mean ± SD at 2.7 ± 0.4 hours). The mean elimination half-life calculated on the basis of the plasma profile after intravenous injection was 7.5 ± 0.99 hours, which is close to the values estimated in volunteers. It should be noted that the doses administered in this animal study were very high.

When one compares the kinetic data on the observed biomarkers of exposure to lambda-cyhalothrin (CFMP and 3-PBA) with those obtained previously in the laboratory from volunteers exposed to permethrin and cypermethrin orally (Ratelle et al., 2015a, 2015b), some similarities are clearly visible. After oral administration in volunteers, the plasma and urine kinetics of the biomarkers of exposure to permethrin and cypermethrin, namely *trans*- and *cis*-DCCA and 3-PBA, revealed apparent absorption half-lives of ≈ 3–4 hours and apparent elimination half-lives of 4.5–9 hours, which is similar to the respective values of 2–3 hours and 4–6 hours obtained after ingestion of lambda-cyhalothrin. In addition, the volume of distribution calculated based on the kinetic profiles of CFMP and 3-PBA in volunteers exposed orally to lambda-cyhalothrin was also similar to that calculated on the basis of the profiles of *trans*- and *cis*-DCCA and 3-PBA after ingestion of permethrin and cypermethrin, suggesting that all these metabolites largely remain in circulation once formed. Moreover, the percentage of the administered dose of lambda-cyhalothrin, found in total in the form of CFMP and 3-PBA in the urine (21% and 30%, respectively), was similar to that observed for DCCA (the total of the *trans*- and *cis*-DCCA forms) and 3-PBA (36% and 27%, respectively) after ingestion of cypermethrin in volunteers (Ratelle et al., 2015a). The corresponding values for DCCA after ingestion of permethrin were also 36% (Ratelle et al., 2015b), whereas the urinary excretion of 3-PBA was slightly higher, representing 47% of the administered dose. Like lambda-cyhalothrin, cypermethrin is a type II pyrethroid with a cyano group in the alpha position of the cyclopropane carboxylate structure, whereas permethrin is a type I pyrethroid without a cyano group. It remains to be verified whether the divergences obtained are due to kinetic differences or simply to variability among participants in this small sample. Overall, these similar kinetic data suggest that both 3-PBA and the more specific metabolites of lambda-cyhalothrin, permethrin and cypermethrin (CFMP, *trans*- and *cis*-DCCA) would be appropriate to assess total pyrethroid exposure.

6.2 Modelling of kinetics in volunteers and prediction in workers

The toxicokinetic modelling done based on the data collected from exposed volunteers confirmed that the kinetics of the biomarkers of exposure to lambda-cyhalothrin is similar to the kinetics of the metabolites of permethrin and cypermethrin. For oral absorption, the conceptual model simulating the fate of lambda-cyhalothrin and its metabolites used as biomarkers of exposure (Figure 3) proved similar to that for permethrin and cypermethrin, although the specific metabolites were different. However, to simulate dermal absorption, the model had to be modified to include three dermal compartments representing: (i) lambda-cyhalothrin on the surface of the skin; (ii) lambda-cyhalothrin inside the skin structures; and (iii) the metabolites CFMP and 3-PBA in the skin structures. This model assumes that a portion of the lambda-cyhalothrin reaches systemic circulation unchanged whereas another portion is in metabolized form. Although there are no *in vitro* or *ex vivo* data that specifically show metabolism of lambda-cyhalothrin in the skin, several authors have shown that pyrethroids were metabolized by the action of carboxylesterases (Crow, Borazjani, Potter, & Ross, 2007; Nishi et al., 2006; Ross, Borazjani, Edwards, & Potter, 2006). Other authors have shown the presence of carboxylesterases in the internal skin structures (Fu, Sadgrove, Marson, & Jay, 2016; Yang, Wang, Chen, Deng, & Yan, 2009). The presence of carboxylesterases in the skin may modify the kinetics of this substance when it is absorbed dermally, as opposed to ingested. It was impossible to model the kinetics of the metabolites without adding a dermal compartment and without considering metabolism in the skin.

The conclusions in terms of the kinetic behaviour of the metabolites of interest for biomonitoring exposure to lambda-cyhalothrin in workers are in accordance with those drawn from the modelling of the kinetics of the biomarkers of exposure to permethrin and cypermethrin (Bouchard et al., 2016). The modelling confirmed the coherence of the toxicokinetic parameters of clearance, absorption speed and elimination speed determined previously on the basis of the profiles observed in volunteers. The speeds for oral absorption, distribution, metabolism and elimination of lambda-cyhalothrin are of the same order of magnitude as those for permethrin and cypermethrin. This modelling confirmed that oral absorption speed is faster than elimination speed. For the dermal absorption of lambda-cyhalothrin, the modelling shows a very low absorption constant from the surface of the skin. This low constant explains the low fraction absorbed by the skin, which is of the same order of magnitude as that for permethrin and cypermethrin and other molecules such as malathion, parathion, chlorpyrifos, and carbaryl (Bouchard et al., 2008; Bouchard et al., 2005; Bouchard et al., 2006; Bouchard et al., 2003; Gosselin et al., 2004). Nevertheless, the modelling of data specific to dermal absorption of lambda-cyhalothrin done in this project suggests the retention of the parent product and its metabolites in the skin. The study also showed a difference in the urinary ratios for both metabolites (CFMP and 3-PBA) between oral and dermal exposure, which could be explained by differences in the metabolism depending on the modelling done.

Indeed, the analysis of the urinary and blood profiles of the metabolites in volunteers and the modelling of these data show that lambda-cyhalothrin is absorbed and eliminated somewhat less rapidly with dermal exposure than with oral exposure. This is reflected in the parameters established during the modelling. During a temporal follow-up of the amounts of metabolites found in serial collections of urine (amounts per hour), it should therefore be possible to differentiate between excretion peaks that are mainly associated with oral versus dermal exposure. Nevertheless the amounts of CFMP and 3-PBA obtained in the volunteers' blood and urine following dermal exposure are close to the limit of detection, which may create uncertainty about the results.

Moreover, these results show that the urine levels of the metabolites of lambda-cyhalothrin are very low following dermal exposure compared with those observed after ingestion. This has a major impact on the interpretation of biomonitoring data in workers exposed to lambda-cyhalothrin by the former route. In this regard, the model was used to reproduce a realistic exposure scenario for a farmworker, who is subject to continuous dermal exposure but also to oral exposure in the form of a bolus dose at lunchtime, reflecting inadvertent occupational exposure due to "hand-to-mouth behaviour" (Figure 15). In this example, we can see that the temporal profiles of metabolites in the urine of a worker, expressed as the excretion rate per hour, are greatly affected by oral exposure. In fact, even though the daily exposure by ingestion is very low compared with the daily exposure by the dermal route (approximately 400 times lower), the peaks of urinary excretion associated with oral exposure were 2.7 times higher than those associated with exposure via the skin.

In addition, the differences in the ratio of the metabolites CFMP and 3-PBA observed in the urine and confirmed by modelling suggest *a priori* that the measurement of those ratios could be used to assess the main exposure route in exposed workers and thus to differentiate between the contribution of the oral route versus the dermal route. Nevertheless, 3-PBA is also a metabolite of other pyrethroids, and thus the use of this ratio alone could lead to a false interpretation. CFMP is also a metabolite of bifenthrin, but that pyrethroid is not used in market gardening in Quebec.

Thus, measuring the specific metabolite CFMP remains a more cautious method of assessing exposure to lambda-cyhalothrin alone.

On the basis of this consideration in particular, the model was used to derive biological reference values (BRV), expressed as the daily amounts of CFMP, not to be exceeded after an episode of acute exposure to lambda-cyhalothrin or repeated long-term exposure. These BRVs were determined by simulating an absorbed dose, the value of which corresponds to the absorbed amounts of the acute reference dose by ingestion (RfD) of 0.0025 mg/kg bw/day or the chronic dose of 0.001 mg/kg bw/day established by the US EPA. The daily exposure scenario used to establish these BRVs is intended to be plausible while allowing protective values to be derived. The scenario retained is therefore the one that simulates the smallest value for the total amounts of CFMP excreted over 24 hours, or continuous daily dermal exposure for a 15-hour period. In the summer, a worker may have shifts lasting up to 15 hours. Since the kinetics by the dermal route is slower than the kinetics for oral or respiratory exposure, the amounts excreted over 24 hours will be lower, thereby leading to a lower BRV. In fact, the set of parametric values used in the model to derive the BRV was generally the one that resulted in the lowest value for BRV (namely the set from volunteer 6). The associated absorption fraction is 0.75; thus, the BRV of 0.2 nmol/kg bw/day for CFMP in the urine for acute exposure or of 0.08 nmol/kg bw/day for chronic exposure is intended to reflect a daily absorbed dose equivalent to 0.75 times the value of the acute or chronic RfD, or 0.0025 or 0.001 mg/kg bw/day. The dermal exposure dose corresponding to this absorbed dose is therefore 0.36 or 0.14 mg/kg bw/day.

In volunteers exposed to Matador® acutely by the dermal route, the dose applied to the skin was 0.25 mg/kg bw/day, or 1.45 times lower than the critical dermal exposure dose used to derive the acute BRV. Note that the RfD is established to prevent systemic health effects rather than local effects. Some volunteers complained of a stinging feeling on the skin during exposure, although these symptoms disappeared a few hours after the treated area was washed. Thus, the BRV is protective only against systemic effects but does not guarantee the absence of reversible local skin effects like those described in certain volunteers. Nevertheless, the volunteers were exposed to Matador® and not to the pure product in an undiluted formulation. Moreover, it is improbable that a worker exposed to a higher skin dose than was used with the volunteers would be able to tolerate the stinging sensation throughout the workday without washing.

7. CONCLUSION

This study has filled the gap in knowledge of the toxicokinetics of the biomarkers of exposure to lambda-cyhalothrin in humans, promoting a better interpretation of biomonitoring data and a better assessment of the risks associated with workers' exposure to this pesticide. It has also enabled us to develop a toxicokinetic model to establish the essential determinants of the biological behaviour of lambda-cyhalothrin and its metabolites, which can be used to reconstruct the doses absorbed on the basis of measurements of biomarkers for different exposure routes and temporal exposure scenarios.

The temporal profiles of the biomarkers of exposure, CFMP and 3-PBA, obtained in the volunteers' plasma and urine, and the subsequent toxicokinetic modelling showed that lambda-cyhalothrin was rapidly absorbed in the body but also rapidly eliminated from the body following oral and dermal exposure. Thus, these biomarkers reflect recent exposure.

In addition, lambda-cyhalothrin seems to be metabolized in the skin to some degree and there appears to be a difference in the metabolism of lambda-cyhalothrin into CFMP compared with 3-PBA inside the skin. There also seems to be some retention of the parent product and its metabolites by the skin. Consequently, lambda-cyhalothrin is absorbed and eliminated less rapidly in the case of dermal exposure than oral exposure. It remains to be verified whether some of the differences observed between the oral and dermal routes are due to kinetic differences or simply to biological variability. One of the limitations of this project was the small number of individuals used to establish the kinetics of the biomarkers in humans, for reasons of feasibility.

Nevertheless, despite these differences, absorption by the skin is limited (low constant of absorption and thus low absorbed fraction), according to the data obtained. The dermal exposure doses must therefore be very high in order to contribute significantly to doses absorbed by multiple routes (combination of oral, dermal and respiratory routes).

In addition, the data from volunteers and the toxicokinetic modelling showed that the kinetics of the biomarkers of exposure to lambda-cyhalothrin was similar to that of the metabolites of permethrin and cypermethrin. These similar kinetic data suggest that both 3-PBA, which is common to several pyrethroids, and the more specific metabolites of lambda-cyhalothrin, permethrin and cypermethrin (CFMP, *trans*- and *cis*-DCCA) are appropriate for assessing overall exposure to pyrethroids.

This new toxicokinetic knowledge and the modelling done in this project can be used directly to interpret biological monitoring data on exposure in workers exposed to lambda-cyhalothrin or other pyrethroids. The project also allowed us to derive a biological reference value that can be used in biomonitoring as a benchmark to assess the risks associated with exposure to lambda-cyhalothrin in workers. During biological monitoring, a 24-hour urine collection period (extending from the start of the shift to the start of the following shift) is preferable because it can easily be compared to the proposed BRV. Exceeding this value would be an indicator that the workplace health and safety practices should be modified to reduce any harmful effects on health.

REFERENCES

Anadon, A., Martinez, M., Martinez, M. A., Diaz, M. J., & Martinez-Larranaga, M. R. (2006). Toxicokinetics of lambda-cyhalothrin in rats. *Toxicology Letters, 165*(1), 47–56. doi:10.1016/j.toxlet.2006.01.014

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. *Toxicology Letters, 211*(1), 1–9. doi:10.1016/j.toxlet.2012.02.012

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, 24*(6), 5841–5856.

Bouchard, M., Carrier, G., & Brunet, R. C. (2008). Assessment of absorbed doses of carbaryl and associated health risks in a group of horticultural greenhouse workers. *International Archives of Occupational and Environmental Health, 81*(3), 355–370. doi:10.1007/s00420-007-0220-1

Bouchard, M., Carrier, G., Brunet, R. C., Bonvalot, Y., & Gosselin, N. H. (2005). Determination of biological reference values for chlorpyrifos metabolites in human urine using a toxicokinetic approach. *Journal of Occupational and Environmental Hygiene, 2*(3), 155–168. doi:10.1080/15459620590922407

Bouchard, M., Carrier, G., Brunet, R. C., Dumas, P., & Noisel, N. (2006). Biological monitoring of exposure to organophosphorus insecticides in a group of horticultural greenhouse workers. *Annals of Occupational Hygiene, 50*(5), 505–515. doi:10.1093/annhyg/mel005

Bouchard, M., Gosselin, N. H., Brunet, R. C., Samuel, O., Dumoulin, M.-J., & Carrier, G. (2003). A toxicokinetic model of malathion and its metabolites as a tool to assess human exposure and risk through measurements of urinary biomarkers. *Toxicological Sciences, 73*(1), 182–194. doi:10.1093/toxsci/kfg061

Bouchard, M., Ratelle, M., & Côté, J. (2016). *Développement et application d'une approche pour l'évaluation de l'exposition des travailleurs agricoles aux pyréthrinoïdes.* IRSST research report 2010-0009 submitted to the Institut de recherche en santé et sécurité du travail du Québec.

Brzak, K. (2000*). A rising dose toxicology study to determine the no-observable-effect-levels (NOEL) for erythrocyte acetylcholinesterase (AChE) inhibition and cholinergic signs and symptoms of chlorpyrifos at three dose levels – Part B* (Report No 981176). Toxicology & Environmental Research and Consulting, Dow Chemical Company.

Canadian Health Measures Survey. (2013). *Second report on human biomonitoring of environmental chemicals in Canada.* Ottawa, ON: Health Canada.

Chester, G., Sabapathy, N., & Woollen, B. (1992). Exposure and health assessment during application of lambda-cyhalothrin for malaria vector control in Pakistan. *Bulletin of the World Health Organization, 70*(5), 615–619.

Choi, J., Rose, R. L., & Hodgson, E. (2002). In vitro human metabolism of permethrin: The role of human alcohol and aldehyde dehydrogenases. *Pesticide Biochemistry and Physiology, 74*(3), 117–128. doi:10.1016/S0048-3575(02)00154-2

Choi, J.-S., & Soderlund, D. M. (2006). Structure–activity relationships for the action of 11 pyrethroid insecticides on rat Na v 1.8 sodium channels expressed in *Xenopus* oocytes. *Toxicology and Applied Pharmacology, 211*(3), 233–244. doi:10.1016/j.taap.2005.06.022

Clark, J. M., & Symington, S. B. (2007). Pyrethroid action on calcium channels: Neurotoxicological implications. *Invertebrate Neuroscience, 7*(1), 3–16.

Côté, J., Bonvalot, Y., Carrier, G., Lapointe, C., Fuhr, U., Tomalik-Scharte, D., … Bouchard, M. (2014). A novel toxicokinetic modeling of cypermethrin and permethrin and their metabolites in humans for dose reconstruction from biomarker data. *PLoS One, 9*(2), e88517. doi:10.1371/journal.pone.0088517

Côté, J., & Bouchard, M. (2018). Dose reconstruction in workers exposed to two major pyrethroid pesticides and determination of biological reference values using a toxicokinetic model. *Journal of Exposure Sciences and Environmental Epidemiology, 28*(6), 599–614.

Couture, C., Fortin, M.-C., Carrier, G., Dumas, P., Tremblay, C., & Bouchard, M. (2009). Assessment of exposure to pyrethroids and pyrethrins in a rural population of the Montérégie area, Quebec, Canada. *Journal of Occupational and Environmental Hygiene, 6*(6), 341–352. doi:10.1080/15459620902850907

Crow, J. A., Borazjani, A., Potter, P.M., & Ross, M. K. (2007). Hydrolysis of pyrethroids by human and rat tissues: examination of intestinal, liver and serum carboxylesterases. *Toxicology and Applied Pharmacology*, *221,* 1–12. doi:10.1016/j.taap.2007.03.002

European Food Safety Authority. (2006). *Guidance for the setting and application of acceptable operator exposure levels (AOELs)*. Brussels: European Commission, Health & Consumer Protection Directorate-General.

European Food Safety Authority. (2014). Conclusion on pesticide peer review. Conclusion on the peer review of the pesticide risk assessment of the active substance lambda-cyhalothrin. *EFSA Journal, 12*(5), 1–170.

Ferland, S., Côté, J., Ratelle, M., Thuot, T., & 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. *Annals of Occupational Hygiene, 59*(9), 1152-1167. doi:10.1093/annhyg/mev059

Fetoui, H., & Gdoura, R. (2012). Synthetic pyrethroid increases lipid and protein oxidation and induces glutathione depletion in the cerebellum of adult rats: Ameliorative effect of vitamin C. *Human and Experimental Toxicology, 31*(11), 1151–1160. doi:10.1177/0960327112444478

Field, L. M., Davies, T. E., O'Reilly, A. O., Williamson, M. S., & Wallace, B. A. (2017). Voltage-gated sodium channels as targets for pyrethroid insecticides. *European Biophysics Journal, 46*(7), 675–679. doi:10.1007/s00249-016-1195-1

Food and Agriculture Organization. (2003). *FAO evaluation report. Lambda-cyhalothrin.* Retrieved from http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation08/Lambda-cyhalotrhin.pdf

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

Fu, J., Sadgrove, M., Marson, L., & Jay, M. (2016). Biotransformation capacity of carboxylesterase in skin and keratinocytes for the penta-ethyl ester prodrug of DTPA. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 44*(8), 1313–1318. doi:10.1124/dmd.116.069377

Gorse, I., & Balg, C. (2013). *Bilan des ventes de pesticides au Québec pour l'année 2010.* Quebec, QC: Ministère du Développement durable, de l'Environnement et des Parcs.

Gosselin, N. H., Bouchard, M., Brunet, R. C., Dumoulin, M.-J., & Carrier, G. (2004). Toxicokinetic modeling of parathion and its metabolites in humans for the determination of biological

reference values. *Toxicology Mechanisms and Methods, 15*(1), 33–52. doi:10.1080/15376520490446392

Gueguen, Y., Mouzat, K., Ferrari, L., Tissandie, E., Lobaccaro, J., Batt, A., … Gourmelon, P. (2006). [Cytochromes P450: Xenobiotic metabolism, regulation and clinical importance.] *Annales de biologie clinique, 64*(6), 535–548.

He, F., Wang, S., Liu, L., Chen, S., Zhang, Z., & Sun, J. (1989). Clinical manifestations and diagnosis of acute pyrethroid poisoning. *Archives of Toxicology, 63*(1), 54–58. doi:10.1007/BF00334635

International Programme on Chemical Safety. (1990). *Cyhalothrin. Environmental Health Criteria 99.* Geneva, Switzerland: World Health Organization.

Kaneko, H., & Miyamoto, J. (2001). Pyrethroid chemistry and metabolism. *Handbook of Pesticide Toxicology, 2,* 1263–1288. doi:10.1016/B978-0-12-374367-1.00076-8

Khemiri, R., Côté, 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. *Toxicology letters*, 276, 115–121. doi:10.1016/j.toxlet.2017.05.022

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

Krieger, R. I., & Thongsinthusak, T. (1993). Captan metabolism in humans yields two biomarkers, tetrahydrophthalimide (THPI) and thiazolidine-2-thione-4-carboxylic acid (TTCA) in urine. *Drug and Chemical Toxicology, 16*(2), 207–225. doi:10.3109/01480549309031997

Leng, G., Leng, A., Kühn, K.-H., Lewalter, J., & Pauluhn, J. (1997). Human dose-excretion studies with the pyrethroid insecticide cyfluthrin: Urinary metabolite profile following inhalation. *Xenobiotica*, *27,* 1273–1283.

Le Quesne, P., Maxwell, I., & Butterworth, S. (1981). Transient facial sensory symptoms following exposure to synthetic pyrethroids: A clinical and electrophysiological assessment. *NeuroToxicology, 2*(1), 1–11.

Ministère de l'Agriculture, Pêcheries & Alimentation. (2016a). Croissance de l'emploi dans l'industrie bioalimentaire au Québec. *BioClips, 24*(34), 2. Retrieved from https://www.mapaq.gouv.qc.ca/SiteCollectionDocuments/Bioclips/Bioclips2016/Volume_24_no34.pdf

Ministère de l'Agriculture, Pêcheries et Alimentation. (2016b). Les travailleurs étrangers temporaires: une présence importante dans le secteur bioalimentaire du Québec. *BioClips, 24*(14), 2. Retrieved from https://www.mapaq.gouv.qc.ca/SiteCollectionDocuments/Bioclips/Bioclips2016/Volume_24_no14.pdfaspx

Ministère du Développement durable, Environnement et Lutte contre les changements climatiques. (2016). *Bilan des ventes de pesticides au Québec 2016: Tableau des ingrédients actifs par groupe chimique.* Retrieved from http://www.mddelcc.gouv.qc.ca/pesticides/bilan/2016/tableau-ia-groupes-chimiques.pdf

National Health and Nutrition Examination Survey. (2009). *Fourth national report on human exposure to environmental chemicals. National Health and Nutrition Examination Survey*. Atlanta, GA. Retrieved from https://www.cdc.gov/exposurereport/pdf/fourthreport.pdf

National Health and Nutrition Examination Survey. (2017). *The fourth report on human exposure to environmental chemicals, updated tables, January 2017*. Atlanta, GA. Retrieved from https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume1_Jan2017.pdf

Nishi, K., Huang, H., Kamita, S. G., Kim, I. H., Morisseau, C., & Hammock, B. D. (2006). Characterization of pyrethroid hydrolysis by the human liver carboxylesterases hCE-1 and hCE-2. *Archives of Biochemistry and Biophysics,* 445, 115–123. doi:10.1016/j.abb.2005.11.005

Oulhote, Y., & Bouchard, M. F. (2013). Urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in Canadian children. *Environmental Health Perspectives (Online), 121*(11–12), 1378–1384. doi:10.1289/ehp.1306667

Pest Management Regulatory Agency. (2004). *Proposed regulatory decision document PRDD2004-02. Lambda-cyhalothrin.* Ottawa, ON: Health Canada. Retrieved from http://publications.gc.ca/collections/collection_2011/sc-hc/H113-24-2010-43-eng.pdf

Pest Management Regulatory Agency. (2017). *Proposed reevaluation decision PRVD2017-03. Lambda-cyhalothrin.* Ottawa, ON: Health Canada. Retrieved from http://publications.gc.ca/collections/collection_2011/sc-hc/H113-24-2010-43-eng.pdf

Ratelle, M., Côté, J., & 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. *Journal of Applied Toxicology, 35*(12), 1586–1593. doi:10.1002/jat.3124

Ratelle, M., Côté, J., & Bouchard, M. (2015b). Toxicokinetics of permethrin biomarkers of exposure in orally exposed volunteers. *Toxicology Letters, 232*(2), 369–375. doi:10.1016/j.toxlet.2014.12.003

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

Renwick, A. G. (2008). Toxicokinetics. In A. W. Hayes (Ed.), *Principles and methods of toxicology* (5th ed., pp. 179–230). New York, NY: Informa Health Care.

Righi, D. A., Xavier, F., & Palermo-Neto, J. (2009). Effects of type II pyrethroid cyhalothrin on rat innate immunity: A flow cytometric study. *International Immunopharmacology, 9*(1), 148–152. doi:10.1016/j.intimp.2008.10.009

Ross, M. K., Borazjani, A., Edwards, C. C., & Potter, P. M. (2006). Hydrolytic metabolism of pyrethroids by human and other mammalian carboxylesterases. *Biochemical Pharmacology*, *71*(5), 657–669. doi:10.1016/j.bcp.2005.11.020

Saillenfait, A. M., Ndiaye, D., & Sabate, J. P. (2015). Pyrethroids: Exposure and health effects – An update. *International Journal of Hygiene and Environmental Health, 218*(3), 281–292. doi:10.1016/j.ijheh.2015.01.002

Schettgen, T., Heudorf, U., Drexler, H., & Angerer, J. (2002). Pyrethroid exposure of the general population – Is this due to diet? *Toxicology Letters, 134*(1), 141–145. doi:10.1016/S0378-4274(02)00183-2

Shelton, J. F., Geraghty, E. M., Tancredi, D. J., Delwiche, L. D., Schmidt, R. J., Ritz, B., … Hertz-Picciotto, I. (2014). Neurodevelopmental disorders and prenatal residential proximity to agricultural pesticides: The CHARGE study. *Environmental Health Perspectives, 122*(10), 1103–1109.

Syngenta. (2014). *Approved pamphlet Matador 120EC 24984 2014-04-30.* Guelph, ON: Syngenta Canada Inc.

Tornero-Velez, R., Davis, J., Scollon, E., Starr, J. M., Setzer, R. W., Goldsmith, M., … DeVito, M. J. (2012). A pharmacokinetic model of cis- and trans-permethrin disposition in rats and humans with aggregate exposure application. *Toxicological Sciences, 130*(1), 33–47. doi:10.1093/toxsci/kfs236

United States Environmental Protection Agency. (2004). *Lambda-cyhalothrin and an isomer gamma-cyhalothrin; Tolerances for residues* (Publication no. EPA OPP-2005-0293). Washington, DC: EPA. Retrieved from https://www.gpo.gov/fdsys/pkg/FR-2004-04-08/pdf/04-7979.pdf

United States Environmental Protection Agency. (2008). *Reregistration eligibility decision (RED) for cypermethrin (revised 01/14/08)* (Publication no. EPA OPP-2005-0293). Washington, DC: EPA. Retrieved from https://nepis.epa.gov/Exe/ZyPDF.cgi/P100BE6X.PDF?Dockey=P100BE6X.PDF

United States Environmental Protection Agency. (2009). *Reregistration eligibility decision (RED) for permethrin* (Publication no. EPA 738-R-06-017). Washington, DC: EPA. Retrieved from https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/red_PC-109701_11-May-09.pdf

Vermeulen, R., Stewart, P., & Kromhout, H. (2002). Dermal exposure assessment in occupational epidemiologic research. *Scandinavian Journal of Work, Environment and Health, 28*(6), 371–385.

Viau, C., Lafontaine, M., & Payan, J. (2004). Creatinine normalization in biological monitoring revisited: The case of 1-hydroxypyrene. *International Archives of Occupational and Environmental Health, 77*(3), 177–185. doi:10.1007/s00420-003-0495-9

Viel, J.-F., Warembourg, C., Le Maner-Idrissi, G., Lacroix, A., Limon, G., Rouget, F., … Chevrier, C. (2015). Pyrethroid insecticide exposure and cognitive developmental disabilities in children: The PELAGIE mother–child cohort. *Environment International, 82,* 69–75. doi:10.1016/j.envint.2015.05.009

Walters, J. K., Boswell, L. E., Green, M. K., Heumann, M. A., Karam, L. E., Morrissey, B. F., & Waltz, J. E. (2009). Pyrethrin and pyrethroid illnesses in the Pacific Northwest: A five-year review. *Public Health Reports, 124*(1), 149–159. doi:10.1177/003335490912400118

Watkins, D. J., Fortenberry, G. Z., Sánchez, B. N., Barr, D. B., Panuwet, P., Schnaas, L., … Hernández-Ávila, M. (2016). Urinary 3-phenoxybenzoic acid (3-PBA) levels among pregnant women in Mexico City: Distribution and relationships with child neurodevelopment. *Environmental Research, 147,* 307–313. doi:10.1016/j.envres.2016.02.025

Woollen, B. H., Marsh, J. R., Laird, W. J., & Lesser, J. E. (1992). The metabolism of cypermethrin in man: Differences in urinary metabolite profiles following oral and dermal administration. *Xenobiotica, 22*(8), 983–991. doi:10.3109/00498259209049904

World Health Organization. (2013). *WHO specifications and evaluations for public health pesticides. Lambda-cyhalothrin.* Geneva, Switzerland: World Health Organization.

Yang, D., Wang, X., Chen, Y.-t., 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. *Toxicology and Applied Pharmacology, 237*(1), 49–58. doi:10.1016/j.taap.2009.02.012

APPENDIX A

The conceptual model of lambda-cyhalothrin is represented here by differential equations. The compartments M(t), MD(t), U(t) and F(t) can be for either the metabolite CFMP or the metabolite 3-PBA. The dose g(t) (g_{oral}, g_{inh}, g_{dermal}) corresponds to the bioavailable dose per unit of time. This dose is equal to D_{exp} (t) x f_{abs}.

$$\frac{dD(t)dt}{dt} = -k_{DDin}D(t) + g_{dermal}(t) \quad (6)$$

$$\frac{dGI(t)}{dt} = -k_{abs\_oral}GI(t) + g_{oral}(t) \quad (7)$$

$$\frac{dRT(t)}{dt} = -k_{abs\_inh}RT(t) + g_{inh}(t) \quad (8)$$

$$\frac{dB(t)}{dt} = k_{abs_{oral}}GI(t) + k_{abs_{inh}}RT(t) + k_{SB}S(t) + k_{DinB}D_{in}(t) - \left(k_{BS} + k_{BM} + k_{BM\_NO}\right)B(t) \quad (9)$$

$$\frac{dS(t)}{dt} = k_{BS}B(t) - k_{SB}S(t) \quad (10)$$

$$\frac{dM(t)}{dt} = k_{BM}B(t) + k_{MDM}MD(t) - (k_{MU} + k_{MF})M(t) \quad (11)$$

$$\frac{dM_{non\ observés}(t)}{dt} = k_{BM\_NO}B(t) + k_{DinMD\_NO}D_{in}(t) \quad (12)$$

$$\frac{dD_{in}(t)}{dt} = k_{DDin}D(t) - (k_{DinMD_{NO}} + k_{DinMD} + k_{DinB})D_{in}(t) \quad (13)$$

$$\frac{dMD(t)}{dt} = k_{DinMD}D_{in}(t) - k_{MDM}MD(t) \quad (14)$$

$$\frac{dU(t)}{dt} = k_{MU}M(t) \quad (15)$$

$$\frac{dF(t)}{dt} = k_{MF}M(t) \quad (16)$$