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Exposition des travailleurs aux résidus de pesticides sur les fleurs coupées et sur les produits horticoles

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Khaoula TOUMI (2018) Exposition des travailleurs aux résidus de pesticides sur les fleurs coupées et sur les produits horticoles (Thèse de doctorat). Université de Liège, Gembloux Agro-Bio Tech, Belgique (259 pages, 14 Figures, 36 Tableaux).

Résumé

Les personnes qui, durant leurs activités professionnelles, entrent en contact avec des résidus de pesticides sont exposées par diverses voies, avec des effets possibles sur leur santé. L'objectif de la thèse est d'évaluer les risques d'exposition de deux catégories de travailleurs indirectement exposés aux résidus de pesticides (composés parents et métabolites) : les fleuristes belges et les travailleurs maraîchers tunisiens. Dans un premier temps, l'exposition des fleuristes belges aux résidus de pesticides présents sur les fleurs coupées a été étudiée. Une enquête auprès des fleuristes révèle que, malgré une durée de travail assez élevée, ils ne se protègent pas assez du contact avec les résidus. L'analyse des résidus sur les fleurs coupées les plus vendues en Belgique a démontré que celles-ci sont fortement contaminées, avec 107 résidus de différents pesticides ont été détectés sur 90 échantillons. Grâce au port de gants en coton par les fleuristes, il a pu être établi que 111 résidus de différents pesticides pouvaient être transférés des fleurs vers les mains. Dans le pire des cas, l'exposition systémique estimée des fleuristes à quatre substances actives dépasse (aux concentrations maximales) les valeurs acceptables (AOEL). L'approche par bio-monitoring menée auprès des fleuristes belges et un groupe de référence ont permis de conclure que les urines de fleuristes sont significativement plus contaminées et qu'une relation linéaire existe entre l'exposition cutanée aux résidus et l'excrétion urinaire. La même approche méthodologique, hormis le biomonitoring, a été utilisée pour évaluer l'exposition de travailleurs maraîchers tunisiens aux résidus de pesticides. L'enquête révèle que la majorité des travailleurs ne se protège généralement pas durant la réalisation des tâches de réentrée malgré un temps de travail journalier prolongé. Le port de gants en coton, combiné à l'échantillonnage et l'analyse des résidus présents sur les produits horticoles manipulés, ont permis de démontrer que les échantillons de piment et de tomate sont contaminés par des résidus de pesticides, avec 7 dépassements de la LMR. Un total de 57 et 63 résidus de différents pesticides ont été détectés respectivement sur les gants portés par les travailleurs durant la récolte des tomates et des piments cultivés sous serre. L'exposition systémique des travailleurs réalisant la récolte de tomates et de piments dépasse (aux concentrations maximales) pour 9 et 15 pesticides, respectivement, leurs valeurs d'AOEL. L'étude a ainsi permis de démontrer que les fleuristes belges et les travailleurs tunisiens sont exposés durant leurs tâches habituelles à des quantités notablement élevées de nombreux résidus de pesticides dont les propriétés toxicologiques permettent de penser qu'ils pourraient engendrer à terme des effets négatifs sur la santé.

Mots-clés : résidus de pesticides, évaluation de l'exposition, caractérisation des risques, fleuristes, travailleurs

Khaoula TOUMI (2018) Worker exposure to pesticide residues on cut flowers and on horticultural products (PhD thesis). Gembloux, Belgium, University of Liege – Gembloux Agro-Bio Tech, (259 pages, 14 Figures, 36 Tables).

Summary

Persons who, during their professional tasks, come in contact with pesticide residues are exposed by various routes, with possible negative effects on their health. The aim of the thesis is to evaluate the exposure risks of two categories of workers indirectly exposed to pesticide residues (parent compounds and metabolites): Belgian florists and Tunisian market gardeners. As a first step, the exposure of Belgian florists to pesticide residues present on cut flowers was studied. A survey of florists reveals that, despite a fairly long work period, they do not protect themselves enough from contact with residues. The analysis of residues on cut flowers most sold in Belgium has shown that they are highly contaminated, with 107 residues of different pesticides were detected on 90 samples. By wearing cotton gloves by florists, it has been established that 111 residues of different pesticides could be transferred from the flowers to the hands. In the worst case, the estimated systemic exposure of florists to four active substances exceeds (at maximum concentrations) the acceptable threshold values (AOEL). The bio-monitoring approach conducted with Belgian florists and a reference group led to the conclusion that the florists' urine is significantly more contaminated and that a linear relationship exists between dermal exposure to residues and urinary excretion. The same methodological approach, except biomonitoring, was used to assess the exposure of Tunisian market garden workers to pesticide residues. The survey reveals that the majority of workers doesn't generally protect themselves during the re-entry tasks despite an extended daily working time. The wearing of cotton gloves, combined with the sampling and analysis of residues on the handled horticultural products, demonstrated that chili pepper and tomato samples are contaminated by pesticide residues, with 7 exceedances of EU MRL. A total of 57 and 63 pesticide residues were detected on gloves worn by workers during harvesting in tomato and chili pepper greenhouses, respectively. The systemic exposure of workers harvesting tomatoes and chili peppers exceeds (at maximum concentrations) their AOEL values, for 9 and 15 pesticides, respectively. The study has thus demonstrated that Belgian florists and Tunisian workers are exposed during their usual tasks to significantly high amounts of pesticide residues whose toxicological properties suggest that they could have on long-term negative effects on health.

Keywords: pesticide residues, exposure assessment, risk characterization, florists, workers.

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Table des matières

Liste des figures

Liste des tableaux

Sigles et abréviations

2CTCA	:	2-Chloro-1, 3-thiazole-5-carboxylic acid
a.s.	:	Active substance
AChE	:	Acétylcholinestérase
ADE	:	Actual Dermal Exposure
AFSCA	:	Agence Fédérale pour la Sécurité de la Chaîne Alimentaire
AOEL	:	Acceptable Operator Exposure Level
ARfD	:	Acute Reference Dose
BROWSE	:	Bystanders, Residents, Operators and WorkerS Exposure Models for plant protection products
CLP	:	Classification, labelling and packaging
DAP	:	Dialkylphosphates
DEP	:	Diethylphosphate
DETP	:	Diethylthiophosphate
DFR	:	Dislodgeable foliar residue
DL50	:	Dose létale 50
DMP	:	Dimethylphophate
DMTP	:	Dimethylthiophosphate
EC	:	European Commission
EFSA	:	European Food Safety Authority
EU	:	European Union
FAO	:	Food and Agriculture Organization of the United Nations
GC-MS/MS	:	Gas chromatography coupled to tandem mass spectrometry
GHS	:	Globally Harmonised System
HBGV	:	Health Based Guidance value
ILO	:	International Labour Organisation
IPM	:	Integrated Pest Management
Kow	:	Coefficient de partage octanol/eau
JMPR	:	Joint Meetings on Pesticide Residues
LC-MS/MS	:	Liquid chromatography coupled to tandem mass spectrometry
LOD	:	Limit of detection
LOQ	:	Limit of quantification
MRL	:	Maximum Residue Level
OECD	:	Organisation for Economic Cooperation and Development
OMS	:	Organisation Mondiale de la Santé
PDE	:	Potential dermal exposure
PHI	:	Preharvest interval
PPE	:	Personal Protective Equipment

PPP	:	Plant Protection Products
PSTI	:	Predictable Short Term Intake
REI	:	Restricted Entry Interval
SD	:	Standard Deviation
SE	:	Systemic Exposure
T	:	Duration of exposure
TC	:	Transfer Coefficient
TCPy	:	3,5,6-trichloro-2-pyridinol
ULg	:	Université de Liège
US EPA	:	United States Environmental Protection Agency
WHO	:	World Health Organization

Chapitre 1

Introduction générale

1. Contexte général de la thèse

1.1. Exposition des travailleurs : l'origine du risque

Sous la pression conjuguée de l'atteinte des exigences des standards de qualité et de viabilité économique sur des marchés très compétitifs, le recours aux produits phytopharmaceutiques (ou « pesticides ») demeure encore le plus souvent le choix de prédilection de la majorité des producteurs, notamment en ce qui concerne les cultures à haute valeur ajoutée comme la culture florale et l'horticulture en général. Les fleurs sont en effet très sensibles et elles peuvent être endommagées par de nombreux bioagresseurs (surtout insectes, acariens, moisissures et agents pathogènes) qui nuisent à leur production et/ou à leur qualité marchande. Afin d'atteindre leurs objectifs de production et de mettre sur le marché des produits floraux en quantité suffisante et de qualité satisfaisante, à des prix compétitifs, les floriculteurs utilisent des produits phytopharmaceutiques de manière intensive jusqu'à la récolte. De même, dans les cultures maraîchères conduites en production intensive, l'utilisation des produits phytopharmaceutiques est jugée nécessaire pour assurer une régularité de production, atteindre les standards de qualité et satisfaire aux exigences du marché national et international pour les fruits et légumes. L'efficacité des produits phytopharmaceutiques, pourvu que le producteur dispose des compétences, des formulations et du matériel d'épandage appropriés, n'est plus à démontrer pour la prévention des attaques des ennemis de ces cultures, ce qui les rend populaires d'autant qu'ils diminuent la pénibilité du travail sans grever le coût de production.

Malgré les avantages offerts par leur utilisation, il demeure important de rappeler que les produits phytopharmaceutiques (PPP) sont, encore aujourd'hui dans leur grande majorité, des produits nocifs ou toxiques (irritation ou corrosion cutanée ou oculaire, sensibilisation cutanée, suspicion d'être cancérogènes, mutagènes ou toxiques pour la reproduction, etc.), même si en Europe, aux USA et dans de nombreux pays les produits les plus toxiques ne peuvent plus être commercialisés. Par conséquent, leur utilisation ne peut s'envisager sans évaluer au préalable le risque de contamination des opérateurs (directement exposés durant le mélange, le chargement, l'application, le nettoyage des appareils d'épandage, l'élimination des déchets, etc.), des travailleurs (indirectement exposés pendant les activités de réentrée après l'application), des riverains (personnes passant à côté des champs au moment du traitement), des résidents (personnes qui séjournent ou habitent en bordure de champs traités), sans oublier le risque éventuel pour les consommateurs et pour l'environnement. Récemment, l'Autorité européenne de sécurité des aliments (EFSA) a publié un document-guide (EFSA, 2014) qui doit être utilisé lors du processus d'approbation de mise sur le marché pour estimer l'exposition de ces groupes potentiellement exposés (opérateurs, travailleurs, passants et résidents). Issu d'un long processus de concertation entre scientifiques et autres parties prenantes, ce document de 2014 représentait une avancée considérable dans ce domaine, même si l'EFSA elle-même reconnaissait que pour certaines situations d'exposition, des

données complémentaires devraient être générées afin d'améliorer la robustesse de plusieurs estimations.

Les risques d'exposition aux PPP sont multiples et de nombreux facteurs peuvent en être responsables, ce qui rend complexe le processus d'évaluation. Ces risques apparaissent dès qu'une personne entre en contact direct ou indirect avec les PPP ou leurs résidus durant ou après l'application. Les agriculteurs, en général, sont souvent portés à croire que ce sont seulement les personnes impliquées dans la préparation et/ou l'application de pesticides qui peuvent être exposés de façon significative aux PPP. Il est certain que la manipulation des PPP sous leur forme concentrée constitue un risque d'exposition plus élevé pour ces travailleurs. Mais, il a aussi été démontré que les personnes qui effectuent des tâches sur un site qui a préalablement été traité peuvent être exposées de façon significative, par voie cutanée principalement, mais aussi respiratoire ou même orale par contact des mains à la bouche.

L'exposition potentielle des travailleurs par contact avec les cultures préalablement traitées par des PPP est donc une préoccupation importante puisqu'ils risquent d'être exposés aux résidus de pesticides non-négligeables. Plusieurs études ont d'ailleurs confirmé le risque d'exposition des travailleurs qui réalisent des activités de réentrée et entrent en contact avec des produits et des feuillages traités avec des PPP. Une attention particulière a ainsi été accordée aux travailleurs qui réalisent des tâches des réentrées, notamment en ce qui concerne les fleurs : les chrysanthèmes (Thongsinthusak *et al.,* 1990), les œillets (Brouwer *et al.,* 1992a, b), les roses (Brouwer *et al.,* 1992c ; Thongsinthusak *et al.,* 1990), les plantes ornementales (Aprea *et al.,* 2001, 2002, 2005, 2009). Pour la culture maraîchère : les concombres (Caffareli et al 2004 ; Jurewicz *et al.,* 2009), les fraises (Caffareli *et al.,* 2004), les tomates (Caffarelli *et al.,* 2004 ; Kasiotis *et al.,* 2017 ; Kittas *et al.,* 2013 ; Ramos *et al.,* 2010) et les piments (Kasiotis *et al.,* 2017). D'autres études ont également rapportés des niveaux d'exposition pour les tâches de réentrée dans les cultures fruitières, comme les agrumes (Iwata *et al.,* 1983, 1982 ; Stamper *et al.,* 1986), les pêches (McCurdy *et al.,* 1994 ; Popendorf *et al.,* 1979 ; Schneider *et al.,* 1994 ; Spencer *et al.,* 1995) et les pommes (De Cock *et al.,* 1998 ; Tielemans *et al.,* 1999 ; Simcox *et al.,* 1999).

Il apparaît que de nombreux pesticides appliqués sont persistants, liposolubles, peuvent être délogés au contact des mains et pénètrent dans les organismes humains. Par conséquent, les travailleurs qui réalisent les activités de réentrée (la taille, l'inspection, l'entretien, la récolte, etc.) peuvent être exposés aux résidus de pesticides, de façon aigüe ou répétée et avec des effets possibles sur leur santé. Depuis 1952, des cas d'empoisonnement ont été signalés parmi des travailleurs qui réalisent des activités d'éclaircissement des pommes (Quinby et Lemmon, 1958). D'autres problèmes de santé ont été signalés chez les travailleurs exposés à long terme aux pesticides lors des activités de rentrée, notamment des dermatites (O'Connell *et al.,* 1987; Maddy and Smith 1985), des problèmes de reproduction (Abell *et al.,* 2000a, b) ou des dommages génétiques (Lander *et al.,* 2000).

Il est donc légitime de craindre des problèmes sur la santé des « travailleurs » exposés par leur métier aux résidus de pesticides dans les secteurs de production où

leur emploi est intensif (floriculture et maraîchage) et il est nécessaire d'entamer des études pour objectiver l'exposition de ces travailleurs.

Cette thèse de doctorat s'inscrit dans la volonté d'évaluer les risques d'exposition des travailleurs qui entrent au quotidien, sans le vouloir et le plus souvent sans le savoir, en contact avec des résidus de pesticides. Il nous a paru intéressant de considérer en parallèle deux groupes de travailleurs a priori très éloignés par leur contexte socio-économique, mais que les circonstances d'exposition rapprochent, à savoir les fleuristes belges, qui sont exposées aux résidus de pesticides durant la manipulation des fleurs et la préparation des bouquets, et les travailleurs maraîchers tunisiens, qui sont exposés aux résidus de pesticides durant la récolte des légumes cultivés sous serre.

Une évaluation objective du risque aura pour but d'émettre une série de recommandations pour approfondir le sujet et pour minimiser le risque d'exposition de ces travailleurs aux résidus de pesticides.

1.2. Le contexte de la production florale

En Europe, comme dans le monde, la floriculture représente une branche importante et dynamique de l'agriculture avec un fort potentiel de croissance et un poids économique majeur dans les échanges internationaux. La floriculture est devenue une profession lucrative avec un rendement potentiel beaucoup plus élevé que les autres cultures horticoles (Sudhagar *et al.,* 2013). La consommation mondiale de fleurs coupées est stupéfiante, puisqu'elle est estimée à 30 milliards d'euros par an, l'Europe et l'Amérique du Nord étant les principaux marchés (Rikken, 2010). La production de fleurs et de plantes ornementales ne cesse d'augmenter en Europe, et celle-ci possède une des plus grandes densités de production de fleurs par hectare au monde : 10 % de la superficie mondiale totale et 44 % de la production mondiale de fleurs et de plantes en pot (Commission européenne, 2017). Les volumes de production ont régulièrement progressés au cours des dernières décennies avec l'internationalisation des marchés et l'apparition progressives de nouvelles origines. Le montant total des importations extra UE de fleurs et de plantes s'élève à 1,68 milliard d'euros. Les fleurs et feuillages coupés représentent 78 % de la valeur des importations et les plantes en pot 15,5 %. Parmi les continents, les pays européens ont représenté la plus grande valeur en dollars des exportations de bouquets de fleurs en 2016, avec des expéditions s'élevant à 4 milliards de dollars (52,3% des exportations mondiales de fleurs) (Word's Top Export, 2017). La consommation européenne s'étale sur toute l'année, mais elle connaît des périodes de pointe (Saint-Valentin, Toussaint, fête des mères) nécessitant l'importation de produits non européens pour faire face à la demande. En conséquence, des millions de fleurs produites généralement au Kenya (27 % des importations de l'UE), en Éthiopie (11%) et en Équateur (11%) (Val'hor, 2017), ou ailleurs dans le monde, transitent en camion et en avion vers des marchés de consommation situés essentiellement dans les pays riches ou émergents de l'hémisphère nord, notamment la Belgique.

Comme toute production intensive sur des espaces réduits, la production florale nécessite l'utilisation de différents produits phytopharmaceutiques pour lutter contre

les nombreux parasites et les diverses maladies qui nuisent à la production et à la qualité marchande. Malgré le développement encore modeste d'un secteur « bio » et de la lutte intégrée en floriculture, l'emploi des pesticides est considéré par la grande majorité des producteurs comme nécessaire pour atteindre leur objectif de production et mettre sur le marché des produits floraux en quantité importante et en qualité satisfaisante à des prix relativement modestes. Pourvu que les produits soient autorisés pour cet usage, l'absence de limites maximales pour les résidus (LMR) pour les fleurs n'impose pas de limites à l'utilisation des pesticides, contrairement aux autres cultures dont les produits récoltés sont consommés. Ceci explique pourquoi les fleurs sont régulièrement traitées jusqu'à la récolte, exposant potentiellement les fleuristes qui les manipulent aux dépôts résiduels de ces pesticides avec des effets possibles sur leur santé. Une étude publiée en 1979 a montré la forte contamination des fleurs coupées et que 18 des 105 lots (17,7%) de toutes les fleurs importées à Miami contenaient des niveaux de résidus de pesticides supérieurs à 5 mg/kg et que trois lots avaient des niveaux supérieurs à 400 mg/kg.

Ainsi, il était légitime de penser que les fleuristes pourraient faire partie de la catégorie des « travailleurs » au même titre que les personnes qui réalisent des activités de réentrée, car lors du triage, de l'effeuillage, de la manipulation manuelle pour préparer les bouquets ou les montages floraux, ils sont en contact avec des fleurs et des verdures préalablement traitées par des pesticides, avec pour eux également des effets possibles sur leur santé.

1.3. Le contexte de la production maraîchère en Tunisie

En Tunisie, le secteur des légumes occupe une superficie d'environ 167.000 ha/an répartie sur 90.000 exploitations. La production nationale des cultures maraîchères est estimée en moyenne à 3,2 millions de tonnes. Elle se caractérise par la diversité des cultures dont les principales sont la tomate (39%), la pastèque (15%), l'oignon (12%), la pomme de terre (11,5%) et le piment (10%) (Gil, 2015). Aujourd'hui, la tomate et le piment sont des composantes essentielles du régime alimentaire tunisien et sont utilisés dans le cadre de préparations crues ou cuites (Jeder *et al.,* 2017). Les cultures maraîchères nécessitent l'utilisation de différents PPP pour faire face aux problèmes créés par les insectes et acariens nuisibles, les maladies fongiques et la prolifération des mauvaises herbes qui nuisent non seulement à la production mais aussi à la qualité marchande. Les herbicides peuvent également supprimer ou limiter le développement de plantes qui génèrent des alcaloïdes toxiques qui peuvent contaminer certaines cultures vivrières. Dans les pays en développement, les programmes de surveillance des résidus de pesticides sont souvent limités en raison de la faiblesse du cadre réglementaire et des ressources disponibles : le manque d'inspecteurs et de formateurs qualifiés, le manque de ressources humaines, financières et matérielles, l'absence d'équipement spécialisé et l'absence quasi-totale de formations et de vulgarisations auprès des travailleurs. La faiblesse de la réglementation locale et des contrôles des résidus de pesticides (respect des LMR) pour la consommation locale explique que, contrairement à ce qui se passe dans d'autres pays développés, il y ait moins de restrictions à l'utilisation des pesticides. En Tunisie, le secteur des pesticides est très marginalisé et pauvre en matière de réglementation. Le seul texte existant dans ce domaine est le décret n° 2010-2793 du

15 novembre 2010, modifiant et complétant le décret n° 92-2246 du 28 décembre 1992, qui fixe simplement les modalités et les conditions d'obtention de l'homologation et de l'autorisation de vente des pesticides. Par conséquent, les fruits et légumes sont pulvérisés plusieurs fois et jusqu'à la récolte, avec des produits chimiques souvent très toxiques qui ne sont plus approuvés en Europe, aux États-Unis et dans de nombreux autres pays grâce à une évaluation des risques étendue et à une réglementation plus restrictive. De plus, les produits récoltés sont mis sur le marché local sans tenir compte de l'intervalle de pré-récolte (DAR - délai avant récolte, en anglais PHI). Par conséquent, des niveaux élevés de résidus de pesticides peuvent être présents sur les fruits et les légumes au moment de la récolte, engendrant un risque potentiel pour la santé des consommateurs (Chourasiya *et al.,* 2015 ; Darko & Akoto, 2008 ; Elgueta *et al.,* 2017 ; Nougadère *et al.,* 2012).

Le risque d'exposition des « travailleurs » dans le secteur maraîcher est également une réalité, et comme il a été peu exploré jusqu'ici en Tunisie, il paraissait opportun de s'y intéresser.

2. Objectif de la recherche

L'objectif principal de la présente thèse de doctorat est d'évaluer les risques d'exposition des travailleurs qui sont indirectement exposés aux produits phytopharmaceutiques en entrant en contact avec des produits végétaux contaminés par des résidus de pesticides.

Deux groupes de travailleurs sont ciblés :

2.1. Les fleuristes belges :

Les fleuristes sont régulièrement en contact avec des fleurs coupées contaminées par des résidus de pesticides dans le cadre de leurs activités professionnelles. L'inquiétude soulevée, au regard des risques pour leur santé qui pourraient découler de leur exposition journalière aux résidus de pesticides, justifiait d'entreprendre une étude. La démarche adoptée repose sur les trois objectifs spécifiques suivants :

- Tout d'abord, évaluer le niveau de contamination moyen des fleurs coupées les plus vendues en Belgique (les roses, les gerberas et les chrysanthèmes) ainsi que le risque subséquent d'exposition des fleuristes lors de la préparation des bouquets.
- Ensuite, étudier l'exposition cutanée potentielle des fleuristes belges aux résidus de pesticides lors de la manipulation des fleurs et la préparation des bouquets et évaluer le risque de cette exposition en utilisant un modèle en vue de calculer l'exposition systémique théorique.
- Enfin, évaluer l'exposition totale réaliste par monitoring biologique.

2.2. Les maraîchers tunisiens :

Parallèlement à l'étude chez les fleuristes belges, une étude a été réalisée en Tunisie dont l'objectif principal était d'évaluer l'exposition des travailleurs effectuant des tâches de réentrée après l'application de pesticides sur des cultures maraichères et d'estimer les risques potentiels pour leur santé. La situation des travailleurs horticoles en Tunisie se rapproche de celle des fleuristes en Belgique. Il était donc intéressant de tenter de réaliser une étude en utilisant la même approche que pour les fleuristes. Seule la bio surveillance ne pouvait être réalisée faute de moyens d'analyse sur place. La démarche adoptée repose sur les deux objectifs spécifiques suivant :

- Tout d'abord, évaluer le niveau de contamination moyen des deux produits maraîchers les plus stratégiques en Tunisie, à savoir les piments et les tomates.
- Ensuite, étudier l'exposition cutanée potentielle des travailleurs aux résidus de pesticides lors de la récolte des piments et des tomates cultivés sous serre et évaluer le risque de cette exposition en utilisant un modèle pour calculer l'exposition systémique théorique.

3. Structure de la thèse

Les chapitres de cette thèse suivent les objectifs cités auparavant. La thèse est présentée en huit chapitres. Le **premier chapitre** présente l'introduction générale de la thèse de doctorat, les problématiques, les objectifs et les grandes lignes de la thèse. Le **chapitre deux** est une large étude bibliographique qui permet de faire une synthèse des connaissances sur les circonstances et sur le risque d'exposition des travailleurs lors de la réalisation des tâches de réentrée. Le **chapitre trois** aborde, à travers une enquête et l'analyse d'une gamme d'échantillons collectés en Belgique, les niveaux moyens de contamination des fleurs coupées les plus vendues en Belgique (les roses, les gerberas et les chrysanthèmes). Le **chapitre quatre** présente la méthodologie utilisée pour l'évaluation de l'exposition cutanée potentielle des fleuristes belges lors de la réalisation de leurs tâches professionnelles (manipulation des fleurs coupées et préparation des bouquets), la détermination du transfert des résidus présents sur les fleurs vers les mains des fleuristes et les calculs effectués pour évaluer l'exposition. Le **chapitre cinq** est consacré à la mesure de l'exposition systémique totale des fleuristes. Il reprend le développement et la validation d'une méthode analytique pour la quantification multi-résidus des pesticides dans l'urine par chromatographie liquide couplée à la spectrométrie de masse d'une part, et rapporte ensuite les résultats de l'évaluation de l'exposition totale des fleuristes belges par le moyen de la surveillance biologique (analyse des urines d'un groupe de professionnels exposés et d'un groupe de référence, au cours de trois périodes de l'année). Dans le **chapitre six** la problématique de l'exposition des travailleurs tunisiens aux résidus de pesticides lors de la récolte des piments et des tomates cultivés sous serre est abordée en suivant la même méthodologie qu'au chapitre quatre, et les expositions cutanées potentielles sont déterminées**.** Le **chapitre sept** est une discussion générale sur l'exposition, des fleuristes belges et des travailleurs horticoles tunisiens, et sur la caractérisation du risque d'exposition des travailleurs.

Enfin, **le chapitre huit** fait un bilan des travaux menés et résume les principaux résultats obtenus durant cette thèse en donnant des perspectives, des recommandations et des suggestions pour de futurs travaux de recherche.

Chapitre 2

Synthèse bibliographique

Exposure of workers to pesticide residues during re-entry activities: A review

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Abstract

Workers may be exposed to pesticide residues when they enter an area that has been previously treated in order to realize different tasks (e.g. for pruning, cutting, picking, harvesting, pest scouting) or to handle a contaminated crop commodity (e.g. sorting, bundling, packing). A review of the scientific literature on workers exposed to pesticide residues during re-entry tasks provides a comprehensive view of possible exposure routes and a better understanding of the risk assessment context, threshold values and calculation methodology. Methods assessing the risk to workers health are also reported and discussed. The impact of re-entry activities on health and factors affecting workers exposure are examined. Finally, solutions and mitigation measures aiming to reduce their exposure to pesticide residues are recommended.

Key Words: pesticide residues, workers, re-entry, risk assessment

1. Introduction

Pesticides (fungicides, herbicides, insecticides, etc.) have undoubtedly helped to control pests and diseases (Aktar *et al.*, 2009) in order to increase agricultural production qualitatively and quantitatively over decades (Alexandratos & Bruinsma, 2012). Pesticide exposure of the human body, which occurs through ingestion, inhalation and skin contact, can result in either acute or chronic effects on health. Despite all provided advantages and extensive use, some pesticides can be

associated with increased risks and human illness (Blair et al., 2014; Sarwar, 2015), including neurological disorders (Van Maele-Fabry *et al.,* 2013; Hernández *et al.,* 2016), reproduction problems (Flocks *et al.,* 2012; Hossain *et al.,* 2010), development of many types of cancers (Koutros *et al.,* 2013; Alavanja *et al.,* 2012) and metabolic diseases (Kim *et al.,* 2014). Therefore effort are made to prevent and reduce exposure and the most toxic pesticides are no more approved in Europe, USA and many other countries thanks to an extended risk assessment and more restrictive regulation.

Main source of exposure of the general population to pesticides occurs primarily through eating food and drinking water contaminated with pesticide residues (Damalas & Eleftherohorinos, 2011). Several groups can be identified based on their directly or indirectly exposure to pesticides, like occupational pesticide users (farmers, sprayers and field workers), but also families of occupational pesticide users, bystanders and residents. Workers are defined as persons who, as part of their employment, enter an area that has been treated previously with a plant protection product (PPP) ((Dong & Beauvais, 2013), or who handle a crop that has been treated with a PPP (Krol *et al.,* 2005; EFSA, 2014), or who come into contact with pesticide residues remaining on work surfaces (Kasiotis *et al.,* 2017). Physical contact with branches, leaves, fruit or vegetables in previously treated crops is responsible for the transfer of pesticides to the worker's skin during re-entry tasks such as irrigating, scouting, thinning, pruning, weeding, roguing, transplanting, staking, tying, swathing and harvesting (Baldi *et al.,* 2014). The vast majority of available studies concern operators who are exposed during loading and mixing operations or during application of the mixture, while reviews about workers are scarcely reported despite their high level of exposure to pesticide residues. However workers may be more at risk: they are working in sprayed areas during several hours compared to the operators who may finish their task within an hour. Operators are trained to prevent their exposure and wear personal protective equipment, while workers may not be informed about the risk of contamination, usually do not wear protective clothes and may not respect re-entrance intervals. Baldi *et al.* (2014) reported that during re-entry tasks their bodies can enter in contact with levels of pesticide residues that exceed those measured during application. Therefore this review focus on identification of the main issues related to pesticide residues during re-entry activities.

The objectives of such a review were to present and discuss: (1) the risk assessment steps; (2) the possible exposure routes for workers; (3) the risk assessment methods for workers; (4) the health effects on workers following re-entry activities; (5) the exposure of workers to pesticides and factors affecting exposure; and (6) the preventive and mitigation measures able to reduce workers exposure to pesticide residues.

2. Risk assessment steps

Risk assessment can be defined as a systematic process for generating a probability distribution or similar quantification that describes uncertainty about the magnitudes, timing or nature of possible health consequences associated with

possible exposure to pesticide residues (Covello *et al.,* 2013). The definition includes quantitative risk assessment, which emphasises reliance on numerical expressions of risk, and also qualitative expressions of risk, as well as an indication of the attendant uncertainties (WHO/FAO, 1995). The risk assessment process includes four steps: hazard identification, hazard characterisation (dose-response assessment), exposure assessment and risk characterisation (figure 1). In the first step (hazard identification) pesticide residues of active substances which can generate detrimental consequences on the health of farm workers after exposure during re-entry activities, according to their biological activity, their mode of action and their toxicity are identified. In the second step (hazard characterisation) the potential adverse health effects and the toxicological profile of the relevant active substances are described with mechanisms by which agents exert their toxic effects, the associated dose, route, duration and timing of exposure (Ferrario *et al.,* 2014; IPCS/OCED, 2003). This should, where possible, include a dose–response assessment and its attendant uncertainties (Ferrario *et al.,* 2014). For workers, the Health Based Guidance value (HBGV) of each active substance (metabolite included) is the Acceptable Operator Exposure Level (AOEL, in mg/kg bw/day)). The AOEL is expressed as milligrams of the chemical per kilogram body weight of the operator[1]. The European Commission (2006) defines the AOEL as follow: "AOEL relates to the internal (absorbed) dose available for systemic distribution from any route of absorption and are expressed as internal levels (mg/kg bw/day)" AOEL is also defined in the Directive 97/57/EC as "the maximum amount of active substance to which the operator may be exposed without any adverse health effects'' and "the AOEL is based on the highest level at which no adverse effect is observed in tests in the most sensitive relevant animal species or, if appropriate data are available, in humans. However, in some cases, serious findings requiring a large assessment factor may drive an AOEL even though less serious effects occur at lower doses in the most sensitive species" (European Commission, 2006). In the Directive, the AOEL specifically refers to operators but it can also be used for workers exposed during re-entry and for residents or bystanders non-intentionally exposed (European Commission, 2006). In the third step (exposure assessment), the intensity, the frequency and the duration of workers exposure to pesticide residues are measured or evaluated in relation to the observed re-entry activities. The exposure assessment will consider realistic and high exposure (or even worst case) scenarios for the proposed good agricultural practices and then non-dietary systemic exposure values can be compared with the appropriate toxicological reference values (AOEL) (EFSA, 2014). In the last step (risk characterisation), the results of the exposure assessment (step 3) are compared to the HBGV values to determine the risk level for health of workers (expressed in % of the AOEL).

[1] La plupart des valeurs d'AOEL considérées dans la thèse sont issues de la base de données européenne (EU Pesticides Database). Il faut cependant noter que les valeurs d'AOEL sont adaptées régulièrement (mises à jour).

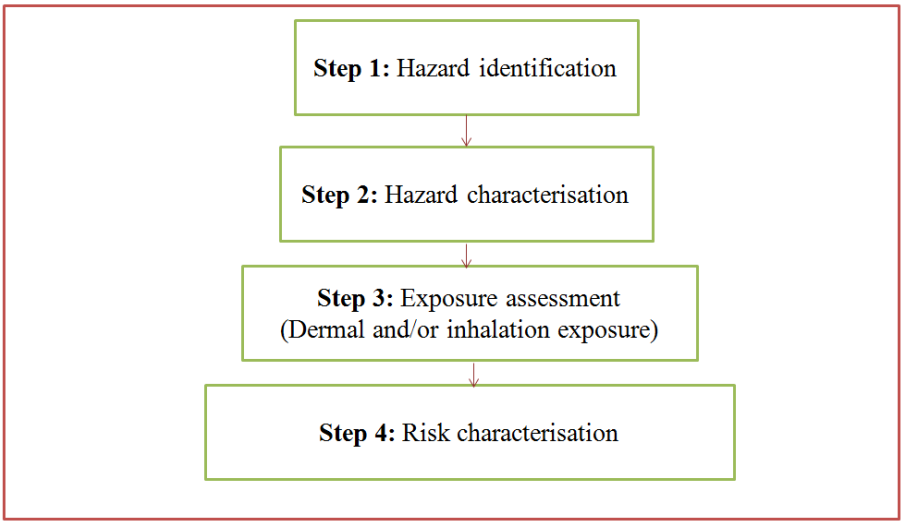

Figure 1: The 4 steps of the risk assessment process

3. Possible exposure routes for workers

The routes of exposure during activities performed by a worker in the field after the application of PPP are the same as those of the operator. Dermal exposure is the most important route of exposure (Brouwer *et al.,* 1992 a, b, c; Van Hemmen & Brouwer, 1997; Jurewicz *et al.,* 2009) but other routes (inhalation and ingestion) can also contribute to the total worker exposure (Aprea, 2001; Cherrie *et al.,* 2006). Therefore, workers may be exposed to pesticide residues by various routes during working in treated fields:

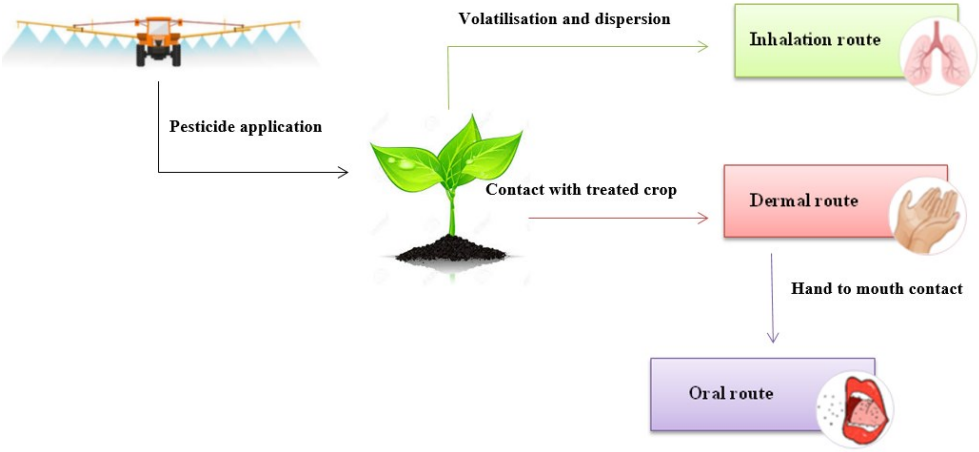

Figure 2: Possible exposure routes for workers

3.1. Dermal route

Dermal exposure occurs by direct and physical contact with the contaminated crop. Pesticides residues on branches, leaves, fruit or vegetables previously treated can be transferred to the skin of workers. Many actives substances are persistent, fat-soluble and therefore could be partly dislodged by contact with the skin (Toumi *et al.,* 2016a, b; 2017a, b; Toumi *et al.,* 2018). The main dermal routes result from re-entry tasks such as harvesting, bending, leaf pulling and tying, etc. During some other activities, a part of the dermal exposure can result from a contact with a contaminated soil. The intensity of the contact with the treated foliage, the amount of residues on the foliage, the duration of the re-entry activity and the port of personal protective equipment are considered to be the main factors influencing the dermal exposure (Sankaran *et al.,* 2015; Kasiotis *et al.,* 2017).

3.2. Inhalation route

Inhalation exposure may result from a concentration of pesticide in the air (vaporization indoor or outdoor) or from airborne particles contaminated with pesticides (Brouwer *et al.,* 1992b). Exposure occurs during re-entry activities by inhalation of contaminated air (e.g. dust) or vapours (e.g. volatile or semi-volatile compounds). After application, pesticide droplets are usually dispersed in the air (Stearns *et al.,* 1952). When the spray has dried, the dust has settled and the vapours are dissipated, most of the particles can be found on the foliage and soil (Krieger *et al.,* 2007). The inhalation exposure mainly depends on the concentration of the substance in the air, the breathing rate and the duration of exposure. Greenhouse temperature, ventilation rate, the vapour pressure of the substance and the ratio between adsorption and volatilisation processes were identified as important factors influencing volatilisation in greenhouses (Doan Ngoc *et al.,* 2015). Nevertheless, during re-entry activities inhalation exposure is very low compared to the dermal exposure (Popendorf *et al.,* 1979; Spear *et al.,* 1977; Aprea *et al.,* 2002), but a good correlation exist between levels of airbone pesticides and levels of dislogeables foliar dust (Popendorf et al., 1975, 1980). The inhalation exposure to dusted pesticides after re-entry is about of the same order of magnitude as during the application itself when adjusted for exposure time (Brouwer *et al.*, 1992a). When the duration of re-entry tasks increases than the exposure of workers through inhalation may probably be still much higher and, in some situations, may also result in health risks.

3.3. Oral route

Oral exposure is the third possible route but is generally considered the least important in a worker exposure scenario. Skin contamination may sometimes lead to oral non-dietary exposure (Aprea *et al.* 2000). This can happen through hand to mouth transfer after facial contact or when pesticide residues on hands contaminate food or tobacco products (Ulenbelt *et al.* 1990; Fenske *et al.* 1993). Oral exposure may also occur secondarily when an air loaded with particles containing active substances enter in the mouth cavity and those are then subsequently ingested.

However, the potential exposure by this route is generally assumed to be negligible for workers in comparison with the ones via skin and inhalation (EFSA 2014; Doan Ngoc *et al.* 2014).

4. Risk assessment methods for workers

Various approaches have been suggested in the scientific literature to assess the risk for the health of workers after exposure to pesticide residues during re-entry activities. The main approaches are summarised in this section.

4.1. Direct methods used for exposure assessment

In the direct method the pesticide residues are trapped when they enter in contact with workers (e.g. contact with dried residue of the diluted product on foliage) (Li *et al.,* 2011). Dermal and respiratory exposures during re-entry tasks are estimated with the same approach than exposure measurements during mixing and loading or application. Direct methods are considered easier to perform than indirect methods, but the total estimated potential exposure is more difficult to link to the absorbed dose (total exposure) because measurement is limited to the amount of pesticides retained on external clothing, patches or gloves (Li *et al.,* 2011). Many practical measurement methods have been developed and are used to estimate the pesticide exposure of workers when re-entering an area after pesticide application. Table 1 presents a list of methods that cover a range of approaches which were reviewed by various authors over time to increase their performance.

Table 1: Direct methods used to estimate the exposure of workers during re-entry activities, sampling method, measured compartment and the references

Method	Measured compartment	Sampling method	References
Dermal exposure of workers			
Dosimetry : Surrogate skin (passive)	Skin	**Gloves** Glove exposure assessment methods measure dermal exposure to hands by analyzing the residues on gloves (cotton, rubber latex, etc.) worn during manual activities in treated crops. At the end of the trial, pesticide residues on gloves are extracted and the amount of pesticide is measured.	Brouwer *et al.,* 1992 a, b, c Aprea *et al.,* 1999 Fenske *et al.,* 1999 Aprea *et al.,* 2002 Jurewicz *et al.,* 2009 Ramos *et al.,* 2010 Li *et al.,* 2011 Sankaran *et al.,* 2015 Kasiotis *et al.,* 2017 Toumi *et al.,* 2018
	Skin	**Patches/pads** Method of measurement using patches (mainly tissues or absorbent substances like cotton or cellulose) distributed over different regions of the worker's body (shoulders, neck, chest, forearm, lower arm, upper and lower thigh) or on work clothes. The patches are attached to clothing before workers enter in the treated area. At the end of the trial, pesticide residues on patches are extracted and the amount of pesticide/cm² is measured. The deposits are then converted to a distribution on the whole body using a table. The method allows a semi-quantitative estimate of the external skin contamination of the workers.	Ware *et al.,* 1973 Spencer *et al.,* 1991 Aprea *et al.,* 1999 Aprea *et al.,* 2001 Aprea *et al.,* 2002 Aprea *et al.,* 2005 Aprea *et al.,* 2009 Jurewicz *et al.,* 2009 Bradman *et al.,* 2009 Baldi *et al.,* 2014

Method	Measured compartment	Sampling method	References
	Skin	**Whole body (coverall)** Cotton or polyester whole body dosimeters are long-sleeved shirts and long-legged pants worn by workers (sometimes internal and external coveralls) to collect by contact the pesticide residues during the tasks. At the end of the trial, the coverall is cut in various pieces on which pesticide residues are extracted, analysed and quantified for each part of the body.	McCurdy *et al.,* 1994 Ramos *et al.,* 2010 Kasiotis *et al.,* 2017
Removal techniques	Skin	**Hand washes and wipes** Pesticide residues deposited on skin can be removed by washing or wiping. Water-surfactant mixture or water-alcohol wash solution are generally used only to assess hand exposure, while wiping techniques can in theory be applied to larger and more diverse skin surfaces. Hand washes and skin wipes have been used for dermal exposure sampling since they collect residues that are potentially available for skin absorption.	Ware *et al.,* 1974 Ware *et al.,* 1975 Spencer *et al.,* 1991 McCURDY *et al.,* 1994 Aprea *et al.,* 1994 Aprea *et al.,* 1999 Fenske *et al.,* 1999 Aprea *et al.,* 2001 Aprea *et al.,* 2002 Curwin *et al.,* 2003 Aprea et al., 2005 Aprea et al., 2009 Bradman et al., 2009 Baldi et al., 2014 Sankaran *et al.,* 2015

Method	Measured compartment	Sampling method	References
Tracer techniques	Skin	**Video imaging technique (Video Imaging Technique to Assess Exposure (VITAE))** A fluorescent imaging system provides an illuminated image of an object that has been subjected to a fluorescent dye, as well as a fluorescence image of the object. This technique can be used to measure transfer of pesticide residues from surfaces to hands during re-entry activities.	Archibald *et al.,* 1994a Archibald *et al.,* 1994b
Inhalation exposure of workers			
Personal air sampling	Air (inhalated)	**Personal air sampler** Personal air sampling is a technique for personal monitoring. The sampling equipment is carried around by the worker during re-entry activities and pesticide in the air is filtered and fixed on an absorbent. Air concentrations of pesticides, measured after desorption with a solvent, are used to calculate the actual respiratory dose and inhalation exposure on the basis of lung ventilation.	Ware *et al.,* 1973 Ware *et al.,* 1974 Ware *et al.,* 1975 Spencer *et al.,* 1991 Brouwer *et al.,* 1992a, b Brouwer *et al.,* 1993 Aprea *et al.,* 1999 Aprea *et al.,* 2001 Aprea *et al.,* 2002 Aprea *et al.,* 2005 Aprea *et al.,* 2009

4.2. Indirect methods used for exposure assessment

Indirect methods provide indirect indication of potential for skin exposure, included environmental monitoring (plant surface-sampling techniques and determination of the Dislodgeable Foliar Residue) and biological monitoring (measurement of a pesticide, its metabolite(s) or reaction product(s) in various biological matrices) (van Hemmen *et al.,* 2006; Li *et al.,* 2011).

4.2.1. Environmental monitoring : the Dislodgeable Foliar Residue (DFR)

Currently the most widely used technique for calculating worker exposure during re-entry activities is by quantifying the dislodgeable foliar residues (DFR), which are indirect estimates of total surface foliar residues "available" after spraying for transfer from leaf and other vegetative surfaces to workers bodies (Chowdhury *et al.,* 2001; Korpalski *et al.,* 2005; Dong & Beauvais, 2013; EFSA, 2014; Sankaran *et al.,* 2015). The dislodgeable foliar residue (DFR) is defined as the amount of pesticide residue that can be removed from both sides (the top and the bottom) of the treated leaves using an extraction procedure with an aqueous surfactant (Iwata *et al.,* 1977). The DFR value is reported in amount of residue per unit of leaf area (µg/cm²). Based on the DFR measurement it is possible to approximate the potential dermal exposures to pesticide residues for workers re-entering treated crops. Re-entry exposures were estimated using the DFR values for many active substances applied to many different crops over the time (Table 2). In absence of specific data, a default value of 3 $\mu g/cm^2/kg$ active substance/ha is used (EFSA 2014), based on the 90th percentile of DFR data extracted from literature (Van Hemmen *et al.,* 2002)

Table 2: DFR (µg/cm^2) of different actives substances reported by many studies over the time, with the crops, the region, the re-entry activities and time since application and the exposure period (presented in chronological order from 1973 to 2017)

Active Substances	**Crop**	**Country (Region)**	**DFR (µg/cm^2)**	**Re-entry Activity**	**Re-Entry Time (Since Application)**	**Exposure duration**	**References**
Azinphos-methyl	Peach	USA (Washington)	2.62	Thinning	-	-	Foster, 1973
	Apple						
	Peach	USA (California)	2.10	Thinning	-	-	Kraus *et al.,* 1977
			2.63		-	-	Richards *et al.,* 1978
Carbofuran	Citrus		0.16	-	8.1day	-	Iwata *et al.,* 1983
3-Hydroxy-carbofuran			0.03		12 day		
Chlorothalonil	Tomato	-	1.13	Harvesting	-	-	Spencer *et al.,* 1991
Azinphos-methyl	Peach	USA (California)	0.82-1.72	Harvesting, Sorting	-	-	Schneider *et al.,* 1991
Abamectin	Rose	The Netherlands	0.0081	Cutting, sorting and bundling	27h	73 min	Brouwer *et al.,* 1992c
Dodemorph			0.26				
Bupirimate			0.64				
Chlorothalonil	Carnations	The Netherlands	4.22		35h	74 min	Brouwer *et al.,* 1992b
Thiophanate-methyl			4.47				
Thiram			1.10				
Zineb			1.21				
Propoxur			0.3	Cutting	-	-	Brouwer *et al.,* 1993
			0.2	Sorting, bundling	-	-	

Active Substances	Crop	Country (Region)	DFR (µg/cm^2)	Re-entry Activity	Re-Entry Time (Since Application)	Exposure duration	References
Azinphos-Methyl	Peach	USA (California)	0.64	Thinning	30 day	21 days	McCurdy *et al.,* 1994
Malathion	Strawberry		0.074	Harvesting	-	-	Hernandez *et al.,* 1997
Azinphos-Methyl	Apple	USA (Washington)	0.5	Thinning	-	-	Simcox *et al.,* 1999
Captan	Strawberry	USA (California)	0.037	Harvesting	-	-	Krieger & Dinoff, 2000
Chlorothalonil	Ornamental Plants	Italy	2.62	-	41h	-	Aprea *et al.,* 2002
			2.15		65h		
			1.66		89h		
			1.44		137h		
Malathion	Strawberry	USA (California)	0.39	-	12h	-	Hernandez *et al.,* 2002
Tetradifon	Strawberry		0.109		1 day		
			0.018		15 day		
Chlorpyrifos-ethyl	Tomato		0.092		1 day		
			0.001		16 day		
Azoxystrobin			0.107		1 day		
			0.117		16 day		
Achrinathrin			0.115		3 day		
			0.008		16 day		

Active Substances	**Crop**	**Country (Region)**	**DFR ($\mu g/cm^2$)**	**Re-entry Activity**	**Re-Entry Time (Since Application)**	**Exposure duration**	**References**
		Italy (Bracciano)				-	Cafferli *et al.,* 2005
Fenarimol	Cucumber		0.082		1 day		
			0.002		16 day		
Metalaxyl			0.116		1 day		
			0.001		16 day		
Azoxystrobin			0.126		1 day		
			0.002		20 day		
Malathion	Strawberry	USA (California)	0.22	-	1 day		Zhang, 2005
			0.014	-	10 day		
Profenofos	Jasmine	Inde (Tamil Nadu)	6.04	Picking	0 day	-	Suganthi *et al.,* 2008
			3.87		1day		
			1.53		3 day		
Malathion	Strawberry	-	0.2	Harvesting	-	-	Salvator *et al.,* 2008
			1	Harvesting		72 hours	Bradman *et al.,* 2009
Imidacloprid	Ornamental Plants	Italy	0.00102-0.80937	Stapling	-	-	Aprea *et al.,* 2009
			0.09987-1.13363				
Malathion	Strawberry	USA (California)	0.132	Harvesting	3 day		Li *et al.,* 2011

Active Substances	**Crop**	**Country (Region)**	**DFR ($\mu g/cm^2$)**	**Re-entry Activity**	**Re-Entry Time (Since Application)**	**Exposure duration**	**References**
Methidathion	Cucumber	-	0.0121-0.2225	Harvesting	7 day	44 hours	Choi *et al.,* 2013
Fenpropathrin	Strawberry	USA (California)	0.023	Harvesting	4 day	-	Sankaran *et al.,* 2015
			0.003		14 day		
Malathion			0.248		4 day		
			0.003		14 day		
Tebufenozide	Tomato	Greece	0.11	Pruning	3.5h	60 min	Kasiotis *et al.,* 2017
	Pepper		0.085	Tying	14.7h		
Bupirimate	Tomato		0.0115	Pruning	3h		
	Pepper		0.1358		16.5h		

USA : United States of America

4.2.2. Biological monitoring

Biomonitoring involves the measurement of a pesticide and its metabolite(s) or reaction product(s) in various biological matrices (urine, hair, nails, blood or blood components and tissues). This approach is often preferred because it allows an integration of all possible sources and routes of exposure and provides a complete picture of the internal dose with a better assessment of the possible associated risks (He, 1993, 1999; Anwar, 1997; Barr *et al.,* 1999, 2006; Albertini *et al.,* 2006; Ferland *et al.,* 2015). Table 3 summarises a list of active substances biomonitored for the workers during re-entry activities over time. The concentrations and its units are kept according to the original articles because each author uses specific creatinine concentrations since this may vary from a person to another.

Table 3: Active substances biomonitored for the workers during re-entry activities (presented in chronological order from 1973 to 2015), their CLP classification (according the EU Pesticides database), their chemical family, their urinary metabolites and the crop, the limit of detection and the mean concentrations ± standard deviation (range) when available

Active substance	CLP classification	Chemical family	Crop	Urinary metabolites	LOD	Mean concentration ± standard deviation (range) when available	References
Azinphos-methyl	H300, H311, H317, H330	Organophosphorus	Peach	DMTP	-	830 µg/L	Foster, 1973
			Apple				
Methyl parathion	H300, H311, H330, H373	Organophosphorus	Cotton	PNP	-	no detectable residues	Ware *et al.,* 1973
Ethyl parathion	H300, H311, H330, H372						
Methyl parathion	H300, H311, H330, H373	Organophosphorus	Cotton	PNP	-	0.50±0.49 (0.15-1.20) mg/48h	Ware *et al.,* 1974
Ethyl parathion	H300, H311, H330, H372				-	0.89±0.14 (0.74-1.01) mg/48h	
Methyl parathion	H300, H311, H330, H373	Organophosphorus	Cotton	PNP	-	1.76±0.47 (1.13-2.31) mg/48h	Ware *et al.,* 1975
Ethyl parathion	H300, H311, H330, H372					0.14±0.07 (0.09-0.25) mg/48h	
						0.12±0.03 (0.09-0.16) mg/48h	
Azinphos-methyl	H300, H311, H317, H330	Organophosphorus	Peach	DMTP	-	2850±2490 µg/L	Kraus *et al.,* 1977
						2430±2730 µg/L	

Active substance	CLP classification	Chemical family	Crop	Urinary metabolites	LOD	Mean concentration ± standard deviation (range) when available	References
						2280±2430 µg/L	
						2080±2080 µg/L	
						1020±1390 µg/L	
Azinphos-methyl	H300, H311, H317, H330	Organophosphorus	Peach	DMTP	-	14100 µg/L	Richards *et al.,* 1978
NA	-	Organophosphorus	Citrus	DMTP	20 µg/L (occasionaly 30 or 40 µg/L)	500 µg/L	Duncan & Griffith 1985
				DMDTP		600 µg/L	
				DMP		1,650 µg/L	
				DEP		650 µg/L	
				DETP		75 µg/L	
				DEDTP		60 µg/L	
				DMTP	20 µg/L (occasionaly 30 or 40 µg/L)	150±83 µg/L	Griffith & Duncan 1985
				DMDTP		250±106 µg/L	
				DMP		390±198 µg/L	
				DEP		90±7µg/L	
				DETP		70±6 µg/L	
				DEDTP		60±6 µg/L	
Propoxur	H301	Carbamates	Carnation	IPP	6 µg/L	158.3±4.4 (10.3-1231.1) µg/24h*	Brouwer *et al.,* 1993

Active substance	CLP classification	Chemical family	Crop	Urinary metabolites	LOD	Mean concentration ± standard deviation (range) when available	References
Chlorpyrifos methyl	H317	Organophosphorus	Peach	DMP DMDTP DMTP	-	3990±2035.1 (1658-8833) nmol/g creatinine	Aprea *et al.,* 1994
Azinphos methyl	H300, H311, H317, H330						
Pirimicarb	H301, H317, H331, H351	Carbamates	Chrysanthe-mum	DDHP	0.05 µg/L[¥]	no detectable residues	Archibald *et al.,* 1994b
				MDHP			
Azinphos-methyl	H300, H311, H317, H330	Organophosphorus	Peach	DMP	-	3.84 µmoles/day**	McCurdy *et al.,* 1994
				DMTP			
				DMDTP			
Chlorpyrifos methyl	H317	Organophosphorus	Vine	TCPy	5 nmol/L	92.4 ± 162.5 (4.5-748.8) nmol/g creatinine	Aprea *et al.,* 1997
				DMP	18 nmol/L	123.0 ±79.0 (22.1-302.6) nmol/g creatinine	
				DMTP	12 nmol/L	489.3 ± 288.3 (139.0-1237.7) nmol/g creatinine	

Active substance	CLP classification	Chemical family	Crop	Urinary metabolites	LOD	Mean concentration ± standard deviation (range) when available	References
Fenitrothion	H302	Organophosphorus	Ornamental plants	DMP DMTP	LOD $_{DMP}$ = 18 nmol/L, LOD $_{DMTP}$ = 12 nmol/L	278.8 ± 143.5 (80.0–270.0) nmol/g creatinine	Aprea *et al.,* 1999
						206.4 ± 117.2 (128.0–444.7) nmol/g creatinine	
						387.4 ± 178.9 (219.8–629.9) nmol/g creatinine	
Azinphos-methyl	H300, H311, H317, H330	Organophosphorus	Apple	DMTP	40 µg/L	530 µg/L	Simcox *et al.,* 1999
						290 µg/L	
						900 µg/L	
				DMDTP	-	(40-290) µg/L	
Captan	H317, H318, H331, H351	Phtalimides	Strawberry	THPI	5 µg/L[¥]	5.3 ± 4.0 (0.4–13.8) µg captan /person/day	Krieger & Dinoff, 2000
Omethoate	H301, H312					92±27 (60-130) nmol/g creatinine	
Fenitrothion	H302					122±33 (72-166) nmol/g creatinine	

Active substance	CLP classification	Chemical family	Crop	Urinary metabolites	LOD	Mean concentration ± standard deviation (range) when available	References
		Organophosphorus	Ornamental plants	DMP DMTP	LOD_{DMP} = 18 nmol/L, LOD_{DMTP} = 12 nmol/L	212±108 (101-335) nmol/g creatinine	Aprea *et al.,* 2001
Tolclofos Methyl	H317					223±54(153-291) nmol/g creatinine	
						123±59 (59-188) nmol/g creatinine	
N.A	-	Organophosphorus	Apple	DMP	0.15 µg/L	33.1 ± 3.3 µg /g creatinine*	Ueyma *et al,.* 2002
						10.8 ± 3.0 µg /g creatinine*	
				DMTP	0.05 µg/L	10.1 ± 3.4 µg /g creatinine*	
						5.8 ± 4.0 µg /g creatinine*	
				DEP	0.07 µg/L	4.2 ± 2.6 µg /g creatinine*	
						4.7 ± 2.4 µg /g creatinine*	
				DETP	0.05 µg/L	1.6 ± 2.6 µg /g creatinine*	
						0.8 ± 2.9 µg /g creatinine*	

Active substance	CLP classification	Chemical family	Crop	Urinary metabolites	LOD	Mean concentration ± standard deviation (range) when available	References
Mancozeb	H317, H361d	Carbamates	Vine	ETU	0.5 µg /g creatinine	12.5 ± 25.9 µg /g creatinine	Colosio *et al.,* 2002[1]
Chlorothalonil	H317, H318, H330, H335, H351	Chloronitriles	Ornamental plants	CHL	0.25 µg/L	1.58 ± 2.13 (0.45-8.3) µg/L	Aprea *et al.,* 2002
Azinphos-methyl	H300, H311, H317, H330	Organophosphorus	Apple	DMP DMTP DMDTP	40 µg/L	27 ± 2.4 (3.5- 310) µg/ kg/day*	Fenske *et al.,* 2003
Omethoate	H301, H312	Organophosphorus	Ornamental plants	DMP	-	(8.38–854) nmol/g creatinine	Aprea *et al.,* 2005
				DMTP		(38.3–2496) nmol/g creatinine	
Dichlorvos	H301, H311, H317, H330	Organophosphorus	N.A	DMP DMTP DMDTP		204 (23-582) nmol/g creatinine**	Bouvier *et al.,* 2006[2]
Fenthion	H302, H312, H331, H341, H372						
Malathion	H302, H317						
Methyl parathion	H300, H311, H330, H373						

Active substance	CLP classification	Chemical family	Crop	Urinary metabolites	LOD	Mean concentration ± standard deviation (range) when available	References
Chlorpyrifos	H301			DEP DETP DEDTP	0.01 µg/L[¥]	15(ND – 107) nmol/g creatinine**	
Diazinon	H302						
Ethyl parathion	H300, H311, H330, H372						
Malathion	H302, H317	Organophosphorus	Strawberry	MDA	0.3 µg/L	44.4 µg/g creatinine*	Salvatore *et al.,* 2008
				DMP	0.4 µg/L	215.4 nmol/g creatinine*	
				DMTP	0.3 µg/L		
				DMDTP	0.08 µg/L		
Ethylenebisdithio-carbamates	-	Carbamates	Flower bulbs	ETU	-	1.27 mg/mmol creatinine*	van Amelsvoort *et al.,* 2008
Malathion	H302, H317	Organophosphorus	Strawberry	MDA	0.3 µg/L	131.2 µg/g creatinine**	Bradman *et al.*, 2009
Permethrin	H302, H332, H335	Pyrethroids	Corn	3-PBA		0.206 µmol/ mol creatinine**	Ferland *et al.,* 2015
						0.449 µmol/ mol creatinine**	
						0.241 µmol/ mol creatinine**	
						0.362 µmol/ mol creatinine**	
						0.072 µmol/ mol creatinine**	

Active substance	CLP classification	Chemical family	Crop	Urinary metabolites	LOD	Mean concentration ± standard deviation (range) when available	References
						0.161 µmol/ mol creatinine**	
						0.183 µmol/ mol creatinine**	
						0.273 µmol/ mol creatinine**	
						0.228 µmol/ mol creatinine**	
						0.802 µmol/ mol creatinine**	
				trans-DCCA	0.1-0.3 µg/L	0.048 µmol/ mol creatinine**	
						0.146 µmol/ mol creatinine**	
						0.268 µmol/ mol creatinine**	
						0.102 µmol/ mol creatinine**	
						0.08 µmol/ mol creatinine**	
						0.058 µmol/ mol creatinine**	
						0.079 µmol/ mol creatinine**	
						0.197 µmol/ mol creatinine**	

Active substance	CLP classification	Chemical family	Crop	Urinary metabolites	LOD	Mean concentration ± standard deviation (range) when available	References
						0.105 µmol/ mol creatinine**	
						0.455 µmol/ mol creatinine**	
Malathion	H302, H317	Organophosphorus	Strawberry	MMA	5 µg/L¥	28.3 nmol/g creatinine	Sankaran *et al.,* 2015
				MDA″	10 µg/L ¥		
Fenpropathrin	H301, H312, H330			DMP	1 µg/L¥	16.4 nmol/g creatinine	
				DMTP	1 µg/L¥		
				DMDTP	1 µg/L¥		

3-PBA: 3-phenoxybenzoic acid, CHL: chlorothalonil urinary, DEP: diethylphosphate, DEDTP: diethyldithiophosphate, DETP: diethylthiophosphate, DDHP: 2-dimethylamino-5,6-dimethyl-4-hydroxypyrimidine, DMP: dimethylphosphate, DMDTP: dimethyldithiophosphate, DMTP: dimethylthiophosphate, ETU: Ethylenethiourea, IPP: 2-isopropoxyphenol, MMA: malathion monoacid, MDA″ : malathion diacids, MDA: malathion dicarboxylic acid, MDHP : 2-methylamino-5,6-dimethyl-4-hydroxypyrimidine, PNP: p-nitrophenol, TCPy : 3,5,6-trichloro-2-pyridinol, THPI: tetrahydrophalimide, Trans-DCCA: trans- 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanel-carboxylic acid metabolites.

1 study conducted among 12 operators and 1 worker

2 study conducted among 12 workers from different occupational places (two greenhouses, three florist shops)

NA: Non applicable

ND : Non detectable

*Geometric mean ± Geometric standard deviation (range) when available

** Median (range) when available

¥ LOQ

H300: Fatal if swallowed; H301: Toxic if swallowed; H302: Harmful if swallowed ; H311: Toxic in contact with skin; H312: Harmful in contact with skin; H317: May cause an allergic skin reaction; H318: Causes serious eye damage; H330: Fatal if inhaled; H331:Toxic if

inhaled; H332: Harmful if inhaled; H335: May cause respiratory irritation; H341: Suspected of causing genetic defects; H351: Suspected of causing cancer; H361d: suspected of damaging the unborn child; H372: Causes damage to organs through prolonged or repeated exposure; H373: May cause damage to organs through prolonged or repeated exposure

4.3. Risk assessment for workers

4.3.1. Dermal exposure

According to the EFSA Guidance Document (EFSA, 2014), dermal exposure from contact with residues on foliage should be estimated as the product of the dislodgeable foliar residue (DFR), the transfer coefficient (TC) and the task duration (T):

Potential dermal exposure (PDE) in mg a.s./day = (DFR [µg/cm^2] × TC [cm^2/h] × T [h/day])/1 000

The default value for time of exposure should be taken as eight hours a day for harvesting and maintenance type activities and two hours for crop inspection (scouting or certification) and irrigation-type activities. The actual dermal exposure is defined as the exposure to the skin that would occur in the presence of clothing and/or personal protective equipment (EFSA, 2014). In contrast to potential and actual exposures, which are external exposures, the internal dose, absorbed dose or systemic dose is the fraction of the external dose that has been absorbed and enters the general circulation (EFSA, 2016). To convert estimated dermal exposures to corresponding systemic exposures, potential dermal exposure should be multiplied by a dermal absorption factor. This factor is based on absorption values obtained in dermal absorption studies performed on the formulation or on default values (EFSA, 2012, 2017).

4.3.2. Inhalation exposure

According to the EFSA Guidance Document (EFSA, 2014), the potential exposure to a volatile substance decreases with time as its concentration decreases by absorption in plants, degradation or losses in the environment. Although in many cases, exposure by inhalation contributes far less to the total potential exposure compared to the dermal route, in some situations (e.g. orchards or greenhouses) the inhalation route is significant and needs to be calculated. For this purpose, task-specific inhalation factors should be used for first tier exposure assessments (e.g. relating to harvesting tasks indoors and to re-entering in greenhouses where pesticide droplets may remain airborne after the treatment). Inhalation exposure for these re-entry scenarios may be predicted by the following:

Potential inhalation exposure [mg a.s./h inhaled] = Application Rate [kg a.s./ha] × Task Specific Factor [ha/h × 10^{-3}]

The Task Specific Factors can be used in the first tier of the exposure and risk assessment: they have been estimated for a small set of exposure data for harvesting and re-entry in ornamental greenhouses.

4.3.3. Oral exposure

According to the BROWSE (Bystanders, Residents, Operators and Workers Exposure) models for plant protection products (BROWSE, 2014) and the EFSA Guidance Document (EFSA, 2014), the dermal exposure on the hands may become

ingested through hand-to-mouth contact. During this contact, a certain amount of PPP is transferred from the hands to the mouth. Oral exposure may be predicted by the following equation:

$$OE = DE_{HANDS} \times A_M/A_H \times SE \times (N \times T)$$

Where OE : Oral exposure (µg/d), DE : hands Dermal exposure of the hands (µg/d), A_M/A_H : Fraction of hand area making contact with the mouth, input on assessment tab of the BROWSE software (default = 7%), SE : Skin-to-mouth transfer factor (%), input on assessment tab (default = 43%), N : Number of hand-to-mouth contacts (contacts/h), fixed at 1 contact/hour and T : Duration of exposure (h/d).

5. Health effects of workers following re-entry activities

The health risk for the agricultural worker using pesticides is an important aspect to consider during the registration procedure of plant protection products. Health effects may resulting from pesticide exposure will vary with the pesticide involved and the route of exposure (dermal, oral or inhalation) (MacFarlane *et al.,* 2013) and can be observed despite that these chemical products are developed under a very strict regulation process which aims to reduce the risk with reasonable certainty and to minimise the negative impacts on human health and the environment (Damalas & Eleftherohorinos, 2011). Despite their popularity and extensive use, previous studies showed that pesticide exposure often induces acute (short-term) health effects, as well as chronic (long-term) health effects on workers that entered in treated fields. Adverse effects of pesticide residues on field workers were already recognised more than fifty years ago (Carman, 1952; Quinby and Lemmon, 1958). Frequent dermal contact with foliage treated with organophosphorous pesticides have led to incidents of illness among fieldworkers in citrus crops in USA (Gunther *et a1.,* 1977). Local effects such as contact dermatitis due to heavy foliar contact and cutaneous exposure to crop associated materials have been reported among California table grape workers (O'Connell *et al.,* 1987; Maddy & Smith 1985). Over the past decades, several studies have pointed exposure to pesticide residues as a potential cause of reproductive problems. Male fecundity (sperm concentration, morphology and viability) may be endangered after repeated exposure to pesticide residues by handling products in greenhouses (Abell *et al.,* 2000a). Other studies underlined the probability that re-entry tasks entail a risk for reduced fecundity in increasing the time to pregnancy (Abell *et al.,* 2000b; Bretveld *et al.,* 2006). In addition, Lander *et al.* (2000) showed some effects on chromosome aberration after an exposure during re-entry activities (such as nipping, cutting, pricking, and potting) to low pesticide concentrations among workers in Denmark. Moreover, neurological disorders could be associated to re-entry tasks in greenhouses or field previously treated with pesticides (Kamel *et al.,* 2003; Baldi *et al.,* 2011). Pesticide residues can also be related to an increase in bladder cancer (Boulanger *et al.,* 2016) and breast cancer (Lemarchand *et al.,* 2016) even if more studies are needed for confirmation. Consequently, serious concerns have been raised about health risks resulting from exposure of workers during re-entry activities. These health effects are different

depending on the contamination level, the type of exposure, the frequency and duration of tasks and the behaviour of the worker (Andersson *et al.,* 2014).

6. Factors affecting worker exposure

Several factors influence the workers' exposure to pesticide residues during re-entry tasks. Dermal exposure is determined by the transfer of the pesticide residue from the surface of the foliage to the skin of the workers resulting from contact with crops previously treated with pesticide residues (Jurweiz *et al.,* 2009; Kasiotis *et al.,* 2017; Toumi *et al.,* 2018). Risk of exposure depends on the amount available for transfer and the frequency and intensity of skin contact with the treated crops (Jurewicz *et al.,* 2009). The amount of pesticide on the leaves available (DFR) depends on the formulation (active substance and its physicochemical properties, such as vapour pressure or solubility), application technique, frequency and rate of required pesticide application, crop height and the re-entry intervals (Brouwer *et al.,* 1992a). Potential toxicity of the PPP, dermal absorption, number and duration of contacts with residues persistency (contact or systemic active substances), use of dermal and respiratory protections, all those factors are important and can affect the exposure of workers who re-enter the pesticide-treated fields or greenhouses. Other factors that can also explain variation in exposure levels are the crop nature and characteristics (e.g. physiological proprieties and composition of the cuticles) (Toumi *et al.,* 2018), the relative humidity and temperature during the working day, previous rainfall, the worker skills and status (seasonal or not) and the worn clothing (Baldi *et al.,* 2014). Temperature and relative humidity seem to be major factors affecting the exposure of workers to pesticide residues. High temperature and humidity facilitate the passage of the pesticide through clothing (Aprea *et al.,* 2005; Aprea *et al.,* 2009). Many studies reported that these conditions associated with poor ventilation in greenhouses affect significantly the level of risk for their health. Moreover, in greenhouses temperature is maintained at about 18 °C and variations are smaller than in field conditions. Therefore greenhouse workers are exposed to higher levels of pesticide in the air compared to other workers (Kittas *et al.,* 2014). Consequently, working in greenhouse increases both dermal and inhalation exposures of workers to pesticide residues during re-entry.

7. Solutions and mitigation measures to reduce worker exposure to pesticide residues

Previous studies showed that pesticide residues remaining available in crops could be an issue for workers entering an area previously treated. Results of the studies reviewed suggest that behavioural interventions are needed and can be effective in reducing pesticide exposures for workers. Greater precautions should be taken to reduce contamination, in particular of the hands and skin because the dermal exposure is an important source of exposure for workers. Lots of studies have showed the efficacy of the personal protective equipment. Gloves (even in latex) can offer a powerful protective barrier against surface residues (Sankaran *et al.,* 2015). Li et al. (2011) reports the use of rubber latex gloves by strawberry harvesters to protect their skin from exposure and to promote food safety.

By urine biomonitoring, Krieger & Dinoff (2000) showed that wearing rubber latex gloves reduces harvester exposure to captan by about 40% compared to bare-handed harvesters during harvesting of strawberries. Bradman *et al.* (2008) and Salvatore *et al.* (2009) showed also that wearing gloves results both in lower levels of pesticide residues on worker's hands and lower absorbed dose. Additionally, hand washing (Curwin *et al.,* 2003; Salvatore *et al.,* 2009) and daily changing of gloves and clothing (Aprea *et al.,* 2009) can reduce skin exposure. But, it should be noted that protective equipment such as gloves on which pesticide can accumulate could lead to a secondary exposure. Study results indicate that normal work clothing provides a 90% reduction in dermal exposure to chlorothalonil (Spencer *et al.,* 1991). Standard work clothing for re-entry activities such as harvesting may include long-sleeve shirts, long pants, shoes and socks (Franklin & Worgan, 2005; Whitmyre *et al.,* 2005). For certain worker re-entry activities such as scouting, coveralls may be worn which impart additional protection Franklin & Worgan, 2005; Whitmyre *et al.,* 2005).

In addition, concerning inhalation exposure, a suggested improvement in worker protection would involve respiratory protection with a face mask to filter out airborne particulates (Aprea *et al.,* 2002). As pesticide residues are normally declining during the days following application, pre-harvest intervals and restricted re-entry intervals indicated on the labels should be strictly respected to lower the potential exposure of workers. The no respect of the period following application before re-entry is illegal and lead to exposure to toxic levels of pesticide residues. Training and education of workers on (personal) hygiene and the use of protective gloves should be advocated in order to reduce exposure (Brouwer *et al.,* 1992b). Finally, the use of pesticides with a higher penetration in plants and lower volatilization could also be useful to decrease the risk level.

8. Conclusion

Workers re-entering in treated fields or greenhouses can be highly exposed to pesticide residues which may result in serious risks for their health. The levels of dermal exposure on a working day due to a manual contact with a contaminated crop can be similar to or higher than those observed for people who handle and sprayed a pesticide. Similarly, the inhalation exposure of workers to pesticide residues after re-entry is of the same order of magnitude than during application of a pesticide. In Europe, placing a PPP on the market is only allowed if a safe use is identified, among other, for the worker. However, in some cases the risk is only acceptable for workers wearing gloves or when mitigations measures are applied. Therefore, a greater attention should be given to raise the awareness of workers about the risk for their health and better preventive measures should be taken to reduce the levels of exposure.

Chapitre 3

Contamination des fleurs coupées par les résidus de pesticides

Introduction

L'identification et la caractérisation du danger se présentent comme les deux premières étapes de la démarche d'évaluation du risque. L'objectif de cette première partie de l'étude est d'établir, par un échantillonnage de diverses fleurs coupées, la liste des substances actives potentiellement dangereuses présentes sur celles-ci et d'en étudier par la suite la nature, les propriétés, la fréquence de détection et les concentrations des dépôts de résidus de pesticides. Ces deux premières étapes sont indispensables pour avoir une idée globale de la gravité du danger en caractérisant les propriétés toxicologiques de ces substances actives et en recensant leurs effets néfastes préoccupants pour la santé en cas d'exposition.

Dans un premier temps, une enquête a donc été menée auprès d'une cohorte de fleuristes, répartis dans tout le pays, chez lesquels ont été prélevés des échantillons de fleurs coupées, en ciblant les espèces les plus vendues et les plus travaillées en Belgique (les roses, les gerberas et les chrysanthèmes). Elle devait permettre de caractériser les pratiques professionnelles d'une part (nature des tâches, durée du travail par exemple), et d'autre part, grâce à une analyse des dépôts au moyen d'une méthode multi-résidus, d'identifier les dangers (substances actives et métabolites) et d'évaluer le niveau de contamination moyen des fleurs pour ainsi aider à évaluer le risque subséquent d'exposition des fleuristes lors de la préparation des bouquets et la manipulation de ceux-ci. Dans un premier temps, une étude d'évaluation du niveau de contamination moyen de 50 échantillons de roses produites en Europe ou ailleurs dans le monde a été effectué (Partie I). Dans un deuxième temps, cette étude a été étendue pour considérer deux autres espèces (gerberas et chrysanthèmes) déclarées également comme les plus vendues en Belgique. L'échantillonnage a donc été complété par 20 échantillons de gerberas et 20 échantillons de chrysanthèmes (Partie II). Les résultats des 50 échantillons de roses repris dans les parties I & II sont les mêmes.

Ce chapitre est une version adaptée des deux articles suivants :

- Toumi, K., Vleminckx, C., Van Loco, J., & Schiffers, B. (2016). A survey of pesticide residues in cut flowers from various countries. (Publié dans *Communications in Agricultural and Applied Biological Sciences*, 81(3), 493-502.)

- Toumi, K., Vleminckx, C., van Loco, J., & Schiffers, B. (2016). Pesticide residues on three cut flower species and potential exposure of florists in Belgium. (Publié dans *International journal of environmental research and public health*, 943-956. DOI: 10.3390/ijerph13100943)

I. A survey of pesticide residues in cut flowers from various countries

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Abstract

As in any intensive culture, flowers require the use of a wide range of pesticides to control diseases and pests which can damage production and marketability. In order to evaluate the average levels of contamination of the cut flowers and to assess the risk for professionals exposed to pesticide residues when handling cut flowers, a survey was carried out with a group of florists from the Belgian largest cities. Fifty samples of roses (5 stems per bouquet) were collected: 45 bouquets were sampled in the 7 largest cities of Belgium (Antwerp, Brussels, Charleroi, Ghent, Leuven, Liege and Namur) and 5 were sampled from 5 supermarkets. Analysis of residual pesticide deposit is made by combining two multi-residue methods (GC-MS-MS and LC-MS-MS) in a laboratory accredited for pesticide residues. For all the samples analysed, a total of 97 active substances were detected, i.e. an average of 14 active substances per bouquet and a total average pesticide load of 26,03 mg/kg per flower sample. Most active substances (a.s.) reached high levels of residues, with concentrations between 10 and 50 mg/kg. Samples from Belgium and The Netherlands have a lower average number of a.s./sample, but the amount of residues is about the same in all samples (20-30 mg/kg) whatever the country of origin , except for the sample from Germany who is the worst case (22 a.s. with a total amount of 92 mg/kg). Most of the detected active substances are fungicides (dodemorph, spiroxamine, cyprodinil, fluopyram, pyrimethanil, benomyl (carbendazim), propamocarb, boscalid and iprodione) which are present on more than 20 of the 50 samples. All of them have a dermal acute toxicity. Consequently, florists who handle a large number of flowers are exposed daily with a potential effect on their health.

Key words: pesticide residues, cut flowers, roses, exposure assessment, florists.

1. Introduction

Floriculture is a most essential and vast parts of Horticulture. The total acreage allocated to cut flower production worldwide is now over 200,000 hectares, with roses, carnations, and chrysanthemums the dominant varieties (ITC, 2001). They are used on all religious festival occasions and given as a birthday presents, wedding gifts or while meeting sick people and even at funerals and especially during the

peak seasons (coinciding with Valentine's Day, Christmas, and other international holidays) (Korovkin, 2003; Palma *et al.,* 2010).

Cut flower production in the world gained importance and has become a very popular commercial activity during last decade of the outgoing millennium, especially after the Second World War (Amar, 1995; Kendirli and Cakmak, 2007) with the globalisation of markets and the gradual appearance of strong players such as Colombia, Kenya, Ecuador and Zimbabwe (ITC, 2001).

Today the European Union market is a high potential importer of floriculture products. The growth trend in import is clear from the fact that the total imports, which were 881 million dollars in 1990, improved to 1.684 million dollars in 2004. Roses accounted for a greater share of EU, whereas Roses shot up to 61.94 % (1,043 million dollars) 2004 from 33.6 % (296 million dollars) in 1990. It is also evident that the roses are the preferred species in EU from the fact that over the period under review it experienced a compound growth rate of 9.41 %, which far exceeds the growth of other species of flowers imported into E.U (Ravinath, 2007).

As any intensive production, pesticide use is a significant strategy to fight against many pests (mainly mites and insects) and various diseases, so that ornamental producers can stay competitive in both national and international markets (Bethke & Cloyd, 2009).

While flowers are susceptible to pests and diseases, they are sprayed several times during their growth considering that no MRL are set for flowers. Therefore, residue deposits can be high and, as a consequence, a health hazard may exist to exposed individuals who handle those flowers contaminated by pesticides during cropping, cutting, sorting or bundling (Brouwer *et al.,* 1992c). The most common general signs and symptoms mentioned after exposure are weakness, fatigue with muscle pain, when other symptoms are often centred on the eye (eye itchiness and blurring of vision), ear, nose and throat or neuralgic (Lu, 2005). Other potential problems are also mentioned such as: reproduction (Restrepo *et al.,* 1990; Weidner *et al.,* 1998, Bell *et al.,* 2001; Garry *et al.,* 2002, 2003; Beard *et al.,* 2003; Hanke *et al.,* 2004), allergic reactions (Sato *et al.,* 1998), increase in certain types of cancer (Dich *et al.,* 1997; Infante-Rivard *et al.,* 1999; Richter *et al.,* 1999; Hardell *et al.,* 2002; De Rose *et al.,* 2003; Alavanja *et al.,* 2003, 2004; Bassil *et al.,* 2007), neurological disorders (Baldi *et al.,* 2003a & 2003b; Elbaz *et al.,* 2004, Alavanja *et al.,* 2004) like Parkinson disease.

In order to assess the risk for professionals exposed to pesticide residues on flowers produced in Belgium or imported from various countries, a study has been carried out with a group of florist representatives of the Belgian largest cities on a voluntary basis.

2. Materials and methods

Fifty samples of roses (at least 5 stems/bouquet) were collected within 3 consecutive days. 45 bouquets were sampled from florists located in the 7 largest cities of Belgium (Antwerp, Brussels, Charleroi, Ghent, Leuven, Liege and Namur) and 5 were sampled from 5 supermarkets to evaluate the average residue levels of

roses which are necessary to estimate the potential exposure of florists preparing bouquets.

During sampling, the bouquets were labelled and the countries of origin identified (asking the florists). After two centimetres of stem have been cut obliquely with a sterilised sharp knife to maintain water absorption, the bouquets were stored in a cool room in vases filled with tap water. All collected samples were transported within 2 days by road from Gembloux to the laboratory in Ghent.

The residual pesticide deposits on the bouquets were analysed in a laboratory holding a BELAC accreditation to ISO/CEI 17025 (PRIMORIS, Technologiepark 2/3, B-9052 Zwijnaarde – Ghent). PRIMORIS is a private, accredited and officially recognised service laboratory for herbal products.

The residues were extracted after the 5 flower stems had been totally crushed, taking a homogenous 10 g sub-sample. The extract was analysed using a combination of two multi-residue methods validated by the laboratory for pesticides residue analysis in foodstuffs. According to the active substances to be determined, the pesticide residue concentration was determined by GC-MS-MS or LC-MS-MS (gas chromatography or liquid chromatography with mass spectrometry). Gas chromatography was used to analyse relatively small, thermally stable, volatile, non-polar molecules. Liquid chromatography was used to analyse larger, thermolabile, non-volatile, polar molecules.

The combination of both methods allows the analysis of approximately 500 active substances in a single run (a screening of almost all pesticides usually sprayed on flowers). For most of the active substances, the quantification limit was ≤ 0.01 mg/kg.

3. Results and Discussion

3.1. Pesticide residues concentrations on roses

All flower samples appeared to be contamined by pesticide residues whatever their origin. Most active substances (a.s.) reached high levels of residues, with concentrations between 10 and 50 mg/kg, about thousand times above the maximum limit value set for foodstuffs (EU MRL values) for most of those a.s. and specialy those which are no more approved in Europe (Table 4).

Table 4 : Pesticide residue concentrations in 50 samples of roses

Total Pesticide Residues Concentration (mg/kg, all a.s. together)	Samples with Pesticide Residues	
	Number of samples	%
0,01-0,99	2	4
1,00-4,99	4	8
5,00-9,99	7	14
10,0-50,00	33	66
>50,00	4	8
Total	50	100

3.2. Frequency of active substance residues on roses

Nine fungicides (dodemorph, spiroxamine, cyprodinil, fluopyram, pyrimethanil, benomyl (carbendazim), propamocarb, boscalid and iprodione) and one insecticide (imidacloprid) are the most frequently detected active substances. They are present on more than 20 of the 50 samples when most of the other active substances (29) are detected only once (Table 5).

97 different active substances were identified on the rose samples. 53 a.s. (roughly a half of all a.s.) with a frequency below 10%, and 29 a.s. (about 30%) were only detected in a single bouquet.

Table 5 : Active substances found in the samples, number of bouquets contamined by each a.s. detected and frequency of detection (in %, with LOQ < 0.01 mg/kg)

Active substances detected in the samples	Number of samples where a.s. are detected (out of 50)	Frequency of detection (in %)
Dodemorph	37	74
Spiroxamine	34	68
Cyprodinil	31	62
Fluopyram, pyrimethanil	23	46
Benomyl (carbendazime), propamocarb	22	44
Imidacloprid	21	42
Boscalid, iprodione	20	40
Fludioxonil	19	38
Flonicamide, procymidone	18	36
Dimethomorph	17	34
Acephate, fluopicolide	15	30
Methamidophos	14	28
Ethirimol, fenhexamide	13	26
Acetamiprid, clofentezine, lufenuron	12	24
Famoxadone	11	22
Pirimicarb	10	20
Bupirimate, kresoxim-methyl, methoxyfenozid, spinosad	9	18
Thiametoxam	8	16
Fipronil, pyraclostrobine, thiacloprid	7	14
Ametoctradin, azoxystrobin, cyhalothrin, cypermethrin, novaluron, pymetrozin	6	12
Fenamidone, iprovalicarb, mandipropamid, metalaxyl (metalaxyl-m), metrafenone, spinetoram	5	10

Active substances detected in the samples	Number of samples where a.s. are detected (out of 50)	Frequency of detection (in %)
Difenoconazole, prochloraz, tebuconazole	4	8
Buprofezin, chlorantraniliprole, chlorothalonil, cyfluthrin, etoxazole, flubendiamide, hexythiazox, indoxacarb, methomyl (thiodicarb), oxycarboxine, triflumizole	3	6
Bifenazate, chlorfenapyr, diazinon, dimethoate, dinotefuran, fenpropidin, furalaxyl, mepanipyrim, picoxystrobin, trifloxystrobin	2	4
6-benzyladenine, acrinatrin, benalaxyl, bifenthrin, bitertanol, carboxin, chloridazon, cyflufenamid, deltamethrin, dicofol, fenarimol, fensulfothion-oxon, fenamiphos, fenvalerate, flufenoxuron, forchlorfenuron, fosthiazate, isocarbofos, methiocarb, myclobutanil, oxamyl, pyridabene, pyridalyl, quinalphos, spirotetramat, tétradifon, thiabendazole, thiophanate-methyl, triforine	1	2

3.3. Pesticide residues on roses by country of origin

Only 20 (40%) of all samples collected were originate from EU countries (8 from Belgium). This table (Table 6) reflects the importance of flower exchanges in the world and how many flowers, mainly roses, are coming from third countries (Latin America and Africa) and sold into the EU market.

Table 6: Country of origin of rose samples, number of samples/country analysed, average number of active substances/sample, average of total amount of pesticide residues/sample (mg/kg) and number of active substances detected in samples

Country of origin (declared by florists)	Number of samples/country	Average number of active substances /sample (Median)	Average of total amount of pesticide residues/sample (mg/kg) (Median)	Number of active substances detected in samples
Belgium	8	10.1 (9.0)	27.7 (19.0)	38
Colombia	2	19.0 (19.0)	31.8 (31.8)	24
Ecuador	9	14.8 (17.0)	18.8 (12.8)	60
Ethiopia	3	12.3 (12.0)	22.9 (22.0)	29
Germany	1	22.0 (22.0)	92.0 (92.0)	22
Israel	2	16.0 (16.0)	29.6 (29.6)	27
Netherlands	11	10.5 (7.0)	20.6 (24.3)	54
Kenya	9	15.6 (15.0)	26.5 (25.0)	48
(Sold in) Supermarkets	5	15.8 (15.0)	28.2 (28.7)	36
Total	50	13.6 (11.5)	26.0 (24.5)	97

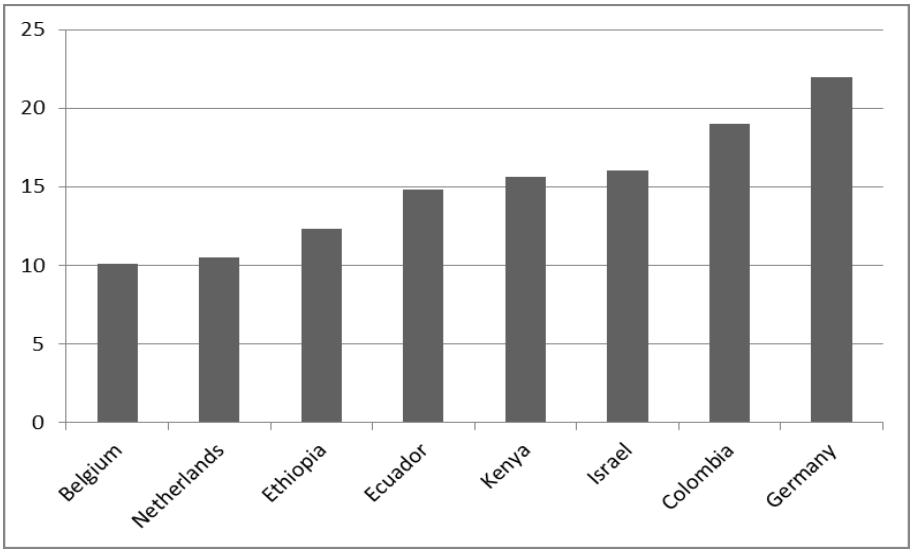

Figure 3: Classification of countries based on the average number of pesticides detected/sample of roses

Samples from Belgium and The Netherlands have a lower average number of a.s./sample, but the amount of residues is about the same in all samples (20-30

mg/kg) except for the sample from Germany which is the worst case (22 a.s. with a total amount of 92 mg/kg) while some countries use a huge number of different pesticides on flowers (60 for Ecuador, 54 for The Netherlands). Anyway, those results indicate an intensive use of pesticides to prevent pests and diseases on flowers.

3.4. Risk assessment for florists

The risk is generated by combination of the "hazard" (mode of action; acute and chronic toxicity of a.s.) and "exposure" (concentration levels on flowers; routes of exposure: oral or dermal). If the main route of exposure to plant protection products is in general the oral route, for operators, workers, bystanders and residents the main exposure routes are dermal and /or inhalation. Therefore, it is necessary to assess the exposure for the operator (i.e. applicators, crop-workers, harvesters) for the different likely routes of exposure.

3.4.1. Classification of active substances according to their biological activity

Forty-seven % of detected active substances are insecticides and 46% of fungicides. Three active substances are growth regulators and one substance is a herbicide (chloridazon). Of the 97 detected active substances, most of the pesticides belong to the following chemical groups: organophosphates (9 a.s); pyrethroids (7 a.s); neonicotinoids (6 a.s); carbamates, triazole and strobilurins (5 a.s. each). Pesticides from those groups are known for their toxicological properties (action on the nervous system after exposure; acute toxicity).

3.4.2. Classification of active substances according to acute toxicity

Table 7: Classification of the active substances detected on oral acute toxicity values (oral route of exposure): number of a.s. for each Category (ILO classification) and number of samples where at least one a.s. belong to this Category

ILO Categories	LD_{50} (mg/kg body weight)	Hazard wording	Number of active substances	Number of samples
1	[0-5]	Fatal if swallowed	1	1
2	] 5-50]	Fatal if swallowed	7	17
3	] 50-300]	Toxic if swallowed	12	36
4	] 300-2000]	Harmful if swallowed	19	47
5	] 2000-5000]	May be harmful if swallowed	58	50

For oral exposure, only a few a.s. (8 a.s.) belong to the most toxic groups but they are not rare: 18 samples from 50 (almost 40%) are contamined with such very toxic pesticide residues (the active substance belonging to Category 1 is the oxon-fensulfothion). 72% of samples contain active substances belonging to the Category 3 "Toxic if Swallowed" and 92% to the Category 4 "Harmful If Swallowed". All rose

samples contain one or several active substances belonging to the less toxic Category 5.

Even if the oral contact is not the usual route of exposure, the risk still exists to have an exposure resulting from a "hand to mouth" contact. This accidental exposure can result from a lack of hygiene (florists do not wash their hands frequently and do not wear gloves systematically as observed in our survey of 25 professionals) or the use of cell phone during working.

Table 8: Classification of the active substances detected on dermal acute toxicity values (cutaneous route of exposure): number of a.s. for each Category (ILO classification) and number of samples where at least one a.s. belong to this Category

Categories	LD_{50} (mg/kg body weight)	Hazard wording	Number of active substances	Number of samples
1	[0-50]	Fatal in contact with skin	2	2
2	] 50-200]	Fatal in contact with skin	1	1
3	] 200-1000]	Toxic in contact with skin	3	16
4	] 1000-2000]	Harmful in contact with skin	5	38
5	] 2000-5000]	May be harmful in contact with skin	86	50

Two active substances oxon-fensulfothion and isocarbofos belong to Category 1 "Fatal in contact with skin". 76% of samples contain active substances belonging to the Category 3 "Harmful in contact with skin". All rose samples contain one or several active substances belonging to the less toxic Category 5 "May be harmful in contact with skin".

For dermal exposure, which is supposed to be the main route of exposure during handling, the risk can be considered as moderate when 94% of a.s. belong to the less toxic Categories, but all substances found on roses have more or less harmful effects in contact with skin.

3.4.3. Classification of active substances according to the EU Pesticides Database

As the florists handle the flowers every day in the course of their work, they are exposed to plant protection products like other "operators". The "Acceptable Operator Exposure Level" (AOEL) is the reference value to consider for professionals exposed to pesticides. AOEL is defined in Regulation (EC) 1107/2009 as "... the maximum amount of active substance to which the operator may be exposed without any adverse health effects." AOEL values relate to the internal (absorbed) dose available for systemic distribution from any route of absorption and

are expressed as internal levels (mg/kg bw/day). When the operator exposure remains below this limit, the risk for them is considered as “acceptable” (Regulation (EC) 1107/2009) (Table 9).

The active substances can also be classified on their hazard category according to the CLP regulation (for "Classification, Labelling and Packaging”) (Regulation (EC) 1272/2008) is a European Union regulation from 2008, which aligns the European Union system of classification, labelling and packaging of chemical substances and mixtures to the Globally Harmonised System (GHS). It is expected to facilitate global trade and the harmonised communication of hazard information of chemicals and to promote regulatory efficiency (Table 10).

Table 9 : Number of active substances detected on the bouquets classified according to the AOEL values (Source: EU Pesticides Database 2016, European Commission/DG HEALTH, Regulation (EC) 1107/2009)

AOEL values (mg/kg bw/d)	**Number**
[0.001-0.01 [	19
[0.01-0.1 [	43
[0.1-1[	18
>1	1
No AOEL*	16

*Active substances which have no AOEL values; not assessed at European level

Table 10 : Number of active substances detected on the cut roses classified in each hazard category according to the CLP regulation (Source: Regulation (EC) 1272/2008)

Class	**Category**	**Code (Hazard)**	**Number of a.s. in the category**
Acute toxicity	Category 1	H310: Fatal in contact with skin	2
	Category 2	H300 : Fatal if swallowed	10
		H330 : Fatal if inhaled	6
	Category 3	H301 : Toxic if swallowed	7
		H311: Toxic in contact with skin	2
		H331 : Toxic if inhaled	10
	Category 4	H302 : Harmful if swallowed	21
		H312: Harmful in contact with skin	7
		H332 : Harmful if inhaled	3
Carcinogenicity	Category 2	H351:Suspected of causing cancer	13

Class	Category	Code (Hazard)	Number of a.s. in the category
Serious eye damage/ eye irritation	Category 1	H318: Causes serious eye damage	2
	Category 2	H319: Causes serious eye irritation	3
Germ cell mutagenicity	Category 1,1A or 1B	H340: May cause genetic defects	1
	Category 2	H341: Suspected of causing genetic defects	1
Reproductive toxicity	Category 1,1A or 1B	H360: May damage fertility or the unborn child.	3
	Category 2	H361: Suspected of damaging fertility or the unborn child.	11
	Additional category for effects on or via lactation	H362: May cause harm to breast-fed children	2
Sensiti-sation of the respiratory tract or the skin	Respiratory sensitisers category 1,1A or 1B	H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled	1
	Skin sensitisers category 1,1A or 1B	H317: May cause an allergic skin reaction	21
Skin corrosion / irritation	Category 1,1A or 1B	H314: Causes severe skin burns and eye damage	1
	Category 2	H315 : Causes skin irritation	6
Specific target organ toxicity (single exposure)	Category 3	H335: May cause respiratory irritation	4
Specific target organ toxicity (repeated exposure)	Category 1	H372: Causes damage to organs through prolonged or repeated exposure	2

Class	Category	Code (Hazard)	Number of a.s. in the category
	Category 2	H373: May cause damage to organs through prolonged or repeated exposure	7

4. Conclusion

The first conclusion after analysis of the residual deposits on roses is the high level of contamination of all samples whatever their origin: 97 active substances detected, i.e. an average of almost 14 active substances/sample and a total average pesticide load of 26.03 mg/kg per flower sample, while the accumulated total of all the residues was as much as 97.03 mg/kg for a single bouquet of 5 Belgian roses. This reflects the intensive use of pesticides on cut flowers in general.

Dodemorph (a fungicide) was not only the most frequently detected substance but also the active substance for which the highest maximum concentration (41.9 mg/kg) was measured on the rose samples analysed.

As the flowers are susceptible to pests and disease, they are regularly treated till harvest time because there are no maximum residue limits (MRLs) for flowers, unlike other cultures. The high levels of pesticide residues in cut flowers are linked to high rates of pesticides but also to repeated sprayings during the growing season.

On the other hand, analyses of roses from Belgian or Dutch origins revealed an abnormal presence of active substances which are not authorised for use in the EU. 19 of the active substances detected on the 50 rose samples analysed are not authorised in EU. The unauthorised active substances are more frequently detected in the Belgian samples. However those results should be put into perspective as it was not possible to have a firm guarantee of the origin of the samples, as they were taken from the premises of the retailers rather than from the producers.

Even if pesticides are generally less toxic by cutaneous compared to the oral route, people who handle a large number of flowers every day are susceptible to be affected by various diseases after contact with skin and percutaneous absorption. All active substances identified on roses could affect the skin of florists if they do not wear protective equipment. Some active substances (acephate, methiocarb, monocrotophos, methomyl, deltamethrin, etc.) on flowers have a direct effect on the nervous system and could cause accidental poisoning by transfer from the hands to the mouth. To better assess the risk, dislogeable residues and potential transfers are still to be investigated at the lab.

To reduce the exposure of florists to pesticide residues, solutions could be recommended: a better management of the pesticide used (IPM at the field or even organic flower production, a potential niche market); a stronger quality control of imported cut flowers. Finally, it could be interesting to set up a Maximum Residue Limit for flowers to decrease the risk for professionals and all other people in contact with flowers.

II. Pesticide residues on three cut flower species and potential exposure of florists in Belgium

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Abstract:

In order to assess the prevalence of pesticide contamination and the risk of florists' exposure when handling cut flowers, sampling and analysis of 90 bouquets of the most commonly sold cut flowers in Belgium (50 bouquets of roses; 20 of gerberas, and 20 of chrysanthemums) were carried out. The bouquets were collected from 50 florists located in the seven largest cities of Belgium (Antwerp, Brussels, Charleroi, Ghent, Leuven, Liege, and Namur) and from five supermarkets located in the different regions. To have a better understanding of the route of exposure and professional practices a questionnaire was also addressed to a group of 25 florists who volunteered to take part in the survey. All florists were interviewed individually when collecting the questionnaire. The residual pesticide deposit values on cut flowers were determined in an accredited laboratory using a multi-residue (QuEChERS Quick Easy Cheap Effective Rugged Safe) method and a combination of gas chromatography (GCMS/MS) and liquid chormatograhphy (LCMS/MS) analysis. A total of 107 active substances were detected from all samples; i.e., an average of about 10 active substances per bouquet. The most severely contaminated bouquet accumulated a total concentration of residues up to 97 mg/kg. Results show that roses are the most contaminated cut flowers; with an average of 14 substances detected per sample and a total concentration per rose sample of 26 mg/kg. Some active substances present an acute toxicity (acephate, methiocarb, monocrotophos, methomyl, deltamethrin, etc.) and exposure can generate a direct effect on the nervous system of florists. Nevertheless, fungicides (dodemorph, propamocarb, and procymidone) were the most frequently detected in samples and had the highest maximum concentrations out of all the active substances analysed. Dodemorph was the most frequently detected substance with the highest maximum concentration (41.9 mg/kg) measured in the rose samples. It appears from the survey that, despite being exposed to high deposits of residues, florists usually do not protect themselves from contact with residues even if they spend several hours handling cut flowers and preparing bouquets (from 2 to 6 h/day, depending on the time of year and/or selling

periods) daily. Bad habits (eating, drinking or smoking at work[2]) and absence of personal protective equipment of most florists also increase the risk of contact with pesticide residues. Keywords: cut flowers; roses; pesticide residues; exposure risk evaluation; florists.

1. Introduction

Flowers are used for beautification purpose or given as an expression of love, friendship, gratitude, or appreciation (Palma and Ward, 2010)They are sold throughout the year, but with peak periods (Valentine's Day, Halloween, Mother's Day, New Year, etc.). Today, the cut flowers world market represents about 30 billion Euros per year. Europe and North America are still the main markets (Rikken, 2016). The European demand of cut flowers (cut flowers and pots) is estimated to 13 billion Euros, representing 50% of the world's demand (Val'hor, 2013). As a result, millions of flowers produced in Africa, India, Israel, or Latin America travel by road and air to consumer markets located essentially in the rich or emerging countries of the Northern hemisphere. Three hundred fifty million cut flowers are imported each year in the United States and similar quantities are imported in Canada and Europe (Morse *et al.,* 1979). Flower production is a dynamic sector in European horticulture with a high growth potential and a major economic weight in international trade (Kendirli & Çakmak, 2007). Traditionally in Europe, floriculture is most strongly developed in The Netherlands and Belgium, but cut flowers are also among the intensive crops grown in greenhouses in Great Britain (Illing, 1997). As in any intensive culture, flowers require the use of a wide range of pesticides to control diseases and pests, which can damage production and marketability. Plants and flowers entering into the European market must meet stringent regulations on plant health designed to prevent introduction of some pests or diseases. Therefore, imported cut flowers receive heavy pesticide applications prior to shipment. In 1977, a sampling of all flowers imported to Miami on three consecutive days showed that 18 bouquets of 105 (17.7%) contained pesticide residue levels superior to 5 mg/kg, and three samples had levels superior to 400 mg/kg (Morse *et al.,* 1979).

The lack of maximum residue limits (MRL) for flowers explains that, unlike other crops which are harvested for consumption, there is no restriction on the use of pesticides and cut flowers are often treated regularly up to harvesting or even after harvest. This also explains the modest development of an "organic" sector or integrated pest management (IPM) in floriculture. Many pesticides applied on flowers are persistent, dislodgeable by contact with the hands, and are fat-soluble. As they can easily be absorbed through skin contact, florists who handle the flowers daily and for several hours can potentially be exposed to residual deposits of pesticides and possibly endanger their health. Health problems have been reported all over the world for workers and professionals exposed to pesticides daily,

[2] L'usage du téléphone portable peut également engendrer des contacts mains-bouche. Cette mauvaise pratique (qui ne faisait pas partie du questionnaire) a été fréquemment observée.

including contact allergies, dermatitis and skin effects (Das *et al.,* 2001; Penagos *et al.,* 2004), neurologic pathologies (Farahat *et al.,* 2003; Baldi *et al.,* 2011) or even increases in certain types of cancers (Alavanja *et al.,* ;2004 ; Alavanja *et al.,* 2003; Bassil *et al.*, 2007), hematotoxic effects (Abu, 2004; Del Prado-Lu, 2007), endocrine disruptor effects (Lacasaña *et al.,* 2010), or cytogenetic damage (Gómez-Arroyo *et al.,* 2000). Hormonal and reproductive problems of workers (abortions, prematurity, stillbirth, and congenital malformations, low fecundity) have also been reported (Abell *et al.,* 2000a; Hanke & Jurewicz, 2004). Various detrimental health disorders were mentioned for female florists and their children in Colombia (Restrepo *et al.,* 1990a; Restrepo *et al.,* 1990b) and other developing countries. Therefore, in Europe, EFSA (European Food Safety Authority) reviews, in close cooperation with EU Member States, the risk of exposure of each active substance for operators, workers, bystanders, and residents before plant protection products are allowed to be used in crops or greenhouses (EFSA, 2014). Nevertheless, despite an important potential exposure and a subsequent high level of risk for this group of workers, only a small amount of information was available in Belgium or in Europe about the contamination of flowers and the exposure of florists in link with their professional practices. This information is crucial when people want to assess the risk. As a first step in developing an exposure assessment framework of florists (hazard identification and characterization) we have investigated the extent and severity of pesticide contamination (nature, frequency, and concentrations of pesticide deposits) of the most commonly sold cut flowers in Belgium and the main activities of florists to prepare the bouquets. This survey will be completed later by results of field and laboratory trials to measure the dislodgeable foliar residues (DFR), the transfer from plant to hands and, finally, to estimate the dermal exposure of florists to pesticides applied on cut flowers.

2. Methods

2.1. Sampling of Cut Flowers

In order to assess the prevalence of pesticide contamination and to evaluate the average levels of contamination of the cut flowers most commonly sold in Belgium (roses, the number one flower sold annually, gerberas, and chrysanthemums) a sampling of 90 bouquets (50 of roses, 20 of gerberas, and 20 of chrysanthemums) was carried out at 50 florist's premises. The sampling size was estimated according to a similar study carried out by Morse *et al.* (1979) who estimated the minimum sample size required to detect 10% of contamination when a 0% level is expected to be 77 samples, and sampled 105 bouquets from 43 different growers to assess flower contamination in the United States.

Fifty samples of roses (at least five stems per bouquet) were collected within three consecutive days in February (the Valentine Day period). The bouquets were sampled from 45 florists located in the seven largest cities of Belgium (Antwerp, Brussels, Charleroi, Ghent, Leuven, Liege, and Namur) and from 5 supermarkets located in the different regions.

Bouquets of gerberas and chrysanthemums were collected in 25 florist's shops located in Brussels and Wallonia within three consecutive days in April.

After collection, the sampled bouquets were kept in a cool room in vases filled with water and two centimetres of stems were cut obliquely using a sterilised sharp knife to maintain water absorption during storage before analysis. Although cut flowers normally last a fortnight in these conditions, the samples were kept for no more than three days before being taken to the analytical laboratory (transport by road from Gembloux to Ghent).

2.2. Analysis of the Residual Pesticide Deposits on the Bouquets

The residual pesticide deposits on the bouquets were analysed by PRIMORIS (formerly FYTOLAB, Technologiepark 2/3, 9052 Zwijnaarde, Belgium) laboratory holding a BELAC (Belgian Accreditation Council) accreditation to ISO/CEI 17025 for pesticide residues on vegetables and herbal products in general. PRIMORIS is an independent, accredited, and officially recognized service laboratory. Samples were analysed with a multi-residue (QuEChERS) method validated by the laboratory for analysis of residues in foodstuffs, which will detect approximately 500 different active substances in a single analysis thanks to a combination of gas chromatography (GC) and liquid chromatography (LC). The QuEChERS method is based on work accomplished and published by Anastassiades *et al.* (2003). After the sample (five flower stems) had been totally crushed, one homogenous 10 g sub-sample is homogenized by vortex mixing in a blender with acetonitrile to extract the residues. After agitation the extract is put through a clean-up column prior to analysis by gas or liquid chromatography with mass spectrometry (GC or LC-MS/MS) according to the active substances to be determined (GC-MS/MS for small, thermally-stable, volatile, non-polar molecules, or LC-MS/MS for larger, thermolabile, non-volatile, and polar molecules). For almost all active substances, the quantification limit (LOQ) was ≤0.01 mg/kg.

2.3. Statistical Analyses

All results of pesticide residues (number of active substances (a.s.) found and the total load of pesticides per sample) were analysed with a Student's t-test using Minitab 16 Statistical Software (Minitab Inc., State college, PA, USA).

2.4. Exposure Scenario of Florists

EFSA has adopted the following definition for "workers": they are persons who, as part of their employment, enter an area that has previously been treated with a plant protection product (PPP) or who handle a crop that has been treated with a PPP. Since worker exposures can vary substantially for a given scenario (e.g., nature of activities and duration of work), it is necessary to have a clear idea of the professional practices in order to be confident that individual exposures will not be importantly underestimated. As the sources of exposure are in contact with foliage, exposure of florists must be estimated for activities that involve significant contact with treated plants. To have a better understanding of the route of exposure and professional practices a questionnaire was also addressed to a group of 25 florists who volunteered to take part in the survey. All florists were interviewed individually

when collecting the questionnaire. The size of the group was considered large enough to be representative as they all sell the same flowers in Belgium and have the same activities to prepare the bouquets. In a similar study in the United States (Morse *et al.,* 1979) 20 flower inspectors participated and only 12 were interviewed. The florists were randomly chosen from professionals located in the Province of Namur (16 florists, i.e., 64%), and the Brussels-Capital Region (nine florists, i.e., 36%). They were asked to answer a detailed questionnaire (five pages, see Annex 1) on their personal history, the flowers sold from their premises (flower species and origins), their usual practices, their estimated working hours, their personal protective equipment (PPE) worn, their hygiene rules, and their perception of health problems linked with their occupation. All of the questionnaires were filled in and collected in the week during which the samples were taken for analysis of residual pesticide deposits.

3. Results

3.1. Origins of the Cut Flowers Collected and Analysed

The 25 florists surveyed purchased flowers from wholesalers. The roses had the widest range of sources: 96% of the florists surveyed purchased roses from Holland, 92% from Belgium, 60% from Kenya, 40% from Israel, 36% from Ecuador or Ethiopia and Morocco (12% together). 80% of the florists purchased chrysanthemums from The Netherlands, 72% from Belgium, and 4% from Israel. Gerberas came, in order of importance, from Belgium, The Netherlands, France, and Israel. As the bouquets were sampled randomly at the shops visited, there was no attempt to reproduce the origins declared in the questionnaires proportionally for the samples analysed. During sampling, the bouquets were labelled and the countries of origin identified by asking the florist. The origin of bouquets collected in the supermarkets was unknown. As expected from the survey, declared countries of origin vary widely for roses (eight countries, Belgium included), while gerberas and chrysanthemums collected were identified as flowers from Belgium and The Netherlands, but the traceability of cut flowers cannot be considered as reliable[3].

3.2. Global Results of Analyses of Residual Deposits

The pesticides residues levels (Table 11) and the number of active substances (Table 12) were determined on the 90 samples of cut flowers.

[3] Selon l'URFB (Union royale des fleuristes belges), la grande majorité des fleurs coupées vendues en Belgique sont importées de pays non-européens.

Table 11: Pesticide residue levels in 90 samples of cut flowers sampled in Belgium (2016)

Total Pesticide Residues Concentration (mg/kg, All a.s. Together)	Samples with Pesticide Residues	
	Number of Samples	%
0.01–0.99	15	17
1.00–4.99	21	23
5.00–9.99	15	17
10.0–50.00	35	39
>50.00	4	4
Total	90	100

Table 12: Total number of active substances (a.s.) detected, average number of a.s. per sample (min-max), average total concentration of residues (mg/kg), median concentration, and maximum cumulated deposit (sample with the highest total amount of pesticide residues, in mg/kg) observed on a bouquet, for the three species

Flower species	Roses	Gerberas	Chrysanthemums
Total number of active substances detected	97	30	31
Average number of active substances/sample (minimum–maximum number)	13.6 (3–28)	4.3 (1–9)	6.2 (0–15)
Total load average in pesticides/sample (mg/kg)	26.03	1.70	3.99
Median concentration/sample (mg/kg)	24.35	1.73	2.65
Maximum cumulated deposit/sample (mg/kg)	97.03	4.41	15.73

A statistical analysis performed on the results showed a significant difference between contamination levels according to the species (Table 13).

Table 13: Statistical analysis (Student's t-test, using Minitab® 16 software) of the contamination levels (number of a.s. found and the total load average in pesticides per sample) and comparison between the three species

Flower species	Number of Active Substances		Total Load in Pesticides (mg/kg)	
	T-Value	*p*-Value	T-Value	*p*-Value
Roses/Gerberas	4.66 [a]	0.000	4.92 [a]	<0.001
Roses/Chrysanthemums	3.42 [a]	0.002	4.42 [a]	<0.001
Gerberas/Chrysanthemums	−2.04 [a]	0.050	−2.36 [a]	0.028

[a] Significant difference between results.

It appears also that the bouquets on which the highest number of different a.s. have been detected are also those which were the most contaminated by residues. This can be interpreted as an index of bad phytosanitary practices (numerous and repeated treatments with several PPP instead of an alternation between them in an Integrated Pest Management scheme) (Figure 4). Whatever their origins, samples are

contaminated by numerous a.s. (22 up to 60 different a.s. according to declared country of origin). A total of 107 a.s. are present (Table 14).

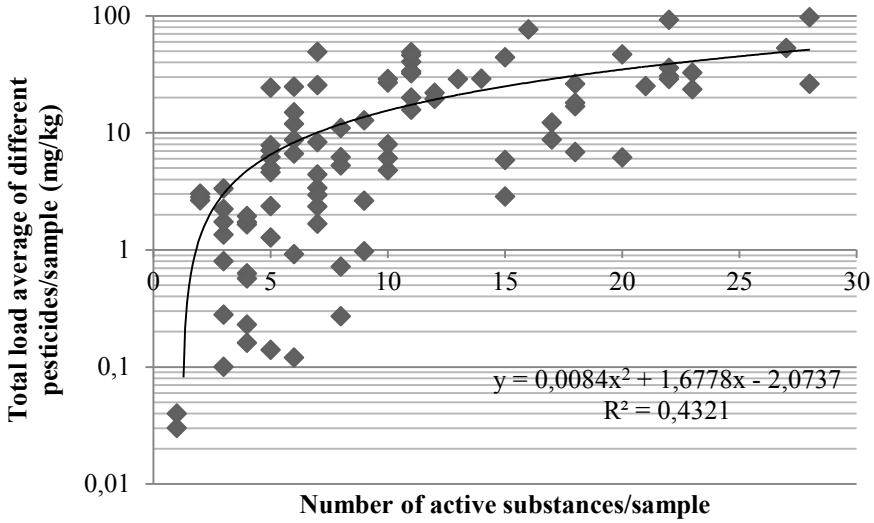

Figure 4: Variation in the total load of pesticides (mg/kg)/sample according to the number of active substances detected/sample

Table 14: Number of different active substances present in the samples of each species, according to country of origin (n = number of samples collected/origin). A total of 107 a.s. have been detected on samples

Origin	Roses	Gerberas	Chrysanthemums
Belgium	38 (*n* = 8)	18 (*n* = 11)	17 (*n* = 2)
Colombia	24 (*n* = 2)	-	-
Ecuador	60 (*n* = 9)	-	-
Ethiopia	29 (*n* = 3)	-	-
Germany	22 (*n* = 1)	-	-
Israel	27 (*n* = 2)	-	-
The Netherlands	54 (*n* = 11)	24 (*n* = 9)	28 (*n* = 18)
Kenya	48 (*n* = 9)	-	-
Unknown (supermarkets)	36 (*n* = 5)	-	-

3.3. Detailed Results of Analyses: Nature and Prevalence of Pesticide Residues

The following table list the 107 active substances found (concentration > 0.01 mg/kg) in the 90 samples of cut flowers and their maximum concentrations (Table 16). Frequency of a.s. in samples varies between the three species: dodemorph (a fungicide) is the most frequent active substance for roses, fluopyram (a fundicide)

for gerberas, and bifenazate, thiamethoxam, and tolclofos-methyl (an acaricide, an insecticide and a fungicide) for chrysanthemums.

Table 15: Number of active substances found in the 90 samples according to their biological activity

Biological activity	Roses	Gerbera	Chrysanthemums
Fungicides	46	15	12
Herbicides	1	-	-
Insecticides	47	14	19
Growth regulators	3	1	-

Table 16: Alphabetic classification of all a.s. present in the 90 samples of roses, gerberas, and chrysanthemums, number of detections (concentrations > LOQ), frequency (samples in % containing the a.s.), and maximum concentration values

Active Substances Detected in the Samples	Roses		Gerberas		Chrysanthemums	
	Number of Detections (out of 50) (Frequency)	Maximum Concentration (mg/kg)	Number of detections (out of 20) (Frequency)	Maximum Concentration (mg/kg)	Number of Detections (out of 20) (Frequency)	Maximum Concentration (mg/kg)
6-Benzyladenine	1 (2%)	0.02	0	<0.01	0	<0.01
Acephate	15 (30%)	21.90	0	<0.01	2 (10%)	2.10
Acetamiprid	12 (24%)	0.71	1 (5%)	0.01	0	<0.01
Acrinatrin	1 (2%)	0.05	0	<0.01	0	<0.01
Ametoctradin	6 (12%)	0.30	0	<0.01	0	<0.01
Azadirachtine	0	<0.01	3 (15%)	0.13	4 (20%)	1.30
Azoxystrobin	6 (12%)	0.06	0	<0.01	0	<0.01
Benalaxyl	1 (2%)	0.14	0	<0.01	0	<0.01
Benomyl (carbendazim)	22 (44%)	27.30	2 (10%)	0.03	0	<0.01
Bifenazate	2 (4%)	0.12	0	<0.01	17 (85%)	0.53
Bifenthrin	1 (2%)	0.69	0	<0.01	0	<0.01
Bitertanol	1 (2%)	0.03	1 (5%)	0.06	0	<0.01
Boscalid	20(40%)	12.90	2 (10%)	0.08	1 (5%)	0.05
Bupirimate	9(18%)	1.80	3 (15%)	0.04	0	<0.01
Buprofezin	3(6%)	0.69	0	<0.01	0	<0.01
Carbosulfan	0	<0.01	0	<0.01	1 (5%)	0.14
Carboxin	1 (2%)	0.03	0	<0.01	0	<0.01
Chlorantraniliprole	3 (6%)	0.03	2 (10%)	0.02	0	<0.01
Chlorfenapyr	2 (4%)	0.04	0	<0.01	0	<0.01
Chloridazon	1 (2%)	0.02	0	<0.01	0	<0.01
Chlorothalonil	3 (6%)	0.12	1 (5%)	2.00	3 (15%)	3.50
Chlorpyrifos	0	<0.01	0	<0.01	2 (10%)	0.31
Clofentezine	12 (24%)	15.30	0	<0.01	0	<0.01
Cyflufenamid	1 (2%)	0.01	0	<0.01	0	<0.01
Cyfluthrin	3 (6%)	0.39	0	<0.01	0	<0.01
Cyhalothrin	6 (12%)	2.40	0	<0.01	0	<0.01

Active Substances Detected in the Samples	Roses		Gerberas		Chrysanthemums	
	Number of Detections (out of 50) (Frequency)	Maximum Concentration (mg/kg)	Number of detections (out of 20) (Frequency)	Maximum Concentration (mg/kg)	Number of Detections (out of 20) (Frequency)	Maximum Concentration (mg/kg)
Cypermethrin	6 (12%)	0.92	0	<0.01	0	<0.01
Cyprodinil	31 (62%)	7.40	0	<0.01	0	<0.01
Deltamethrin	1 (2%)	0.22	0	<0.01	6 (30%)	1.30
Diazinon	2 (4%)	0.05	0	<0.01	0	<0.01
Dicofol	1 (2%)	1.00	0	<0.01	0	<0.01
Difenoconazole	4 (8%)	0.02	0	<0.01	0	<0.01
Dimethoate	2 (4%)	0.33	0	<0.01	0	<0.01
Dimethomorph	17 (34%)	1.90	0	<0.01	0	<0.01
Dinotefuran	2 (4%)	2.10	0	<0.01	0	<0.01
Dodemorph	37 (74%)	41.90	2 (10%)	0.02	0	<0.01
Ethirimol	13 (26%)	0.36	0	<0.01	0	<0.01
Etoxazole	3 (6%)	1.20	0	<0.01	3 (15%)	1.60
Etridiazole	0	<0.05	0	<0.05	7 (35%)	3.50
Famoxadone	11 (22%)	3.30	1 (5%)	0.04	0	<0.01
Fenamidone	5 (10%)	1.10	1 (5%)	0.02	0	<0.01
Fenamiphos	1 (2%)	3.30	0	<0.01	0	<0.01
Fenarimol	1 (2%)	0.03	0	<0.01	0	<0.01
Fenhexamid	13 (26%)	19.50	0	<0.01	2 (10%)	0.90
Fenpropathrin	0	<0.01	1 (5%)	0.02	0	<0.01
Fenpropidin	2 (4%)	0.02	0	<0.01	0	<0.01
Fensulfothion-Oxon	1 (2%)	0.02	0	<0.01	0	<0.01
Fenvalerate	1 (2%)	0.06	0	<0.01	5 (25%)	1.90
Fipronil	7 (14%)	0.68	0	<0.005	1 (5%)	0.75
Flonicamid	18 (36%)	1.40	11 (55%)	3.30	4 (20%)	0.45
Flubendiamide	3 (6%)	0.28	0	<0.01	0	<0.01
Fludioxonil	19 (38%)	2.00	1 (5%)	0.03	1 (5%)	0.02
Flufenoxuron	1 (2%)	0.02	0	<0.01	0	<0.01
Fluopicolide	15 (30%)	1.60	0	<0.01	0	<0.01
Fluopyram	23 (46%)	12.40	15 (75%)	3.00	4 (20%)	6.40
Forchlorfenuron	1 (2%)	0.19	0	<0.01	0	<0.01
Fosthiazate	1 (2%)	0.02	0	<0.01	0	<0.01
Furalaxyl	2 (4%)	9.90	0	<0.01	0	<0.01
Hexythiazox	3 (6%)	0.16	0	<0.01	0	<0.01
Imidacloprid	21 (42%)	3.00	0	<0.01	3 (15%)	0.93
Indoxacarb	3 (6%)	1.20	2 (10%)	0.16	0	<0.01
Iprodione	20 (40%)	17.40	7 (35%)	0.65	0	<0.01
Iprovalicarb	5 (10%)	5.40	0	<0.01	0	<0.01
Isocarbofos	1 (2%)	0.01	0	<0.01	0	<0.01
Kresoxim-methyl	9 (18%)	1.40	0	<0.01	0	<0.01
Lufenuron	12 (24%)	1.90	0	<0.02	5 (25%)	0.87
Mandipropamid	5 (10%)	6.70	1 (5%)	0.01	1 (5%)	0.02
Mepanipyrim	2 (4%)	5.20	0	<0.01	0	<0.01
Metalaxyl and Metalaxyl-M	5 (10%)	0.29	0	<0.01	1 (5%)	0.02
Methamidophos	14 (28%)	5.40	0	<0.01	1 (5%)	0.57

Active Substances Detected in the Samples	Roses		Gerberas		Chrysanthemums	
	Number of Detections (out of 50) (Frequency)	Maximum Concentration (mg/kg)	Number of detections (out of 20) (Frequency)	Maximum Concentration (mg/kg)	Number of Detections (out of 20) (Frequency)	Maximum Concentration (mg/kg)
Methiocarb	1 (2%)	13.60	0	<0.01	4 (20%)	6.00
Methomyl and Thiodicarb	3 (6%)	4.50	0	<0.01	0	<0.01
Methoxyfenozide	9 (18%)	5.20	0	<0.01	1 (5%)	0.02
Metrafenone	5 (10%)	10.30	0	<0.01	0	<0.01
Myclobutanil	1 (2%)	0.13	0	<0.01	0	<0.01
Novaluron	6 (12%)	2.20	0	<0.01	0	<0.01
Oxadixyl	0	<0.01	0	<0.01	2 (10%)	0.03
Oxamyl	1 (2%)	0.01	0	<0.01	0	<0.01
Oxycarboxin	3 (6%)	0.11	0	<0.01	0	<0.01
Paclobutrazol	0	<0.01	1 (5%)	0.01	0	<0.01
Picoxystrobin	2 (4%)	1.80	0	<0.01	0	<0.01
Piperonyl-butoxide	0	<0.01	1 (5%)	0.27	4 (20%)	0.07
Pirimicarb	10 (20%)	0.26	0	<0.01	0	<0.01
Prochloraz	4 (8%)	3.10	0	<0.01	0	<0.01
Procymidone	18 (36%)	35.30	1 (5%)	0.35	0	<0.01
Propamocarb	22 (44%)	35.40	4 (20%)	0.16	0	<0.01
Pymetrozine	6 (12%)	0.56	0	<0.01	1 (5%)	0.03
Pyraclostrobin	7 (14%)	1.30	1 (5%)	0.02	0	<0.01
Pyridaben	1 (2%)	0.08	0	<0.01	0	<0.01
Pyridalyl	1 (2%)	0.01	0	<0.01	0	<0.01
Pyrimethanil	23 (46%)	13.70	0	<0.01	0	<0.01
Quinalphos	1 (2%)	0.05	0	<0.01	0	<0.01
Spinetoram	5 (10%)	0.13	0	<0.01	0	<0.01
Spinosad	9 (18%)	0.58	3 (15%)	0.40	0	<0.01
Spirotetramat	1 (2%)	0.03	3 (15%)	2.30	2 (10%)	0.10
Spiroxamine	34 (68%)	15.00	1 (5%)	0.03	1 (5%)	0.02
Tebuconazole	4 (8%)	5.20	0	<0.01	0	<0.01
Tetradifon	1 (2%)	0.08	0	<0.01	0	<0.01
Thiabendazole	1 (2%)	4.20	0	<0.01	0	<0.01
Thiacloprid	7 (14%)	5.80	0	<0.01	0	<0.01
Thiamethoxam	8 (16%)	4.20	1 (5%)	0.80	17 (85%)	2.20
Thiophanate-methyl	1 (2%)	9.90	2 (10%)	0.02	0	<0.01
Tolclofos-methyl	0	<0.01	0	<0.01	17 (85%)	5.60
Trichlofron	0	<0.02	6 (30%)	0.05	0	<0.02
Trifloxystrobin	2 (4%)	0.09	0	<0.01	1 (5%)	0.03
Triflumizole	3 (6%)	0.54	4 (20%)	0.24	0	<0.01
Triforine	1 (2%)	0.79	0	<0.01	0	<0.01

Among the 90 flower samples analysed, the highest maximum concentrations out of all the active substances analysed were for dodemorph, propamocarb, and procymidone, with 41.9, 35.4, and 35.3 mg/kg, respectively. Regarding the three species, the highest average concentrations were found:

On roses, for methiocarb, thiophanate-methyl, and furalaxyl (13.60, 9.90 and 8.90 mg/kg, respectively).

On gerberas, for chlorothalonil, flonicamid, and spirotetramat (2.00, 1.71, and 1.37 mg/kg, respectively). Four of the 30 active substances detected in the 20 gerbera samples present maximum concentrations of 2 mg/kg and above. Flonicamid and fluopyram present the highest maximum concentrations, with 3.3 and 3.0 mg/kg, respectively.

On chrysanthemums, for acephate, chlorothalonil, etridiazole, methiocarb, and fluopyram (1.06, 1.21, 1.52, 1.80, and 2.57 mg/kg, respectively). Eleven of the 31 active substances detected in the 20 chrysanthemums samples presented maximum concentrations of 1 mg/kg and more. Tolclofos-methyl, methiocarb, and fluopyram presented the highest maximum concentrations, with 5.6, 6.0, and 6.4 mg/kg, respectively.

3.4. Hazard Characterization: Classification of Active Substances According to Their Toxicity

The risk for workers to develop adverse health effects is the combination of health hazards (mode of action; acute and chronic toxicity of a.s.) of pesticides with the likelihood of exposure (concentration levels on flowers; routes of exposure; mitigation measure such as PPE). Both acute and chronic toxicity are of concern for florists. The biological activity is often linked with the toxicity in animals and humans. Insecticides are, in general, the most acutely toxic products, whereas fungicides are considered as less toxic compounds. Many other properties (such as solubility and cutaneous absorption) may interfere with exposure. Of the 107 detected active substances, most belong to groups known for their toxicological properties: organophosphates (12 a.s.); pyrethroids (8 a.s.), and carbamates (7 a.s.) are all pesticides with an action on the nervous system. Since florists are mainly exposed by the dermal route, it was interesting to consider the classification of all a.s. found on cut flowers for acute dermal toxicity according to the CLP (Classification, Labelling and Packaging) Regulation (EC) No. 1272/2008 (Table 17). Most a.s. have a LD50 >2000 mg/kg bw and are not classified for that property (86 a.s. of 97 for roses; 27 a.s. of 30 for gerberas; 28 a.s. of 31 for chrysanthemums)

Table 17: Number of a.s. detected on cut flowers belonging to each category of acute toxicity hazard for the dermal route of exposure (CLP classification)

Categories	LD_{50} (mg/kg bw)	Hazard Wording	Roses	Gerberas	Chrysanthemums
1	(0–50)	Fatal in contact with skin	2	-	-
2	(50–200)	Fatal in contact with skin	1	-	-
3	(200–1000)	Toxic in contact with skin	3	1	1
4	(1000–2000)	Harmful in contact with skin	5	2	2

In addition, classification according to CLP regulations for the different health hazards is reported in Table 18. According to this table, the number of sensitizing active substances detected in the roses, chrysanthemums, and gerberas were 16, 11, and 12, respectively.

Table 18: Number of active substances detected on the various cut flower species classified in each hazard category according to CLP regulation (with the corresponding code of hazard (only relevant categories for florist exposure are listed)

Category	Code	Roses	Gerberas	Chrysanthemums
Acute toxicity				
Category 1	H310: Fatal in contact with skin	2	-	-
Category 2	H300: Fatal if swallowed	10	-	2
	H330: Fatal if inhaled	6	2	3
Category 3	H301: Toxic if swallowed	7	2	4
	H311: Toxic in contact with skin	2	-	2
	H331: Toxic if inhaled	10	1	3
Category 4	H302: Harmful if swallowed	21	7	9
	H312: Harmful in contact with skin	7	2	2
	H332: Harmful if inhaled	3	4	1
Carcinogenicity				
Category 2	H351:Suspected of causing cancer	13	5	4
Serious eye damage/eye irritation				
Category 1	H318: Causes serious eye damage	2	1	2
Category 2	H319: Causes serious eye irritation	3	1	1
Germ cell mutagenicity				
Category 1, 1A or 1B	H340: May cause genetic defects	1	1	-
Category 2	H341: Suspected of causing genetic defects	1	1	-
Reproductive toxicity				
Category 1, 1A or 1B	H360: May damage fertility or the unborn child	3	3	-
Category 2	H361: Suspected of damaging fertility or the unborn child	11	5	3
Additional category for effects on or via	H362: May cause harm to breast-fed children	2	-	-

Category	Code	Roses	Gerberas	Chrysanthemums
lactation				
Sensitisation of the respiratory tract or the skin				
Respiratory sensitizers Category 1, 1A or 1B	H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled	1	-	1
Skin sensitizers Category 1, 1A or 1B	H317: May cause an allergic skin reaction	21	13	11
Skin corrosion/irritation				
Category 1, 1A or 1B	H314: Causes severe skin burns and eye damage	1	1	-
Category 2	H315: Causes skin irritation	6	3	1
Specific target organ toxicity (single exposure)				
Category 3	H335: May cause respiratory irritation	4	3	2
Specific target organ toxicity (repeated exposure)				
Category 1	H372: Causes damage to organs through prolonged or repeated exposure	2	1	1
Category 2	H373: May cause damage to organs through prolonged or repeated exposure	7	3	3

As the florists handle the flowers every day in the course of their work, the exposure risk is also chronic. The reference value considered for this category of workers is the AOEL (Acceptable Operator Exposure Level) (Table 19). The AOEL is the maximum amount of a.s. to which the worker (in this case) may be exposed without any adverse health effect. It is expressed in mg/kg bw/day.

Table 19: Number of active substances detected on the three species of cut flowers classified according to their AOEL values (Source: EU Pesticides Database 2015, European Commission/DGSANCO, Regulation (EC) 1107/2009)

AOEL Values (mg/kg bw/day)	Roses	Gerberas	Chrysanthemums
(0.001–0.01)	19	3	6
(0.01–0.1)	43	15	13
(0.1–1)	18	9	7
>1	1	-	-
No AOEL *	16	3	5

* Active substances, which have no AOEL values; not assessed at the European level.

3.5. Lesson Learned from the Florist Observations and Interviews

The heads of the businesses filled in the great majority (79%) of the questionnaires. Fifty-six percent of the 25 Belgian florists interviewed were male. Twenty-four percent of the florists were aged between 20 and 30 years, 44% between 30 and 50, and 32% were over 50. Sixty-eight percent of the florists worked alongside other people (employees or family members who are also occasionally exposed). Florist exposure can arise from their activities and can vary according to the working time spent on handling cut flowers. According to the survey, they all have similar activities, such as handling, sorting, pruning, bundling of flowers, and preparation of bouquets. Activities were carefully observed to be repeated later at the laboratory. Sixty percent of the florists worked between 6 and 7 h a day (40% more than 8 h). The time spent preparing bouquets and handling flowers vary greatly over the year, but is always quite high, varying on average from 2 to 6 h a day for 80% of the florists in the low season, and for 40% of the florists in the high season. This handling time could be in excess of 6 h for 8% of the florists in the low season, but during the high season or special occasions, an intense working period, 60% spent more than 6 h a day on this work. Only 12% of the florists worked fewer than 2 h a day in the low season. In addition, the majority of the florists (18 out of the 25 respondents) worked six or even seven days a week. The others worked five days a week. Regarding the potential long term exposure of florists, the survey showed that 44% of the respondents had been working as florists for less than 10 years, but more than 30% had been working as florists for more than 30 years.

With regard to the use of personal protection equipment, 96% of the florists wear no special clothing. Only 20% of the florists surveyed use occasionally latex gloves when preparing bouquets and handling flowers. With regard to hygiene practices, 84% wash only their hands after handling flowers; 20% wash their hands and arms, and 8% their hands, arms, and faces after working.

Sixty-five percent wash thoroughly all over after their day's work. Eighty-eight percent of the florists eat and drink and 12% smoke, during working. None of the florists surveyed use PPP themselves (some used CHRYSAL®, an aluminium sulphate, to lengthen the life of the cut flowers).The main routes of exposure during post-application activities are dermal and by inhalation (EU Pesticide database). Inhalation could be later investigated because some pesticides are rather volatile and the plants are stored directly on the premises of the shop where florists are working. This could lead to a significant concentration of active substances in the air. Oral exposure may also occur secondarily to dermal exposure, through hand to mouth transfer.

However, for workers, maximum potential exposure by this route is generally assumed to be negligible in comparison with that via the dermal route and by inhalation (EU Pesticide database). Sixty percent of the florists surveyed had not received any information regarding the presence of residual pesticides on cut flowers. Thirty-six percent of them had received information through the media. Only 4% had received information from health workers. With regard to health, four persons declared that they had eye problems, one declared respiratory problems, and

four declared irritations and itching of the skin. Only one florist mentioned headaches and recurrent tiredness. Of the 25 florists surveyed, two suffered from cancers, seven had skin allergy problems, and one suffered from thyroid problems.

4. Discussion

From the results of this survey, cut flowers (roses, gerberas, and chrysanthemums) sold in Belgium were found to be heavily contaminated by pesticide residues. The first significant result is the overall contamination of cut flowers. Only a single sample analysed (chrysanthemums from the Netherlands) was free from detectable residues, rather than 16 (15.2 per cent) of 105 lots that did not contain any pesticide residues in the study of Morse *et al.* published in 1979. On the contrary, most active substances (a.s.) reached high levels of residues, with concentrations between 10 and 50 mg/kg, about a thousand times above the maximum limit value set for residues in foodstuffs. Sixty percent of flowers had total pesticide residues >5 mg/kg and 4% had concentrations >50 mg/kg.

The second lesson learned from the analyses is the large number of a.s. detected on flowers. A total of 107 active substances (almost 10 active substances/sample) were detected in the 90 cut flower samples (roses, gerberas and chrysanthemums) with a total pesticide load average of 15.72 mg/kg per flower sample. The high pesticide levels on cut flowers are apparently bound to the use of a large number of different pesticides on flowers by growers and can be explained by the pressure of pests and diseases, the lack of alternative pest control methods, the commercial value of flowers, which should be perfect at harvest, and the absence of maximum residue limits. The analyses of samples declared of Belgian or Dutch origins reveal the abnormal presence of 15 active substances, which are not authorised for use in the EU. These results should, however, be put into perspective as we have no firm guarantee of the origin of the samples taken from the retailer premises rather than from the producers. Nevertheless, the frequency of the presence of active substances not authorised in the EU is significantly higher in the Belgian samples, regardless of the species involved, which could be alarming if flowers were produced in Belgium but, generally, the Belgian official controls do not reflect a misuse of pesticides (AFSCA, 2015).

Of the three species, roses are the most heavily contaminated by pesticide residues, with an average total load of active substance per sample of 26.03 mg/kg. No fewer than 97 pesticide residues were found in the rose samples (on average 13.56 active substances per sample). For chrysanthemums and gerberas, pesticide residues detected were lower: an average of 6.25 active substances per chrysanthemum sample and an average of 4.35 active substances per gerbera sample with an average total load of 1.70 mg/kg. Statistical analysis confirmed that the roses were very significantly more contaminated than gerberas and chrysanthemums. The cumulated total of all the residues was as high as 97.03 mg/kg for a single bouquet of five Belgian roses. Clearly, the largest number of different a.s. and the highest total concentration of residues were detected on the rose samples.

All detected active substances are insecticides (50%) and fungicides (46%), except four growth regulators and one herbicide (chloridazon). The most frequently

detected substances are the fungicides fluopyram (42 samples out of 90), dodemorph, propamocarb, and procymidone and their residues reached the highest concentrations on the rose samples (e.g., 41.9 mg/kg for dodemorph). Nevertheless, a certain number of the active substances detected are highly acutely toxic (acephate, methiocarb, monocrotophos, methomyl, deltamethrin, etc.) and can generate a direct effect on the nervous system (e.g., in the case of handling flowers, transfer from the hands to the mouth could cause accidental poisoning and affect the florist's health). Even if pesticides are generally less toxic by dermal contact than by the oral route, people who handle a large number of contaminated flowers daily are exposed via dermal absorption, especially in the case of fat-soluble pesticides, and subjected to long term effects on their health. In the study of published by Morse *et al*. in 1979, the insecticide monocrotophos was also one of the most important contaminants (detected in nine of 105 lots), with residue levels from 7.7 up to 4750 mg/kg. Other toxic insecticides (such as endosulfan and diazinon) were also frequently detected. Nevertheless, the comparison between active substances detected on flowers in both studies is poorly relevant as many new active substances are used by growers today with lower dosages.

From the survey of 25 Belgian florists it is concluded that florists may be exposed to residual deposits from contaminated flowers, especially when preparing bouquets. Contact with foliage may deposit residues onto the skin of a worker. The exposure is assumed to depend on the task duration (h/day) (EU Pesticide data base). The length of florists' exposure varies greatly within the year, but remains high regardless of the season (the working day varies from 2 to 6 h). The task duration of florists, which is an important factor to consider when building exposure scenarios for a specific group of workers, is lower than the default value for time of exposure (8 h) in the EFSA Guidance Document 2014 (EFSA, 2014). However, bad habits (eating, drinking, or smoking at work) and the absence of wearing personal protective equipment of most of the florists could increase the risk of contact with the pesticide residues.

Regarding the effect of residues on the florists' health in Belgium, it was not possible to conclude only on the basis of personal feelings and declarations. The Belgian florists are not directly involved with pesticide handling and spraying. However, analytical results show that they can be exposed to high levels of residues during handling. According to their answers in the survey, they seem to be mainly affected by skin allergy problems. Only one had mentioned headaches and recurrent tiredness. Those observations are consistent with their usual professional practices and toxicological properties of the compounds (see Tables 17 and 18). The survey of Lu in 2005 has shown that frequent contact with residues of pesticide applied on flowers can generate detrimental health effects: workers who re-entered a recently sprayed area were 20 times more likely to get ill than those who did not. Moreover, Abell *et al.* demonstrated in 2000a that male fecundity could be decreased after exposure to pesticides in the manual handling of ornamental flowers in greenhouses.

5. Conclusions

In summary, overall the samples of cut flowers (roses, gerberas, and chrysanthemums) sold in Belgium contain high pesticide residue levels. Thus, florists who handle a large number of flowers are exposed daily, with a potential effect on their health. Therefore, to reduce the exposure of florists to pesticide residues, sensitisation of professionals to better practices and hygiene rules is highly recommended. The European Regulation on Maximum Residue Limits (Regulation (EC) N° 396/2005) could be extended to the control of pesticide residues on flowers and MRLs (Maximum Residue Limits) could be set up for flowers to decrease the risk of exposure of florists and the general population.

This survey will be completed later by results of field and laboratory trials to measure the dislodgeable foliar residues (DFR, $\mu g/cm^2$), the transfer from plant to hands and, finally, to estimate the dermal exposure of florists to pesticides applied on cut flowers.

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Author Contributions

This research was undertaken as part of khaoula Toumi's Doctor of Phytopharmacy thesis. Bruno Schiffers is the promoter of this thesis. All authors contributed significantly to the successful completion of this research work both intellectually and financially. Accordingly, they conceived and designed the study plan. Khaoula Toumi conducted sampling, performed the interviews, analyzed the data and wrote the intial manuscript. Bruno Schiffers guided this study and provided revisions on the manuscript. Christiane Vlemincks and Joris van Loco provided feedback on the manuscript. Finally, all the authors have read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Chapitre 4

Exposition cutanée potentielle des fleuristes belges aux résidus de pesticides

Introduction

L'évaluation de l'exposition est une étape clé parmi les quatre principales étapes de l'évaluation des risques. L'évaluation de la quantité de résidus de pesticides présente sur les mains des travailleurs constitue souvent la principale mesure de l'exposition cutanée. Dans ce chapitre, le transfert des résidus de pesticides présents sur les fleurs coupées vers les mains de fleuristes durant la manipulation des fleurs coupées et la préparation des bouquets a été étudié afin d'évaluer les risques d'exposition cutanée potentielle des fleuristes belges aux résidus de pesticides.

Ce chapitre est une version adaptée des deux articles suivants :

- Toumi, K., Joly, L., Vleminckx, C., & Schiffers, B. (2017). Potential demal exposure of florists to fungicide residues on flowers and risk assessment. (Publié dans *Communications in Agricultural and Applied Biological Sciences*, 82(2), 49-60.)

- Toumi, K., Joly, L., Vleminckx, C., & Schiffers, B. (2017). Risk assessment of florists exposed to pesticide residues through handling of flowers and preparing bouquets. (Publié dans *International journal of environmental research and public health*, 14(5), 526-344. DOI: 10.3390/ijerph14050526)

I. Potential dermal exposure of florists to fungicide residues on flowers and risk assessment

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Abstract

Flowers are susceptible to many pests and diseases. Therefore, they can be sprayed several times during their growth considering that no MRL are set for flowers. High levels of pesticide residues potentially expose daily the florists who handle cut flowers and possibly could endanger their health. A study was carried out to evaluate the risk for florists exposed to fungicide residues during normal professional tasks. Cotton gloves were distributed to 20 florists (two pairs to each florist) and worn during two consecutive half days during normal professional tasks (from min 2 hours to max 3 hours/day) to measure their potential dermal exposure (PDE). Samples were analyzed with a multi-residue (QuEChERS) method validated by a laboratory accredited for pesticide residues and with a combination of gas and liquid chromatography tandem mass spectrometry. It appears from the results that a total of 54 fungicides with different toxicity classes were detected on cotton gloves. An average of 15.53 mg/kg fungicide residues per glove sample was measured. Six of 54 are suspected of causing cancer after prolonged or repeated exposure. Boscalid was both the active substance for which the highest maximum and average concentrations (26.21 and 3.47 mg/kg, respectively). Famoxadone had the most critical PDE (156% AOEL for the maximum concentration). As a consequence, this study leads to the conclusion that Belgian florists, who work for several years and handle a large number of flowers contaminated by high concentrations of pesticide residues, are exposed daily with a potential effect on their health. This suggests that safety standards should be set for residue levels on cut flowers.

Key words: dermal exposure, risk assessment, cut flowers, florists.

1. Introduction

As in any intensive culture, flowers require the use of a wide range of fungicides to control fungal diseases, which can damage production and marketability and to stay competitive in both national and international markets (Cooper Dobson, 2007). Fungicides use provides many benefits to ornamental producers, including the consistent availability, the reliable control. They are in general less expensive than alternatives and may reduce plant pathogenic transmission (Bethke and Cloyd, 2009). However, a large majority of growers still consider pesticides, as vital tool in floriculture that can improve and maintain crop more productive and profitable in the face of costs and quantities of floral products, meaning they will be able to market large quantities of floral products with an acceptable quality and relatively modest prices. The vast majority of European florists is not actively engaged in social and environmental standards, not as a purchasing criterion, nor in their communication towards consumers (Rikken, 2010). Moreover, the flower industry practices have been completely unregulated with lack of maximum residue limits (MRL) for flowers explains that, unlike other crops which are harvested for consumption, resulting in the use of highly toxic chemicals which are no more approved in Europe, USA and many other countries thanks to an extended risk assessment and more restrictive regulation, regularly up to harvesting. A recent study, conducted on cut flowers (roses, gerberas, and chrysanthemums) sold in Belgium, showed that flowers are heavily contaminated by fungicide residues. The most frequently detected active substance was the fungicide fluopyram (42 samples out of 90) (Toumi *et al.,* 2016a). In another study, the fungicide dodemorph was the most frequently detected active substance with the highest maximum concentration (41.9 mg/kg) measured in the rose samples (Toumi *et al.,* 2016b). The great majority of fungicides sprayed on cut flowers are actually dislodgeable by human contact (hands, gloves, and clothing). These active substances are persistent, fat-soluble and can be absorbed through skin contact (Toumi *et al.,* 2016a). Consequently, Belgian florists who handle a large number of flowers, daily and for several hours can potentially be exposed to residual deposits of pesticides and possibly endanger their health (Toumi *et al.,* 2016a). Pesticides have been closely linked to a wide range of serious health concerns for exposed floriculturist operators and workers, ranging from short-term impacts such as weakness and fatigue, muscle pain, chills and fever, blurred vision, dizziness and headache (Lu, 2005) to chronic impacts like cancer (Fleming *et al.,* 1999), genetic damage (Munnia *et al.,* 1999; Bolognesi, 2003) and reproductive harm (abortion, prematurity, and congenital malformations) (Restrepo *et al.,* 1990). Following the exposure problems, dermal risk assessment is deemed necessary for Belgian florists handling daily contaminated flowers and preparing bouquets. Assessing the amount of pesticide residues on workers' hands is often the main measure of dermal exposure. Hand exposure often accounts for a significant proportion of the total exposure has been documented many times (US EPA 1986; OECD 1997). As a matter of fact, gloves, hand washes or hand wipes (McCurdy *et al.,* 1994; Baldi *et al.,* 2006; Aprea *et al.,* 2009; Baldi *et al.,* 2014) are often used to sample dermal hand exposure. Gloves worn during normal professional tasks act as a reservoir for active substances that come into contact with the skin (Brouwer *et al.,* 1992 a, b, c; Jurewicz *et al.,* 2009; Li *et al.,* 2011). In order to assess the risk for

professionals exposed to pesticide residues on flowers, a study has been carried out with a group of florist on a voluntary basis.

2. Materials and methods

To evaluate the potential dermal exposure of florists preparing bouquets, twenty samples of cotton gloves (two pairs of cotton gloves/sample) were distributed to Belgian volunteer florists and worn during two consecutive half days when handling flowers (from min 2 h to max 3 h/day) to measure the potential transfer of pesticides from treated flowers to hands. The two pairs were collected as a single sample (four gloves/sample), weighed, cut in small pieces with scissors and stored in freezing bags at −18 °C. The samples were kept for no more than three days before being taken to the analytical laboratory (transport by road from Gembloux to Ghent).

The residual fungicide deposits on the gloves were analysed in a laboratory holding a BELAC accreditation to ISO/CEI 17025 for pesticide residues (PRIMORIS, Technologiepark 2/3, B-9052 Zwijnaarde – Ghent). PRIMORIS is a private, accredited and officially recognised service laboratory. Samples were analyzed using a multi-residue Quick Easy Cheap Effective Rugged Safe (QuEChERS) method validated by the laboratory for the analysis of residues in foodstuffs. The extraction procedure is based on the AOAC (Association of Official Analytical Chemists) Official Method 2007.01 (Lehotay, 2007). According to the active substances to be determined, the pesticide residue concentration was quantified by GC-MS/MS or LC-MS/MS (gas chromatography or liquid chromatography tandem mass spectrometry). Gas chromatography was used to analyse relatively small, thermally stable, volatile, non-polar molecules. Liquid chromatography was used to analyse larger, thermolabile, non-volatile, polar molecules. The combination of both methods allows the analysis of approximately 500 active substances in a single analysis. For most of the active substances, the quantification limit was ≤ 0.01 mg/kg. Therefore, the analytical results were corrected accordingly for all active substances with a recovery ratio between 50–130% (only few substances had a percentage of recovery below or above these values; in this case, the results remain uncorrected in the tables 20 & 23).

The potential dermal exposure (PDE) values were estimated as the amount of pesticide residues with low adhesion that were transferred from flowers to gloves. For each active substance, PDE was calculated as follows:

PDE (in mg a.s./kg bw per day) = ((C_T (mg/kg) × GW (kg)) × 3)/bw (kg)

where C_T is the concentration of active substance in the sub-sample during the task duration of the trial (2 h), GW is the average weight of the cotton gloves samples (57 g ± 0.17 g), 3 is a correction factor (total task duration value equal to 6 h/day) and bw is the body weight (60 kg). A recent publication mentioned that 60% of the Belgian florists worked between 6 and 7 h/day (Toumi *et al*., 2016a). A default body weight (bw) value of 60 kg is used in line with the recent EFSA Guidance Document to cover a range of professionally exposed adults (EFSA, 2014). The risk characterisation is obtained as the ratio of the exposure level to the reference value of each active substance, the AOEL (Acceptable Operator Exposure Level; in mg

a.s./kg·bw per day). Several prediction levels of the PDE were considered, including the mean, 75th percentile, 90th percentile, and the maximum (worst case) (in mg/kg bw per day) to assess the risk for florists.

3. Results and Discussion

3.1. Fungicide residues concentrations on cotton gloves

All glove samples appeared to be contaminated by high levels of fungicide residues for most active substances (Figure 5). A total of 54 fungicides were identified, with an average of about 21.05 a.s./sample and an average total fungicide residue concentrations per glove sample of 15.53 mg/kg (Table 20).

Seventeen active substances (azoxystrobine, benomyl, boscalid, cyprodinil, dimethomorph, dodemorph, famoxadone, fenhexamid, fludioxonil, fluopyram, iprodione, mandipropamid, methoxyfenozide, prochloraz, procymidone, propamocarb and spiroxamine) are the most frequently detected fungicides. They are present on more than 12 of the 20 samples (60%).

Table 20: Total number of a.s. detected per sample and fungicide residues concentrations in 20 samples of gloves

Samples from florists	Total number of detected a.s. (LOQ ≤ 0.01 mg/kg)	Total fungicide residues concentrations (mg/kg)
Sample N°1	25	89.87
Sample N°2	24	19.21
Sample N°3	27	2.69
Sample N°4	31	27.46
Sample N°5	36	35.50
Sample N°6	23	7.41
Sample N°7	13	4.71
Sample N°8	34	33.92
Sample N°9	17	6.11
Sample N°10	9	1.08
Sample N°11	25	18.41
Sample N°12	15	6.18
Sample N°13	33	20.89
Sample N°14	10	3.31
Sample N°15	25	16.89
Sample N°16	18	4.56
Sample N°17	14	0.96
Sample N°18	21	6.10
Sample N°19	13	4.10
Sample N°20	8	1.18
Mean	**21.05**	**15.53**
Median	**22.00**	**6.15**

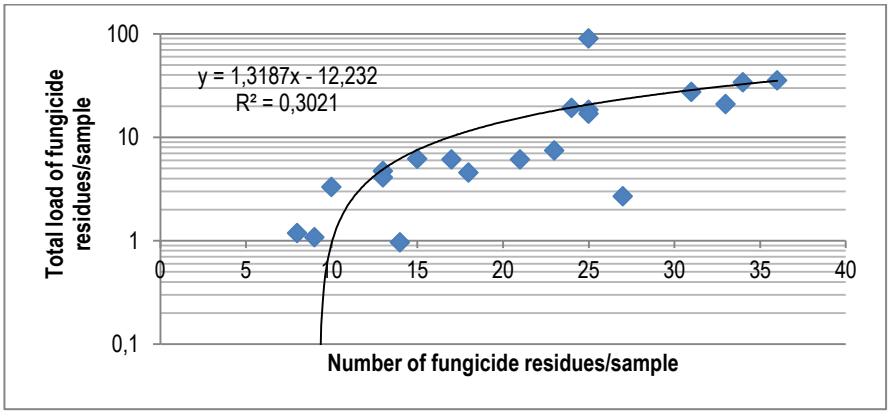

Figure 5: Variation in the total load of pesticides (mg/kg)/sample according to the number of active substances detected/sample.

3.2. Risk assessment for florists

The risk is generated by combination of the "hazard" (mode of action; acute and chronic toxicity of a.s.) and "exposure" (concentration levels on flowers; routes of exposure: oral or dermal). In general, the main route of exposure to plant protection products is the oral route, yet most exposures to operators, workers, bystanders and residents will be via dermal and /or inhalation routes. Therefore, it is necessary to assess the exposure for the operators (i.e. applicators, crop-workers, harvesters) for the different likely routes of exposure.

3.3. Hazard identification and characterisation

3.3.1. Classification of active substances according to the average and maximum concentrations

Five active substances (boscalid, iprodione, mandipropamid, fludioxonil and fenhexamid) of the 54 detected fungicides in the 20 cotton gloves samples have an average concentration higher than 1 mg/kg. Boscalid was the active substance with both the highest maximum and average concentrations (26.21 and 3.47 mg/kg, respectively) (Figure 6).

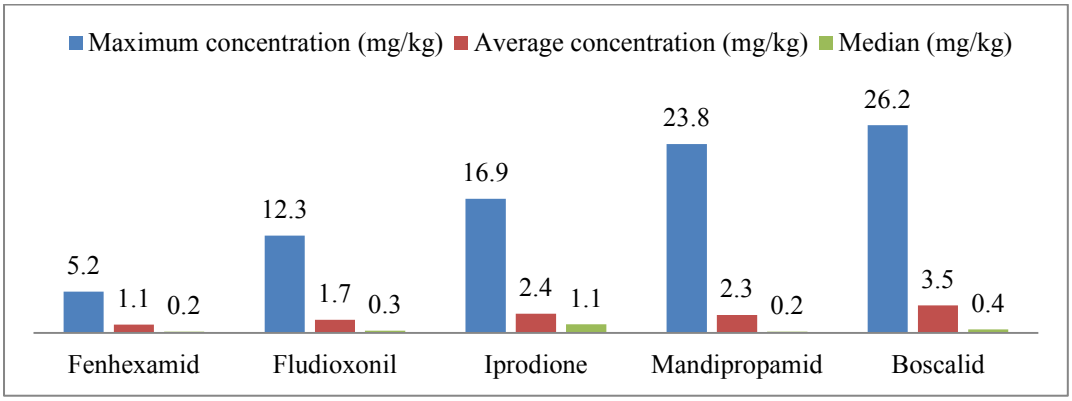

Figure 6: Fungicides with the highest average and maximum residues concentrations measured on 20 samples of cotton gloves

3.3.2. Classification of active substances according to the chemical family

Of the 54 detected active substances, most of the fungicides belong to the following chemical groups: triazoles (12 a.s); strobilurins (6 a.s); anilinopyrimidines and benzimidazoles (3 a.s. each); carbamates, dicarboximides and phenylpyrroles (2 a.s. each). Pesticides from those groups are known for their toxicological properties (action on the nervous system after exposure; acute toxicity, etc).

3.3.3. Classification of active substances according to the EU Pesticides Database

As the florists handle the flowers every day in the course of their work, they are exposed to plant protection products like other “operators”. Worker exposure rates can be similar to those of operators. However, it should be taken into account that workers are often exposed for extended periods of time and usually don’t take any protective measures. The “Acceptable Operator Exposure Level” (AOEL) is the reference value to consider for professionals exposed to pesticides. AOEL is defined in Regulation (EC) 1107/2009 as "... the maximum amount of active substance to which the operator may be exposed without any adverse health effects". AOEL values relate to the internal (absorbed) dose available for systemic distribution from any route of absorption and are expressed as internal levels (mg/kg bw/day). When the operator exposure remains below this limit, the risk for them is considered as “acceptable” (Regulation (EC) 1107/2009) (Table 21).

The active substances can also be classified on their hazard category according to the CLP regulation (for "Classification, Labelling and Packaging”) (Regulation (EC) 1272/2008) is a European Union regulation from 2008, which aligns the European Union system of classification, labelling and packaging of chemical substances and mixtures to the Globally Harmonised System (GHS). It is expected to facilitate

global trade and the harmonised communication of hazard information of chemicals and to promote regulatory efficiency (Table 22).

Table 21: Number of fungicides detected on the gloves worn by florists classified according to their AOEL values (Source: EU Pesticides Database 2017, European Commission/DG HEALTH and Regulation (EC) 1107/2009)

AOEL values (mg/kg bw/d)	Number of active substances
[0.001-0.01 [	4
[0.01-0.1 [	27
[0.1-1[	20
>1	1
No AOEL*	2

**Active substances which have no AOEL values; not assessed at European level.*

Table 22: Number of fungicides detected on the gloves worn by florists classified by hazard category according to the CLP regulation (Source: Regulation (EC) 1272/2008)

Class	Category	Code (Hazard)	Number of a.s. in the category
Acute toxicity	Category 2	H330: Fatal if inhaled	1
	Category 3	H331: Toxic if inhaled	3
	Category 4	H302: Harmful if swallowed	12
		H312: Harmful in contact with skin	1
		H332: Harmful if inhaled	4
Carcinogenicity	Category 2	H351:Suspected of causing cancer	6
Serious eye damage/	Category 1	H318: Causes serious eye damage	4
eye irritation	Category 2	H319: Causes serious eye irritation	1
Germ cell mutagenicity	Category 1,1A or 1B	H340: May cause genetic defects	1
	Category 2	H341: Suspected of causing genetic defects	1
Reproductive toxicity	Category 1,1A or 1B	H360: May damage fertility or the unborn child.	2

Class	Category	Code (Hazard)	Number of a.s. in the category
	Category 2	H361: Suspected of damaging fertility or the unborn child.	6
Sensitisation of the respiratory tract or the skin	Skin sensitisers category 1,1A or 1B	H317: May cause an allergic skin reaction	14
Skin corrosion / irritation	Category 1,1A or 1B	H314: Causes severe skin burns and eye damage	1
	Category 2	H315: Causes skin irritation	4
Specific target organ toxicity (single exposure)	Category 3	H335: May cause respiratory irritation	2
Specific target organ toxicity (repeated exposure)	Category 2	H373: May cause damage to organs through prolonged or repeated exposure	2

According to the CLP classification (Table 22), the majority of detected active substances have potential hazardous chronic effects. Twenty one have an acute toxicity. Six are also suspected of causing cancer. Many fungicides detected in the glove samples may affect the skin of the florists after exposure by contact (allergic reaction: 14; skin irritation: 4; harmful in contact with skin: 1; severe skin burns and eye damage: 1). Furthermore, five cause serious eye damage and eye irritation. Moreover, one active substance may cause genetic defects and one is suspected of a similar effect. In addition, eight active substances have a reproductive toxicity, two may cause respiratory irritation and two may cause damage to organs through prolonged or repeated exposure.

3.3.4. Dermal exposure assessment and risk characterisation

Risk characterization is the fourth step of the risk assessment process, integrating information from the hazard characterization and the exposure assessment to produce scientific advice for risk managers (Renwick *et al.,* 2003).

Risk is estimated by comparing potential dermal exposure to the Acceptable Operator Exposure Level (AOEL)[4].

Table 23: Classification of the active substances (and metabolites) according to the PDE expressed in percentages of their respective AOEL values (mean, 75th percentile, 90th percentile, and maximum) for all identified fungicides in the 20 samples of gloves worn by the Belgian florists

PDE in % of AOEL	PDE (Mean)	PDE (75th P)	PDE(90th P)	PDE (Max)
[0-50% [	Ametoctradin Azoxystrobine Benomyl (carbendazim) Bitertanol Boscalid Bupirimate Captan (tetrahydrophta-limide) Chlorothalonil Cyproconazole Cyprodinil Difenoconazole Dimethomorph Diphenylamine Dodemorph Famoxadone Fenamidone Fenhexamid Fluazinam Fludioxonil Fluopicolide Fluopyram Fluoxastrobin Flusilazole Flutolanil Flutriafol Fluxapyroxad Iprodione Iprovalicarb Kresoxim-methyl Mandipropamid Mepanipyrim Metalaxyl (metalaxyl-M) Methoxyfenozide	Ametoctradin Azoxystrobine Benomyl (carbendazim) Bitertanol Boscalid Bupirimate Captan (tetrahydrophta-limide) Chlorothalonil Cyproconazole Cyprodinil Difenoconazole Dimethomorph Diphenylamine Dodemorph Famoxadone Fenamidone Fenhexamid Fluazinam Fludioxonil Fluopicolide Fluopyram Fluoxastrobin Flusilazole Flutolanil Flutriafol Fluxapyroxad Iprodione Iprovalicarb Kresoxim-methyl Mandipropamid Mepanipyrim Metalaxyl (metalaxyl-M) Methoxyfenozide	Ametoctradin Azoxystrobine Benomyl (carbendazim) Bitertanol Boscalid Bupirimate Captan (tetrahydrophta-limide) Chlorothalonil Cyproconazole Cyprodinil Difenoconazole Dimethomorph Diphenylamine Dodemorph Fenamidone Fenhexamid Fluazinam Fludioxonil Fluopicolide Fluopyram Fluoxastrobin Flusilazole Flutolanil Flutriafol Fluxapyroxad Iprodione Iprovalicarb Kresoxim-methyl Mandipropamid Mepanipyrim Metalaxyl (metalaxyl-M) Methoxyfenozide Metrafenone	Ametoctradin Azoxystrobine Bitertanol Bupirimate Captan (tetrahydrophta-limide) Chlorothalonil Cyproconazole Cyprodinil Difenoconazole Dimethomorph Diphenylamine Dodemorph Fenamidone Fenhexamid Fluazinam Fludioxonil Fluopicolide Fluopyram Fluoxastrobin Flusilazole Flutolanil Flutriafol Fluxapyroxad Iprodione Iprovalicarb Kresoxim-methyl Mandipropamid Mepanipyrim Metalaxyl (metalaxyl-M) Methoxyfenozide Metrafenone Myclobutanil Penconazole Picoxystrobin

[4] La comparaison se fait préférentiellement au PDE (Potential Dermal Exposure) et non à l'ADE (Actual Dermal Exposure) car la toute grande majorité des fleuristes ne portent aucun équipement de protection comme l'ont démontré l'enquête et nos observations.

PDE in % of AOEL	PDE (Mean)	PDE (75th P)	PDE(90th P)	PDE (Max)
	Metrafenone Myclobutanil Penconazole Picoxystrobin Prochloraz Procymidone Propamocarb Propiconazole Pyraclostrobin Pyrimethanil Spiroxamine Tebuconazole Tetraconazole Thiabendazole Thiophanate methyl Tolclofos-methyl Triadimefon (triadimenol) Trifloxystrobin Triflumizole	Metrafenone Myclobutanil Penconazole Picoxystrobin Prochloraz Procymidone Propamocarb Propiconazole Pyraclostrobin Pyrimethanil Spiroxamine Tebuconazole Tetraconazole Thiabendazole Thiophanate methyl Tolclofos-methyl Triadimefon (triadimenol) Trifloxystrobin Triflumizole	Myclobutanil Penconazole Picoxystrobin Prochloraz Propamocarb Propiconazole Pyraclostrobin Pyrimethanil Spiroxamine Tebuconazole Tetraconazole Thiabendazole Thiophanate methyl Tolclofos-methyl Triadimefon (triadimenol) Trifloxystrobin Triflumizole	Prochloraz Propamocarb Propiconazole Pyraclostrobin Pyrimethanil Spiroxamine Tebuconazole Tetraconazole Thiabendazole Thiophanate methyl Tolclofos-methyl Triadimefon (triadimenol) Trifloxystrobin Triflumizole
[50-100% [	-	-	Procymidone Famoxadone	Boscalid
≥ 100%	-	-	-	Procymidone Benomyl (carbendazim) Famoxadone

The potential dermal exposures of florists were estimated for the average, for different percentiles, and for the maximum concentration of residues in samples (Table 23). The results from the different percentiles used to estimate PDE vary by orders of magnitude. As was shown in Table 23, no fungicide exceeds the AOEL for PDE mean, PDE P75 and PDE P90 values. However, at the maximum (PDE_{MAX} or worst case) values of PDE, three fungicides (benomyl (carbendazim) (128% AOEL), famoxadone (156 % AOEL) and procymidone (100 % AOEL)) exceed the AOEL indicating risk situations.

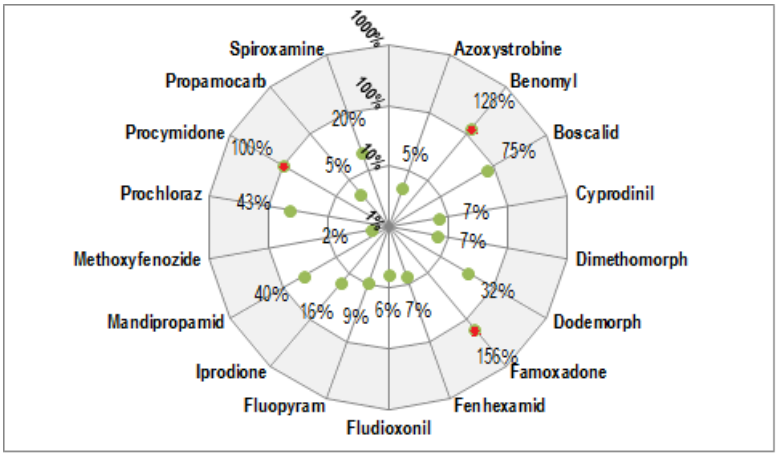

Figure 7: The maximum potential exposure (PDE_{MAX}) of the seventeen most frequently detected active substances on gloves worn by florists as a percentage of the AOEL

Three of 17 fungicides mostly detected on gloves exceed the AOEL indicating risk situations. Famoxadone has the most critical PDE (156% AOEL for the maximum concentration) (Figure 7).

4. Conclusion

The first conclusion after analysis of the fungicide residual deposits on cotton gloves worn by florists is the high level of contamination of all samples: 54 fungicides detected, i.e. an average of almost 21 active substances/sample and a total average pesticide load of 15.53 mg/kg per cotton gloves sample. This reflects the intensive use of fungicides on cut flowers in general. As the flowers are susceptible to fungal diseases, they are regularly treated till the harvest time without any restriction on the fungicide use because there are no maximum residue limits (MRLs) for flowers, unlike other cultures. The high levels of fungicide residues in gloves worn by the florists are linked to high rates of pesticides but also to repeated sprayings during the growing season. The results from this study have shown that boscalid was the active substance for which the highest maximum and average concentrations (26.21 and 3.47 mg/kg, respectively) measured on the glove samples analysed. This study illustrates that the majority of these fungicides have an acute toxicity (21 s.a.) and potential hazardous chronic effects (carcinogenicity (6 s.a.), serious eye damage or irritation (5 s.a.), germ cell mutagenicity (2 s.a), reproductive toxicity (8 s.a.), sensitisation of the skin (14 s.a.), skin corrosion or irritation (5 s.a.), specific target organtoxicity (single exposure) (2 s.a.) and specific target organtoxicity (repeated exposure) (2 s.a.)).

Concerning the PDE, no fungicide exceeds the AOEL for PDEmean, PDE P75 and PDE P90 values. However, at the maximum (or worst case) values of PDE, three fungicides (benomyl (carbendazim) (128% AOEL), famoxadone (156 % AOEL) and procymidone (100 % AOEL)) exceed the AOEL indicating risk situations for people who doesn't wear a protective equipment. These fungicides are known for their toxicological properties and have potential hazardous chronic effects, e.g. benomyl (and its metabolite carbendazim), which is not approved in EU, may affect the skin

of the florists after exposure by contact (allergic reaction, skin irritation), may cause genetic defects and respiratory irritation and may damage fertility or the unborn child. In addition, famoxadone may cause damage to organs through prolonged or repeated exposure.

Thus, Belgian florists who will handle a large number of flowers without protective equipment may be at risk of exposure to pesticide residues with potential effects on their health. Moreover, it should be taken into account that the majority of florists do not wear gloves, or any other PPE, even if they spend 2 to 6 h per day handling cut flowers and preparing bouquets (Toumi *et al*., 2016a). To better assess the risk, bio-monitoring of the florists with analysis of their blood, urines, and hairs is still to be investigated in the lab. To reduce the exposure of florists to pesticide residues, solutions could be recommended: protective gloves during handling, hygiene rules, better pesticides management at the field (IPM or even organic flower production, a potential niche market) and a quality control of imported cut flowers. Finally, it could be interesting to set up a Maximum Residue Limit for flowers to decrease the risk for professionals and all other people in contact with flowers.

II. Risk Assessment of Florists Exposed to Pesticide Residues through Handling of Flowers and Preparing Bouquets

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Abstract

Flowers are frequently treated with pesticides and, as a result, florists handling daily a large number of flowers can be exposed to pesticide residues. A study was conducted among twenty volunteer florists located in Namur Province and in the Brussels Capital Region of Belgium in order to assess their potential dermal exposure to dislodgeable pesticide residues transferred from flowers to hands. Two pairs of cotton gloves were worn during two consecutive half days while handling flowers and preparing bouquets (from min 2 h to max 3 h/day). The residual pesticide deposits on the glove samples were extracted with a multi-residue Quick Easy Cheap Effective Rugged Safe (QuEChERS) method and analyzed by a combination of gas and liquid chromatography tandem mass spectrometry (GC-MS/MS and LC-MS/MS) by an accredited laboratory. A total of 111 different active substances (mainly insecticides and fungicides) were detected, with an average of 37 active substances per sample and a total concentration per glove sample of 22.22 mg/kg. Several predictive levels of contamination were considered to assess the risk. The potential dermal exposures (PDE) of florists were estimated at the average, for different percentiles, and at the maximum concentration of residues in samples. At the PDE P90 and at the PDE_{MAX} (or worst case) values, three and five active substances respectively exceed the Acceptable Operator Exposure Level (AOEL), indicating risk situations. For the systemic exposure (SE), one active substance (clofentezine) exceeds the AOEL at the P90 predictive level. In the worst case, SE_{MAX} (at the maximum concentrations), four active substances (clofentezine, famoxadone, methiocarb, and pyridaben) exceed their respective AOEL values. Among the 14 most frequently detected active substances, two have SE_{MAX} values exceeding the AOEL. Exposure could be particularly critical for clofentezine with an SE_{MAX} value four times higher than the AOEL (393%). The exposure of florists appeared to be an example of a unique professional situation in which workers are exposed regularly to both a very high number of toxic chemicals and rather high concentration levels. Therefore, the priority should be to raise the level of awareness among the florists who must change their habits and practices if they want to minimize their exposure.

Keywords: pesticide residues; dermal exposure; risk assessment; cut flowers; florists

1. Introduction

Flower production generally involves frequent use of a wide range of pesticides to control diseases and pests in an effort to reduce or eliminate yield losses and maintain high product quality (Cooper and Dobson, 2007; Bethke and Cloyd, 2009). A great majority of producers consider the use of pesticides as necessary to achieve their production targets and the only way to be able to market large quantities of floral products with an acceptable quality and relatively modest price. Research published in 1979 showed that 18 of 105 lots (17.7%) of all flowers imported into Miami contained pesticide residue levels greater than 5 mg/kg and that three lots had levels greater than 400 mg/kg (Morse *et al.,* 1979). Thirty-seven years later, recent studies on cut flowers (roses, gerberas, and chrysanthemums) sold in Belgium showed that flowers are heavily contaminated. One hundred and seven active substances (a.s.) were detected, i.e., an average of almost 10 active substances per sample and a total average pesticide load of 15.72 mg/kg of flowers (Toumi *et al.,* 2016a). In another study, a total of 97 actives substances were detected on 50 bouquets of roses (Toumi *et al.,* 2016b). On one hand, weakness of local regulations and the lack of maximum residue limits (MRL) for flowers explains that, unlike other crops which are harvested for consumption, there is less restriction on the use of pesticides on flowers. Cut flowers tend to be sprayed at the maximum allowed dosage up to the time of harvest, often with rather toxic chemicals, and then shipped directly to the markets with no interval between treatment and harvest. According to Rikken (2017), a vast majority of the producers and European florists are not actively engaged in social and environmental standards, either when they purchase flowers or in communication with their clients. When selling products via the auction system, there are no mandatory requirements with respect to certifications such as the MPS-ABC (Milieu Project Sierteelt A, B and C) Standard, FFP (Fair Flowers-Fair Plants), or Florimark TraceCert (Rikken, 2017). Despite their popularity and extensive use, pesticides may present serious health concerns for exposed operators and workers. Many research studies have demonstrated both acute and chronic toxic effects after exposure during spraying or in post-harvest operations. Moreover, a recent study has shown that pesticides may also have negative impacts on the public health in general (Baldi *et al.,* 2013). The United Nations Environment Program (UNEP) estimates that, in the whole world, approximately 20,000 workers die every year from pesticide poisoning after direct or indirect exposure (WHO, 2004; Dasgupta & Meisner, 2005). No one can deny today that pesticides can be an important source of injury and illness among workers and other professionals who are not informed about the toxicity of the plant protection products, not properly protected, and exposed regularly to pesticides during their usual activities. Over the past decade, several studies have pointed to exposure to pesticides as a potential cause of prostate and testicular cancers among male floriculturist pesticide applicators and of cervical cancer among females in Florida (Fleming *et al.,* 1999). In Italy, early-stage cancers have been observed in 60

percent of long-term workers in the floriculture industry (Munnia *et al.,* 1999). Around the world, genetic damage has been reported in more than 71 percent of cut flower growers (Bolognesi, 2003). The prevalence of reproductive problems (abortion, prematurity, and congenital malformations) has been reported in Colombian workers who had been working in the floriculture industry for at least six months (Restrepo *et al.,* 1990). In the Philippines, a study assessed the risk factors to pesticide exposure and reported that the most commonly associated health symptoms among cut-flower farmers are weakness and fatigue, muscle pain, chills and fever, blurred vision, dizziness, and headache (Lu, 2005). The exposure of workers must be estimated for activities that involve contact with treated crops or products (e.g., picking, harvesting, cutting, maintenance, inspection, irrigation). Worker exposure can arise from other activities such as packaging, sorting, and bundling (EFSA, 2014). Considering the numerous and high levels of pesticide residues found on cut flowers in our previous study (Toumi *et al.,* 2016), the florists could be a group more severely exposed to serious hazards than other workers. This is a unique risk situation where workers could be exposed almost every day to many different pesticide residues during their professional activities. This is the reason why a risk assessment was deemed necessary to evaluate the health risks of people who manipulate contaminated flowers. Based on the results, it will be considered if recommendations to reduce the exposure by appropriate prevention and protection measures need to be established. The amount of pesticide residues on the hands of workers represents the main measure of dermal exposure (U.S. EPA, 1986). As skin is generally recognized as the primary route of exposure to pesticides (Brouwer *et al.,* 1992a, b; Kangas *et al.,* 1995; Methner *et al.,* 1996; Illing *et al.,* 1997; Ecobichon, 1998;), the transfer of pesticide residues to the hands could contribute significantly to the total exposure. According to their physical and chemical properties (physical state, vapor pressure, Henry constant, solubility, hydrolysis rate), many pesticides sprayed on cut flowers are in the form of persistent, fat-soluble pesticide residue, which can be dislodged from the two-sided foliar surface of a plant or after spraying. The active substances which are adsorbed and fixed on the surface of the plant could therefore be dislodged by contact with hands[5]. The actual dermal exposure has been defined previously as the amount of pesticide coming into contact with the skin of workers that becomes available for absorption through the skin (Van Hemmen & Brouwer, 1995; Rajan-Sithamparanadarajah *et al.,* 2004; Lesmes-Fabian *et al.,* 2012). Recently, the EFSA (European Food Safety Authority) (EFSA, 2014) has harmonised the approach of pesticide exposure assessment for workers. The EFSA has proposed various transfer coefficients (U.S. EPA, 2000; U.S. EPA, 2001) to be applied for different scenarios (nature and duration of the activity during re-entry), including activities in

[5] En fonction de leurs propriétés physico-chimiques, les substances actives sont plus ou moins absorbées dans les cires cuticulaires ; d'autres substances (systémiques) sont plutôt à l'intérieur des tissus de la plante et sont par conséquent moins accessibles aux mains. Pour ces substances, le transfert sera donc moindre que pour les produits de contact (voir Annexe 2).

ornamentals (EFSA, 2014). In this paper, we have attempted to measure the transfer of pesticide residues from flowers to hands and to assess the potential dermal exposure of florists. Finally, the risk level for each active substance was established by comparison to the Acceptable Operator Exposure Level (AOEL (EU Pesticides Database, 2017).

2. Materials and Methods

2.1. Assessment of Florist Hands Exposure Using Cotton Gloves

Cotton gloves can be used to assess the potential dermal exposure of workers through hands. Gloves worn during normal professional tasks act as a reservoir for active substances that come into contact with the skin (Brouwer *et al.,* 1992a, b; OCDE, 1997). A study was conducted among twenty volunteer florists located in Namur Province and in the Brussels Capital Region of Belgium to evaluate their potential dermal exposure, measuring the potential transfer of pesticides from treated flowers to hands. Two pairs of 100% cotton gloves were distributed to each florist and worn during two consecutive half days when handling flowers and preparing bouquets (from min 2 h to max 3 h/day). The two pairs were collected as a single sample (four gloves/sample), weighed, cut in small pieces with scissors, and stored in freezing bags at −18 ◦C until transport (by road, from Gembloux to Ghent) and analysis.

2.2. Extraction and Analysis of Pesticide Residues

The residual pesticide deposits on the gloves were analysed by PRIMORIS (formerly FYTOLAB, Technologiepark 2/3, 9052 Zwijnaarde, Belgium) laboratory holding a BELAC (Belgian Accreditation Council) accreditation to ISO/CEI 17025 for pesticide residues. PRIMORIS is an independent, accredited, and officially recognized service laboratory (accreditation number 057-TEST). Samples were analyzed using a multi-residue Quick Easy Cheap Effective Rugged Safe (QuEChERS) method validated by the laboratory for the analysis of residues in foodstuffs. It detects approximately 500 different active substances (a.s.) in a single analysis thanks to a combination of gas and liquid chromatography. The extraction procedure is based on the AOAC (Association of Official Analytical Chemists) Official Method 2007.01. (AOAC, 2007). Briefly, a homogenous 10.0 g sub-sample (small pieces of gloves) is weighted into a 50 mL polypropylene tube. Then, 10 mL of acidified acetonitrile (1% acetic acid), 4 g of anhydrous magnesium sulfate (MgSO4), and 1 g of sodium acetate (NaOAc) are added. After shaking and sonication in an ultrasonic bath, the polypropylene tube is centrifuged. A portion of the acetonitrile phase (upper layer) is transferred to vials and further analyzed by gas or liquid chromatography tandem mass spectrometry (GC-MS/MS (Thermo Fischer Scientific, Interscience Belgium, Louvain-la-Neuve, Belgium) or LC-MS/MS (Waters Corporation, Zellik, Belgium)), according to the active substances to be determined (GC-MS/MS for small, thermally stable, volatile, non-polar molecules, or LC-MS/MS for larger, thermolabile, non-volatile, or polar molecules). For almost

all active substances, the limit of quantification (LOQ) was ≤0.01 mg/kg. A similar method has been previously used to measure the pesticide deposits on flowers (Toumi *et al.,* 2016a). Considering that extraction from flowers could differ from the one from cotton gloves, a preliminary multi-residue recovery study has been carried out. Therefore the analytical results were corrected accordingly for all active substances with a recovery ratio between 50–130% (only few substances had a percentage of recovery below or above these values; in this case, the results remain uncorrected in the tables).

2.3. Multi-Residue Recovery Preliminary Study

To assess the recovery percentages of pesticide residues, two multi-pesticide solutions were spiked on cotton gloves, which were allowed to dry for 24 h, cut in small pieces, stored, and analyzed with a similar multi-residue method. The recovery is obtained as a ratio between the amount of residue measured in the extract after analysis and the amount spiked on gloves (% Recovery = (amount of extracted residues (mg)/amount of active substance placed on the gloves (mg)) × 100). Three replicates of the recovery trial were conducted on three different days. For each trial, four samples were prepared (5 g of gloves/sample); one sample with pieces of untreated gloves (blank sample) and three samples used to estimate recoveries. These ones were spiked with two multi-residue solutions containing 240 active substances in methanol and 155 active substances in acetone. Solutions were prepared in an accredited ISO17025 laboratory (Sciensano, chemical residues and contaminants) according to an accredited internal procedure. Stability and variability tests were passed according to the quality criteria of the SANTE/11945/2015 document (European Commission, 2015). All the solutions (individual, intermediate mix, and spiking) were stocked and aliquoted at −20 °C. The spiking was done by spraying small droplets of 250 µL and 100 µL of the two solutions (concentration 2 µg/mL and 5 µg/mL respectively) using a 250 µL and 100 µL calibrated syringes, respectively. No pesticides were detected in the three blanco samples, proving an absence of pesticides in the gloves themselves. The spiking was calculated to reach an average concentration of about 0.1 mg/kg gloves for each active substance. Twenty-four hours after deposit, the samples were cut into small pieces and stored in freezing bags at −18 °C until analysis. The recoveries were calculated using statistical analysis software developed by Verplaetse in 1998, modified by Van Loco in 2003. The preliminary study allowed a percentage of recovery for 395 substances to be determined, but the recoveries of nine active substances (azadirachtin, captan, cyflumetofen, etoxazole, fluoxastrobin, pymetrozine, spinetoram, tetrahydrophtalimide, and thiophanate methyl) that were not available in the spiking solutions could not been determined.

2.4. Florists Exposure Assessment Calculation

Classically, exposure is described as the amount of an agent that contacts the outer boundary of the body. However, this definition of exposure is limited because the real interest in risk assessment is the amount of an agent that breeches the outer

boundary of the body (dose) and is capable of being distributed to one or more organs to exert a toxic effect (target dose) (U.S. EPA, 2007). For dermal exposure to occur, an individual must have contact with the chemical in a given medium. The amount of exposure will depend on the concentration of the chemical contacting a given area of skin, the dermal loading or skin adherence, the ability of the chemical to penetrate and pass through intact skin, the dermal dose, and the duration and frequency of contact in terms of the intervals of contact and the number of intervals per day, weeks, months, or even a lifetime (U.S. EPA, 2007).

The exposure of workers can be estimated for activities that involve contact with treated crops or products. The main route of exposure for florists who handle daily cut flowers and ornamentals is skin contact and subsequent dermal absorption.

The potential dermal exposure (PDE) values were estimated as the amount of pesticide residues with low adhesion that were transferred from flowers to gloves. For each active substance, PDE was calculated as follows:

PDE (in mg a.s./kg bw per day) = ((C_T (mg/kg) × GW (kg)) × 3)/bw (kg)

where C_T is the concentration of active substance in the sub-sample during the task duration of the trial (2 h), GW is the average weight of the cotton gloves samples (57 g $\pm$ 0.17 g), 3 is a correction factor (total task duration value equal to 6 h/day) and bw is the body weight (60 kg).

A total task duration value of 6 h/day was used to assess the dermal exposure of florists. A recent survey in Belgium (Toumi *et al.,* 2016) showed that 60% of the florists worked between 6 and 7 h/day. The time spent preparing bouquets and handling flowers vary greatly over the year, but is always quite high, varying on average from 2 to 6 h/day for 80% of the florists in the low season and for 40% of the florists in the high season. This handling time could be in excess of 6 h for 8% of the florists in the low season, but during the high season or special occasions, an intense working period, 60% spent more than 6 h/day on this work. Only 12% of the florists worked less than 2 h/day in the low season. A default body weight (bw) value of 60 kg is used in line with the recent EFSA Guidance Document to cover a range of professionally exposed adults (EFSA, 2014).

The PDE values were then converted into systemic doses using an appropriate dermal absorption percentage of 75% (default value) (EFSA, 2012). To obtain the actual dermal exposure (ADE), the potential dermal exposure (PDE) values in absence of protection can be reduced by 90%, the penetration factor being equal to 10% skin sorption when workwear and gloves are worn (EFSA, 2014).

The risk characterisation is obtained as the ratio of the exposure level to the reference value of each active substance, the AOEL (Acceptable Operator Exposure Level; in mg a.s./kg·bw per day), which should not be exceeded to avoid any detrimental effect on florists' health. Several prediction levels of the PDE were considered, including the mean, 75th percentile, 90th percentile, and the maximum (in mg/kg bw per day) to assess the risk for florists. Therefore, the systemic exposure (SE) values (mean, 75th percentile, 90th percentile, and maximum) were expressed as percentage of the AOEL. It has been assumed that the most appropriate level to cover and assess the risk is the maximum value of the SE (SE_{MAX}or worst case).

3. Results

3.1. Pesticide Residues Identified on Glove Samples

All glove samples appeared to be contaminated by high levels of pesticide residues for most active substances. A total of 111 different a.s. were identified, with an average of about 37 a.s./sample and an average total pesticide residue concentrations per glove sample of 22.22 mg/kg (Table 24). Fourteen active substances (azoxystrobine (80%), benomyl (95%), boscalid (90%), clofentezine (90%), fenhexamid (85%), flonicamid (90%), fludioxonil (85%), fluopyram (80%), imidacloprid (75%), iprodione (95%), lufenuron (90%), methiocarb (75%), procymidone (85%), and spiroxamine (80%)) are the most frequently detected. They are present on more than 15 of the 20 samples (75%).

Table 24: Total number of active substances (a.s.) detected and total pesticide residue concentrations (mg/kg) in 20 samples of gloves

Samples from florists (2 pairs of gloves/sample)	Total number of active substances detected (LOQ ≤ 0.01 mg/kg)	Total pesticide residues concentrations (mg/kg)*
Sample N°1	47	113.44
Sample N°2	48	24.75
Sample N°3	43	5.69
Sample N°4	54	31.23
Sample N°5	68	70.41
Sample N°6	40	8.88
Sample N°7	19	5.08
Sample N°8	59	41.54
Sample N°9	24	13.92
Sample N°10	12	1.43
Sample N°11	40	24.07
Sample N°12	31	7.35
Sample N°13	51	28.83
Sample N°14	23	7.88
Sample N°15	52	36.18
Sample N°16	41	9.38
Sample N°17	21	1.36
Sample N°18	35	6.85
Sample N°19	20	4.50
Sample N°20	20	1.62
Mean	37.40	22.22
Median	40.00	8.11

* All the active substances below the LOQ were not taken into account in the sum

3.2. Pesticide Residues Hazard Characterisation

The intrinsic toxicological properties (acute and chronic toxicity including mutagenic, carcinogenic and reproductive hazards) of each substance identified on the gloves were collected in pesticide databases (European Union Pesticides Database, Directorate-General for Health and Food Safety) and JMPR (Joint Meeting on Pesticide Residues)reports (FAO (Food and Agriculture Organization) Joint Meeting on Pesticide Residues) (Table 25) (EU Pesticides Database, 2017; AGP, 2017; FAO, 2017; JMPR, 2017; European Commission, 2009).

3.3. Florists Exposure Assessment

Table 26 presents the number of detection (N), the Potential Dermal Exposure (mean, 75th percentile, 90th percentile, and maximum values) in mg/kg bw per day, and the systemic exposure as a percentage of the AOEL calculated for SE (mean, 75th percentile, 90th percentile, and maximum values), for all active substances detected on the gloves of florists. All values exceeding 100% of the AOEL indicate a potential risk situation.

The actual dermal exposure (ADE) values have been calculated for the same prediction levels of risk. None of these values exceed the AOEL. Therefore the detailed results were not reported here.

3.4. Most Frequently Detected Active Substances and AOEL Exceedance

Fourteen active substances were the most frequently detected in the glove samples (frequency > 75%). Therefore, it was considered that a great part of the risk could be related to the repeated exposure of the florists to these specific 14 substances. Figure 8 presents the percentage of their respective AOEL (from 0 to more than 100% AOEL) for their SE_{MAX} values.

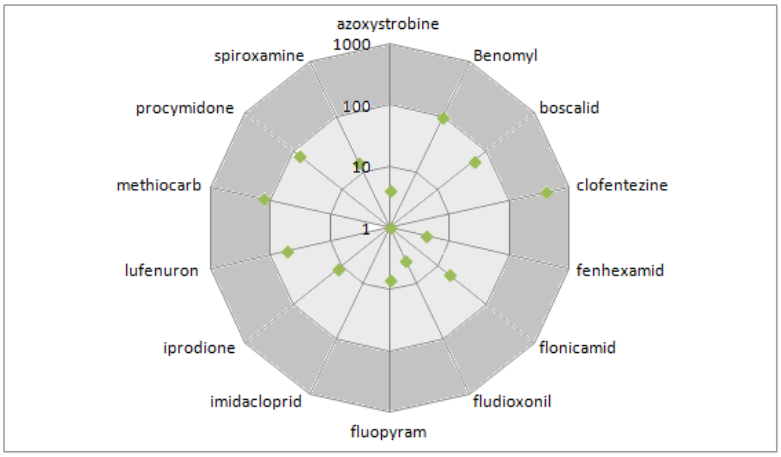

Figure 8: The maximum systemic exposure (SE_{MAX}) of the fourteen most frequently detected active substances on gloves worn by florists as a percentage of the Acceptable Operator Exposure Level (AOEL), Green Symbol: SE_{MAX} as a percentage of the AOEL

Table 25: Alphabetic classification of all a.s. detected in the 20 samples of gloves with their chemical family, biological activity, detection frequency (% samples where the a.s. is detected), average (± SD) and range of concentrations (mg/kg gloves, LOQ < 0.01 mg/kg) in the samples and their toxicological properties (Dermal LD50, AOEL values and CLP Classification according the EU Pesticides database)

Active substance	Chemical family	Biologi-cal activity	Detection frequency (%)	Average concentration (mg/kg) ± SD	Range (mg/kg)	Dermal LD_{50} (mg/kg bw)	AOEL values (mg/kg bw/day)	CLP Classification
Acephate*	Organophosphates	I	10%	0.055 ±0.055	<LOQ-0.094	>10000	0.0300	H302
Acetamiprid	Neonicotinoids	I	30%	0.219±0.263	<LOQ-0.602	>2000	0.0700	H302
Acrinathrin	Pyrethroids	I	10%	0.285±0.372	<LOQ-0.547	>2000	0.0070	-
Ametoctradin	Triazolopyrimidine	F	55%	0.859±1.921	<LOQ-6.477	>2000	2.0000	-
Azadirachtin*	limonoid	I	20%	0.169±0.137	<LOQ-0.350	>2000	0.1000	-
Azoxystrobine	Strobilurins	F	80%	0.617±0.917	<LOQ-3.287	>2000	0.2000	H331
Benomyl (carbendazim)*	Benzimidazole	F	95%	0.739±2.040	<LOQ-9.000	>10000	0.0200	H315, H317, H335, H340, H360
Bifenazate*	Carbazates	I	15%	0.132±0.164	<LOQ-0.320	>5000	0.0028	H317, H373
Bifenthrin	Pyrethroids	I	15%	0.059±0.048	<LOQ-0.108	>2000	0.0075	H300, H317, H331, H351, H372
Bitertanol	Triazoles	F	20%	0.043±0.038	<LOQ-0.097	>5000	0.0100	-
Boscalid	Carboxamides	F	90%	3.471±6.810	<LOQ-26.213	>2000	0.1000	-
Bupirimate	Pyrimidine	F	35%	0.170±0.184	<LOQ-0.565	>2000	0.0500	H317, H351
Buprofezin	Thiadiazine	I	35%	0.488±1.093	<LOQ-2.963	1635– 3847	0.0400	-
Captan*	Phthalimides	F	10%	0.510±0.240	<LOQ-0.680	>2000	0.1000	H317, H318, H331, H351
Carbofuran	Carbamates	I	5%	0.012±0.000	<LOQ-0.012	>500	0.0003	H300, H330
Chlorantraniliprole	Diamides	I	15%	0.215±0.878	<LOQ-0.373	>5000	0.3600	-
Chlorothalonil*	Organochlorine	F	25%	0.197±0.190	<LOQ-0.420	>5000	0.0090	H317, H318, H330 H335, H351
Chlorpyrifos (-ethyl)	Organophosphates	I	10%	0.041±0.012	<LOQ-0.049	>2000	0.0010	H301
Clofentezine	Quinoxalines	I	90%	2.881±5.604	<LOQ-18.373	>2100	0.0100	-
Cyflumetofen*	Benzoylacetonitriles	I	60%	0.116±0.204	<LOQ-0.750	>5000	0.1100	-

Active substance	Chemical family	Biological activity	Detection frequency (%)	Average concentration (mg/kg) ± SD	Range (mg/kg)	Dermal LD_{50} (mg/kg bw)	AOEL values (mg/kg bw/day)	CLP Classification
Cyhalothrin	Pyrethroids	I	35%	0.107±0.158	<LOQ-0.452	632	0.0025	-
Cypermethrin	Pyrethroids	I	50%	0.135±0.161	<LOQ-0.455	>1600	0.0600	H302, H332, H335
Cyproconazole	Triazoles	F	5%	0.043±0.000	<LOQ-0.043	>2000	0.0200	H301, H360, H373
Cyprodinil	Anilinopyrimidines	F	70%	0.132±0.219	<LOQ-0.745	>2000	0.0300	H317
Deet		I	85%	0.146±0.078	<LOQ-0.299	-	-	-
Deltamethrin	Pyrethroids	I	40%	0.074±0.066	<LOQ-0.221	>2000	0.0075	H301, H331
Dicofol	Organochlorine	I	5%	0.035±0.000	<LOQ-0.035	>5000	-	H302, H312, H315, H317
Difenoconazole	Triazoles	F	50%	0.120±0.175	<LOQ-0.552	2010	0.1600	-
Diflubenzuron	Benzoylureas	I	10%	0.036±0.021	<LOQ-0.051	>10000	0.0330	-
Dimethoate	Organophosphates	I	5%	0.016±0.000	<LOQ-0.016	>7000	0.0010	H302, H312
Dimethomorph	Cinnamic acid	F	70%	0.476±0.878	<LOQ-3.485	>2000	0.1500	-
Diphenylamine	Amides	F	25%	0.144±0.139	<LOQ-0.392	1700	0.1000	H315, H317
Dodemorph*	Morpholine	F	65%	0.503±1.017	<LOQ-3.700	>2000	0.0330	H314, H317, H361, H373
Endosulfan	Organochlorine	I	25%	0.092±0.071	<LOQ-0.183	500	-	H300, H312, H330
Etoxazole*	Oxazolines	I	45%	0.301±0.552	<LOQ-1.700	>2000	0.0300	-
Famoxadone	Oxazolidinediones	F	60%	0.563±0.779	<LOQ-2.627	>2000	0.0048	H373
Fenamidone	Imidazolinones	F	40%	0.215±0.366	<LOQ-1.056	>2000	0.3000	-
Fenazaquin	Quinazolines	I	10%	0.689±1.039	<LOQ-1.364	>2000	0.0100	H301, H332
Fenhexamid	Phenylpyrroles	F	85%	1.052±1.713	<LOQ-5.195	>5000	0.2000	-
Fenoxycarb	Carbamates	I	5%	0.047±0.000	<LOQ-0.047	>2000	0.1000	H351
Fenpyroximate	Pyridazinones	I	5%	1.268±0.000	<LOQ-1.268	>2000	0.0050	H301, H317, H330
Fenvalerate	Pyrethroids	I	10%	0.198±0.201	<LOQ-0.339	5000	-	-
Fipronil	Phenylpyrazoles	I	40%	0.281±0.407	<LOQ-1.199	>2000	0.0035	H301, H311, H331, H372
Flonicamid	Pyridinecarboxamides	I	90%	0.379±0.563	<LOQ-1.964	>5000	0.0250	H302
Fluazinam	Phenylpyridylamines	F	20%	0.098±0.069	<LOQ-0.197	5500	0.0040	H317, H318, H332, H361
Flubendiamide	Keto-Enol	I	15%	0.128±0.164	<LOQ-0.317	>2000	0.0060	-

Active substance	Chemical family	Biologi-cal activity	Detection frequency (%)	Average concentration (mg/kg) ± SD	Range (mg/kg)	Dermal LD_{50} (mg/kg bw)	AOEL values (mg/kg bw/day)	CLP Classification
Fludioxonil	Phenylpyrroles	F	85%	1.665±3.125	<LOQ-12.278	>2000	0.5900	-
Flufenoxuron	Benzoyl urea	I	15%	0.317±0.385	<LOQ-0.762	>2000	0.0100	H362
Fluopicolide	Acylpicolides	F	30%	0.257±0.430	<LOQ-1.100	>5000	0.0500	-
Fluopyram	Pyridines	F	80%	0.360±0.428	<LOQ-1.624	>2000	0.0500	-
Fluoxastrobin*	Strobilurins	F	5%	0.031±0.000	<LOQ-0.031	>2000	0.0300	-
Flusilazole	Triazoles	F	5%	0.631±0.000	<LOQ-0.631	>2000	0.0050	H302, H351, H360
Flutolanil	Phenylamides	F	5%	0.207±0.000	<LOQ-0.207	>5000	0.5600	-
Flutriafol	Triazoles	F	20%	0.107±0.173	<LOQ-0.365	>2000	0.0500	-
Fluxapyroxad	Pyrazole carboxamides	F	15%	0.147±0.119	<LOQ-0.242	>2000	0.0400	-
Hexythiazox	Carboxamides	I	55%	0.344±0.840	<LOQ-2.854	>5000	0.0090	-
Imidacloprid	Neonicotinoids	I	75%	0.072±0.046	<LOQ-0.203	>5000	0.0800	H302
Indoxacarb	Oxadiazines	I	40%	0.078±0.104	<LOQ-0.322	>5000	0.0040	H301, H317, H332, H372
Iprodione	Dicarboximides	F	95%	2.422±3.969	<LOQ-16.931	>2000	0.3000	H351
Iprovalicarb	Carbamates	F	40%	0.236±0.367	<LOQ-1.085	>5000	0.0150	-
Kresoxim-methyl	Strobilurins	F	50%	0.589±1.097	<LOQ-3.471	>2000	0.9000	H351
Lufenuron	Benzoylureas	I	90%	0.289±0.557	<LOQ-2.462	>2000	0.0100	H317
Malathion	Organophosphates	I	5%	0.018±0.000	<LOQ-0.018	>2000	-	H302, H317
Mandipropamid	Mandelic acid	F	60%	2.272±6.804	<LOQ-23.836	>5000	0.1700	-
Mepanipyrim	Anilinopyrimidines	F	20%	0.217±0.234	<LOQ-0.470	>2000	0.0700	H351
Metalaxyl (metalaxyl-M)	Acylamines	F	15%	0.348±0.457	<LOQ-0.875	>3100	0.0800	H302, H317, H318
Methiocarb	Carbamates	I	75%	1.209±2.387	<LOQ-7.664	>5000	0.0130	H301
Methoxyfenozide	Diacylhydrazines	F	70%	0.164±0.180	<LOQ-0.676	>5000	0.1000	-
Metrafenone	Benzophenones	F	15%	0.086±0.064	<LOQ-0.139	>5000	0.4300	-
Myclobutanil	Triazoles	F	20%	0.068±0.030	<LOQ-0.097	>5000	0.0300	H302, H319, H361
Nitrothal-isopropyl	-	F	5%	0.087±0.000	<LOQ-0.087	>5000	-	-

Active substance	Chemical family	Biological activity	Detection frequency (%)	Average concentration (mg/kg) ± SD	Range (mg/kg)	Dermal LD_{50} (mg/kg bw)	AOEL values (mg/kg bw/day)	CLP Classification
Novaluron	Benzoylureas	I	5%	3.382±0.000	<LOQ-3.382	>2000	-	-
Oxycarboxin	Anilides	F	5%	0.017±0.000	<LOQ-0.017	16000	-	H302
Paclobutrazol	Triazoles	R	15%	0.291±0.451	<LOQ-0.811	>1000	0.1000	-
Penconazole	Triazoles	F	5%	0.131±0.000	<LOQ-0.131	>3000	0.0300	H302, H361
Permethrin	Pyrethroids	I	5%	0.037±0.000	<LOQ-0.037	>2000	-	H302, H332, H335
Picoxystrobin	Strobilurins	F	25%	0.223±0.451	<LOQ-1.030	>2000	0.0430	-
Piperonyl-butoxyde	-	I	45%	0.065±0.111	<LOQ-0.351	>2000	-	-
Pirimicarb	Carbamates	I	15%	0.023±0.012	<LOQ-0.039	>2000	0.0350	H301, H317, H331, H351
Pirimiphos-methyl	Organophosphates	I	5%	0.024±0.000	<LOQ-0.024	>2000	0.0200	H302
Prochloraz	Imidazoles	F	65%	0.476±0.837	<LOQ-3.049	>2000	0.0200	H302
Procymidone	Dicarboximides	F	85%	0.729±1.352	<LOQ-4.207	>2500	0.0120	-
Profenofos	Organophosphates	I	5%	0.013±0.000	<LOQ-0.013	>2000	-	H302, H312, H332
Propamocarb*	Carbamates	F	65%	0.770±1.462	<LOQ-5.100	>2000	0.2900	-
Propiconazole	Triazoles	F	20%	0.032±0.022	<LOQ-0.064	>4000	0.1000	H302, H317
Pymetrozine*	Pyridine-azométhrine	I	45%	0.072±0.084	<LOQ-0.280	>2000	0.0300	H351
Pyraclostrobin	Strobilurins	F	50%	0.589±0.845	<LOQ-2.222	>2000	0.0150	H315, H331
Pyridaben	Pyridazinones	I	30%	0.654±1.103	<LOQ-2.804	>2000	0.0050	H301, H331
Pyridalyl	Dihalopropenes	I	65%	0.175±0.177	<LOQ-0.625	>5000	0.0200	-
Pyrimethanil	Anilinopyrimidines	F	25%	0.094±0.111	<LOQ-0.283	>5000	0.1200	-
Pyriproxyfen	Pyridines	I	5%	0.015±0.000	<LOQ-0.015	>2000	0.0400	-
Simazine	Triazines	H	5%	0.021±0.000	<LOQ-0.021	-	-	H351
Spinetoram*	Spinosyns	I	25%	0.018±0.005	<LOQ-0.024	>5000	0.0065	-
Spinosad*	Spinosyns	I	35%	0.149±0.306	<LOQ-0.840	>5000	0.0120	-
Spirodiclofen*	Keto-Enol	I	5%	2.000±0.000	<LOQ-2.000	>2000	0.0090	-
Spiromesifen	Keto-Enol	I	5%	0.025±0.000	<LOQ-0.025	>2000	0.0150	-
Spirotetramat*	Keto-Enol	I	30%	0.072±0.047	<LOQ-0.140	>2000	0.0500	H317, H319, H335, H361

Active substance	Chemical family	Biologi-cal activity	Detection frequency (%)	Average concentration (mg/kg) ± SD	Range (mg/kg)	Dermal LD_{50} (mg/kg bw)	AOEL values (mg/kg bw/day)	CLP Classification
Spiroxamine	Spirocétalamines	F	80%	0.363±0.323	<LOQ-1.031	1068	0.0150	H302, H312, H315, H317, H332, H361, H373
Tebuconazole	Triazoles	F	40%	0.323±0.511	<LOQ-1.494	>2000	0.0300	H302, H361
Tebufenozide	Diacylhydrazines	I	5%	0.155±0.000	<LOQ-0.155	>5000	0.0080	-
Tebufenpyrad	Pyrazoles	I	10%	0.161±0.197	<LOQ-0.300	>2000	0.0100	H301, H317, H332, H373
Tetraconazole	Triazoles	F	5%	0.037±0.000	<LOQ-0.037	-	0.0300	H302, H332
Tetramethrin*	Pyrethroids	I	5%	0.020±0.000	<LOQ-0.020	-	-	-
Thiabendazole*	Benzimidazoles	F	10%	0.042±0.020	<LOQ-0.056	>5000	0.1000	-
Thiacloprid	Neonicotinoids	I	45%	0.347±0.596	<LOQ-1.777	>2000	0.0200	H301, H332, H336, H351, H360
Thiametoxam	Neonicotinoids	I	55%	0.294±0.360	<LOQ-1.031	>2000	0.0800	H302
Thiophanate methyl*	Benzimidazoles	F	35%	0.087±0.083	<LOQ-0.230	>10000	0.0800	H317, H332, H341
Tolclofos-methyl	Organophosphates	F	20%	0.035±0.017	<LOQ-0.057	>5000	0.2000	H317
Triadimefon (triadimenol)	Triazoles	F	5%	0.053±0.000	<LOQ-0.053	>5000	0.0500	H302, H317, H360, H362
Trifloxystrobin	Strobilurins	F	50%	0.161±0.169	<LOQ-0.529	>2000	0.0600	H317
Triflumizole	Triazoles	F	5%	0.027±0.000	<LOQ-0.027	>5000	0.0500	H302, H317, H360, H373

H300: Fatal if swallowed - **H301**: Toxic if swallowed - **H302**: Harmful if swallowed- **H310**: Fatal in contact with skin- **H311**: Toxic in contact with skin- **H312**: Harmful in contact with skin- **H314**: Causes severe skin burns and eye damage- **H315**: Causes skin irritation- **H317**: May cause an allergic skin reaction- **H318**: Causes serious eye damage- **H319**: Causes serious eye irritation- **H330**: Fatal if inhaled- **H331**: Toxic if inhaled- **H332**: Harmful if inhaled- **H334**: May cause allergy or asthma symptoms or breathing difficulties if inhaled- **H335**: May cause respiratory irritation- **H340**: May cause genetic defects- **H341**: Suspected of causing genetic defects- **H351**:Suspected of causing cancer- **H360**: May damage fertility or the unborn child- **H361**: Suspected of damaging fertility or the unborn child- **H362**: May cause harm to breast-fed

children- **H372**: Causes damage to organs through prolonged or repeated exposure - **H373**: May cause damage to organs through prolonged or repeated exposure; **F**: Fungicide - **H**: Herbicide - **I**: Insecticide - **R** : Growth regulator; *: active substances with non-corrected recovery due to recovery below or above 50-130%

Table 26: All a.s. present in the 20 samples of gloves and the corresponding calculation: number of detection (N), Potential Dermal Exposure (Mean, 75th Percentile, 90th Percentile and Maximum values) in mg/kg bw per day, and the Systemic Exposure in % of the AOEL calculated for SE (Mean, 75th Percentile, 90th Percentile and Maximum values), for all active substances detected on the gloves of florists (*: Value exceeds the AOEL)

Active Substance	N	PDE (Mean) (mg/kg bw per day)	SE (Mean) in % of AOEL	PDE (75th P) (mg/kg bw per day)	SE (75th P) in % of AOEL	PDE (90th P) (mg/kg bw per day)	SE (90th P) in % of AOEL	PDE (Maximum) (mg/kg bw per day)	SE (Maximum) in % of AOEL
Acephate	2	0.00016	0%	0.00021	1%	0.00025	1%	0.00027	1%
Acetamiprid	6	0.00062	1%	0.00115	1%	0.00158	2%	0.00172	2%
Acrinathrin	2	0.00081	9%	0.00118	13%	0.00141	15%	0.00156	17%
Ametoctradin	11	0.00245	0%	0.00181	0%	0.00389	0%	0.01846	0%
Azadirachtin	4	0.00048	0%	0.00068	1%	0.00087	1%	0.00100	1%
Azoxystrobine	16	0.00176	1%	0.00260	1%	0.00466	2%	0.00937	4%
Benomyl (carbendazim)	19	0.00210	8%	0.00168	6%	0.00342	13%	0.02565 *	96%
Bifenazate	3	0.00038	10%	0.00053	14%	0.00076	20%	0.00091	24%
Bifenthrin	3	0.00017	2%	0.00023	2%	0.00028	3%	0.00031	3%
Bitertanol	4	0.00012	1%	0.00016	1%	0.00023	2%	0.00028	2%
Boscalid	18	0.00989	7%	0.00943	7%	0.02767	21%	0.07471	56%
Bupirimate	7	0.00048	1%	0.00049	1%	0.00096	1%	0.00161	2%
Buprofezin	7	0.00139	3%	0.00040	1%	0.00370	7%	0.00845	16%
Captan	2	0.00145	1%	0.00170	1%	0.00184	1%	0.00194	1%

Active Substance	N	PDE (Mean) (mg/kg bw per day)	SE (Mean) in % of AOEL	PDE (75th P) (mg/kg bw per day)	SE (75th P) in % of AOEL	PDE (90th P) (mg/kg bw per day)	SE (90th P) in % of AOEL	PDE (Maximum) (mg/kg bw per day)	SE (Maximum) in % of AOEL
Carbofuran	1	0.00003	8%	0.00003	8%	0.00003	8%	0.00003	8%
Chlorantraniliprole	3	0.00061	0%	0.00087	0%	0.00099	0%	0.00106	0%
Chlorothalonil	5	0.00056	5%	0.00105	9%	0.00114	10%	0.00120	10%
Chlorpyrifos (-ethyl)	2	0.00012	9%	0.00013	10%	0.00014	11%	0.00014	11%
Clofentezine	18	0.00821	62%	0.00623	47%	0.02862 *	215%	0.05236 *	393%
Cyflumetofen	12	0.00033	0%	0.00029	0%	0.00042	0%	0.00214	1%
Cyhalothrin	7	0.00030	9%	0.00029	9%	0.00074	22%	0.00129	39%
Cypermethrin	10	0.00039	0%	0.00074	1%	0.00093	1%	0.00130	2%
Cyproconazole	1	0.00012	0%	0.00012	0%	0.00012	0%	0.00012	0%
Cyprodinil	14	0.00038	1%	0.00033	1%	0.00112	3%	0.00212	5%
Deet	17	0.00042	-	0.00054	-	0.00068	-	0.00085	-
Deltamethrin	8	0.00021	2%	0.00023	2%	0.00040	4%	0.00063	6%
Dicofol	1	0.00010	-	0.00010	-	0.00010	-	0.00010	-
Difenoconazole	10	0.00034	0%	0.00041	0%	0.00085	0%	0.00157	1%
Diflubenzuron	2	0.00010	0%	0.00012	0%	0.00014	0%	0.00014	0%
Dimethoate	1	0.00005	4%	0.00005	4%	0.00005	4%	0.00005	4%
Dimethomorph	14	0.00136	1%	0.00090	0%	0.00158	1%	0.00993	5%
Diphenylamine	5	0.00041	0%	0.00026	0%	0.00078	1%	0.00112	1%

Active Substance	N	PDE (Mean) (mg/kg bw per day)	SE (Mean) in % of AOEL	PDE (75th P) (mg/kg bw per day)	SE (75th P) in % of AOEL	PDE (90th P) (mg/kg bw per day)	SE (90th P) in % of AOEL	PDE (Maximum) (mg/kg bw per day)	SE (Maximum) in % of AOEL
Dodemorph	13	0.00143	3%	0.00086	2%	0.00295	7%	0.01055	24%
Endosulfan	5	0.00026	-	0.00043	-	0.00049	-	0.00052	-
Etoxazole	9	0.00086	2%	0.00108	3%	0.00202	5%	0.00485	12%
Famoxadone	12	0.00160	25%	0.00167	26%	0.00412	64%	0.00749 *	117%
Fenamidone	8	0.00061	0%	0.00044	0%	0.00175	0%	0.00301	1%
Fenazaquin	2	0.00214	16%	0.00319	24%	0.00381	29%	0.00423	32%
Fenhexamid	17	0.00300	1%	0.00261	1%	0.01058	4%	0.01483	6%
Fenoxycarb	1	0.00014	0%	0.00014	0%	0.00014	0%	0.00014	0%
Fenpyroximate	1	0.00361	54%	0.00361	54%	0.00361	54%	0.00361	54%
Fenvalerate	2	0.00056	-	0.00077	-	0.00089	-	0.00097	-
Fipronil	8	0.00080	17%	0.00082	18%	0.00203	44%	0.00342	73%
Flonicamid	18	0.00108	3%	0.00140	4%	0.00358	11%	0.00560	17%
Fluazinam	4	0.00028	5%	0.00032	6%	0.00046	9%	0.00056	11%
Flubendiamide	3	0.00036	5%	0.00051	6%	0.00075	9%	0.00090	11%
Fludioxonil	17	0.00474	1%	0.00541	1%	0.01162	1%	0.03499	4%
Flufenoxuron	3	0.00090	7%	0.00124	9%	0.00180	14%	0.00217	16%
Fluopicolide	6	0.00073	1%	0.00073	1%	0.00204	3%	0.00314	5%
Fluopyram	16	0.00103	2%	0.00126	2%	0.00228	3%	0.00463	7%

Active Substance	N	PDE (Mean) (mg/kg bw per day)	SE (Mean) in % of AOEL	PDE (75th P) (mg/kg bw per day)	SE (75th P) in % of AOEL	PDE (90th P) (mg/kg bw per day)	SE (90th P) in % of AOEL	PDE (Maximum) (mg/kg bw per day)	SE (Maximum) in % of AOEL
Fluoxastrobin	1	0.00009	0%	0.00009	0%	0.00009	0%	0.00009	0%
Flusilazole	1	0.00180	27%	0.00180	27%	0.00180	27%	0.00180	27%
Flutolanil	1	0.00059	0%	0.00059	0%	0.00059	0%	0.00059	0%
Flutriafol	4	0.00030	0%	0.00031	0%	0.00075	1%	0.00104	2%
Fluxapyroxad	3	0.00042	1%	0.00061	1%	0.00066	1%	0.00069	1%
Hexythiazox	11	0.00098	8%	0.00055	5%	0.00103	9%	0.00813	68%
Imidacloprid	15	0.00021	0%	0.00025	0%	0.00031	0%	0.00058	1%
Indoxacarb	8	0.00022	4%	0.00018	3%	0.00050	9%	0.00092	17%
Iprodione	19	0.00690	2%	0.00550	1%	0.01569	4%	0.04825	12%
Iprovalicarb	8	0.00067	3%	0.00080	4%	0.00169	8%	0.00309	15%
Kresoxim-methyl	10	0.00168	0%	0.00135	0%	0.00449	0%	0.00989	1%
Lufenuron	18	0.00082	6%	0.00064	5%	0.00125	9%	0.00702	53%
Malathion	1	0.00005	-	0.00005	-	0.00005	-	0.00005	-
Mandipropamid	12	0.00647	3%	0.00127	1%	0.00395	2%	0.06793	30%
Mepanipyrim	4	0.00062	1%	0.00111	1%	0.00125	1%	0.00134	1%
Metalaxyl (metalaxyl-M)	3	0.00099	1%	0.00140	1%	0.00205	2%	0.00249	2%
Methiocarb	15	0.00345	20%	0.00176	10%	0.01306 *	75%	0.02184 *	126%
Methoxyfenozide	14	0.00047	0%	0.00063	0%	0.00088	1%	0.00193	1%

Active Substance	N	PDE (Mean) (mg/kg bw per day)	SE (Mean) in % of AOEL	PDE (75th P) (mg/kg bw per day)	SE (75th P) in % of AOEL	PDE (90th P) (mg/kg bw per day)	SE (90th P) in % of AOEL	PDE (Maximum) (mg/kg bw per day)	SE (Maximum) in % of AOEL
Metrafenone	3	0.00025	0%	0.00035	0%	0.00038	0%	0.00040	0%
Myclobutanil	4	0.00019	0%	0.00025	1%	0.00027	1%	0.00028	1%
Nitrothal-isopropyl	1	0.00025	-	0.00025	-	0.00025	-	0.00025	-
Novaluron	1	0.00964	-	0.00964	-	0.00964	-	0.00964	-
Oxycarboxin	1	0.00005	-	0.00005	-	0.00005	-	0.00005	-
Paclobutrazol	3	0.00083	1%	0.00120	1%	0.00187	1%	0.00231	2%
Penconazole	1	0.00037	1%	0.00037	1%	0.00037	1%	0.00037	1%
Permethrin	1	0.00011	-	0.00011	-	0.00011	-	0.00011	-
Picoxystrobin	5	0.00063	1%	0.00009	0%	0.00180	3%	0.00294	5%
Piperonyl-butoxyde	9	0.00019	-	0.00016	-	0.00042	-	0.00100	-
Pirimicarb	3	0.00008	0%	0.00010	0%	0.00010	0%	0.00011	0%
Pirimiphos-methyl	1	0.00007	0%	0.00007	0%	0.00007	0%	0.00007	0%
Prochloraz	13	0.00136	5%	0.00118	4%	0.00300	11%	0.00869	33%
Procymidone	17	0.00225	14%	0.00178	11%	0.00744	47%	0.01199	75%
Profenofos	1	0.00004	-	0.00004	-	0.00004	-	0.00004	-
Propamocarb	13	0.00219	1%	0.00177	0%	0.00553	1%	0.01454	4%
Propiconazole	4	0.00009	0%	0.00010	0%	0.00015	0%	0.00018	0%
Pymetrozine	9	0.00021	1%	0.00021	1%	0.00041	1%	0.00080	2%

Active Substance	N	PDE (Mean) (mg/kg bw per day)	SE (Mean) in % of AOEL	PDE (75th P) (mg/kg bw per day)	SE (75th P) in % of AOEL	PDE (90th P) (mg/kg bw per day)	SE (90th P) in % of AOEL	PDE (Maximum) (mg/kg bw per day)	SE (Maximum) in % of AOEL
Pyraclostrobin	10	0.00168	8%	0.00197	10%	0.00576	29%	0.00633	32%
Pyridaben	6	0.00186	28%	0.00195	29%	0.00524 *	79%	0.00799 *	120%
Pyridalyl	13	0.00050	2%	0.00049	2%	0.00116	4%	0.00178	7%
Pyrimethanil	5	0.00027	0%	0.00031	0%	0.00061	0%	0.00081	1%
Pyriproxyfen	1	0.00004	0%	0.00004	0%	0.00004	0%	0.00004	0%
Simazine	1	0.00006	-	0.00006	-	0.00006	-	0.00006	-
Spinetoram	5	0.00005	1%	0.00006	1%	0.00006	1%	0.00007	1%
Spinosad	7	0.00042	3%	0.00018	1%	0.00109	7%	0.00239	15%
Spirodiclofen	1	0.00570	48%	0.00570	48%	0.00570	48%	0.00570	48%
Spiromesifen	1	0.00007	0%	0.00007	0%	0.00007	0%	0.00007	0%
Spirotetramat	6	0.00020	0%	0.00030	0%	0.00037	1%	0.00040	1%
Spiroxamine	16	0.00103	5%	0.00179	9%	0.00232	12%	0.00294	15%
Tebuconazole	8	0.00092	2%	0.00092	2%	0.00247	6%	0.00426	11%
Tebufenozide	1	0.00044	4%	0.00044	4%	0.00044	4%	0.00044	4%
Tebufenpyrad	2	0.00046	3%	0.00066	5%	0.00077	6%	0.00085	6%
Tetraconazole	1	0.00011	0%	0.00011	0%	0.00011	0%	0.00011	0%
Tetramethrine	1	0.00006	-	0.00006	-	0.00006	-	0.00006	-
Thiabendazole	2	0.00012	0%	0.00014	0%	0.00015	0%	0.00016	0%

Active Substance	N	PDE (Mean) (mg/kg bw per day)	SE (Mean) in % of AOEL	PDE (75th P) (mg/kg bw per day)	SE (75th P) in % of AOEL	PDE (90th P) (mg/kg bw per day)	SE (90th P) in % of AOEL	PDE (Maximum) (mg/kg bw per day)	SE (Maximum) in % of AOEL
Thiacloprid	9	0.00099	4%	0.00065	2%	0.00292	11%	0.00506	19%
Thiametoxam	11	0.00084	1%	0.00065	1%	0.00252	2%	0.00319	3%
Thiophanate methyl	7	0.00025	0%	0.00037	0%	0.00055	1%	0.00066	1%
Tolclofos-methyl	4	0.00010	0%	0.00012	0%	0.00015	0%	0.00016	0%
Triadimefon (triadimenol)	1	0.00015	0%	0.00015	0%	0.00015	0%	0.00015	0%
Trifloxystrobin	10	0.00046	1%	0.00067	1%	0.00106	1%	0.00151	2%
Triflumizole	1	0.00008	0%	0.00008	0%	0.00008	0%	0.00008	0%

4. Discussion

A linear relationship exists between the levels of dislodgeable residue and the dermal exposure (Popendorf and Leffingwell, 1982; Nigg *et al.,* 1985; Zweig & Leffingwell, 1985). Contact with contaminated flowers resulted in the transfer of pesticide residues to gloves worn by florists. All glove samples appeared to be highly contaminated by many different pesticide residues (111 active substances detected with an average of about 37 active substances per sample and a total concentration per glove sample of 22.22 mg/kg). These concentrations are 1000 times higher than the concentrations which are usually detected on foodstuffs. Half of the detected active substances are insecticides and the other half are fungicides. Only one substance is a growth regulator (paclobutrazol) and another one is a herbicide (simazine). Three fungicides (benomyl and its metabolite carbendazim, boscalid, and iprodione) and three insecticides (clofentezine, lufenuron, and flonicamid) are the most frequently detected active substances (90% of the samples). Twenty eight active substances (25%) are detected only once.

The maximum residue concentrations are measured for boscalid, clofentezine, iprodione, and mandipropamid (26.21, 18.37, 16.93, and 16.50 mg/kg, respectively). Boscalid, novaluron, clofentezine, iprodione, and spirodiclofen present the highest average concentrations, with 3.47 and 3.38, 2.88, 2.42, and 2 mg/kg, respectively. Boscalid is the active substance that has both the highest average and maximum concentrations out of all the active substances analysed.

Of the 111 detected active substances, most of the pesticides belong to the following chemical groups: triazoles (13 a.s.); pyrethroids (8 a.s.); organophosphates (7 a.s.); carbamates and strobilurins (6 a.s.); and benzoylurea, keto-enol, and neonicotinoids (4 a.s.). Pesticides from these families are known for their toxicological properties (acute toxicity, with an action on the nervous system). Many active substances detected in the glove samples may affect the skin of the florists after exposure by contact. According to the CLP (classification, labelling, and packaging) classification (Table 25), some of these active substances have potential hazardous and/ or chronic effects. The potential health effects of these hazards are in accordance with symptoms recorded in various publications. In the survey of Morse *et al.* (1979) the symptoms most frequently reported by the florists after exposure are headaches (20%), skin irritation (20%), and watery eyes (20%). In the survey of Restrepo *et al.* (1990), a moderate increase in the prevalence of abortion, prematurity, and congenital malformations for pregnant female workers in floriculture was noted. During the interviews conducted by Toumi *et al.* (2016a), only one florist mentioned frequent headaches and recurrent tiredness, but many of them declared suffering from various symptoms like skin allergy, eye irritation, itching of their skin, respiratory problems, thyroid problems and even, for two out of 25, cancer. These testimonies should of course be considered with caution when no diagnosis can support such declarations. Based on repeated observations of workers who are regularly exposed to pesticides at concentrations above their AOEL, such exposures can result in adverse health effects. The potential dermal exposures of florists were estimated for the average, for different percentiles, and for the

maximum concentration of residues in samples (Table 26). The results from the different percentiles used to estimate PDE vary by orders of magnitude. As was shown in Table 26, no active substance exceeds the AOEL for PDE mean and PDE P75 values. However, at the P90 and at the maximum (or worst case) values of PDE, three and five active substances respectively exceed the AOEL indicating risk situations. The potential dermal exposure values are in accordance with Thongsinthusak *et al.* (1990) and Brouwer *et al.* (1992c). They have respectively reported contamination levels of 0.0005 mg/kg bw per day after handling chrysanthemums and roses and 0.1714 mg/kg bw per day during cutting, sorting, and bundling of roses. For a vast majority of active substances, PDE values obtained in our trial have the same order of magnitude. Even for the worst cases of exposure (e.g., clofentezine or methiocarb), the values are rather similar to previously reported data. Only results of dermal exposure reported by Brouwer *et al.* (1992a) for workers in contact with flowers at the field were significantly higher. Average exposures of 0.8571 mg/kg bw per day during cutting and 0.6000 mg/kg bw per day during sorting and bundling of carnations were observed. The short elapsed time between application of pesticides and the re-entry of workers as well as the application rates and the nature of their activities could explain a higher transfer of residues after contact. Nevertheless, the comparison between those previous studies and our results should be considered with caution as local situations are different and practices have evolved with time.

For the systemic exposure, one active substance (clofentezine) exceeds the AOEL at the P90 predictive level. In the worst case, SE_{MAX} (at the maximum concentrations), four active substances (clofentezine, famoxadone, methiocarb, and pyridaben) exceed their respective AOEL values. Among the 14 most frequently detected active substances, two have SE_{MAX} values exceeding the AOEL. Exposure could be particularly critical for clofentezine with SE_{MAX} values that are four times higher the AOEL (393%).

For the actual dermal exposure (ADE), whatever the PDE values considered, it is interesting to confirm that no active substances exceed the AOEL when the florists are wearing PPE. A few studies on workers have confirmed that protective clothing (McCurdy *et al.,* 1994; Krieger and Dinoff, 2000) and gloves (Gomes, 1999) can reduce the amount of pesticides reaching the skin. It is assumed today that their potential dermal exposure can be reduced by 90% when workers protect themselves with appropriate PPE (EFSA, 2014). Nevertheless, the survey conducted in Belgium (Toumi *et al.,* 2016a) showed that this scenario is not representative of their habits; the majority of florists do not wear gloves, or any other PPE, even if they spend 2 to 6 h per day handling cut flowers and preparing bouquets.

5. Conclusions

In conclusion, the exposure of florists is an example of a unique situation in which a professional is exposed regularly to both a very high number of toxic chemicals and rather high concentration levels. According to the results of the risk assessment, Belgian florists who handle a large number of flowers are at risk of exposure to pesticides residues with potential effects on their health. To better assess the risk,

bio-monitoring of the florists with analysis of their blood, urines, and hairs is still to be investigated.

Meanwhile, to reduce their exposure, solutions could be recommended. The priority should be to raise the level of awareness among the florists who can change their habits and practices if they want to minimize their exposure. Wearing gloves, washing their hands and their arms, and respecting hygiene rules could be effective. In the near future, it is necessary to promote a better pesticide management at the field level (integrated pest management, certification schemes, and labels) or even organic flower production if clients are ready to pay. Moreover, extending the European regulation on maximum residue limits (Regulation (EC) N°396/2005) for pesticide residues on flowers or controlling the residue levels on cut flowers could also be discussed.

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Author Contributions

This research was undertaken as part of Khaoula Toumi's Doctor of Phytopharmacy thesis. Bruno Schiffers is the promoter of this thesis. All authors contributed significantly to the successful completion of this research work both intellectually and financially. Accordingly, they conceived and designed the study plan. Khaoula Toumi conducted sampling, analyzed the data, and wrote the intial manuscript. Bruno Schiffers guided this study and provided revisions on the manuscript. Laure Joly and Christiane Vleminckx provided feedback on the manuscript. Finally, all the authors have read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest

Chapitre 5

Exposition totale des fleuristes belges aux résidus de pesticides

Introduction

La peau permet de protéger l'organisme des agressions externes. Mais, elle ne constitue toutefois pas une barrière étanche puisque différents éléments sont capables de la traverser. La peau peut être une cible ou une porte d'entrée privilégiée pour de nombreuses substances actives qui ont des effets toxiques (selon la classification CLP, on distingue des substances ayant les propriétés suivantes : irritation ou corrosion cutanée ou oculaire, sensibilisation cutanée, suspicion d'être cancérogènes, mutagènes ou toxiques pour la reproduction, etc.). Par conséquent, plusieurs substances actives peuvent être absorbées et passer dans l'organisme humain et être excrétées dans les urines. La surveillance biologique reste souvent l'approche privilégiée car elle présente l'avantage d'intégrer toutes les voies d'exposition et de fournir une image complète de la dose interne provenant de l'exposition.

Le présent chapitre présente d'abord le développement et la validation d'une méthode multi-résidus par chromatographie liquide couplée à la spectrométrie de masse. Ce préalable était indispensable pour l'identification et la quantification des résidus de pesticides et des métabolites urinaires. Les résidus de pesticides et leurs métabolites urinaires spécifiques à rechercher ont été choisis sur la base des résultats obtenus lors de l'analyse des fleurs coupées les plus vendues en Belgique et de l'identification des substances détectées sur les gants en coton portés par les fleuristes durant leurs activités professionnelles. Par la suite, la méthode d'analyse a été appliquée, durant trois périodes consécutives, à des échantillons d'urine collectés auprès de fleuristes et de groupes contrôles. Elle devait permettre d'évaluer l'exposition systémique totale des fleuristes belges aux résidus de pesticides durant la manipulation des fleurs et la préparation des bouquets.

Ce chapitre est une version adaptée des deux articles suivants :

- Toumi, K., Szternfeld, P., Schiffers, B. & Joly, L. (2018). Multi-residue quantification of pesticides in urine by liquid chromatography coupled to mass spectrometry (LC-MS/MS) for the evaluation of human exposure (Article soumis dans *International Journal of Environmental Analytical Chemistry*).

- Toumi, K., Joly, L., Vleminckx, C., & Schiffers, B. (2018). Biological monitoring of exposure to pesticide residues among Belgian florists (Article soumis dans *Human and Ecological Risk Assessment: An International Journal*).

I. Multi-residue quantification of pesticides in urine by liquid chromatography coupled to mass spectrometry (LC-MS/MS) for the evaluation of human exposure

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Abstract

A new multi-residue method for the simultaneous identification and quantification of pesticide residues and their metabolites in human urines by means of liquid chromatography - tandem mass spectrometry (LC-MS/MS) is presented. After extraction with acidified ethyl acetate, an aliquot of the extract is concentrated under nitrogen gas and redissolved in methanol before injection on LC-MS/MS. The LC-MS determination was performed on a methanol/water concentrated extract (0.5 ml) using a UHPLC-ESI-MS/MS system consisting of an ACQUITY UPLC coupled to Quattro Premier MS (Waters) and equipped with 3 different columns : an ACQUITY™ BEH C18 column (1.7 µm, 2.1 x 100 mm,), an ACQUITY™ BEH phenyl column (1.7 µm; 2.1 × 100 mm) and a SeQuant® ZIC®-HILIC HPLC column (3.7 µm, 2.1 × 100 mm). The method was successfully validated according to the SANTE 11813/2017 guidelines for 110 different pesticides and metabolites, except for the practical limits of quantification (LOQs) which were fixed at the first point of the calibration curve with a minimum S/N of 6 (0.02 µg/L for the majority of the pesticides). Mean recoveries were, with a few exceptions, in the range 80-120% with a relative standard deviation below 20%. Finally, the method was tested on urine samples of several Belgian healthy volunteers. A total of 37 pesticide residues and metabolites were identified in 28 urine samples with average of 4.5 pesticide residues and metabolites per sample for an average total concentration of 2.8 µg/L.

Keywords: Pesticide residues, pesticide metabolites, human urine, validation, LC-MS/MS

1. Introduction

The use of pesticides is considered as necessary by the majority of farmers to prevent and control pests and diseases that can markedly reduce the productivity and the quality of products. As a result, pesticides are widely and extensively used in conventional agriculture (Aktar *et al*., 2009). Extensive use of some of them, has been proven to induce acute and chronic health problems (Damalas & Eleftherohorinos, 2011; Blair *et al.,* 2014 ; Kim *et al.,* 2017), including increases of multiple cancer types (Amr *et al.,* 2015 ; Arrebola *et al.,* 2015 ; Koutros *et al.,* 2015), reproductive problems (Mehrpour *et al.,* 2014 ; Cremonese *et al.,* 2017), neurology disorders (Baldi *et al.,* 2003 ; Sanchez-Santed *et al.,* 2016), respiratory problems (Hernández *et al.,* 2011 ; Amaral *et al.,* 2014) and diabetes (Sylvie *et al.,* 2013 ; Jaacks *et al.,* 2015).

Faced with exposure problems, risk assessment is considered essential step to characterize the nature and magnitude of health risks to humans, then eliminate hazards or minimise the level of risks by applying control measures. Various approaches have been used in health risks evaluation from exposure to pesticide residues (Reffstrup *et al.,* 2010). However, bio monitoring is a valuable, powerful and reliable assessment tool that offers the advantage of integrating all of the possible sources and routes of exposure and of representing the total exposure (Cortéjade *et al.,* 2016; López *et al.,* 2016; Appenzeller *et al.,* 2017). For this purpose, urine remains one of the favourite biological matrix that has been used to date for the bio monitoring of exposure to pesticide residues and metabolites, because it can be easily collected and is a non-invasive sampling method (Esteban *et al.,* 2009). However, only few works of multi residue pesticide analysis methods using liquid chromatography-tandem mass spectrometry (LC-MSMS) applied to urine samples for monitoring have been reported. In 2004, Olsson *et al.* (2004) developed a multi residue method that measured 19 different compounds (seven specific metabolites of organophosphorus pesticides, five metabolites of synthetic pyrethroids, six herbicides or their metabolites, and one insect repellant). Jayatilaka *et al.* (2011), developed a method to monitor 4 organophosphorus pesticides and 2 fungicides in urine. Reemtsma *et al.* (2011), described a determination of 14 organophosphorus pesticides in a single run. Davis *et al.* (2013) quantified 12 specific metabolites of organophosphorus, pyrethroids and herbicides in urine. Despite their efficiency and their good limit of quantification (between 0.004 µg/L and 0.5 µg/L), these methods have a common characteristic, they possessed a restricted scope, covering only two or three classes of pesticides.

High resolution mass spectrometry (HRMS) have been used in the field of biomonitoring such as orbitrap or quadrupoletime-of-flight (Q-TOF) mass spectrometer allowing to extend considerably the scope of pesticide (classes) covered. Roca *et al.* (2014) used liquid chromatography coupled to an orbitrap to develop an analytical method including the quantification of 29 biomarkers in urine belonging to various pesticides classes (organophosphorus and pyrethroid insecticides, carbamate fungicides, phenoxy and chloroacetanilide herbicides) with LOQs ranging from 0.8 to 3.2 $\mu g.L^{-1}$ and a post-target screening of 60 metabolites including 12 pesticide metabolites. Cortéjade et al. (2016) used a Q-TOF for both

targeted and non-targeted analysis in order to study the exposome by analysing urine samples with LOQs ranging between 4.3 µg.L^{-1} and 113.2 µg. L^{-1}. They succeeded to quantify 12 pesticides and 1 pesticide metabolite among 38 selected contaminants and have simultaneously screened some other associated metabolites. Lopez *et al.* (2016) based their strategy on a quantitative target analysis in combination with a retrospective screening of pesticides metabolites using a large database containing 263 compounds. LC-HRMS can be applied to the analysis of biological matrices to identify a wide range of biomarkers of exposure. It is also possible to make retrospective analysis by the use of different databases on existing acquired data. Despite these advantages, all LC-HMRS methods suffer from the same drawbacks. Even if existing spectrum databases allow screening, a huge number of compounds belonging to various classes and their metabolites at the same time, it is not always possible to confirm accurately their identity due to the absence of analytical standards, or the lack of isotopic patterns at low concentration Cortéjade *et al.* (2016) et Lopez *et al.* (2016). For the same reasons, it doesn't allow the quantification which is primordial for an exposure measurement.

The present study aimed to develop and validate a cost effective analytical method for multi-residue quantification of pesticide residues and metabolites in urine by LC-MSMS. This method covers a broad scope of 110 pesticides and metabolites thanks to its configuration, allowing the use of three different chromatographic columns without changing the instrument set-up.

In order to evaluate the method performances, it has been applied to 24h urine samples of healthy subjects. Initially, the goal of this method validation was to assess the risk of Belgian florists exposure to pesticide residues present in cut flowers, but it can be easily adapted for the use of bio monitoring purpose of other people in contact with pesticides directly or indirectly such as operators, workers, bystanders or residents.

2. Materials and methods

2.1. Chemicals and reagents

All analytical standards were purchased from Dr. Ehrenstorfer (Ausburg, Germany) and Sigma Aldrich (ST.Louis, MO, USA) and were of purity exceeding 95%. Solvents (acetone, acetonitrile, ethyl acetate, and methanol) and formic acid were from Biosolve (The Netherlands). Ammonium acetate was of analytical grade and obtained from Fluka (Sigma, Boenem, Belgium). Water was purified using a Millipore Milli-Q system (Millipore Corp., Bedford, MA, USA). Synthetic urine according to DIN EN 1616 is from Synthetic Urine e.k. (Schloβstr. 33 71735 Eberdingen-Nuβdorf, Germany).

2.2. Selection of pesticides

The selected pesticides included all the insecticides, fungicides and herbicides, which were detected in cut flowers the most sold in Belgium (Toumi *et al.,* 2016a, b) and gloves worn by florists during daily professional activities (Toumi *et al.,* 2017a, b). A total of 110 pesticides and urinary metabolites were thus considered for

this study for LC–MS/MS analysis. These pesticides and urinary metabolites are presented in Table 28.

2.3. Preparation of stock solutions

Stock solutions of individual pesticide standards were prepared at 1 mg/ml by accurately weighing 25 mg (±0.01 mg) of each compound and dissolving in 25 mL acetonitrile, acetone or methanol depending on the pesticide solubility. These standard solutions were stored in the dark at -20°C and were used within 5 years. Intermediate, working, and spike standard mixtures were prepared in acetone by mixing the appropriate quantities of the individual stock solutions followed by requisite volume makeup. Internal standard stock solution of oxfendazole was prepared at 0.1 mg/mL in acetonitrile with 0.1% acetic acid.

2.4. Extraction procedure

Ten millilitre of an artificial urine sample was pipetted in a 50 ml Falcon® tube. The first extraction was carried out with 10 ml of ethyl acetate, 2g of NaCl and 1 ml of formic acid. The homogenate was vortexed 15 min and centrifuged for 5 min (3,900 rpm). The ethyl acetate layer was removed by pipetting and the extraction procedure was repeated with 5 ml of ethyl acetate. The first and second ethyl acetate layers were combined and 2 g of Na_2SO_4 was added and evaporated using a gentle nitrogen stream until 10 ml. Then, 5 ml were allocated for LC-MS/MS analysis and evaporated using a gentle stream of nitrogen down to 100 µl. 350 µl of methanol and 50 µl of oxfendazole (internal standard IS) were added. Finally the volume was adjusted to 1 ml with the mobile phase A (water/methanol 90/10 v/v) and gently shacked. 500 µl of extract was transferred in a filter vial and further analysed (Figure 9).

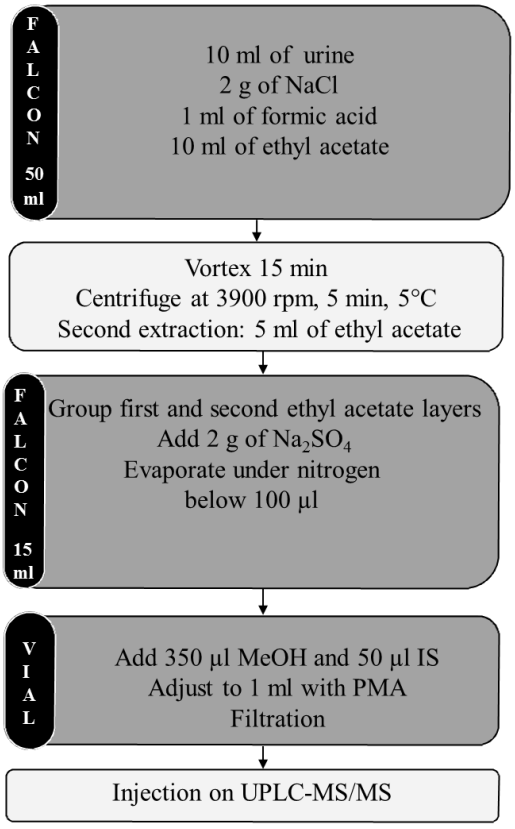

Figure 9: Analytical procedure of urinary extraction

2.5. LC-MS/MS analysis

Chromatographic separation of pesticides was performed using ultra-high-pressure liquid chromatography coupled with a mass spectrometer Quattro Premier TM (Waters). Flow rate was set constant at 0.45 ml min^{-1} during the whole process, with the oven temperature of 45 °C. The electrospray source and the desolvatation temperatures were set at 130 °C and 500 °C, respectively. Nitrogen gas flow rates were 50 L/h for the cone and 800 L/h for desolvatation. After a common extraction step, three different columns were used according to the properties of the residues to separate: an ACQUITY™ BEH C18 column (1.7 µm, 2.1 × 100 mm), an ACQUITY™ BEH phenyl column (Phenyl 1.7 µm, 2.1 mm X 100 mm) and a SeQuant®ZIC®-HILIC HPLC column (3.7 µm, 2.1 × 100 mm) (Table 27).

Table 27: Characteristic of the 4 acquisition runs

ACQUITY™ BEH C18 column (1.7 µm; 2.1 × 100 mm)					
Ionization mode	**Injected volume**	**Run Time**	**Gradient elution**		
ES+	5 µL	15 min	Time	%PMA	%PMB
			Initial	99.9	0.1
			10.00	0.1	99.9
ES-	5 µL	15 min	12.00	0.1	99.9
			12.10	99.9	0.1
			15.00	99.9	0.1
ACQUITY™ BEH phenyl (1.7 µm; 2.1 × 100 mm)					
Ionization mode	**Injected volume**	**Run Time**	**Gradient elution**		
ES+ and ES-	5 µL	10 min	Time	%PMA	%PMB
			Initial	80	20
			7.00	0.1	99.9
			7.10	80	20
			10.00	80	20
SeQuant® ZIC®-HILIC HPLC column (3.7 µm; 2.1 × 100 mm)					
Ionization mode	**Injected volume**	**Run Time**	**Gradient elution**		
ES+ and ES-	3 µL	15 min	Time	%PMC	%PMD
			Initial	0.1	99.9
			2.5	0.1	99.9
			10.00	35.0	65.0
			15.00	0.1	99.9
The mobile phases A (**PMA**) and B (**PMB**) were 5 mM ammonium acetate in water/methanol (90/10; v/v) and in methanol/water (90/10; v/v), respectively. The mobile phase C (**PMC**) were composed of 100 % water with 5 mM ammonium formate and the mobile phase D (**PMD**) composed of 100 % acetonitrile, both containing 0.1% formic acid.					

2.6. Validation of the method

Validation was carried out according to European SANTE guideline 11813/2017 (European commission, 2017). The validation of method was conducted using a within laboratory protocol employing spiked artificial urine samples. The procedure was validated in terms of the limits of detection (LOD) and quantification (LOQ), selectivity, calibration curve, matrix effect, inter- and intra-day precision, and accuracy. The limit of detection (LOD) is defined as the lowest concentration of the compound that can be detected (Cortéjade et al., 2016 ; Taverniers et al., 2004 ;

Bijlsma et al., 2009) which gave a signal to noise ratio (S/N) ≥ 3, and the limit of quantification (LOQ) is associated with the smallest concentration that can be quantified (S/N ≥ 6). LOQ and LOD were estimated for a signal to noise ratio from the chromatograms of samples spiked at the lowest level. Method selectivity was evaluated through possible interference peak in the 2 MS/MS transitions at the retention time of each pesticide. Calibration curves on artificial urine extract were obtained from eight concentration levels, in the range from 0.02 µg/L to 100 µg/L, depending on the sensitivity reached for each analyte. Linearity was considered acceptable when the correlation coefficient (r^2) was > 0.995. The matrix effect was evaluated by preparing seven calibration levels ranging from 0.1 to 100 µg/L both in solvent and in matrix, in order to compare the responses in neat solvent and in matrix. Repeatability (intra-day precision) and reproducibility (inter-day precision) were evaluated by analysing five spiked samples at low level (0.5 µg/L or 5 µg/L) and high level (50 µg/L) for each parameter. To ensure the accuracy and robustness of the method, recovery tests at two levels of concentrations: a low level (0.5 µg/L or 5 µg/L) and a higher level (50 µg/L) were performed. Recoveries between 80–120% with RSD lower than 20% were considered satisfactory according the SANTE/11813/2017 criteria. Recoveries between 30-140% are considered to be acceptable with correction (European commission, 2017).

3. Results and discussion

3.1. Method development

3.1.1. Optimisation of extraction procedure

The method was based on an intra lab validated method to analyse pesticides in water and other published methods for pesticides extraction in urine with ethyl acetate were reported (Honda & kannan, 2018 ; Dulaurent *et al.,* 2006) . A series of preliminary trials were performed looking to the optimization of extraction procedure. The addition of acid makes it possible to obtain the most polar pesticides in the organic phase. In the first step of extraction, two type of acid (formic acid and sulfuric acid) were tested. Recoveries with formic acid were very reliable and reproducible compared to those with sulfuric acid which were unacceptable for the majority of compounds

Then, formic acid at various concentrations (0.5, 1 and 2 ml) was tested. No difference was found between the different tests, despite the pH differences of extracts that were of 2.5, 2 and 1.5 for 0.5, 1 and 2 ml of formic acid, respectively. That is the reason why, 1 ml of formic acid was used throughout the method development and validation.

3.1.2. Optimisation of LC–MS/MS parameters

Some preliminary information (molar mass, parent ion and daughter ions) were collected from the official website of Europe "Reference Laboratory for residues of pesticides (EURL, 2018)''. For each compound, MS/MS conditions were selected individually with direct infusion of 10 µg/ml standard solution prepared in methanol at a flow rate of 5µl/min. Then, two transitions were selected and monitored per

compound from the product ion: the first and more abundant ion was used for quantification and the second for confirmation (Lazartigues et al., 2011). For some compounds, only one product ion was obtained, while the fragmentation was difficult due to the stability of its structure. Optimized parameters for all compounds analysed, are shown in Table 28: ionization mode, mass spectrometry transitions and associated cone and collision cell voltages.

Following Hanot *et al.* (2015), the protocol of injection using BEH C18 was adopted in correspondence with the published method of pesticide residues analysis in fruits and vegetables, available in the laboratory. All pesticides were tested with a reverse phase BEH C18. For very polar metabolites with no retention on column BEH C18, many others columns were tested such as: Shodex® RSpak DE-413 (4.6mmI.D. x 150mm), ACQUITY™ BEH phenyl column (1.7 µm; 2.1 × 100 mm), SeQuant® ZIC®-HILIC HPLC column (3.7 µm, 2.1 × 100 mm) and HYPERCARB column (5 µm, 100 mm × 2.1 mm). Finally, two columns were necessary to obtain a good retention for very polar compounds. As a result, the columns BEH phenyl and Hilic were changed for some of these pesticide residues. Six pesticides and two metabolites were injected using Hilic and BEH phenyl columns, respectively.

The addition of ammonium formate in mobile phases suppresses the formation of sodium adducts ($[M^+Na^+]^+$), which are more common under acidic conditions (formic acid), and therefore, pesticides formed predominantly $[M^+H^+]^+$ and $[M^+NH4^+]^+$, which showed higher sensitivity and more consistent responses for certain pesticides such as 3-hydroxycarbofuran, clofentezine, hexythiazox, kresoxim-methyl and triforine (Hiemstra De kok, 2007; Rizzetti *et al.,* 2016). At the last stage of extraction, concentrate samples around 100 µL remove the major part of ethyl acetate and improves the shape of the peaks.

3.2. Method validation

3.2.1. LOD and LOQ

Usually, limit of quantification (LOQ) is determined as the lowest concentration which gave a signal to noise ratio (S/N) ≥ 6. However quantitative results below the lowest point of the calibration curve are not reliable. Consequently, the lowest LOQs were fixed at the first point of the calibration curve when the S/N ≥ 6 criteria were also fulfilling at this level. For the less sensitive compounds the LOQ level is defined only by a S/N ≥ 6. Pesticide LOQs are resumed in Table 28. LODs were set for each compound as the LOQ / 2.

3.2.2. Selectivity

Urine samples from healthy subjects were used in order to develop and validate the method, but all of them were contaminated of one or more of pesticide residues. As a consequence, artificial urine was used to validate the method, to prepare the calibration curve.

3.2.3. Calibration curve

Based on the results obtained from calibration curves, the majority of the compounds showed coefficients of determination (r^2) ≥ 0.995 at linear or quadratic

equations that are considered as satisfactory. For compounds which did not present $r^2 \geq 0.995$ with the linear model, the quadratic model was suitable in every case.

3.2.4. Matrix effects

Matrix effects present a significant challenge to multi-residue pesticide analysis by LC–MS/MS, showing signal increase and suppression in matrix (Hajšlová *et al.,* 2003; Rizzetti *et al.,* 2016). In this study, the matrix effect was prominent for a large number of pesticide residues. Hence, the method has been validated using matrix matched calibration.

3.2.5. Inter-day and intra-day precision and recovery

Repeatability was investigated by extraction and analysis of blank samples spiked at each recovery level on the same day. Reproducibility has been studies at two levels (0.5 µg/L or 5 µg/L) and 50 µg/L). Replicate (n = 5 for each concentration) samples were analysed and RSD was calculated for each pesticide (Table 28). The method was shown to be precise; with RSD values ranging from 5 to 26 % for all the pesticide residues and metabolites studied at all spiking levels. Recovery outside the range 80-120% was corrected by validation data during sample analysis. 79% and 86% of pesticides residues and metabolites have a recoveries range between 80-120% for low and high level, respectively. Only Dimethylphosphate (DMP), which is urinary metabolites of many organophosphate pesticides, had a recovery below 30%. DMP is kept in method because of it importance in terms of risk assessment (Figure 10).

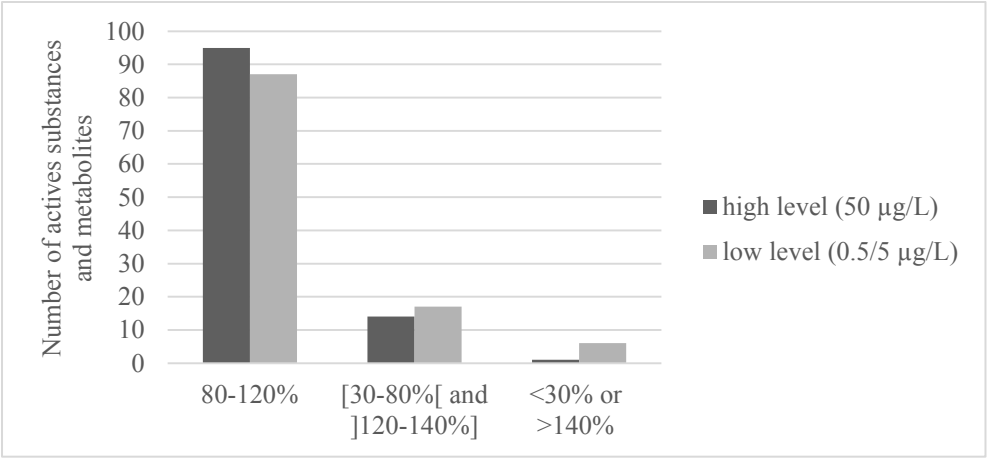

Figure 10: Distribution of recovery range yield of pesticide residues and metabolites in urine at two levels (low (0.5 µg/L (or 5 µg/L)) and high (50 µg/L) levels)

Table 28: Analytical conditions of the studies pesticide residues (retention time (RT), Cone voltage (V), Transition 1 (m/z), collision energy 1 (V), Transition 2 (m/z), collision energy 2 (V)), Ionization, column), LOQs and recoveries at different levels (0.5 (or 5 µg/L) and 50 µg/L

Compound	Ionization mode	column	RT (min)	Cone Voltage (V)	Transition 1 (m/z)	Collision energy 1 (V)	Transition 2 (m/z)	Collision energy 2 (V)	LOQ (µg/L)	Recovery (%) ± RSD (%)	
										0.5 (or 5 µg/L*)	50 µg/L
2 chloro 1.3 thiazole 5 carboxilic acid	ES-	Hilic	2.44	8	162 > 117.8	12	162 >162	5	0.06	106±19	105±16
3-hydroxy carbofuran	ES+	BEH C 18	3.53	25	220.1 > 163.1	10	-	-	0.02	84±15	91±10
				10	-	-	254.96 > 219.94	10			
3-phenoxybenzoic acid	ES+	BEH phenyl	2.42	14	213>169	15	213 > 92.9	25	0.4	104±16	89±13
3, 5, 6-trichoro-2-pyridinol	ES-	BEH C 18	4.72	25	195.8 > 195.8	2	197.8 > 197.8	2	0.08	93±17	102±18
4 bromo 2 chlorophenol	ES-	BEH phenyl	4.03	30	204.9>78.7	25			80	-	111±17
			-	20	-	-	204.9 > 78.7	30	5	97±20*	87±7
6 chloronicotinic acid	ES-	Hilic	1.47	20	155.9 > 111.8	10	-	-			
6 chloronicotinic acid	ES+	Hilic	1.46	20	157.8 > 121.8	20	-	-	5	49±16*	91±17
Acephate	ES+	BEH C 18	1.27	16	184.1 > 143	8	184.1 > 125.0	17	0.02	46±8	46±10
Acetamiprid	ES+	BEH C 18	3.49	26	223 > 125.8	21	223 > 89.9	35	0.02	108±16	92±10
Acetamiprid-n-desmethyl	ES+	BEH C 18	3.4	25	209 > 125.8	20	209 >72.8	50	0.02	90±21	93±7
Ametoctradin	ES+	BEH C 18	9.03	44	276.1 > 149	36	276.1 > 176	37	0.02	101±13	91±10
Azoxystrobin	ES+	BEH C 18	7.17	23	404 > 372	15	404 > 329.2	30	0.02	101±14	90±7
Bitertanol	ES+	BEH C 18	8.81	15	338.25 > 269.1	9	338.25 > 98.9	15	0.04	97±15	93±11

Compound	Ionization mode	column	RT (min)	Cone Voltage (V)	Transition 1 (m/z)	Collision energy 1 (V)	Transition 2 (m/z)	Collision energy 2 (V)	LOQ (µg/L)	Recovery (%) ± RSD (%)	
										0.5 (or 5 µg/L*)	50 µg/L
Boscalid	ES+	BEH C 18	7.38	33	342.79 > 307.1	20	342.79 > 271.2	30	0.2	89±20	92±9
Bupirimate	ES+	BEH C 18	8.22	39	317.2 > 107.9	27	317.2 > 166.1	27	0.02	99±14	92±11
Buprofezin	ES+	BEH C 18	9.58	22	306 > 57	22	306 > 115.8	16	0.02	99±19	90±10
Carbendazim	ES+	BEH C 18	3.74	25	192.1 > 160.1	18	192.1 > 132.1	30	0.02	82±16	76±12
Carbofuran	ES+	BEH C 18	5.36	22	222.1 > 165	12	222.1 > 123	23	0.02	95±20	92±13
Carbosulfan	ES+	BEH C 18		27	381 > 118.1	25	381 > 161.1	15	0.02	92±10	92±10
Chlorantraniliprole	ES+	BEH C 18	6.84	20	484 > 452.9	18	484 > 285.9	14	0.02	89±19	91±10
Chloridazon	ES+	BEH C 18	3.43	38	222.08 > 104	20	222.08 > 92	26	0.08	99±13	93±10
Clofentezin	ES+	BEH C 18	8.73	20	303.1 > 138	21	303.1 > 102	39	0.02	110±16	104±7
Cyflumetofen	ES+	BEH C 18	9.36	20	465.3 > 173.1	19	465.3 > 249.1	20	0.02	109±13	93±19
Cyproconazol 1	ES+	BEH C 18	7.56	25	291.8 > 69.9	19	291.8 > 124.9	31	0.02	124±25	95±10
Cyproconazol 2	ES+	BEH C 18	7.78	25	291.8 > 69.9	19	291.8 > 124.9	31	0.02	90±24	94±8
Cyprodinil	ES+	BEH C 18	8.39	45	226.2 > 108.1	25	226.2 > 93.1	25	0.02	136±16	91±12
Deet	ES+	BEH C 18	6.43	26	192.09 > 118.9	18	192.09 > 90.9	28	0.02	102±18	92±6
DEP	ES+	Hilic	7.64	22	155 > 98.5	15	155 > 127	8	10	-	60±21
DETP	ES-	Hilic	1.29	25	169 > 94.7	18	169 > 140.8	15	2	-	88±7
Difenconazole	ES+	BEH C 18	8.99	37	406 > 251	24	408 > 253	25	0.02	107±12	92±7
Diflubenzuron	ES+	BEH C 18	8.12	35	311 > 158.1	16	311 > 140.9	30	0.2	112±23	94±13
Dimethoate	ES+	BEH C 18	3.33	17	230.1 > 199.1	10	230.1 > 125.1	17	0.02	97±19	106±14
Dimethomorph 1	ES+	BEH C 18	7.32	26	388 > 300.9	23	388 > 164.9	32	0.02	90±20	92±10

Compound	Ionization mode	column	RT (min)	Cone Voltage (V)	Transition 1 (m/z)	Collision energy 1 (V)	Transition 2 (m/z)	Collision energy 2 (V)	LOQ (µg/L)	Recovery (%) ± RSD (%)	
										0.5 (or 5 µg/L*)	50 µg/L
Dimetomorph 2	ES+	BEH C 18	7.61	26	388 > 300.9	23	388 > 164.9	32	0.02	104±14	92±8
Dinotefuran	ES+	BEH C 18	1.59	15	203.1 > 129	12	203.1 > 156.9	7	0.02	57±17	59±7
DMP	ES+	Hilic	8.64	30	127 > 108.8	15	127 > 94.7	18	0.04	-	14±7
Dodemorphe	ES+	BEH C 18	11.05	30	282.4 > 116	20	282.4 > 98	30	0.02	87±12	88±10
Ethirimol	ES+	BEH C 18	6.47	38	210.2 > 140	22	210.2 > 97.9	28	0.02	94±15	83±8
Famoxadone	ES+	BEH C 18	8.67	16	392.01 > 331.15	10	392.01 > 238	20	0.2	87±19	95±15
Fenamidone	ES+	BEH C 18	7.32	20	311.9 > 102.7	19	311.9 > 235.9	26	0.04	93±25	91±13
Fenamiphos sulfone	ES+	BEH C 18	5.76	30	336.23 > 266	20	336.23 > 308.1	16	0.02	95±12	91±8
Fenamiphos sulfoxide	ES+	BEH C 18	5.62	34	320.26 > 233	26	320.26 > 107.9	40	0.02	108±14	92±10
Fenhexamid	ES+	BEH C 18	7.84	35	302.1 > 97	25	302.1 > 55.1	35	0.02	98±19	92±6
Fenoxycarb	ES+	BEH C 18	8.26	20	302.1 > 88	20	302.1 > 116.1	10	0.02	115±15	99±11
Fenpropidin	ES+	BEH C 18	8.29	45	274 > 147	28	274 > 86	26	0.06	101±19	91±5
Fenpyroximate	ES+	BEH C 18	10.11	28	422 > 366.2	16	422 > 231.2	24	0.02	129±23	86±20
Fipronil	ES-	BEH C 18	8.25	20	435 > 330.01	35	435 > 250.05	22	0.04	97±19	96±9
fipronil desulfinyl	ES-	BEH C 18	8.1	20	386.7 > 350.8	20	386.7 > 281.8	35	0.04	98±16	96±12
fipronil sulfone	ES-	BEH C 18	8.6	26	450.85 > 414.7	15	453 > 414.3	15	0.04	83±14	97±11
Flonicamid	ES+	BEH C 18	2.23	30	230 > 203	15	230 > 147.9	25	0.08	104±16	89±11
Fluazinam	ES-	BEH C 18	9.24	26	462.6 > 416	18	462.6 > 398.0	14	1	99±13*	88±18
Flubendiamide	ES+	BEH C 18	8.41	14	683.1 > 408.2	13	683.1 > 274.2	35	0.4	123±9	86±18
Fludioxonil	ES-	BEH C 18	7.31	45	247 > 180.1	28	247 > 126.1	35	0.2	74±18	105±21

Compound	Ionization mode	column	RT (min)	Cone Voltage (V)	Transition 1 (m/z)	Collision energy 1 (V)	Transition 2 (m/z)	Collision energy 2 (V)	LOQ (µg/L)	Recovery (%) ± RSD (%)	
										0.5 (or 5 µg/L*)	50 µg/L
Flufenoxuron	ES+	BEH C 18	10.04	25	488.9 > 158.1	18	488.9 > 141	50	0.02	90±21	97±23
Fluopyram	ES+	BEH C 18	7.84	37	397.1 > 173	32	397.1 > 208.1	22	0.02	107±20	92±9
Flutolanil	ES+	BEH C 18	7.5	30	323.8 > 261.8	20	323.8 > 241.8	26	0.02	99±15	95±12
Flutriafol	ES+	BEH C 18	6.35	28	301.99 > 70.07	16	301.99 > 123.05	26	0.02	72±17	90±18
Fluxapyroxad	ES-	BEH C 18	7.54	24	380.2 > 248.1	19	380.2 > 131	26	0.08	79±38	99±16
Forchlorfenuron	ES+	BEH C 18	6.37	20	248>128.9	20	248 > 92.9	42	0.02	96±10	92±7
Forchlorfenuron	ES-	BEH C 18	6.37	20	248> 128.8	15	246 > 126.8	20	0.04	72±10	107±26
Fosthiazate	ES+	BEH C 18	6.01	21	284.32 > 103.7	22	284.32 > 227.9	10	0.02	99±15	91±8
Furalaxyl	ES+	BEH C 18	7.19	26	301.9 > 242.1	16	301.9 > 94.7	11	0.02	102±16	93±7
Hexythiazox	ES+	BEH C 18	9.79	27	353.1 > 168	25	353.1 > 228	25	0.06	120±18	95±19
Imidacloprid	ES+	BEH C 18	3.02	22	256.1 > 175.1	20	256.1 > 209.2	16	0.04	88±19	95±6
Indoxacarb	ES+	BEH C 18	9.13	28	527.9 > 218.1	20	527.9 >149.8	22	0.4	116±18	98±14
Iprovalicarb	ES+	BEH C 18	7.82	20	321.05 > 119.1	20	321.05 > 203.2	10	0.02	90±17	96±14
Isocarbophos	ES+	BEH C 18	6.55	15	273.2 > 231	10	273.2 > 121	25	0.08	104±17	70±11
Lufenuron	ES-	BEH C 18	9.69	18	509 > 339	12	509 > 326	18	0.4	92±16	80±11
malathion dicarboxilic acid	ES-	Hilic	1.72	14	273 > 141	15	273 > 157	18	40	-	95±17
Mandipropamid	ES+	BEH C 18	7.49	22	412.1 > 327.9	15	412.1 > 124.9	35	0.02	99±9	90±6
Mepanipyrim	ES+	BEH C 18	7.71	44	223.8 > 105.9	26	223.8 > 130.9	24	0.04	101±18	94±8
Metalaxyl	ES+	BEH C 18	6.52	20	280.1 > 220.2	13	280.1 > 192.2	17	0.02	100±16	91±7
Methamidophos	ES+	BEH C 18	1.03	22	141.8 > 93.8	14	141.8 > 124.9	13	0.02	58±13	60±10

Compound	Ionization mode	column	RT (min)	Cone Voltage (V)	Transition 1 (m/z)	Collision energy 1 (V)	Transition 2 (m/z)	Collision energy 2 (V)	LOQ (µg/L)	Recovery (%) ± RSD (%)	
										0.5 (or 5 µg/L*)	50 µg/L
Methiocarb	ES+	BEH C 18	7.07	20	225.9 > 168.9	10	225.9 > 121	20	0.02	64±8	79±13
Methiocarb sulfon	ES+	BEH C 18	3.76	25	257.9 > 200.9	9	257.9 > 122	12	0.02	111±16	97±8
Methiocarb sulfoxid	ES+	BEH C 18	3.37	26	242 > 185	14	242 > 122	28	0.02	112±13	102±21
Methomyl	ES+	BEH C 18	2.17	15	162.9 > 87.8	8	162.9 > 105.9	10	0.04	88±25	100±19
Methoxyfenozide	ES+	BEH C 18	7.57	14	369 > 148.7	16	369 > 90.7	44	0.02	90±15	84±11
Metrafenone	ES+	BEH C 18	8.84	21	409.2>209.1	14	411.2 > 209.1	16	0.02	116±10	93±8
Novaluron	ES+	BEH C 18	9.29	25	492.95 > 158	24	492.95 > 140.9	45	0.02	98±17	89±22
Oxadixyl	ES+	BEH C 18	4.87	23	278.9 > 218.9	12	278.9 > 101.8	10	0.02	97±18	94±8
Oxamyl	ES+	BEH C 18	1.99	11	237 > 71.8	13	-	-	0.02	85±12	95±21
				16	-	-	219.89 > 72.0	16			
Oxycarboxin	ES+	BEH C 18	3.84	18	268.15 > 175	16	268.15 > 146.9	22	0.02	102±17	90±7
Paclobutrazole	ES+	BEH C 18	7.45	28	293.95 > 70.1	18	293.95 > 124.9	34	0.04	124±14	97±13
Piperonil-butoxide	ES+	BEH C 18	9.58	38	177 > 119.1	16	177 > 149	15	0.06	91±19	87±13
Pirimicarb	ES+	BEH C 18	6.15	28	239.1 > 72	18	239.1 > 182.1	15	0.02	-	52±8
Pirimicarb-desmethyl	ES+	BEH C 18	4.71	25	225.1 > 71.97	18	225.1 > 168	14	0.02	40±19	38±12
Prochloraz	ES+	BEH C 18	8.81	22	375.94 > 307.88	12	375.94 > 70.15	26	0.02	112±19	91±9
Pyraclostrobine	ES+	BEH C 18	8.7	14	387.87 > 194	14	387.87 > 163.2	22	0.02	118±11	92±10
Pyridaben	ES+	BEH C 18	10.4	19	364.9 > 147	25	364.9 > 309.1	15	0.02	94±18	90±21
Pyrimethanil	ES+	BEH C 18	7.05	42	200.1 > 107	22	200.1 > 82	25	0.02	94±13	92±9
Pyriproxyfen	ES+	BEH C 18	9.64	18	322.05 > 95.9	16	322.05 > 184.9	24	0.02	123±20	100±11

Compound	Ionization mode	column	RT (min)	Cone Voltage (V)	Transition 1 (m/z)	Collision energy 1 (V)	Transition 2 (m/z)	Collision energy 2 (V)	LOQ (µg/L)	Recovery (%) ± RSD (%)	
										0.5 (or 5 µg/L*)	50 µg/L
Quinalphos	ES+	BEH C 18	8.23	18	298.8 > 96.9	30	298.8 > 147	22	0.02	89±20	92±10
Simazin	ES+	BEH C 18	5.19	30	202.08 > 124	18	202.08 > 103.9	24	0.04	84±15	89±6
Spinetoram 1	ES+	BEH C 18	10.89	41	748.4 > 142	40	748.4 > 97.2	90	0.02	104±8	89±7
Spinetoram 2	ES+	BEH C 18	11.2	41	760.3 > 142	40			0.02	96±17	88±6
Spinosad A	ES+	BEH C 18	10.64	43	732.2 > 141.9	31	732.5 > 98	55	0.02	76±19	90±7
Spinosad D	ES+	BEH C 18	10.91	43	746.5 > 142	31	746.5 > 98	55	0.02	88±22	91±9
Spirodiclofen	ES+	BEH C 18	10.16	18	410.69 > 71.1	14	410.69 > 312.9	10	0.02	127±22	80±16
Spirotetramat	ES+	BEH C 18	7.9	23	374.2 > 216.15	34	374.2 > 302.2	18	0.02	96±7	94±10
spirotetramat-enol	ES-	BEH C 18	3.86	45	300.2 > 214	31	300.2 > 145	44	1	111±16*	108±17
Spirotetramat-enol-glucoside	ES+	BEH C 18	3.09	31	464.2 > 302.2	13	464.2 > 216.2	48	0.02	65±9	54±19
Spirotetramat-ketohydroxy	ES+	BEH C 18	6.65	21	318.2 > 300.2	14	318.2 > 268.2	20	0.02	86±14	88±9
Spirotetramat-mono-hydroxy	ES+	BEH C 18	5.27	29	304.2 > 211.2	19	304.2 > 254.9	18	0.02	109±19	90±8
Spiroxamine	ES+	BEH C 18	9.47	30	298.3 > 144.1	20	298.3 > 100.1	32	0.02	91±19	77±12
Tebuconazol	ES+	BEH C 18	8.48	30	309.8 > 70	20	307.8 > 70	20	0.02	95±27	93±7
Tebufenozid	ES+	BEH C 18	8.26	13	353.2 > 133	20	353.2 > 297.2	8	0.02	101±17	98±13
Tebufenpyrad	ES+	BEH C 18	9.5	46	333.9 > 117	36	333.9 > 145.1	27	0.02	114±9	92±16
Tetraconazole	ES+	BEH C 18	8	39	371.5 > 158.9	32	371.5 > 70.1	22	0.04	90±26	93±10
Thiabendazol-5-hydroxy	ES+	BEH C 18	2.99	30	218 > 146.8	35	218 > 80.8	40	0.02	54±18	52±10
Thiabendazole	ES+	BEH C 18	4.42	40	202 > 175.1	25	202 > 131	32	0.02	82±18	72±7

Compound	Ionization mode	column	RT (min)	Cone Voltage (V)	Transition 1 (m/z)	Collision energy 1 (V)	Transition 2 (m/z)	Collision energy 2 (V)	LOQ (µg/L)	Recovery (%) ± RSD (%)	
										0.5 (or 5 µg/L*)	50 µg/L
Thiacloprid	ES+	BEH C 18	3.97	29	252.76 > 125.7	22	252.76 > 186	14	0.02	97±13	93±8
Thiamethoxam	ES+	BEH C 18	2.35	19	292.26 > 211	12	292.26 >180.9	24	0.02	91±15	87±9
Thiodicarb	ES+	BEH C 18	5.98	21	355 > 87.9	17	355 > 107.9	15	0.02	88±20	83±21
Trichlorfon	ES+	BEH C 18	3.35	28	258.6 > 108.9	18	-	-	0.08	96±18	97±9
				25	-	-	256.7 > 108.9	18			
Triforine	ES-	BEH C 18	6.84	19	432.9 > 315	8	432.9 > 313	8	1	87±20*	96±26

3.3. Method application to real urine samples

In order to show the applicability and reliability of the method to real samples, 28 urine samples of healthy subjects were analysed. Different quality assurance criteria have been set to guarantee the quality of delivered results.

A procedural blank and a spiked sample with all pesticides are processed in parallel for each batch of analysis. Control samples recoveries were between 70 and 120%. In real sample, results were regarded as positively identified when the tolerances specified in the EU legislation and other criteria specific to the Laboratory were respected: signal-to-noise ratio (S/N) was > 3; correlation coefficient (r^2) was > 0.995; RSD of the respective ion ratios was equal to or less than 30%; the difference between retention times of calibration curve and real sample was of 0.1 min; and blank sample was free of pesticide residues (not contaminated). Additionally, the efficiency of the extraction procedure was checked by recovery experiments.

A total of 37 pesticide residues and metabolites were identified in the 28 urine samples with average of 4.5 pesticide residues and metabolites per sample and average total concentration of 2.8 µg/L.

Table 29: Pesticide residues and metabolites detected in 28 urine samples, number and percentage of detections and maximum concentration in µg/L

Pesticide residues and metabolites	Number and percentage of detections	Maximum concentration in µg/L
Acetamiprid	2 (7%)	0.03
Acetamiprid-n-desmethyl*	10 (36%)	6.68
Ametoctradin	4 (14%)	0.09
Bitertanol	3 (11%)	0.06
Cyflumetofen	4 (14%)	0.09
Cyproconazol	2 (7%)	0.08
Diethyl phosphate (DEP)*	1 (4%)	11.66
Diflubenzuron	1 (4%)	0.20
Dimetomorph	4 (14%)	0.03
Dodemorphe	1 (4%)	0.07
Fenhexamid	2 (7%)	0.29
Fenoxycarb	4 (14%)	0.04
Fenpyroximate	5 (18%)	0.08
Fipronil	4 (14%)	0.15
Fipronil desulfinyl*	5 (18%)	0.08

Pesticide residues and metabolites	Number and percentage of detections	Maximum concentration in µg/L
Fipronil sulfone*	12 (43%)	0.33
Fludioxonil	1 (4%)	0.15
Flutolanil	5 (18%)	0.14
Hexythiazox	1 (4%)	0.15
Iprovalicarb	1 (4%)	0.03
Mandipropamid	2 (7%)	0.09
Metalaxyl	1 (4%)	0.05
Methoxyfenozide	2 (7%)	0.05
Novaluron	1 (4%)	0.01
Oxamyl	4 (14%)	0.20
Paclobutrazole	1 (4%)	0.14
Pirimicarb	6 (21%)	9.60
Pirimicarb-desmethyl*	3 (11%)	0.36
Pyraclostrobine	19 (68%)	0.27
Pyriproxyfen	2 (7%)	0.02
Spirotetramat	1 (4%)	0.05
Spirotetramat-enol*	3 (11%)	10.99
Spirotetramat-ketohydroxy*	1 (4%)	1.65
Tebuconazol	3 (11%)	0.04
Tebufenpyrad	2 (7%)	0.07
Thiabendazole	1 (4%)	0.17
Thiacloprid	2 (7%)	0.03

*: metabolites of pesticide residues

4. Conclusion

The study reported herein presents a reliable and cost effective multi residue method for the determination of a wide range of different pesticides and metabolites in urine. This method is either used in order to evaluate the total exposure of florists or other people in contact of pesticides directly or indirectly such as operators, workers, bystanders or residents. This powerful multiclass method has many benefits like fit-for-purpose, high recoveries, good reproducibility, and wide analytical scope), but also, in terms of practical aspects (low cost, labor, waste, glassware and high sample throughput). The procedure includes ethyl acetate extraction and extracts measurement using a UHPLC-ESI-MS/MS system, consisting of an ACQUITY UPLC coupled to Quattro Premier MS (Waters) and

equipped with 3 different columns: an ACQUITY™ BEH C18 column (1.7 µm, 2.1 x 100 mm), an ACQUITY™ BEH phenyl column (1.7 µm; 2.1 × 100 mm) and a SeQuant® ZIC®-HILIC HPLC column (3.7 µm, 2.1 × 100 mm). A total run time of about 50 min (4 runs of 10/15 min in both positive and/or negative ionisation modes) was sufficient to detect all pesticides and their metabolites with a good retention and sensitivity. It has been proved that the method complies with the validation requirements: it was successfully validated according to the SANTE 11813/2017guidelines. Analytes recovery was typically in the 80–120% range, with reproducibility typically <20% for the majority of pesticide residues and metabolites. The scope of this analytical method can be easily expanded to other pesticides.

Disclosure statement

No potential conflict of interest was reported by the authors.

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II. Biological monitoring of exposure to pesticide residues among Belgian florists

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Abstract

Many pesticides applied in cut flowers can be readily absorbed through the skin of florists during preparing bouquets and handling contaminated flowers. A study was conducted among volunteer Belgian florists in order to assess their total exposure by measuring concentrations of pesticides (parent compounds and metabolites) in their urines. A total of 42 urine samples (24-hour urines) were collected from florists during their professional activities, on the three most important commercial periods. The concentrations of pesticide residues and metabolites in urine samples were analysed with a multi-residue liquid chromatography tandem mass spectrometry method, after an ethyl acetate extraction. The results are compared with those of a control group of 42 subjects not occupationally exposed to pesticides, collected in the same periods. A total of 70 residues (56 pesticides and 14 metabolites) were identified, with an average of about 8 pesticide residues and metabolites per florist's urine sample and an average total concentration per sample of 4.3 µg/g creatinine, ranging from 0.2 to 67 µg/g creatinine. Significantly higher urinary excretion of metabolites (t-test) was found in florists than in control group. These results demonstrate that Belgian florists are exposed daily to pesticide residues with a potential effect on their health.

1. Introduction

As flowers are very susceptible to a large number of diseases and various pests (Pirone, 1978), pesticides are used worldwide in floriculture to avoid reaching qualitatively damaging levels and economic losses (Bethke &Cloyd, 2009). The lack of maximum residue limits (MRL) for flowers and the weakness of local regulations often explain the intensive use of pesticides in cut flowers. A vast majority of producers consider their use as a part of their usual practices to improve yield and quality (Bethke & Cloyd, 2009). Over the last four decades, a study conducted in Miami has documented the occurrence of sporadic but high-levels of pesticide concentrations on flowers imported into the United States from South and Central

America (Morse *et al.,* 1979). Another recent survey in Belgium has also reported a rather high contamination of cut flowers by a wide range of various pesticide residues whatever their origin (Toumi *et al.,* 2016a b).

Therefore, florists who handle daily cut flowers and prepare bouquets could be more severely exposed to different pesticide residues than other workers who enter an area that has been treated previously with pesticides. Florists should cope with a unique risk situation where they are exposed almost every day to many different residues during their tasks (Toumi *et al.,* 2016a, b; Toumi *et al.,* 2017, b). This evidence of a higher risk level was strengthened by a recent assessment among Belgian florists. For several active substances commonly detected on flowers (e.g. clofentezine, famoxadone, methiocarb and pyridaben) the systemic exposure (SE) values estimated through model calculations were found to exceed the Acceptable Operator Exposure Level (AOEL) (Toumi *et al.,* 2017a).

Repeated exposures to pesticides have been associated with a variety of health effects among workers harvesting crops or working in the field of floriculture. Health problems includes cancer (Fleming *et al.,* 1999), genetic damage (Munnia *et al.,* 1999; Bolognesi, 2003), effects related to endocrine disruption (Blanco-Muñozet *et al.,* 2016), reproductive problems (abortion, prematurity and congenital malformations) (Restrepo *et al.,* 1990a) and other various detrimental health disorders such as weakness and fatigue, muscular pain, chills and fever, blurred vision, dizziness and headache (Lu, 2005; Defar & Ali, 2013).

Lipophilic pesticides are rapidly absorbed through the skin, metabolized into more polar products and excreted primarily in urine. Therefore, urine is one of the preferable fluid used for biological monitoring (i.e. biomonitoring). It is not an invasive method; it is easy to collect and generally abundant Kapka-Skrzypczak *et al.,* 2011). Biomonitoring became an essential component of any comprehensive assessment of exposure (Cocker *et al.,* 2002) as it helps to determine the systemic exposure to pesticide residues. Biomonitoring data incorporates exposures from all routes for florists: principally dermal through handling of contaminated flowers and greens, inhalation of volatile active substances, secondarily oral through contact of contaminated hands to mouth. Many scientific papers have reported the determination of parent compounds or metabolites in urine of workers. Duncan and Griffith (1985) assessed the exposure of citrus fieldworkers to organophosphates insecticides analyzing their metabolites in urine. Brouwer *et al.* (1993) studied the relationship between respiratory and dermal exposure levels and the total amount of the metabolite 2-isopropoxyphenol in urines from carnation harvesters. Biomonitoring was also used to monitor exposure of workers in a peach orchard after spraying (Aprea *et al.,* 1994), during manual leaf thinning (Aprea *et al.,* 1997), apple pruning (Simcox *et al.,* 1999), strawberries (Krieger & Dinoff, 2000; Salvatore *et al.,* 2008; Sankaran *et al.,* 2015) or corn harvesting (Ferland *et al.,* 2015).. The urinary excretion of alkylphosphates by workers during reentry in ornamental plant greenhouses treated with omethoate, fenitrothion, and tolclofos-methyl was determined by Aprea *et al.* (2001, 2005).

In order to better evaluate the risk level for the florists' health, a biomonitoring study was deemed necessary to measure the real total body burden of pesticides

picked from cut flowers by the analysis of residues and metabolites in urine. Their concentrations were determined for three decisive periods, both in florists and control groups and over a single 24 hours sampling period, to establish the link between the estimated potential dermal exposure and the real systemic exposure.

2. Materials and methods

2.1. Study Participants

A convenience study was conducted among fourteen volunteer florists (7 men and 7 women) to evaluate their total exposure through analysis of selected pesticide residues and related specific metabolites in their urines in comparison with non-professional Belgian residents. The 14 selected florists represent 56% of the total number of florists surveyed at the beginning of the florists' risk assessment study in Belgium (Toumi *et al.,* 2016a). As such, the worst case are recommended and normally used in risk assessment. The aim of these experiments is to evaluate florist's exposure under worst-case scenarios. As a result, this study was conducted in Namur Province (Belgium) during the most intensive working periods: Valentine's Day (February, 14th), Mother's Day (May, 14th) and All Saints' Day (November, 1st)[6]. It is known that 60% of florists spend more than 6 hours at work per day to prepare bouquets during these 3 special occasions (Toumi *et al.,* 2016a). Florists were asked to perform their normal daily activities during the study. During the same periods, control groups of 14 volunteers (7 men and 7 women) were also asked to participate and to collect their urines.

2.2. Questionnaire

A two-page questionnaire was addressed to participants at the time of sampling to have a better understanding of the sources of exposure and to provide additional information about their habits. The questionnaire includes the preference for organic food consumption (never, rarely, sometimes, often or always), the frequency of shopping at specific stores (because each retailer has its own requirements regarding pesticide residues), the presence of companion animals and the use of plant protection products at home. Additionally, florists were asked about the protective equipment they usually wear during handling flowers and preparing bouquets. All volunteers were aware of the purpose of the research study and signed an informed consent form.

[6] Ces trois périodes sont considérées par les professionnels interrogés comme étant parmi les plus intenses en activité, même si fondamentalement ce sont les mêmes types de fleurs coupées (au contraire des potées) qui servent à la préparation des bouquets et les mêmes manipulations qui sont effectuées toute l'année.

2.3. Twenty-four hour's urine sample collection

Spot urine samples were collected from each participant. A total of 84[7] urine samples were collected from all participants within 3 days. A 24 hours urine collection was done by using special containers that could cover a full 24 hours period (3 liters for men and 3 separate containers of 1 liter for women). The entire 24 hours urine volume was well mixed and the total volume was recorded. Six sub-samples were then transferred in 10 ml polypropylene cryovials and stored in freezers at -20°C until analysis.

2.4. Analysis of Samples

2.4.1. Choice of active substances

The selection of pesticides analyzed in the urines of occupational and non-occupational groups was made based on the results obtained in previous studies conducted in Belgium. A total of 107 active substances were detected from 90 bouquets of the most commonly sold cut flowers in Belgium (Toumi *et al.,* 2016a) and a total of 111 active substances were detected in glove samples worn by florists during their professional activities (Toumi *et al.,* 2017a). A preliminary bibliography search was carried out to identify each active substance and its metabolites.

2.4.2. LCMS-MS analysis

The residual pesticide excreted on urine samples were identified and analyzed according to a validated internal procedure in a Belgian laboratory[8] accredited ISO/IEC 17025:2017 for chemical residues and contaminants. Stability and variability tests were passed according to the quality criteria of the SANTE/11813/2017 document (European Commission, 2017). Solutions were prepared according to an accredited internal procedure. All the solutions (individual, intermediate mix and spiking) were stocked and aliquoted at −20 °C. Blank urine (not spiked) and a spiked sample were analyzed in each of 5 batches to ensure reliability of results. Samples were analysed with a multi-residue method, which will detect approximately 110 different active substances in a single analysis. LC analysis was performed with an UPLC™ (Waters, Milford, MA) equipped with a mass spectrometer Quattro Premier ™ (Waters). Ten milliliter of a urine sample was pipetted into a 50 ml Falcon® tube. Ten ml of ethyl acetate, 2 g of NaCl and 1 ml of formic acid were added. The sample was vortexed 15 min and then centrifuged for 5 min (3,900 rpm). The ethyl acetate layer was removed by pipetting and the extraction procedure was repeated with 5 ml of ethyl acetate. The first and second ethyl acetate layers were combined and 2 g of Na_2SO_4 was added and evaporated using a gentle stream of nitrogen until 10 ml. Five milliliters of the extract was pipetted into another Falcon® tube and evaporated using a gentle stream of nitrogen below 100 µl. Three hundred fifty µl of methanol and 50 µl of oxfendazole (internal

[7] 84 échantillons ont été collectés : 3 périodes x 28 (2 groupes : 14 fleuristes + 14 non professionnels du groupe de référence)

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standard) were added. Finally the volume was adjusted to 1 ml with the mobile phase A (water/methanol 90/10 v/v) and gently shacked. 500 µl of extract was transferred in a filter vial and further analysed.

After a common extraction step, three different columns were used according to the properties of the residues to separate: an ACQUITYT^{M} BEH C18 column (1.7 µm, 2.1 × 100 mm), an ACQUITY T^{M} BEH phenyl column (Phenyl 1.7 µm, 2.1 mm X 100 mm) and a SeQuant® ZIC®-HILIC HPLC column (3.7 µm, 2.1 × 100 mm). For the C18 and phenyl columns, the mobile phases were composed of water/methanol (90/10 v/v) (phase A) and methanol/water (90/10 v/v) (phase B) both containing 5 mM of ammonium acetate. For the HILIC column, the mobile phase A was 100% water with 5 mM of ammonium formate and 0.1% formic acid, and the mobile phase B was 100% acetonitrile with 0.1% formic acid. For the vast majority of the pesticide residues, the limit of determination (LOD) is equal to 0.01 µg/L. Only samples with detectable (≥ LOD) levels of residues were considered when discussing the data.

2.5. Creatinine adjustment

In order to conduct the best adjustment for the concentrations of pesticide residues in urines, the most widely used method is the creatinine adjustment: the pesticide residue concentration (in µg/liter urine) is divided by the creatinine concentration in urine (in g/liter). The results are then reported as weight of analyte per gram of creatinine (µg analyte per gram of creatinine) (Barr *et al.,* 2005; Barr *et al.,* 2006).

Creatinine concentrations in urine were determined according to a recent method (Fraselle *et al.,* 2015). Briefly, after homogenization, urine samples were diluted 104 times in four successive steps with 0.1% NH_4OH in water. The final dilution was done directly in a glass vial by mixing 100 µl of the third dilution, 100 µl of creatinine-d3 at 1 µg/ml and 800 µl of 0.1% NH_4OH in water. LC analysis was performed with the same equipment using an ACQUITY TM UPLC HSS T3 (2.1 x 100 mm, 1.8 µm) analytical column.

2.6. Statistical analysis

The statistical analyses were carried out using Minitab 18 Statistical Software (Minitab Inc., State college, PA, USA). Comparisons between periods (Valentine's Day, Mother's Day and All Saints' Day) and groups (florists and control group) were performed with Student's t-test and correlations between results from bio-monitoring and organic food consumption were tested using Spearman correlation coefficient. A P-value below 0.05 was considered as statistically significant.

3. Results and discussion

3.1. Selected characteristics of participants

Table 30: Characteristics reported by florists and control group during test participations

	Florists	Control group
Use of plant protection products		
No	100%	100%
Companion animals		
No	29%	81%
Use of personal protective equipment during professional tasks		
Apron	21%	-
Gloves	-	
Special clothes	-	
Organic food consumption		
Never	14%	10%
Rarely	36%	29%
Occasionally	36%	50%
Often	7%	10%
Always	7%	2%
Most frequented store		
Aldi	-	19%
Carrefour	21%	2%
Clemenceau market	-	2%
Colruyt	36%	57%
Delhaize	14%	5%
Lidl	-	10%
Intermarché	14%	-
Match	7%	-
Organic stores	7%	5%

All study participants were Belgian residents. For each sampling period, the professional and non-professional populations were heterogeneous (50% males and 50% females). None of the participants reported working in areas (e.g., farmers: workers and operators etc.) where they could be exposed to pesticides or to use plant protection products during their participation to the survey. Considering the presence of companion animals, 29% of the florists and 81% of the people of the control group reported not to have domesticated animals.

Regarding food consumption and the presence of residues, 36% of the florists consume occasionally organic food, 36% of them consume it rarely, 14% never consume it and 7% consume only organic food. Fifty % of the control group consumes occasionally organic food. All stores and foodstuffs sold in Belgium are controlled by Afsca (Federal Agency for the Safety of the Food Chain) for the residue levels and respect of EU MRL and for many of them an internal and external quality audit, by an independent partner, are also conducted. With regard to the most

frequent attendance at stores, Colruyt is the most frequented store by the florists (36%) and the control group (57%), followed by Carrefour (21%) for the florists and Aldi (19%) for the control group. Nevertheless the attendance frequency may be mainly affected by the proximity of the retailer and not necessary by its reputation.

With regard to the use of personal protective equipment, 100% of the florists wear no special clothing and gloves. Only 21% of the surveyed florists use occasionally apron when preparing bouquets and handling flowers.

3.2. Pesticide residues in samples of urines collected among florists

All samples of florists' urine appeared to be contaminated by pesticide residues (Table 31). The total pesticide residues and metabolites concentration varies from less than 1 µg/g creatinine (8 of 42 florist or 19% samples) to more than 5 µg/g creatinine (7 of 42 samples or 17% samples), but the majority (64% samples) had a total pesticide residues and metabolites concentration between 1 and 5 µg/g creatinine. Nevertheless, significant higher concentrations could be observed, particularly during the All Saints' Day period where a maximum cumulated concentration of residues reached 67 µg/g creatinine. It should be noted that these values are almost similar to the range of concentrations usually observed in urine of farm workers who enter in contact with freshly treated ornamental plants during re-entry into greenhouses: a study conducted in Italy reported concentration levels of chlorothalonil in urine of plant ornamental workers between 0.24 µg/g creatinine and 6.34 µg/g creatinine (Aprea *et al.,* 2002). Another study conducted in Ecaduor among floriculture workers, showed a median level of ethylenethiourea of 6.2 µg/g creatinine ranging from 1.5 to 26.5 µg/g creatinine (Colosio *et al.,* 2003). In Japan, Ueyama *et al.* (2012) observed in the urine of apple farmers geometric mean concentration values of 33.1 and 10.8 µg/g creatinine for dimethylphosphate (DMP), 10.1 and 5.8 µg/g creatinine for dimethylthiophosphate (DMTP), 4.2 and 4.7 µg/g creatinine for diethylphosphate (DEP) and 1.6 and 0.8 µg/g creatinine for diethylthiophosphate (DETP) in summer and winter, respectively. Another study conducted in Italy among 12 vine applicators and 1 re-entry workers reported ethylenethiourea excretion of 12.5 µg/g creatinine (Colosio *et al.,* 2002).

The concentrations measured in our study are negligible compared to those measured in strawberry harvesters for which the median malathion dicarboxylic acid levels in urine was 131.2 µg/g creatinine (Bradman *et al.,* 2009). These levels are higher, but the source of exposure (harvesting) and the frequency of exposure (daily tasks) can also explain the difference. For example, during re-entry in chrysanthemum greenhouses, the biomonitoring of four workers revealed no detectable residues (Archibald *et al.,* 1994b). Nevertheless, the comparison between those previous studies and our results should be considered with caution as local situations are different and practices have evolved with time (Toumi *et al.,* 2017a).

A total of 70 residues (56 pesticides and 14 metabolites) were identified (Table 32), with an average of about 8 pesticide residues and metabolites per sample. Considering all pesticide residue concentrations in florists'urine samples (Table 31),

the average residue concentration reaches 4.3 µg/g creatinine with a median of 1.7 µg/g creatinine.

Table 31: Total number of pesticide residues and metabolites detected (N) and total pesticide residue and metabolite concentrations (C) in the 42 samples of florists'urines adjusted with creatinine (µg/g creatinine) for the three most intensive working periods[9]

	Valentine's Day		**Mother 's Day**		**All Saints' Day**	
Florists	**[C]**	**N**	**[C]**	**N**	**[C]**	**N**
1	3.99	10	2.50	9	1.12	7
2	0.38	5	1.35	11	6.15	17
3	5.83	12	4.91	6	7.81	12
4	1.93	8	2.72	10	1.09	7
5	1.09	8	1.27	5	1.29	6
6	2.75	8	3.00	15	1.54	10
7	4.86	7	2.55	8	2.24	11
8	1.20	2	6.41	7	15.48	14
9	1.41	5	7.34	10	67.21	10
10	1.22	6	0.75	6	0.94	7
11	3.54	4	1.15	6	1.69	12
12	0.67	6	0.74	9	1.62	8
13	3.30	9	0.22	3	1.83	5
14	0.89	7	0.65	5	1.29	9
Mean	**2.36**	**7**	**2.54**	**8**	**7.95**	**10**
Median	**1.67**	**7**	**1.93**	**8**	**1.65**	**10**
Range	**0.38-5.83**	**2-12**	**0.22-7.34**	**3-15**	**0.94-67.21**	**5-17**

A great variety of pesticide residues were observed in the urine samples obtained from the group of florists. The most contaminated urine sample contained 12, 15 and 17 pesticide residues and metabolites, collected in Valentine's Day, Mother's Day and All Saints' Day, respectively (Figure 11).

Urine of florists appear to be slightly more contaminated by pesticide residues during All Saints' Day compared to other periods, with an average number of 10 pesticide residues and metabolites per sample. A statistical analysis confirmed that these urines are significantly more contaminated than samples collected during

[9] Les données relatives aux groupes de référence pour les 3 périodes sont reprises à l'Annexe 3.

Valentine's Day (P=0.024). However, no significant differences appear between samples collected in Valentine's Day and Mother's Day (P= 0.394) or between Mother's Day and All Saints' Day results (P= 0.154).

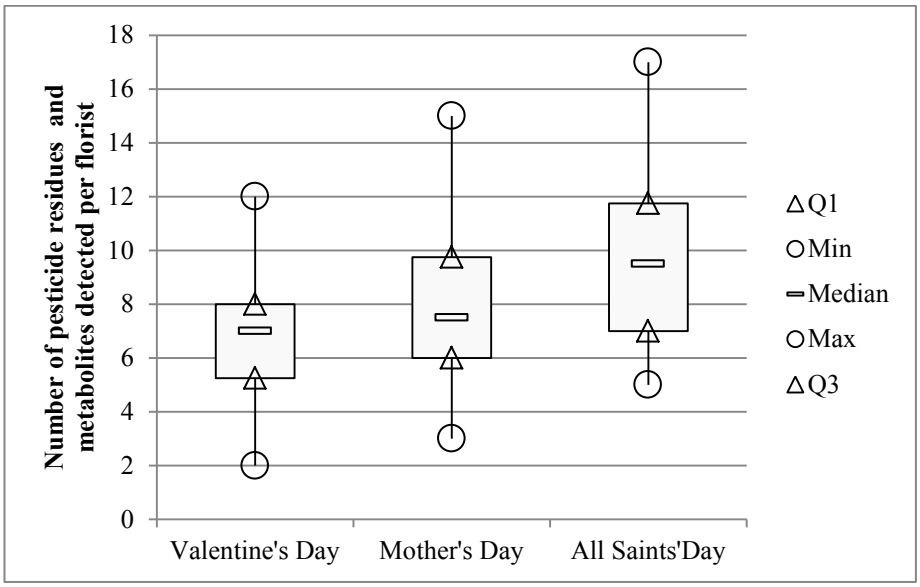

Figure 11: Box plots of pesticide residues and metabolites excreted per florist showing the median, the range (min-max), the first quartile (Q1) and the third quartile (Q3) range of the data for the three most intensive working periods

3.3. Pesticide residues hazard characterisation

Table 32 lists the 70 residues (56 pesticides and 14 metabolites) found (concentration ≥ LOD) in the 42 urine samples and their number of detection (N), their chemical class, biological activity (BA), average and range of concentrations (µg/L) in the samples and their toxicological properties (CLP, Classification according the EU Pesticides database).

Table 32: Alphabetic classification of all pesticide residues and metabolites detected (LOD= 0.01 µg/L) in the 42 urine samples obtained from florists with their number of detection (N), their chemical class, biological activity (BA), average and range of concentrations (µg/L) in the samples and their toxicological properties (CLP (Classification, Labelling and Packaging), classification according the EU Pesticides database)

Pesticide residues and metabolites	N (in 42 samples)	BA	Chemical class**	Mean in µg/L (range)			CLP classification
				Valentine's Day	Mother's Day	All Saints' Day	
2CTCA* (M)	4	I	neonicotinoid	0.55 [LOD-1.12]	-	-	-
3-Hydroxy-carbofuran (M)	4	I	carbamate	0.08 [LOD-0.09]	0.01 [LOD-0.01]	0.03 [LOD-0.03]	-
Acetamiprid	4	I	neonicotinoid	-	0.03 [LOD-0.03]	0.02 [LOD-0.02]	H302
Acetamiprid-n-desmethyl (M)	14	I	neonicotinoid	0.60 [LOD-0.98]	0.40 [LOD-1.20]	0.19 [LOD-0.34]	-
Ametoctradin	5	F	triazolopyrimidine	0.05 [LOD-0.07]	0.03 [LOD-0.03]	0.17 [LOD-0.17]	-
Azoxystrobin	12	F	strobin	-	0.05 [LOD-0.11]	-	H331
Boscalid*	6	F	anilide	0.82 [LOD-0.82]	0.49 [LOD-0.81]	0.58 [LOD-0.68]	-
Bupirimate	6	F	pyrimidine	0.01 [LOD-0.01]	-	0.05 [LOD-0.11]	H317, H351
Buprofezin	5	I	-	-	0.06 [LOD-0.06]	0.09 [LOD-0.14]	-
Carbendazim (M)	4	F	benzimidazole	-	0.05 [LOD-0.05]	0.04 [LOD-0.08]	H340, H360FD
Carbofuran	2	I	carbamate	0.04 [LOD-0.04]	-	-	H300, H330
Chlorantraniliprole	1	I	anthranilic diamide	-	-	0.01 [LOD-0.01]	-
Clofentezine	23	I	tetrazine	0.33 [LOD-0.91]	0.27 [LOD-1.03]	0.10 [LOD-0.16]	-
Cyflumetofen	1	I	-	-	-	0.01 [LOD-0.01]	-
Cyproconazole	12	F	azole	0.06 [LOD-0.13]	0.02 [LOD-0.03]	-	H301, H360D, H373

Cyprodinil	5	F	pyrimidine	0.32 [LOD-0.51]	0.27 [LOD-0.27]	-	H317
DETP* (M)	2	I	organophophate	2.54 [LOD-3.21]	-	-	
Difenoconazole	3	F	azole	-	-	0.04 [LOD-0.07]	-
Diflubenzuron*	6	I	benzoylurea	-	0.27 [LOD-0.27]	0.59 [LOD-1.20]	-
Dimethomorph	10	F	morpholine	0.03 [LOD-0.05]	-	0.06 [LOD-0.14]	-
Dinotefuran	1	I	neonicotinoid	-	6.35 [LOD-6.35]	-	-
DMP* (M)	1	I	organophosphate	-	-	53.05 [LOD-53.05]	
Famoxadone*	1	F	dicarboximide	-	-	0.80 [LOD-0.80]	H373
Fenamiphos sulfone (M)	3	I	organophosphate	-	0.02 [LOD-0.02]	0.02 [LOD-0.02]	-
Fenhexamid	10	F	anilide	1.97 [LOD-5.45]	0.46 [LOD-0.66]	0.10 [LOD-0.13]	-
Fenoxycarb	2	I	carbamate	-	0.04 [LOD-0.04]	0.01 [LOD-0.01]	H351
Fenpropidin*	4	F	-	0.32 [LOD-0.45]	-	0.10 [LOD-0.12]	-
Fenpyroximate	6	I	pyrazole	0.10 [LOD-0.12]	-	0.17 [LOD-0.17]	H301, H317, H330
Fipronil*	8	I	pyrazole	-	-	0.81 [LOD-3.14]	H301, H311, H331, H372
Fipronil sulfone* (M)	1	I	pyrazole	-	0.14 [LOD-0.14]	-	-
Flonicamid*	1	I	-	0.62 [LOD-0.62]	-	-	H302
Flubendiamide*	1	I	anthranilic diamide	-	-	1.70 [LOD-1.70]	-
Flufenoxuron	7	I	benzoylurea	0.14 [LOD-0.14]	-	0.43 [LOD-1.51]	H362
Fluopyram	3	F	pyridine	-	-	0.05 [LOD-0.06]	-
Flutolanil	2	F	anilide	0.01 [LOD-0.01]	-	0.49 [LOD-0.49]	-
Flutriafol	2	F	azole	-	0.47 [LOD-0.76]	-	-
Fosthiazate	6	I	organophosphate	0.08 [LOD-0.08]	-	0.01 [LOD-0.01]	H301, H312,

							H317, H331
Furalaxyl	3	F	xylylalanine	2.05 [LOD-2.05]	4.20 [LOD-4.20]	1.09 [LOD-1.09]	H302
Hexythiazox*	2	I	-	-	0.68 [LOD-0.68]	0.20 [LOD-0.20]	-
Imidacloprid*	1	I	neonicotinoid	-	-	0.58 [LOD-0.58]	H302
Indoxacarb*	4	I	-	-	-	0.31 [LOD-0.44]	H301, H317, H332, H372
Isocarbophos*	2	I	organophosphate	-	0.09 [LOD-0.11]	-	-
Mandipropamid	4	F	-	-	0.03 [LOD-0.03]	0.09 [LOD-0.17]	-
Metalaxyl and/or Metalaxyl-M	2	F	xylylalanine	0.26 [LOD-0.40]	-	-	H302, H317, H318
Methamidophos	3	I	organophosphate	0.15 [LOD-0.15]	0.11 [LOD-0.12]	-	H300, H311, H330
Methiocarb	2	I	carbamate	-	0.03 [LOD-0.04]	-	H301
Methiocarb sulfon (M)	1	I	carbamate	-	-	0.01 [LOD-0.01]	-
Methiocarb sulfoxid (M)	4	I	carbamate	0.08 [LOD-0.08]	0.03 [LOD-0.03]	0.09 [LOD-0.09]	-
Methomyl*	2	I	carbamate	-	-	0.02 [LOD-0.02]	H300
Methoxyfenozide	2	F	diacylhydrazine	-	0.10 [LOD-0.10]	-	-
Metrafenone	9	F	-	0.05 [LOD-0.06]	0.05 [LOD-0.05]	0.02 [LOD-0.04]	-
Novaluron	7	I	benzoylurea	0.11 [LOD-0.18]	0.12 [LOD-0.12]	0.03 [LOD-0.04]	-
Oxamyl	7	I	carbamate	0.68 [LOD-1.02]	1.27 [LOD-1.70]	0.05 [LOD-0.05]	H300, H312, H330
Piperonil-butoxide*	4	S	-	0.07 [LOD-0.07]	0.22 [LOD-0.28]	0.60 [LOD-0.60]	-
Pirimicarb	27	I	carbamate	0.46 [LOD-0.46]	0.68 [LOD-2.11]	0.40 [LOD-3.79]	H301, H317, H331, H351
Pirimicarb-desmethyl (M)	11	I	carbamate	0.10 [LOD-0.21]	0.08 [LOD-0.16]	0.18 [LOD-0.21]	-
Prochloraz	8	F	azole	0.02 [LOD-0.04]	0.01 [LOD-0.01]	-	H302

Pyraclostrobin	4	F	strobin	0.03 [LOD-0.03]	0.05 [LOD-0.05]	-	H315, H331
Pyridaben	5	I	-	0.19 [LOD-0.19]	0.37 [LOD-0.43]	-	H301, H331
Pyrimethanil	3	F	pyrimidine	0.24 [LOD-0.25]	0.52 [LOD-0.52]	-	-
Quinalphos	1	I	organophosphate	0.20 [LOD-0.20]	-	-	H301, H312
Spinosad sum (A+D)	6	I	spinosyn	-	0.02 [LOD-0.02]	0.03 [LOD-0.04]	-
Spirodiclofen	1	I	keto-enol	0.39 [LOD-0.39]	-	-	-
Spirotetramat	2	I	keto-enol	0.16 [LOD-0.16]	0.12 [LOD-0.12]	-	H317, H319, H335, H361fd
Spirotetramat-enol * (M)	1	I	keto-enol	-	1.37 [LOD-1.37]	-	-
Spirotetramat-enol-glucoside (M)	2	I	keto-enol	-	0.09 [LOD-0.09]	0.26 [LOD-0.26]	-
Spiroxamine	5	F	-	-	0.02 [LOD-0.04]	-	H302, H312, H315, H317, H332, H361d, H373
TCPy* (M)	12	I	organophosphate	-	-	1.13 [LOD-4.42]	-
Tebufenpyrad	3	I	pyrazole	0.07 [LOD-0.07]	0.07 [LOD-0.08]	-	H301, H317, H332, H373
Thiabendazole	4	F	benzimidazole	0.07 [LOD-0.08]	0.10 [LOD-0.17]	-	-

*0.01< LOD ≤1 µg/L
**: according to the PAN (Pesticide Action Network) Pesticides Database

I: insecticide (including acaricide, molluscicide and nematicide, etc); F: fungicide; S: synergist ; M: metabolite (BA and chemical class were classified according to the parent compound, if necessary)
2CTCA : 2-Chloro-1,3-thiazole-5-carboxylic acid : urinary metabolite of thiamethoxam
TCPy : 3,5,6-trichoro-2-pyridinol: urinary metabolite of both chlorpyrifos and chlorpyrifos-methyl
DMP: Dimethylphosphate : urinary metabolite of organophosphates
DETP: Diethylthiophosphate : urinary metabolite of organophosphates

H300: Fatal if swallowed; H301: Toxic if swallowed; H302: Harmful if swallowed; H311: Toxic in contact with skin; H312: Harmful in contact with skin; H315: Causes skin irritation; H317: May cause an allergic skin reaction; H318: Causes serious eye damage; H330: Fatal if inhaled; H331: Toxic if inhaled, H332: Harmful if inhaled; H335: May cause respiratory irritation; H340: May cause genetic defects; H341: Suspected of causing genetic defects; H351: Suspected of causing cancer; H360FD: May damage fertility. May damage the unborn child; H361d: suspected of damaging the unborn child; H361fd: suspected of damaging fertility. Suspected of damaging the unborn child; H362: May cause harm to breast-fed children; H372: Causes damage to organs through prolonged or repeated exposure; H373: May cause damage to organs through prolonged or repeated exposure

Sixty three percent of the detected pesticide residues (active substances and metabolites) are insecticides and 36% are fungicides and only piperonil butoxide is a synergist. Of the 70 detected residues (56 pesticides and 14 metabolites), most of the pesticides belong to carbamates, organophosphates or neonicotinoids. Pesticides from these families are known for their acute toxicity, with an action on the nervous system. According to the CLP classification (Table 32), the majority of the detected active substances in urine samples of florists are potentially hazardous with acute and/or chronic effects. The analysis of urines confirmed that Belgian florists are exposed to both a very high number of toxic chemicals which are no more approved in Europe, USA and many other countries, and to rather high concentration levels (Toumi *et al.,* 2016a, b; Toumi *et al.,* 2017a, b).

Eight insecticides and metabolites (3-hydroxy-carbofuran, acetamiprid-n-desmethyl, clofentezine, methiocarb sulfoxid, novaluron, oxamyl, pirimicarb and pirimicarb-desmethyl), five fungicides (ametoctradin, boscalid, fenhexamid, furulaxyl and metrafenone) and one synergist (piperonil-butoxide) have been detected in urine samples of florists during the three periods (Table 32).

DMP, which is a metabolite of phosphamidon, mevinphos, dicrotophos, monocrotophos, dichlorvos, and trichlorfon (Tarbah *et al.,* 2004; Ghosh *et al.,* 2015), has the highest maximum concentrations (53 µg/L) for all periods. Regarding the three periods, the highest average concentrations were found for fenhexamid, furalaxyl and DETP (1.97, 2.05 and 2.54 µg/L, respectively) in Valentine's Day, for oxamyl, spirotetramat-enol, furulaxyl and dinotefuran (1.27, 1.37, 4.20 and 6.35 µg/L, respectively) in Mother's day, for furalaxyl, TCPy, flubendiamide and DMP (1.09, 1.13, 1.70 and 53.05 µg/L, respectively) in All Saints' Day. These pesticide residues and metabolites presented average concentrations of 1 µg/L and more (Table 32). These concentration levels are in accordance with Aprea *et al.* (2002) which conducted a study among ornamental plants workers during re-entry and the concentration of chlorothalonil in urines ranged from 0.45 to 8.30 µg/L.

The lack of alternative pest control methods, the commercial value of flowers which must be perfect at harvest, and the absence of maximum residue limits (Toumi *et al.,* 2016a, b; Toumi *et al.,* 2017a, b) can explain the large number of different pesticides used by growers to control pests and diseases. Moreover, the analyses of urine samples of florists helped to reveal the presence of active substances not allowed for use in the EU (3-hydroxy-carbofuran, carbendazim, carbofuran, dinotefuran, fipronil, fipronil sulfone, flufenoxuron, furalaxyl, isocarbophos, methamidophos, novaluron and quinalphos)[10]. This is a consequence of importation of flowers into Belgium from all over the world (Rikken, 2010; Val'hor, 2013).

[10] Les traces de fipronil et de ses divers métabolites ont pu se trouver accidentellement dans les urines suite à la contamination des oeufs d'origine frauduleuse en 2017. Une autre origine possible est le contact avec certains animaux traités par le fipronil.

3.4. Source of pesticide residues observed in the urine of florists

Measurement of pesticide residues in urine is used to assess actual exposure of workers and to provide an integrated assessment of exposure through all routes-of-entry (Ferland *et al.,* 2015) (non dietary and dietary).

Workers in contact with treated crops can be contaminated by pesticides remaining on the crop after application (Krol *et al.,* 2005; Dong & Beauvais, 2013; Toumi *et al.,* 2018). Therefore, the mixture of pesticide residues observed in urine samples of florists resulted from a dermal exposure after contact with flowers heavily treated. The linear relationship between the dermal exposure of workers and the urinary pesticide excretion has been demonstrated by several studies (Ware *et al.,* 1975; Brouwer *et al.,* 1993; Aprea *et al.,* 1994, 1997, 1999, 2005; McCurdy *et al.,* 1994; Bradman *et al.,* 2009) and our results also confirmed that residues (parent coumponds and metabolites) found in urine samples and active substances sprayed on cut flowers are strictly the same. In a recent study, Toumi *et al.* (2017a) showed that azoxystrobin, boscalid, clofentezine, fenhexamid and methiocarb were among the most frequently detected pesticides in the glove samples worn by florists during handling flowers and preparing bouquets.

In urine samples, the most frequently detected residues are fungicides (azoxystrobine (29% of samples), cyproconazole (29%), dimethomorph (24%), fenhexamid (24%)), insecticides (pirimicarb (64%), clofentezine (55%)) and various metabolites (acetamiprid-n-desmethyl (33%), pirimicarb desmethyl (26%), TCPy (29%)).

The fungicide furalaxyl is among the detected substances over three sampling periods, and among the substances with the highest average concentration. A recent study, conducted on cut flowers (roses, gerberas, and chrysanthemums) sold in Belgium, showed that furalaxyl was among the pesticide residues having the highest average concentration in roses with 8.90 mg/kg (Toumi *et al.,* 2016a).

Using the Spearman's test no correlation (P= 0.328) appeared between the number of pesticide residues found in urines and the food consumption habit of florists declared in the questionnaires[11]. No influence of the food consumption, organic or not, can be noted on the results suggesting that absorption by contact is far higher compared to dietary intake.

3.5. Global Results of Analyses of residual pesticides in urine samples obtained from control group

Non-occupational exposure originating from pesticide residues in food, air and drinking water generally involves low doses and is chronic (or semi-chronic) (Damalas & Eleftherohorinos, 2011). Almost all urine samples of control groups (95%) appeared to be contaminated by one or more pesticide residues. A total of 41

[11] Sur base des habitudes alimentaires de chacun des participants à l'enquête, une notation a été obtenue (Jamais (de consommation d'aliments bios) =0; Rarement=1; Parfois=2; Souvent=3; Toujours=4) et utilisée pour réaliser le test statistique de Spearman.

actives substances and metabolites were identified, with an average of about 4 pesticide residues and metabolites per sample.

Considering all pesticide residue concentrations in control urine samples, the average residue concentration reaches 2.0 µg/g creatinine per sample with a median of 0.3 µg/g creatinine.

Fourteen pesticide residues (33 %) are detected only once but the broad-spectrum fungicide pyraclostrobin is present in 50% of the urine samples. This active substance which has a high level of efficacy and important residual activity is applied on many crops (fruits, vegetables, cereals, etc.) (Fytoweb, 2018).

The insecticide fipronil and its metabolites (fipronil desulfinyl and fipronil sulfone) were also detected in urine samples of the control group with detection frequencies of 17%, 12% and 40%, respectively. The presence of fipronil and its metabolites (fipronil sulfone and fipronil desulfinyl) suggests a possible dietary exposure route (fipronil used illegally in 2017 in the poultry sector to control red mites in laying hens has contaminated eggs and egg-derived) (EFSA, 2018; AFSCA, 2017) or via contact with domestic animals (Dyk *et al.,* 2012; Poché *et al.,* 2017; Gupta & Anadón, 2018; Aerts *et al.,* 2017). Pesticides which are not approved for plant protection use in the EU were detected in urine samples of the control group: bitertanol, novaluron and fipronil and its metabolites (fipronil sulfone and fipronil desulfinyl). A recent study conducted under Belgium conditions found also five active substances not approved for use in Europe such as fipronil and its metabolites (Aerts *et al.*, 2017).

3.6. Difference between occupational and non-occupational groups

The contamination of urine samples of florists is clearly higher, qualitatively and quantitatively, compared to that of the control group (Figures 12 & 13). The t test confirmed that the number of pesticide residues and metabolites detected per urine sample of florist is statistically very significantly higher ($P < 0.001$). The result is in accordance with Aprea *et al.* (1994, 1997) who reported that urine samples of occupational groups were more contaminated than the one from control. In contrast, a study conducted in Paris among 21 workers from different occupational places (two greenhouses, three florist shops and three veterinary departments) reported that dialkylphosphates (DAP) detection frequencies and levels were not different between workers and the general population (Bouvier *et al.,* 2006). Studies conducted among North Carolina Latino migrant farmworkers showed that detections and concentrations for the pesticide urinary metabolites were similar for farmworkers and nonfarm workers groups (Arcury *et al.,* 2016, 2018).

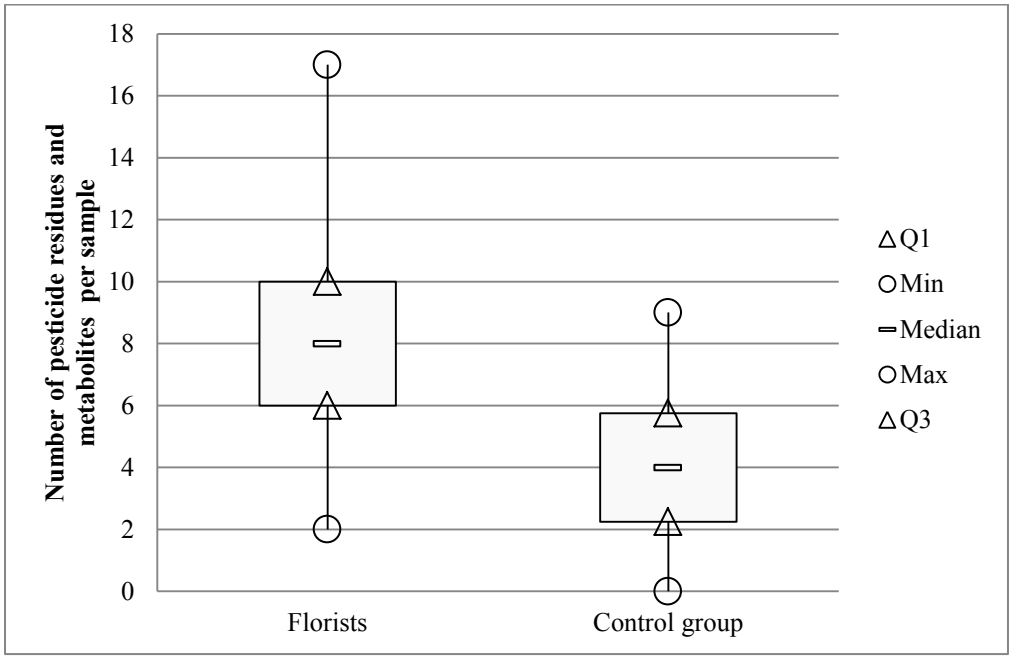

Figure 12: Box plots of number of pesticide residues and metabolites in urine samples of the two groups collected during three periods showing the median, the range (min-max), the first quartile (Q1) and the third quartile (Q3) range of the data

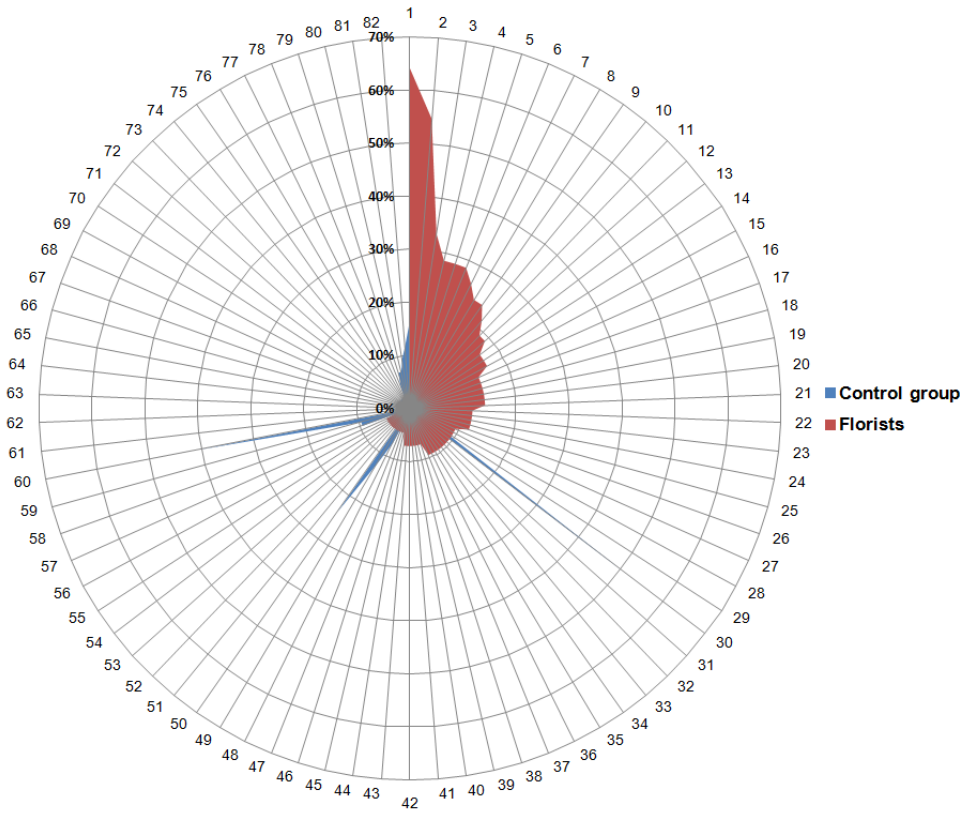

Figure 13: Distribution of pesticide residues and metabolites as a function of their frequency of detection in urine samples of both groups: florists and control group (see table of data in Annex 4)

Legend: (1) pirimicarb – (2) clofentezine – (3) acetamiprid-n-desmethyl – (4) cyproconazole – (5) azoxystrobin – (6) TCPy – (7) pirimicarb-desmethyl – (8) fenhexamid – (9) dimethomorph – (10) metrafenone – (11) fipronil – (12) prochloraz – (13) novaluron – (14) oxamyl – (15) flufenoxuron – (16) diflubenzuron – (17) fenpyroximate – (18) boscalid – (19) bupirimate – (20) fosthiazate – (21) spinosad – (22) cyprodinil – (23) spiroxamine – (24) ametoctradin – (25) buprofezin – (26) pyridaben – (27) thiabendazole – (28) acetamiprid – (29) mandipropamid – (30) pyraclostrobin – (31) 2CTCA (32) carbendazim – (33) 3-Hydroxy-carbofuran – (34) fenpropidin – (35) indoxacarb – (36) methiocarb sulfoxid – (37) piperonil-butoxide – (38) tebufenpyrad (39) difenoconazole (40) fenamiphos sulfone – (41) fluopyram – (42) furalaxyl – (43) methamidophos – (44) pyrimethanil – (45) hexythiazox – (46) metalaxyl (47) spirotetramat – (48) methoxyfenozide – (49) flutolanil – (50) fenoxycarb – (51) carbofuran – (52) DETP – (53) flutriafol – (54) isocarbophos – (55) methiocarb – (56) methomyl – (57) spirotetramat-enol-glucoside – (58) cyflumetofen – (59) spirotetramat-enol – (60) fipronil sulfone – (61) chlorantraniliprole – (62) dinotefuran – (63) DMP – (64) famoxadone – (65)

flonicamid – (66) flubendiamide – (67) imidacloprid – (68) methiocarb sulfon – (69) quinalphos – (70) spirodiclofen – (71) DEP – (72) dodemorph – (73) fludioxonil – (74) iprovalicarb – (75) mepanipyrim – (76) paclobutrazole – (77) spirotetramat-ketohydroxy – (78) pyriproxyfen – (79) bitertanol – (80) thiacloprid – (81) tebuconazol – (82) fipronil desulfinyl.

This biomonitoring study highlights the need to promote mitigation measures to reduce pesticide exposure among florists. Belgian florists who handle a large number of flowers daily are at risk of exposure to pesticides residues with potential effects on their health. Bad habits and absence of personal protective equipment of most florists (Table 30) do not contribute to reduce their exposure to pesticide residues. Furthermore, florists had insufficient knowledge about safety practices and use of basic protective equipment. Concerted efforts are required to create awareness and changes in attitude among the florists to better practices and hygiene rules. Moreover, the use of biological control products should be promoted in ornamental plants, such as products based on sweet orange essential oil to control thrips or based on *Metarhizium anisopliae* ((Metchnikoff) Sorokin, 1883) to control whiteflies. Furthermore, European regulation on Maximum Residue Limits (Regulation (EC) N° 396/2005) could be extended to pesticide residues on cut flowers.

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Author Contributions

This research was undertaken as part of Khaoula Toumi's Doctor of Phytopharmacy thesis. Bruno Schiffers is the promoter of this thesis. All authors contributed significantly to the successful completion of this research work both intellectually and financially. Accordingly, they conceived and designed the study plan. Khaoula Toumi conducted sampling, designed the experiment and wrote the initial manuscript. Khaoula Toumi and Laure Joly performed the chemical analyses and analyzed the data. Bruno Schiffers guided this study and provided revisions on the manuscript. Laure Joly and Christiane Vleminckx and provided feedback on the manuscript. Finally, all the authors have read and approved the final manuscript.

Notes

The authors declare no conflict of interest

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Chapitre 6

Exposition des travailleurs tunisiens aux résidus de pesticides lors de la réalisation des tâches de réentrée

Introduction

Par bien des égards, il est apparu que, en termes d'exposition, la situation des travailleurs horticoles en Tunisie pouvait se rapprocher de celle des fleuristes en Belgique. Il était donc intéressant de tenter de réaliser une étude parallèle, en utilisant la même approche que pour les fleuristes (analyse des résidus sur les produits, analyse de gants portés durant le travail, calcul de l'exposition potentielle). Seule la bio- surveillance n'a pu être réalisée faute de moyens d'analyse sur place.

Les travailleurs qui effectuent des activités de réentrée telles que le sarclage, l'effeuillage ou la récolte peuvent être exposés de façon significative lors d'un contact cutané avec les végétaux et les produits horticoles préalablement traités. L'évaluation de l'exposition cutanée des travailleurs horticoles aux pesticides est donc également nécessaire car les résidus auxquels ils sont exposés peuvent engendrer des effets néfastes pour la santé. L'évaluation des expositions lors de la réentrée repose principalement sur l'estimation de l'exposition par voie cutanée. Dans cette partie de l'étude, on devait observer les pratiques professionnelles des ouvriers tunisiens, collecter et analyser les tomates et les piments cultivés sous serre en Tunisie qu'ils manipulent pendant la récolte. Ce chapitre rapporte les principaux résultats de l'évaluation de l'exposition cutanée des travailleurs maraîchers tunisiens aux résidus de pesticides.

Ce chapitre est une version adaptée de l'article suivant :

- Toumi, K., Joly, L., Tarchoun, N., Souabni, L., Bouaziz, M., Vleminckx, C., & Schiffers, B. Risk assessment of Tunisian consumers and farm workers exposed to residues after pesticide application in chili peppers and tomatoes. (Article accepté dans *Tunisian Journal of Plant Protection*)

Risk assessment of Tunisian consumers and farm workers exposed to residues after pesticide application in chili peppers and tomatoes

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Abstract

In Tunisia, to prevent and control pests and diseases during cultivation under greenhouses, chili pepper and tomato require the use of a wide range of plant protection products potentially toxic and thus presenting a possible risk for farm operators, workers or consumers. A study has been carried out in the Sahel region of Tunisia to assess the risk for farm operators and workers exposed, by contact during harvest tasks, to possible pesticide residues remaining in tomato and chili pepper cultures, and for the Tunisian consumers (adults and children) after intake. A questionnaire was addressed to a group of 73 market gardeners to better understand the local professional practices and to determine the main route of exposure to pesticide. Twenty samples of cotton gloves (2 pairs / sample) were distributed to 20 volunteers who worn them for two consecutive half-days during the harvest of chili peppers or tomatoes before analysis of the dislodgeable pesticide residues which could be transferred from crops to hands. Using models predictive exposures values were calculated for consumers and farm workers. The highest exposure of consumers was observed for chlorpyrifos residues on tomatoes (with 82% and 312% of the Acute Reference Dose (ARfD), for adults and children respectively). The systemic exposure (SE) of farm workers was estimated for the median, the 90th percentile and the maximum concentration. At the highest observed concentrations, 15 pesticide residues (active substance and metabolites) used in pepper greenhouses, and 9 in tomato crops, exceed the Acceptable Operator Exposure Level (AOEL). Exposure appeared to be particularly critical for chlorothalonil sprayed in chili pepper greenhouses with SE_{MAX} values 113 times higher than the AOEL (11285%). Long task duration (8 hours/day) after re-entry in greenhouse, limited access to personal protective equipment (PPE), lack of hygiene and bad habits (eating, drinking, or smoking at work) have also been observed and discussed as risk factors.

Keywords: pesticide residues; risk assessment; consumers; farm workers; dermal exposure.

1. Introduction

In Tunisia, horticulture is an important, dynamic and vast agricultural sector. Today the total area of vegetable crops (field, tunnel and greenhouse) exceeds 160,000 ha. Tunisia produces around 3.2 million tons of vegetables crops (Gil 2015), mainly tomato (39%), watermelon (15%), onion (12%), potato (11.5%) and chili pepper (10%) (Agricultural Investment Promotion Agency 2015; Gil 2015). Tomato and green pepper are a basic component of the Tunisian diet and are used almost on a daily basis as part of raw or home cooked preparations (Jeder *et al.,* 2017).

During cultivation, chili pepper and tomato require the use of a broad range of pesticides to prevent and control pests and diseases. Fruits and vegetables are sprayed several times and up to the final harvest. Pesticides are considered necessary by farmers to provide high crop yields ensuring food security, high agriculture productivity and good quality products. Despite their popularity, pesticides are potentially toxic to humans (farm operators, workers or consumers) and can generate both acute and chronic health effects (WHO, 2005), mostly in developing countries (Ortiz *et al.,* 2002). Harvested products are often put onto the markets without consideration of the pre-harvest interval (PHI). As a consequence, the pesticide residues left on fruits can generate a potential health hazard for consumers (Chourasiya *et al.,* 2015; Darko & Akoto, 2008; Elgueta *et al.,* 2017; Nougadère *et al.,* 2012).

Many pesticides sprayed on tomatoes and chili peppers leave persistent, fat-soluble pesticide residues which can be dislodged from the two-sided foliar surface of a plant through contact. Workers who enter treated areas for pruning or who handle products during harvesting can absorb residues through their skin (EFSA 2014), are potentially exposed daily and thereby possibly endangering their health. Potential exposure of workers through contact with treated foliage is a significant concern in greenhouse production. High humidity, temperature and poor ventilation in greenhouses promote dermal exposure during working (Hanke *et al*., 2004). Several studies have assessed dermal exposure to pesticides during re-entry of workers in greenhouses for various crops: cucumbers (Caffareli *et al.,* 2004; Jurewicz *et al.,* 2009), strawberries (Caffareli *et al.,* 2004), tomatoes (Caffarelli *et al.,* 2004; Kasiotis *et al.,* 2017; kittas *et al.,* 2013; Ramos *et al.,* 2010) and peppers (Kasiotis *et al.,* 2017). Health problems have been reported for workers exposed to pesticides during re-entry activities, including reproductive problems (Abell *et al.,* 2000a, Abell *et al.,* 2000b), genetic damage (Lander *et al.,* 2000), neurological disorders (Baldi *et al.,* 2011) increases in bladder cancer (Boulanger *et al.,* 2016) and even breast cancer (Lemarchand *et al.,* 2016).

In this context, a study was carried out in the Sahel region of Tunisia to estimate the residue concentrations pesticide residues on tomatoes and chili peppers collected in greenhouses in order to assess the potential exposure for some consumers groups and farm workers during the harvest tasks through models.

2. Materials and methods

2.1. Fruit sampling for residue analysis

A random sampling of tomatoes (10 samples) and chili peppers (10 samples) was carried out from 25 to 28 April 2017 in Sousse governorate, according to the guidelines of the European Directive 2002/63/EC (EU Commission 2002) (sampling at the precise time of harvest and sample size of about 1 kg). Samples of 1.00 kg ± 0.04 kg (with at least 10 units) were collected in 20 greenhouses and weighed. The average unit weight (U, the smallest discrete portion in each lot, Directive 2002/63/EC) was about 106 g for tomatoes and 70 g for chili peppers. All samples were labelled and all useful information was collected for each sample (sample number, origin, sampling date, plant protection product applied, etc.).

2.2. Analytical procedures

The residual pesticide deposits were analysed by PRIMORIS (formerly FYTOLAB, Technologiepark 2/3, 9052 Zwijnaarde, Belgium) laboratory holding a BELAC (Belgian Accreditation Council) accreditation to ISO/CEI 17025 for pesticide residues on vegetables and herbal products in general. Food and glove samples were analysed with a multiple-residue Quick Easy Cheap Effective Rugged Safe (QuEChERS) method validated by the laboratory for analysis of residues in foodstuffs, which will detect approximately 500 different pesticide residues (active substances and metabolites) thanks to a combination of gas chromatography (GC) and liquid chromatography (LC) according to the active substances to be determined (GC-MS/MS for small, thermally stable, volatile, non-polar molecules or LC-MS/MS for larger, thermolabile, non-volatile, and polar molecules). For almost all active substances, the quantification limit (LOQ) was ≤ 0.01 mg/kg. The extraction procedure is based on the AOAC Official Method (Lehotay 2007). Briefly, a homogenous 10.0 g sub-sample (crushed fruits or small pieces of gloves) is weighted into a 50 mL polypropylene tube. Then, 10 mL of acidified acetonitrile (1% acetic acid), 4 g of anhydrous magnesium sulfate (MgSO4), and 1 g of sodium acetate (NaOAc) are added. After shaking and sonication in an ultrasonic bath, the polypropylene tube is centrifuged. A portion of the acetonitrile phase (upper layer) is transferred to vials and further analyzed (Toumi *et al.,* 2016 a, b; Toumi *et al.,* 2017a, b). The analytical results were corrected when necessary with the previously determined recovery rates (Toumi *et al.,* 2017a).

2.3. Consumer risk assessment

The human health risk was evaluated based on the concentration of pesticide residues in chili peppers and tomatoes at harvest. To evaluate the acute risk for child and adult consumers, we used a Predicted Short Term Intake (PSTI) values calculated with the general following formula:

$$PSTI = (LP \times OR \times v) / bw$$

where: **LP** is the 97.5th percentile of the portion size taken by people consuming tomatoes or chili peppers, in kg food per day, **OR** is the observed residue level the sample (in mg/kg), **bw** is the mean body weight for the target population subgroup (in kg) and **v** variability factor, the factor applied to the composite residue to approximate the residue level in a high-residue single unit.

For samples with pesticide residues, PSTI values were calculated with the EFSA Primo Model (RASFF, 2016; excel file version 11, 17/04/2017) and the European food consumption database (LP values) was used since Tunisia has no national data for large portions. The risk level for each active substance was established by comparison to the Acute Reference Dose (ARfD): according to FAO (2002) a risk exists when the PSTI > ARfD. When no ARfD value was available, no calculation of the acute risk was performed.

2.4. Exposure scenario of farm operators and farm workers

To have a better understanding of the route of exposure and professional practices, a survey through questionnaires was carried out among 73 farmers which were randomly chosen from professionals located in the Sahel region of Tunisia, more precisely in governorates of Sousse (59 market gardening farmers, i.e., 81%) and Monastir (14 market gardening farmers, i.e., 19%). Farmers were contacted with the help of the heads of Extension Territorial Cells (ETC) and met individually. The size of the group was considered large enough to be representative as all of the participants have the same activities.

The survey was conducted between February and April 2017. It consisted on face-to-face interviews with farm operators and farm workers in rural areas in the Sahel region where horticultural crops (vegetables, fruits) were mainly cultivated. A questionnaire was addressed to two professional categories: operators who are directly exposed to plant protection products (PPP) and workers who are indirectly exposed to PPP during re-entry activities (pruning, tying, leaf pulling, harvesting, etc.). They were asked to answer a detailed questionnaire (fourteen pages) on their socio-demographic data (identity, age, sex, level of education, … – see Annex 5), their horticultural production, their estimated working hours, the personal protective equipment (PPE) they worn, their hygiene rules, and their perception of health problems linked to their occupation, their management of PPP, their knowledge of pesticide residues and their suggestions and recommendations related to this subject.

Assessment of workers hands exposure using cotton gloves

Dermal exposure was determined according to a previously published procedure (Toumi *et al.,* 2017a, b). Twenty volunteers working in tomato or chili pepper greenhouses located in Sousse governorate were chosen at random to measure the transfer of pesticide residues from treated fruits to hands and to evaluate their potential dermal exposure (PDE). Two pairs of 100% cotton gloves were distributed to each worker and worn during two consecutive half days during harvesting fruits in 10 tomato and 10 chili pepper greenhouses (from min 2 h to max 3 h/day). The two pairs were collected as a single sample (four gloves/sample), weighed, cut in small pieces, and stored in freezing bags at -18 °C until transport and analysis.

Based on the determination of pesticide residues detected on gloves, the potential dermal exposure values were estimated. For each substance, a PDE value was calculated as follows:

PDE (mg / kg bw per day) = (C_T (mg / kg) × GW (kg) × 4) / bw (kg)

where C is the concentration of the substance in the sub-sample (5 g), GW is the average weight of the cotton gloves samples (61 g ± 3.27 g), T is the task duration (2 h during the trial; 8 h per day), and bw is the body weight (conventionally, 60 kg).

The duration of the task used to evaluate the dermal exposure of workers is 8 hours per day (EFSA 2014) and the local survey showed an average harvesting time close to 8 hours.

The PDE values were then converted into systemic exposure values (SE) using an appropriate dermal absorption percentage of 75% (default value) (EFSA 2012) as follows:

SE (mg / kg bw per day) = PDE (mg / kg bw per day) × 0.75

The risk characterisation is obtained as the ratio of the systemic exposure level to the reference value of each active substance, the AOEL (Acceptable Operator Exposure Level; in mg a.s./kg bw per day). It should not be exceeded to avoid any adverse effect to farm operators' and workers' health. To assess the risk, several prediction levels of the SE were considered: the median, 90th percentile, and the maximum (in mg/kg bw per day). Therefore, the SE values were expressed as percentage of the AOEL. It has been assumed that the most appropriate level to cover and assess the risk is the maximum value of the SE (SE_{MAX} or worst case).

3. Results

3.1. Lessons learnt from observation of practices and interviews

According to the survey, the majority of the 73 interviewed people were plot owners (86%), predominantly adult male aged from 20 to 78 years (mean age: 47 years ± 12 years). A vast majority of the respondents (77%) can be considered as workers (people who enter in treated areas or who handle treated crops) as well as operators (people involved in mixing, loading, spraying or emptying/cleaning operations) (categories defined by EFSA, 2014). Sixteen respondents should only be considered as workers and one as an operator applying PPP. The main crops in the Tunisian Sahel region were tomatoes (26%), chili peppers (28%) and potatoes (25%), evenly distributed between greenhouses (45%) and open fields (44%). A small part of the production is carried out under shelter (8%) or in tunnels (3%). Tomatoes and chili peppers are grown in greenhouses and are exposed to various pests (*Tuta absoluta*, whiteflies, soil nematodes, mites, psylles and thrips) and diseases (*Phytophthora* sp. and *Botrytis* sp.). Almost all preventive and/or curative treatments are systematic, with PPP obtained from the local authorized suppliers. The majority of respondents (53%) have a rather low level of education but an average working experience of 30 years. Bad habits (smoking) and lack of hygiene rules (36% workers eat and 60% drink while working) observed during the survey

contribute to increase the risk of exposure of operators and farm workers to pesticide residues through direct or indirect contact.

Behavioral observations of operators made during the survey show that 42% of them don't read the labels on PPP packaging and 9% don't understand the instructions of use. More than 20% have no idea about the recommended dosage of the PPPs and use them based on their experience or according to their supplier indications. The majority harvest their products without respect of the PHI, sometimes the day after the treatment. Regarding security, 57 operators never wear Plant Protection Equipment (PPE) during mixing and loading or cleaning of the spray equipment. During application, some of them wear a protective coverall (2%), gloves (23%), masks (16%), boots (12%), goggles (11%) or blouse (14%). After application, 47% wash only their hands; 19% wash their hands and arms; 28% their hands, arms, and faces. A relatively high percentage of operators (84%) take a shower when they return to home.

The survey indicates that working time in greenhouses can vary according to the season and the crop. Activities extend over the entire week, with an average daily duration of 8 h ± 1 h (n = 73). The observed contact duration of workers with crops is 5 h ± 2 h per day (n = 72 workers). Most workers (65%) return to plots or greenhouses immediately or a few hours after treatment. The majority of them wear long (68%) or short sleeve (39%) shirts and long (68%) or short leg (14%) trousers, but very few wear appropriate protective equipment such as gloves (8.3%), aprons (15%) or special clothing (22%). After working, 44% of workers wash only their hands; 18 % wash their hands and arms, 38% their hands, arms, and faces. Nevertheless 74% of workers made full body toilet (shower) at home.

Some operators report various health problems such as: eye problems (21); respiratory problems (13); skin problems: irritation (13) and dry (5) and other symptoms: stomach cramps (5); nosebleeds (4); nausea (4); dizziness (3); headaches (2); and sweating (2); repetitive fatigue (2); fevers (1) and dry mouth (1) sneezing (1). Farm workers complain about: eye problems (8); respiratory problems (5); skin problems: irritation (7) and dry (3) and other symptoms: nausea (3); stomach cramps (2); repeated strain (1); nosebleeds (1); headaches (1) and dizziness (1). Despite all reported problems, the majority of respondents have a passive attitude regarding pesticide use and no proposal for improvement was formulated from the survey. Problems are mainly linked to regulation weaknesses, the lack of awareness and monitoring, but also to inefficacy of some PPP leading them to increase the dosage or application frequency. Workers have proposed that PPE (including gloves) be distributed, or offered with purchased PPP, to encourage them to wear protective equipment and to improve their behavior.

3.2. Results of analysis of residual deposits in fruit samples

Pesticide residues have been detected in almost all tomato and chili pepper samples. Only two samples (one of tomato and one of chili pepper) were free from

detectable residues (concentrations below the analytical limit of quantification). Eighteen different active substances have been detected on 10 chili pepper samples (average: 2.9 a.s./sample), with an average total pesticide load of 0.41 mg a.s. /kg. Two fungicides, proquinazide and benomyl (and its metabolite, carbendazim), had the highest detection frequency (30%) (Table 33).

Fifteen different active substances have been detected in 10 tomato samples (average: 2.4 a.s/ sample), with an average total pesticide load of 0.38 mg a.s./kg. The most frequently detected residue on tomatoes is the fungicide propamocarb (6 samples out of 10) (Table 34).

3.3. Consumer risk assessment

Tables 33 and 34 summarize the detected active substances and their concentrations (in mg/kg) in the fruit samples, the concentration expressed as percentage of EU MRL (Maximum Residue Limit), the PSTI (in mg/kg bw/day) and the PSTI value expressed as a percentage of ARfD for both adults and children. Seven EU MRL exceedances were reported: six exceedances in chili pepper (Table 33) and one exceedance for chlorpyrifos ethyl (insecticide) in tomatoes (Table 34). The EU MRL exceedances appeared particularly critical for propargite (chili pepper) and chlorpyrifos-ethyl (tomato) with concentration values respectively 20 (2000% of EU MRL) and 26 (2685% of EU MRL) times higher than the EU MRL values.

Table 33: Results of 10 chili pepper samples analysed: detected active substances; concentrations expressed as a percentage of EU MRL; PSTI values; PSTI expressed as a percentage of ARfD, for adults and children

Chili pepper samples	Active substance	Concentration (mg/kg)	Concentration in % EU MRL	PSTI (mg/kg bw/day)		% ARfD	
				Adults	Children	Adults	Children
Sample 1	bifenazate	0.1800	6.0%	0.00025	0.00029	n.a	n.a
	proquinazid	0.0100	50.0%	0.00001	0.00002	0.01%	0.01%
Sample 2	acetamiprid	0.0649	21.6%	0.00009	0.00011	0.09%	0.11%
	carbendazim and benomyl	0.0121	12.1%	0.000017	0.00002	0.08%	0.10%
	indoxacarb	0.0453	15.1%	0.00006	0.00007	0.05%	0.06%
	proquinazid	0.0682	341.0%	0.00009	0.00011	0.05%	0.06%
	thiophanate methyl	0.2983	298.3%	0.00004	0.00005	0.21%	0.24%
Sample 3	spiromesifen	0.0139	2.8%	0.00002	0.00002	0.00%	0.00%
	carbendazim and benomyl	0.0455	45.5%	0.00006	0.00007	0.32%	0.37%
	fluopicolide	0.0349	3.5%	0.00005	0.00006	0.03%	0.03%
	myclobutanil	0.4776	95.5%	0.00066	0.00078	0.21%	0.25%
	propamocarb	0.1548	5.2%	0.00021	0.00025	0.02%	0.03%
	proquinazid	0.0901	450.5%	0.00013	0.00015	0.06%	0.07%
	thiophanate methyl	0.3235	323.5%	0.00004	0.00005	0.22%	0.26%
Sample 4	imidacloprid	0.0549	5.5%	0.00008	0.00009	0.10%	0.11%
	tebuconazole	0.0809	13.5%	0.00011	0.00013	0.37%	0.44%
	tebufenpyrad	0.1865	37.3%	0.00026	0.00030	1.29%	1.52%
Sample 5	-	-	-	-	-	-	-

Chili pepper samples	Active substance	Concentration (mg/kg)	Concentration in % EU MRL	PSTI (mg/kg bw/day)		% ARfD	
				Adults	Children	Adults	Children
Sample 6	acetamiprid	0.4668	155.6%	0.00065	0.00076	0.65%	0.76%
Sample 7	carbendazim and benomyl	0.0655	65.5%	0.00009	0.00011	0.45%	0.53%
	indoxacarb	0.0103	3.4%	0.00001	0.00002	0.01%	0.01%
	spirotetramat	0.0170	0.9%	0.00002	0.00003	0.00%	0.00%
Sample 8	tebuconazole	0.3706	61.8%	0.00051	0.00060	1.71%	2.01%
Sample 9	cyproconazole	0.0218	43.6%	0.00003	0.00004	0.15%	0.18%
	spinosad	0.0630	3.2%	0.00009	0.00010	n.a	n.a
Sample 10	propagite	0.2000	2000.0%	0.00028	0.00033	0.93%	1.08%
	bupirimate	0. 3411	17.1%	0.00047	0.00055	n.a	n.a

(n.a.: not available; EU MRL and ARfD values from EU Pesticides database)

Table 34: Results of 10 tomato samples analysed: detected active substances; concentrations expressed as a percentage of EU MRL; PSTI values; PSTI expressed as a percentage of ARfD, for adults and children

Tomato samples	Active substance	Concentration (mg/kg)	Concentration in % EU MRL	PSTI (mg/kg bw/day)		% ARfD	
				Adults	Children	Adults	Children
Sample 1	chlorantraniliprole	0.0147	2.5%	0.00022	0.00085	n.a	n.a
	propamocarb	0.2629	6.6%	0.00400	0.01529	0.40%	1.53%
Sample 2	propamocarb	0.0475	1.2%	0.00072	0.00276	0.07%	0.28%
Sample 3	propamocarb	0.1003	2.5%	0.00153	0.00583	0.15%	0.58%
Sample 4	indoxacarb	0.0295	5.9%	0.00045	0.00172	0.36%	1.37%

Tomato samples	Active substance	Concentration (mg/kg)	Concentration in % EU MRL	PSTI (mg/kg bw/day)		% ARfD	
				Adults	Children	Adults	Children
Sample 5	acetamiprid	0.1058	21.2%	0.00161	0.00615	1.61%	6.15%
	flubendiamide	0.0260	13.0%	0.00040	0.00151	0.40%	1.51%
	myclobutanil	0.0141	4.7%	0.00021	0.00082	0.07%	0.26%
	pirimicarb	0.0232	4.6%	0.00035	0.00135	0.35%	1.35%
	propamocarb	0.0135	0.3%	0.00021	0.00078	0.02%	0.08%
Sample 6	propamocarb	0.5206	13.0%	0.00793	0.03027	0.79%	3.03%
Sample 7	-	-	-	-	-	-	-
Sample 8	azoxystrobine	0.0226	0.8%	0.00034	0.00131	n.a	n.a
	chlorantrraniliprole	0.0311	5.2%	0.00047	0.00181	n.a	n.a
	difenoconazole	0.1231	6.2%	0.00187	0.00716	1.17%	4.47%
	indoxacarb	0.1020	20.4%	0.00155	0.00593	1.24%	4.74%
Sample 9	chlorpyrifos-ethyl	0.2685	2685.0%	0.00409	0.01561	81.75%	312.24%
	carbendazin and benomyl	0.0649	21.6%	0.00099	0.00377	4.94%	18.87%
	propamocarb	0.0326	0.8%	0.00050	0.00190	0.05%	0.19%
	pyrimethanil	0.5877	58.8%	0.00895	0.03417	n.a	n.a
	thiophanate methyl	0.9763	97.6%	0,00149	0,00568	7.43%	28.38%
Sample 10	boscalid	0.0242	0.8%	0.00037	0.00141	n.a	n.a
	spinosad	0.0360	5.1%	0.00055	0.00209	n.a	n.a

(n.a.: not available; EU MRL and ARfD values from EU Pesticides database)

3.4. Results of analyses of residual deposits in glove samples

All active substances detected on vegetables were also measured at rather high concentrations on cotton gloves. For people working in chili pepper greenhouses, 63 a.s. were identified (average: 18 a.s./sample), with an average total concentration of 148 ± 285 mg/kg. Four main active substances were identified: thiophanate-methyl (100%), benomyl (and its metabolite carbendazim) (90%), acetamiprid (70%) and propamocarb (70%)). A total of 57 a.s.. were detected on all the gloves worn by people working in tomato greenhouses (average: 18 a.s./sample), with an average total concentration of 111 ± 193 mg/kg. Propamocarb was detected in all samples, followed by diafenthiuron (90%) and thiophanate methyl (80%). DEET (N, N-diethyl-3-methylbenzamide) was also detected on all glove samples as it is used as a biocide in textile sector/industry.

3.5. Risk characterization for farm operators and farm workers

Tables 35 and 36 present the systemic exposure values (SEmedian, 90th percentile, and maximum values, in mg/kg bw per day) and the systemic exposure expressed as a percentage of the AOEL for all active substances detected on the cotton gloves worn by workers in chili pepper greenhouses (Table 35) and tomato greenhouses (Table 36) and having a SE exceeding their respective AOEL value.

Table 35: Active substances detected on the gloves worn by workers in chili pepper greenhouses and having a SE exceeding their AOEL values, the corresponding systemic exposure (median, 90th percentile, and maximum values) in mg/kg bw per day, the systemic exposure as a percentage of the AOEL and their toxicological properties (AOEL values, and CLP classification according the EU Pesticides database)

Active substance		**AOEL (mg/kg bw/day)**	**SE (Median) (mg/kg bw per day) (in % of AOEL)**	**SE (90th P) (mg/kg bw per day) (in % of AOEL)**	**SE (Maximum) (mg/kg bw per day) (SE in % of AOEL)**	**CLP classification**
Acetamiprid		0.07	0.0006 (1%)	0.0307(44%)	0.0700(100%)	H302
Bifenazate		0.0028	0.0044 (159%)	0.0163(583%)	0.0195(697%)	H317, H373
Benomyl and carbendazim		0.02	0.0055 (27%)	0.0939(470%)	0.2745(1373%)	H315, H317, H335, H340, H360FD
Chlorothalonil		0.009	0.5079 (5644%)	0.9141(10157%)	1.0157(11285%)	H317, H318, H330, H335, H351
Cyhalothrin*	Gamma	0.0003	0.0005 (181%)	0.0005(181%)	0.0005(181%)	-
	Lambda	0.00063	0.0005 (86%)	0.0005(86%)	0.0005(86%)	H301, H312, H330
Cypermethrin		0.06	0.0002 (0%)	0.0572(95%)	0.0949(158%)	H302, H332, H335
Dimethoate		0.001	0.0046 (456%)	0.0082(817%)	0.0091(907%)	H302, H312
Flubendiamide		0.006	0.0342 (569%)	0.0614(1024%)	0.0683(1138%)	-
Indoxacarb		0.004	0.0005 (13%)	0.0679(1699%)	0.0883(2208%)	H301, H317, H332, H372
Omethoate		0.0003	0.0004 (131%)	0.0004(131%)	0.0004(131%)	H301, H312
Proquinazid		0.02	0.0043 (21%)	0.0241(120%)	0.0315(158%)	H351
Spiromesifen		0.015	0.0092 (61%)	0.0996(664%)	0.1551(1034%)	-
Tebuconazole		0.03	0.0053 (18%)	0.0326(109%)	0.0421(140%)	H302, H361d
Tebufenpyrad		0.01	0.0038 (38%)	0.0592(592%)	0.0961(961%)	H301, H317, H332, H373

Active substance	AOEL (mg/kg bw/day)	SE (Median) (mg/kg bw per day) (in % of AOEL)	SE (90th P) (mg/kg bw per day) (in % of AOEL)	SE (Maximum) (mg/kg bw per day) (SE in % of AOEL)	CLP classification
Thiophanate-methyl	0.08	0.0044 (6%)	0.4392 (549%)	1.6470 (2059%)	H317, H332, H341

H301: Toxic if swallowed; H302: Harmful if swallowed; H312: Harmful in contact with skin; H315: Causes skin irritation; H317: May cause an allergic skin reaction; H318: Causes serious eye damage; H330: Fatal if inhaled; H332: Harmful if inhaled; H335: May cause respiratory irritation; H340: May cause genetic defects; H341: Suspected of causing genetic defects; H351: Suspected of causing cancer; H360FD: May damage fertility. May damage the unborn child; H361d: suspected of damaging the unborn child; H372: Causes damage to organs through prolonged or repeated exposure; H373: May cause damage to organs through prolonged or repeated exposure

* The analytical method is unable to identify cyhalothrin (lambda or gamma), therefore the risk assessment was performed for both cases

Table 36: Active substances detected on the gloves worn by workers in tomato greenhouses and having a SE exceeding their AOEL values, the corresponding systemic exposure (median, 90th percentile, and maximum values) in mg/kg bw per day, the systemic exposure as a percentage of the AOEL and their toxicological properties (AOEL values, and CLP classification according the EU Pesticides database)

Active substance		AOEL (mg/kg bw/day)	SE (Median) (mg/kg bw per day) (in % of AOEL)	SE (90th P) (mg/kg bw per day) (in % of AOEL)	SE (Maximum) (mg/kg bw per day) (SE in % of AOEL)	CLP classification
Bifenazate		0.0028	0.0060 (215%)	0.0097 (348%)	0.0107(381%)	H317, H373
Benomyl and carbendazim		0.02	0.0005 (2%)	0.0621 (311%)	0.1220(610%)	H315, H317, H335, H340, H360FD
Chlorothalonil		0.009	0.0150 (167%)	0.0431 (479%)	0.0488(542%)	H317, H318, H330, H335, H351
Chlorpyrifos-ethyl		0.001	0.0001 (8%)	0.0052 (524%)	0.0104(1036%)	H301
Cyhalothrin*	Gamma	0.0003	0.0001 (40%)	0.0009 (291%)	0.0012(398%)	-
	Lambda	0.00063	0.0001 (19%)	0.0009 (138%)	0.0012(189%)	H301, H312, H330
Flubendiamide		0.006	0.0390 (651%)	0.0702 (1170%)	0.0780(1300%)	-
Indoxacarb		0.004	0.0163 (408%)	0.0938 (2344%)	0.1019(2548%)	H301, H317, H332, H372
Spinosad		0.012	0.0106 (88%)	0.0182 (152%)	0.0201(168%)	-
Thiophanate-methyl		0.08	0.0005 (1%)	0.5716 (714%)	1.6989(2124%)	H317, H332, H341

H301: Toxic if swallowed; H312: Harmful in contact with skin; H315: Causes skin irritation; H317: May cause an allergic skin reaction; H318: Causes serious eye damage; H330: Fatal if inhaled; H331:Toxic if inhaled; H332: Harmful if inhaled; H335: May cause respiratory irritation; H340: May cause genetic defects; H341: Suspected of causing genetic defects; H351: Suspected of causing cancer; H372: Causes damage to organs through prolonged or repeated exposure; H373: May cause damage to organs through prolonged or repeated exposure

* The analytical method is unable to identify cyhalothrin (lambda or gamma), therefore the risk assessment was performed for both cases

4. Discussion

Among all vegetable samples analysed only two (one tomato sample and one chili pepper sample) have residue levels below the limit of quantification (0.01 mg/kg). Most often multiple residues were detected in the samples (up to seven pesticides). These results are a direct consequence of local poor practices and bad pest management, as reported for many other countries over the world (Murcia & Stashenko, 2008; Arias *et al.,* 2014).

Chili peppers appear to be slightly more contaminated than tomatoes (higher number of different residues and more EU MRL exceedances). Even though the two vegetables belong to the Solanaceae and are produced according to similar practices, the difference may result from the physiological characteristics of each species and the difference in composition of each cuticle. It is known that the lipophilicity of the cuticle can help some pesticides to enter into the plant (Trapp, 2004). Stronger and thicker cuticle of chili peppers could better retain the residues, and the bigger surface area could intercept more pesticide drift than tomatoes fruits (Riederer and Schönherr, 1984). However, it is difficult to predict the cuticle absorption and the degradation of chemical ingredient as they depend on many factors such as the physicochemical characteristics of the chemical, the contact area, the cuticle composition and its surface (Bonmatin *et al.,* 2015). Four samples of chili peppers (40%) had pesticide residues above the maximum residue limits (EU MRLs). A total of 6 EU MRL exceedances were observed for a single collection of 10 samples. Residues of proquinazid, thiophanate methyl, acetamiprid and propargite exceed dramatically the MRLs (for 156% up to 2000%). A study conducted in Egypt in 2015 showed that only one of 31 pepper samples had acetamiprid residue levels higher than the MRL value (Alla, 2015).

Only one sample of tomato reported an EU MRL violation for the insecticide chlorpyrifos-ethyl by 2685%. Similar trends in results was also reported by Bojacà *et al.,* in 2011 that conducted a monitoring study for tomatoes in Colombia and indicated that almost all the samples of greenhouse tomatoes positive for acephate, cymoxanil, hexaconazole or thiocyclam exceeded the MRLs, on average, by 356, 525, 606 and 1375%, respectively. Chlorpyrifos-ethyl in tomatoes has been detected in different countries around the world including India and Ghana (Essumang *et al., 2008*; Singh, 2012). In contrast, Alla *et al.* did not report any pesticide residue exceeded the MRL on 19 tomato samples (Alla, 2015).

The active substances detected on vegetables, with higher concentrations above their respective EU MRL values (e.g. chlorpyrifos-ethyl, acetamiprid, thiophanate methyl, propagite or proquinazid), are known for their potential detrimental effects to health ; therefore vegetables should be considered as non-compliant for the market. Nevertheless, PSTI calculation consists to estimate the actual risk to consumer group (adults and children) and whether an observed violation of an MRL can lead to a risk to the consumers (Łozowicka, 2012). As shown in Table 33, no values were above the ARfD for chili pepper samples. The PSTI values in chili-peppers samples were in the range of 0.00-1.71% and 0.00-2.01% ARfD for adults and children, respectively (Table 33). Only in one tomato sample, the PSTI of the insecticide chlorpyrifos-ethyl exceeds the ARfD with a factor of 3.1 times (312%).

This exceedance of the ARfD was observed for children but not confirmed for adults. The PSTI values in tomato samples were in the range of 0.02-82% and 0.08-312% ARfD for adults and children, respectively (Table 34).

These results demonstrate that despite the high level of residues in some samples of vegetables, the Tunisian consumers don't face a serious acute risk, except with insecticides such as chlorpyrifos-ethyl. Nevertheless considering the number of detected residues, their chronic exposure through the consumption of raw vegetables could be associated with a health risk if it could be also demonstrated that vegetables reach usualy the same levels of contamination. Moreover, it should be borne in mind that dietary pesticide exposure estimated in this study, considered only exposures through consumption of chili peppers and tomatoes, and did not include other food products such as fruits, other vegetables, grains, dairy products, fish and meat. Furthermore, the estimated risk assessment is based on toxicological evaluation of the single compounds and not based on an evaluation of cumulative exposure to multiple pesticide residues in crops. In addition, these horticultural commodities are also essential ingredients in Tunisian diet, more consumed than in Europe. As a result, the global consumer exposure should be higher than in the present evaluation.

Workers who come into contact with the crop or handle treated products, will be contaminated through contact with pesticides that are still available on the crop after application (Krol *et al.,* 2005; Dong &Beauvais 2013). Previous studies (Nigg *et al.,* 1984; Zweig *et al.,* 1985) have showed the relationship between the levels of residues on the crops and the dermal exposure of workers during harvesting activities. Similarly, in this study all pesticide residues measured in chili pepper and tomato samples were also detected in glove samples worn by farm workers during harvesting. Contact with contaminated vegetable samples resulted in the transfer of pesticide residues to gloves worn by the workers allowing their measurement. All glove samples appeared to be highly contaminated by many different pesticide residues (63 active substances detected with an average of about 18 active substances per sample and an average total concentration per glove sample of 148 mg/kg for chili peppers and 57 active substances detected with an average of about 18 active substances per sample and an average total concentration per glove sample of 111 mg/kg for tomato). These concentrations are 1000 times higher than the concentrations which are usually detected on foodstuffs. The systemic exposures of workers were estimated for the median, for P90, and for the maximum concentration of residues in samples (Tables 35 and 36).

For chili peppers, six, thirteen and fifteen active substances exceed the AOEL respectively at the median, the P90 and maximum values of SE indicating risk situations. However, for tomato samples, four active substances exceed the AOEL at the SE median values. At P90 and the maximum (or worst case), nine active substances exceeds the AOEL indicating potential risk situations.

A recent study was conducted in Greece to assess a worker dermal exposure during re-entry activities in greenhouses reported that the total worker PDE levels ranged from 0.16 to 0.72 mg/kg bw per day and from 0.09 to 0.17 mg/kg bw per day for tomato and chili pepper crops, respectively (Kasiotis *et al.,* 2017).

Exposure could be particularly critical for chlorothalonil, with SE_{MAX} values that are 113 times higher than the AOEL (11285%) for chili peppers, followed by

indoxacarb and thiophanate methyl that are above 20 times higher than the AOEL: 2208% and 2059%, respectively for chili peppers and 2548% and 2124% for tomatoes. At SE_{MAX}, five active substances are above 10 times higher than the AOEL: benomyl and carbendazim (1373%), flubendiamide (1138%) and spiromesifen (1034%) for chili peppers and chlorpyrifos-ethyl (1036%) and flubendiamide (1300%) for tomatoes. Even when wearing personal protection equipment that will minimize exposure by at least 90%, SE values will always exceed the AOEL at the worst case for these active substances. The systemic exposure values are in accordance with the results of a study conducted in Italy to evaluate the risk of pesticide dermal exposure: the highest absorbed doses for workers re-entering in tomato greenhouse in % of AOEL are 288% and 7959% for azoxystrobin and chlorpyrifos-ethyl, respectively (Cafferli *et al.,* 2004). Several reasons are behind these violations of the health based guidance values including, applying higher dose than the recommended ones, not respecting the pre-harvest interval, etc.

According to the CLP classification (Table 35 and 36), the majority of the active substances detected in chili pepper and tomato samples and having a SE exceeding their AOEL value, have potential hazardous acute and/or chronic effects. The results of the observed levels of dermal exposure after re-entry of greenhouses led to the conclusion that a health hazard may exist, especially after application of high rates of relatively toxic pesticides which easily penetrate the skin.

From the survey of 73 farmers, it is concluded that workers and operators may be exposed during usual pesticide handling and re-entry activities. The task duration for harvesting for farm workers, which is an important factor to consider when building exposure scenarios for a group of workers, is equal to the default value of 8 h proposed in the EFSA Guidance Document 2014 (EFSA, 2014). A considerable number of the farmers reported not using protective equipment on a regular basis. The obtained results were agreeing with those reported in Nepal (Shrestha *et al.,* 2010), Palestine (Sa'ed *et al.,* 2010), Lesotho (Mokhele *et al.,* 2011), Iran (Hashemi *et al.,* 2012), Tanzania(Lekei *et al.,* 2014), Uganda (Oesterlund *et al.,*2014), Indonesia (Yuantari *et al.,* 2015), Ghana (Okoffo *et al.,* 2016), Gambia (Idowu *et al.,* 2017) and Burkina Faso (Son *et al.,* 2017). Bad personal behavioral habits (eating, drinking, or smoking at work) were reported by many farmers (operators and workers). Thus, oral exposure may occur secondarily to dermal exposure, through hand to mouth transfer. Health risks can be due to mishandling and habits exhibited during pesticide application and re-entry activities. According to their answers in the survey, workers seem to be affected by many health problems. While, it was not possible to conclude only on the basis of personal feelings and declarations, analytical results and the estimations of exposure confirmed that Tunisian farm operators and workers in the study area are at high risk.

In conclusion, observations completed by analytical results indicate multiple pesticide applications leading to MRL exceedances and probable acute risk for Tunisian consumers. It's a pity that exposure was assessed using a European food consumption database while chilli-peppers and tomatoes are among the staple foods in Tunisia with a consumption significantly higher than in Europe. Thus, these results stress the need for a national consumption survey and continuous monitoring

programs that cover all food commodities consumed locally, especially fruits and vegetables. According to systemic exposure values, workers who spend several hours on a daily basis in greenhouses are at risk during re-entry activities, with potential effects on their health. It appears that lack of awareness, bad habits and absence of personal protective equipment increase their exposure level and their health risks. There is an urgent need for awareness raising amongst professionals' and training on good practices and hygiene rules to avoid their excessive exposure. This survey should be completed later by a bio-monitoring of the operators during spraying and workers during re-entry activities, with analysis of blood, urine and hair samples. Moreover, considering that the concentration of pesticides in the air may be of high concern in greenhouses, the evaluation of the inhalation exposure is highly recommended in the future.

Chapitre 7

Discussion générale

1. Exposition des fleuristes belges aux résidus de pesticides

1.1. La contamination des fleurs coupées par les résidus de pesticides

La consommation mondiale de fleurs coupées est aujourd'hui estimée à 30 milliards d'euros par an, l'Europe et l'Amérique du Nord étant les principaux marchés (Rikken *et al.,* 2010). Face à la demande du marché européen, des millions de fleurs produites en Amérique Latine, en Afrique, en Inde ou ailleurs dans le monde, transitent en camion et en avion vers des marchés de consommation situés essentiellement dans les pays riches de l'hémisphère nord, notamment la Belgique. Les fleurs les plus vendues en Belgique sont les roses, les gerberas et les chrysanthèmes. Ces trois espèces sont très sensibles aux différents ravageurs de ces cultures et à diverses maladies, c'est pourquoi elles sont traitées de manière intensive jusqu'à la récolte afin de mettre sur le marché des produits floraux en quantité importante, en qualité satisfaisante et à des prix relativement modestes (Bethke & Cloyd, 2009).

L'analyse des dépôts résiduels de substances actives pesticides sur 90 échantillons de fleurs coupées (roses, gerberas et chrysanthèmes) révèle que ces derniers sont fortement contaminés, quels que soient leurs origines, avec 107 résidus de différents pesticides détectés, soit une moyenne par échantillon de fleurs de presque 10 résidus de pesticides et une charge totale moyenne, toutes substances confondues, de 16 mg/kg. La concentration totale cumulée de tous les résidus peut atteindre 97 mg/kg pour un seul bouquet de 5 roses belges.

Parmi les trois espèces étudiées, les roses sont généralement plus contaminées que les deux autres espèces de fleurs : 97 résidus de différents pesticides ont été trouvés sur les 50 échantillons de roses analysés, avec une moyenne d'environ 14 résidus par échantillon et une charge totale moyenne par bouquet de roses de 26 mg/kg. Même si cette comparaison n'a pas beaucoup de sens, il est intéressant de noter que les concentrations en résidus mesurées sur les fleurs coupées sont mille fois plus élevées que celles détectées le plus souvent sur les denrées alimentaires. Bien que les pesticides soient généralement moins toxiques pour un contact cutané que pour une exposition par voie orale, le risque est bien réel pour les personnes qui manipulent régulièrement un grand nombre de fleurs aussi fortement contaminées par des résidus de pesticides à cause de l'absorption percutanée d'une partie de ces résidus.

Les niveaux très élevés de résidus de pesticides sur les fleurs coupées (une haute concentration mesurée et un nombre important de pesticides différents détectés) peuvent s'expliquer par :

- L'utilisation répétée d'un grand nombre de pesticides différents par les producteurs des fleurs coupées à cause de la pression des ravageurs et des maladies pour atteindre les hauts standards de qualité exigés et des niveaux

de production économiquement rentables sur un marché très concurrentiel entre origines et entre producteurs.

- L'absence de lutte intégrée, le manque de méthodes alternatives efficaces ou de connaissance de ces méthodes (tels que l'emploi d'agents de biocontrôle, l'utilisation de substances sémio-chimiques, l'emploi d'éliciteurs, etc.).
- L'absence de contrôle des résidus de pesticides sur les fleurs coupées (pas de LMR), contrairement aux autres cultures dont les produits récoltés sont consommés.
- La nécessité de traiter des produits végétaux qui, pour être autorisés à l'importation en Belgique, doivent être absolument exempts d'organismes nuisibles réglementés (dits « de quarantaine »).

En ce qui concerne l'activité biologique des substances détectées sur les fleurs, 50% des résidus sont des insecticides et 46% des fongicides. Trois substances actives sont des régulateurs de croissance et une seule substance est un herbicide. Selon la classification CLP, la majorité des substances actives détectées présentent une toxicité aiguë et/ou chronique avec des effets rapportés tels que : irritation ou corrosion cutanée ou occulaire, sensibilisation cutanée, suspicion d'être cancérogènes, mutagènes ou toxiques pour la reproduction ou encore d'avoir une toxicité spécifique pour certains organes cibles (exposition unique et répétée) non négligeable. Certaines substances actives retrouvées sur les fleurs (acéphate, méthiocarbe, méthomyl, deltaméhrine) agissent sur le système nerveux.

Les résultats des analyses signalent aussi la présence d'un certain nombre de substances actives non approuvées au niveau de l'UE, que ce soit dans les échantillons considérés (sur base des étiquettes et des factures d'achat par les fleuristes) comme d'origine belge ou ceux déclarés d'origine néerlandaise, ce qui est interpellant. Toutefois, il faut noter que ces résultats sont à relativiser car aucune certitude définitive et absolue n'a pu être apportée sur l'origine exacte des échantillons de fleurs coupées collectés puisqu'ils ont été prélevés chez les détaillants et non pas directement chez les producteurs.

Ainsi, l'étude a d'abord montré que les trois espèces de fleurs les plus vendues en Belgique sont porteuses de nombreux résidus différents. Par conséquent, lors de la manipulation des fleurs et la préparation des bouquets, les fleuristes belges sont potentiellement et journellement exposés à de nombreuses substances actives différentes, présentes à des niveaux de contaminations très élevés et dotées de propriétés toxicologiques dont les effets peuvent être significatifs sur la santé.

1.2. Exposition cutanée potentielle des fleuristes belges aux résidus de pesticides

1.2.1. Scénario d'exposition

L'exposition des fleuristes aux résidus des pesticides peut varier en fonction de plusieurs facteurs importants qui peuvent expliquer l'augmentation ou la diminution des risques, tels que la durée des tâches (ou temps de travail), le port ou non des équipements de protection individuels (EPI), les comportements individuels ou encore le respect des règles d'hygiène. Une enquête a donc été menée pour observer les pratiques usuelles des fleuristes.

Grâce à l'enquête réalisée auprès des professionnels en Belgique, il est apparu que les fleuristes ont tous des activités similaires, telles que la manipulation, le triage, l'élagage et le groupage des fleurs. Elle a montré que la majorité des fleuristes consacre journellement un temps assez élevé à la préparation des fleurs et des bouquets. Cette durée varie en moyenne de 2 à 6 heures/jour pour la majorité des fleuristes. De plus, les fleuristes, qui sont des travailleurs indépendants, travaillent 6, voire 7 jours, par semaine. Le temps de travail réservé à la manipulation des fleurs coupées est donc apparu très élevé, et plus élevé que la moyenne des autres « travailleurs » (workers dans le Document Guidance de l'EFSA). Or, le temps de travail conditionnera fortement l'exposition potentielle de ces travailleurs.

En ce qui concerne le port d'équipement de protection individuel, 96 % des fleuristes ne portent aucun vêtement ou équipement spécial. Seuls 20% des fleuristes interrogés utilisent des gants (en latex, a priori peu efficaces) au cours de la préparation des bouquets et la manipulation des fleurs. Si les mains des fleuristes peuvent être particulièrement contaminées, ces derniers peuvent aussi être exposés aux résidus de pesticides par voie orale, le plus souvent par un contact des mains contaminées avec la bouche car 88% d'entre eux boivent et mangent, et 12% des fleuristes fument au cours du travail. Ce type de comportement conditionnera également l'exposition et peut générer des intoxications accidentelles, avec des conséquences non négligeables pour la santé.

1.2.2. Transfert des résidus de pesticides délogeables sur les mains des fleuristes

En ce qui concerne les travailleurs, la voie cutanée constitue toujours la principale voie d'entrée des résidus de pesticides dans l'organisme humain (Brouwer *et al.,* 1992a, b ; Illing *et al.,* 1995; Ecobichon *et al.,* 1998). Et la quantité de résidus de pesticides présents sur les mains des travailleurs représente la principale mesure de l'exposition cutanée (US.EPA, 1986). Or, les fleurs coupées étant fortement contaminées par des résidus de pesticides et de nombreux pesticides pulvérisés sur les fleurs coupées ayant une faible adhérence, étant persistants et liposolubles, ils peuvent facilement être délogés après la pulvérisation par contact des mains avec les

surfaces foliaires. L'exposition cutanée des fleuristes s'explique donc par le contact prolongé de leurs mains (ou d'autres parties du corps) avec des fleurs coupées ayant en surface des résidus plus ou moins facilement « délogeables » (selon les substances).

Pour mesurer le transfert des résidus délogeables au cours des tâches habituelles, des fleuristes ont porté, pour une durée de travail déterminée, des gants en coton qui ont servi de collecteurs des dépôts résiduels. L'analyse de ceux-ci a permis de conclure que les fleuristes belges risquent d'être exposés à des niveaux de résidus de pesticides non négligeables. Tous les échantillons de gants sont apparus fortement contaminés et par de nombreux résidus de pesticides : 111 résidus de pesticides ont été détectés, avec une moyenne d'environ 37 résidus de pesticides par échantillon et une concentration totale moyenne par échantillon de gants de 22 mg/kg. La moitié des substances actives détectées sont des insecticides et l'autre moitié sont des fongicides. Une seule substance est un régulateur de croissance et une autre est un herbicide. Selon la classification CLP, la majorité des substances actives détectées présentent une toxicité aiguë et/ou chronique.

Il a été démontré par divers auteurs qu'une relation linéaire existait entre les niveaux de résidus présents sur les cultures et l'exposition cutanée des travailleurs occupés à des activités de récolte (Popendorf & Leffingwell, 1982 ; Nigg *et al.,* 1984; Zweig *et al.,* 1985). De même, dans notre étude, environ 70% des résidus de pesticides ont été détectés à la fois sur les fleurs coupées et sur les gants portés par les fleuristes lors de la manipulation de ces fleurs et la préparation des bouquets (Annexe 2).

1.2.3. Exposition cutanée potentielle des fleuristes

Les résultats des expositions cutanées potentielles des fleuristes ont été présentés pour la moyenne, différents percentiles et pour la concentration maximale de résidus dans les échantillons. Les résultats des différents percentiles utilisés pour estimer la SE (exposition systémique) varient d'un ordre de grandeur. L'exposition systémique d'une substance active (clofentézine) dépasse l'AOEL établi au niveau prédictif P90. Dans le pire des cas, SE_{MAX} (aux concentrations maximales), l'exposition systémique à quatre substances actives (clofentézine, famoxadone, méthiocarbe et pyridabène) dépasse leurs valeurs AOEL respectives. L'exposition pourrait être particulièrement critique pour la clofentézine avec des valeurs SE_{MAX} quatre fois plus élevées que l'AOEL (393%).

Les valeurs d'exposition cutanée des fleuristes belges obtenues dans notre étude sont du même ordre de grandeur que celle d'autres travailleurs qui réalisent des activités de réentrée. Les valeurs potentielles d'exposition cutanée sont conformes aux valeurs rapportées par Thongsinthusak *et al.* (1990) durant la manipulation de roses et de chrysanthèmes et aux résultats obtenus par Brouwer *et al.* (1992c) pendant la coupe, le triage et le groupage des roses.

1.3. Détermination de l'exposition systémique totale des fleuristes

La peau permet de protéger l'organisme des agressions externes. Mais, elle ne constitue toutefois pas une barrière étanche puisque différents éléments sont capables de la traverser. La peau peut être une cible ou une porte d'entrée privilégiée pour de nombreuses substances actives qui ont des effets toxiques. L'exposition cutanée aux produits phytopharmaceutiques dépend des propriétés physico-chimiques (le coefficient de partition octanol/eau.) et des poids moléculaires de ces molécules : le pourcentage d'absorption percutanée d'une molécule est inversement proportionnel à sa masse molaire (Alikhan *et al.,* 2009). Par conséquent, le fait de manipuler des fleurs contaminées par des résidus de pesticides sans se protéger les mains, et de manière générale la peau, favorise ce type d'exposition.

Quand elle peut être mise en œuvre, la surveillance biologique s'avère être un excellent outil pour évaluer le niveau d'exposition aux résidus de pesticides, notamment parce qu'elle permet une approche intégrée des différentes sources et voies d'exposition, et ainsi de mieux appréhender l'exposition totale et réaliste, la dose interne et les risques sanitaires encourus (Albertini *et al.,* 2006; Cortéjade *et al.,* 2016; Lopez *et al.,* 2016; Appenzeller *et al.,* 2017). Un monitoring biologique a été réalisé dans notre étude à 3 reprises (période de la Saint-Valentin, période de la Fête des Mères, période de la Toussaint) sur les mêmes groupes de participants volontaires.

L'analyse des urines est la méthode qui offre le plus de souplesse. Il s'agit d'une méthode non-invasive. Elle nécessite toutefois de s'assurer notamment de la pleine collaboration des participants (des « volontaires » anonymes), de la comparaison avec un groupe dit « de référence », de la réalisation d'un questionnaire alimentaire (pour distinguer la part des apports alimentaires), de la collecte des urines et leur stockage au froid avant analyse, de disposer d'une méthode d'analyse non seulement des molécules parents mais aussi des métabolites connus.

1.3.1. Mise au point d'une méthode d'analyse des urines

Une méthode d'analyse des résidus de pesticides et des métabolites urinaires a été développée et validée afin d'effectuer l'évaluation de l'exposition totale des fleuristes belges aux résidus de pesticides. Les résidus de pesticides et des métabolites urinaires spécifiques recherchés ont été choisis sur la base des résultats obtenus suite à l'analyse des fleurs coupées les plus vendues en Belgique et des gants en coton portés par les fleuristes durant leurs activités professionnelles. La procédure comprend l'extraction à l'acétate d'éthyle et la mesure des extraits à l'aide d'un système UHPLC-ESI-MS/MS. Cette méthode multi-résidus est très fiable et rentable pour la détermination d'un large éventail de différents pesticides et métabolites dans l'urine qui sont utilisés pour évaluer l'exposition totale des fleuristes ou d'autres personnes entrant en contact direct ou indirect avec des

pesticides tels que les opérateurs, les travailleurs, les riverains ou les résidents, etc. Elle présente de nombreux avantages, non seulement en termes de qualité des résultats (taux de récupération élevé, bonne reproductibilité et large portée analytique), mais aussi en termes d'aspects pratiques (faible coût, main-d'œuvre, déchets, verrerie et espace). La méthode a été validée avec succès selon les directives SANTE 11813/2017 pour 110 résidus de pesticides et métabolites différents. Les taux moyens de récupération (résultats obtenus à des jours différents) étaient, avec quelques exceptions, compris entre 80 et 120% avec un écart-type relatif inférieur à 20% pour la majorité des résidus de pesticides et des métabolites urinaires. Cette méthode est jugée satisfaisante et peut être facilement étendue à d'autres pesticides et métabolites, si nécessaire.

1.3.2. Concentrations mesurées dans les urines

Les concentrations des résidus de pesticides et de métabolites mesurés dans les urines des fleuristes au cours des trois plus importants périodes de vente des fleurs coupées choisies dans notre étude confirment la forte exposition des fleuristes belges aux résidus de pesticides lors de la manipulation des fleurs et la préparation des bouquets : 70 résidus (56 pesticides et 14 métabolites) ont été identifiés au cours des trois périodes étudiées, avec une moyenne d'environ 8 résidus de pesticides et métabolites par échantillon et une concentration moyenne totale de résidus de pesticides par échantillon de 4,3 µg/g de créatinine (valeurs allant de 0,2 à 67 µg/g créatinine).

Ces concentrations sont similaires aux concentrations des résidus de pesticides et des métabolites urinaires mesurées chez des travailleurs qui entrent en contact avec des cultures traitées lors de la réalisation des activités de réentrée et d'application des produits phytopharmaceutiques. On trouve des concentrations du même ordre par exemple dans l'étude menée en Italie chez des travailleurs dans les cultures ornementales (Aprea *et al.,* 2002), en Equateur chez des travailleurs en floriculture (Colosio *et al.,* 2003), au Japon chez des cultivateurs de pommes (Ueyama *et al.,* 2012), et en Italie chez des applicateurs et un travailleur dans la vigne (Colosio *et al.,* 2002).

Les résultats de bio surveillance montrent que les urines des fleuristes sont significativement plus contaminées que celles du groupe de référence (résidents belges non exposés professionnellement aux pesticides).

Les travailleurs en contact avec les cultures traitées peuvent être contaminés par les pesticides restant sur la culture après l'application (Dong & Beauvais, 2013 ; Krol *et al.,* 2005; Toumi *et al.,* 2018). Par conséquent, le mélange de résidus de pesticides observé dans les échantillons d'urine de fleuristes résulte principalement d'une exposition cutanée après le contact avec des fleurs traitées. La relation linéaire entre l'exposition cutanée des travailleurs et l'excrétion urinaire des pesticides a été démontrée par plusieurs études (Aprea *et al.,* 1994, 1997, 1999, 2005; Bradman *et al.,* 2009; Brouwer *et al.,* 1993; McCurdy *et al.,* 1994; Ware *et al.,* 1975). Nos résultats ont également confirmé une corrélation positive entre les résidus de

pesticides et les métabolites dans les échantillons d'urine ; en effet, tous les résidus et métabolites observés dans les urines des fleuristes avaient préalablement été détectées sur les fleurs coupées. Ainsi, sur les mains des fleuristes, l'azoxystrobine, le boscalide, la clofentézine, le fenhexamide et le méthiocarbe figuraient parmi les pesticides les plus fréquemment détectés lors de la manipulation des fleurs et de la préparation des bouquets. Et dans les échantillons d'urine, les résidus les plus fréquemment détectés sont des fongicides (l'azoxystrobine, le cyproconazole, le diméthomorphe et le fenhexamide) et des insecticides (le pirimicarbe et le clofentézine) ainsi que divers métabolites (l'acétamipride-n-desméthyl, le pirimicarbe desméthyl, le 3,5,6-trichloro-2-pyridinol (le chlorpyrifos-éthyle et le chlorpyrifos-méthyle).

Le fongicide furalaxyl figure à la fois parmi les substances détectées au cours des trois périodes d'échantillonnage et parmi les substances ayant la concentration moyenne la plus élevée. Sur les trois espèces les plus vendues en Belgique (roses, gerberas et chrysanthèmes), le furalaxyl faisait partie des résidus de pesticides dont la concentration moyenne était la plus élevée (avec 8,90 mg/kg pour les échantillons de roses).

Notons enfin qu'aucune influence de la consommation alimentaire (organique ou non) n'a pu être notée sur les résultats d'analyse des urines, suggérant que l'absorption par contact est beaucoup plus élevée en regard de l'apport alimentaire.

2. Exposition des travailleurs horticoles tunisiens aux résidus de pesticides

En Tunisie, l'horticulture représente une branche importante et dynamique de l'agriculture avec un fort potentiel de croissance (GIL, 2015). Les tomates et les piments sont des composantes fondamentales du régime alimentaire tunisien (Jeder *et al.,* 2017), c'est pourquoi nous nous sommes plus spécialement intéressés à ces deux cultures très populaires. Ces légumes sont utilisés presque quotidiennement dans le cadre de préparations crues ou cuites (Jeder *et al.,* 2017). Comme pour les fleurs coupées, afin d'atteindre les standards exigés par les consommateurs et des niveaux de production économiquement viables, les producteurs maraîchers doivent utiliser de nombreux produits phytopharmaceutiques pour maintenir la pression exercée par les mauvaises herbes, les insectes nuisibles ou les maladies fongiques à des niveaux acceptables.

Cette constatation nous a amené à nous interroger sur le fait de savoir si les mêmes causes pouvaient produire les mêmes effets, dans un contexte économique et culturel différent. En effet, si l'utilisation des produits phytopharmaceutiques est le plus souvent jugée nécessaire en Tunisie par les producteurs pour atteindre leurs objectifs de production, il n'en reste pas moins que les travailleurs horticoles qui réalisent chez eux durant de longues heures de nombreuses taches de réentrée, pourraient de ce fait être exposés à des niveaux élevés de résidus de pesticides dont ils subiraient alors les effets sur leur santé. D'autant que la pression exercée par les patrons, ou

indirectement par le marché, les rends plus vulnérables (absence d'information, manque de respect des délais de réentrée le plus souvent inconnus). Il nous a donc paru intéressant d'aborder la question avec une démarche identique à celle adoptée pour les fleuristes belges.

2.1. La contamination de légumes récoltés par des résidus de pesticides

La première question était d'évaluer le niveau de contamination des deux légumes (tomates et piments) au moment de la récolte, c'est-à-dire au stade où les mains des travailleurs sont le plus directement en contact avec les produits.

Une campagne d'échantillonnage de tomates et de piments a donc été menée dans la région du Sahel Tunisien (région de Tunisie où les cultures maraîchères sous serres abondent) pour estimer les concentrations résiduelles de pesticides sur les tomates et les piments récoltés dans des serres afin d'évaluer l'exposition potentielle pour certains groupes de consommateurs et de travailleurs au travers de modèles. L'analyse des piments et des tomates révèle la présence de 7 dépassements de LMR (6 sur les piments et 1 sur les tomates), avec des concentrations qui peuvent atteindre 2000% des LMR (20 fois les valeurs de LMR). Cependant, l'estimation de l'ingestion d'un pesticide donné au cours d'un repas/journée (Predicted Short Terme Intake [PSTI]) effectué suivant les méthodes de calcul actuellement en vigueur dans l'UE (RASFF, 2016 ; Excel file version 11, 17/04/2017) montre que seul l'insecticide chlorpyrifos-éthyl dépasse la dose de référence aiguë (ARfD), par un facteur de 3 (312% ARfD). Néanmoins, ce dépassement a été observé uniquement chez les enfants.

La contamination des piments et des tomates par des résidus de pesticides et les dépassements de LMR et des valeurs toxicologiques de références sont probablement et principalement liés à :

- Le non-respect du délai de réentrée. Il faut noter que le délai avant récolte (DAR) n'est pas mieux respecté, expliquant les sept dépassements de LMR constatés pour les produits horticoles échantillonnés (dont les résidus de l'insecticide chlorpyrifos-éthyle pour lesquels la valeur calculée du PSTI dépasse l'ARfD pour les enfants).
- La faiblesse de la réglementation en Tunisie sur l'utilisation des produits phytopharmaceutiques.
- Le manque de sensibilisation au risque sanitaire et de surveillance des pratiques agricoles.
- L'inefficacité de certains PPP qui amène les producteurs tunisiens à augmenter la dose ou la fréquence d'application.
- Le manque de méthodes alternatives (lutte biologique, utilisation des substances sémio-chimiques et d'éliciteurs, etc.),
- L'absence de contrôle systématique pour les résidus de pesticides sur les fruits et légumes en Tunisie.

2.2. Évaluation de l'exposition cutanée potentielle des travailleurs tunisiens aux résidus de pesticides présents sur les legumes

2.2.1. Scénario d'exposition

De nombreux facteurs influencent le niveau de contamination et l'exposition des travailleurs horticoles aux résidus de pesticides, durant les tâches de réentrée dans les cultures. Pour étudier l'importance de ces facteurs, une enquête complétée d'une série d'observations sur sites a été réalisée au cours d'une période de récolte. Plusieurs enseignements peuvent en être tirés.

En Tunisie, la majorité des travailleurs maraîchers sont occupés à des activités similaires, car les cultures nécessitent souvent les mêmes soins, et ils adoptent des comportements assez semblables, car ces travailleurs ne sont pas formés autrement que par l'expérience acquise progressivement et le contact avec les autres travailleurs. L'enquête a montré que la durée de travail observée (en moyenne 8 heures), qui est un facteur très important à considérer pour le scenario d'exposition, elle est sensiblement égale à la valeur par défaut fixée pour ce type de travailleurs dans le Document Guidance de l'EFSA (EFSA, 2014).

Par leur métier, les travailleurs horticoles de Tunisie sont exposés de façon chronique à un ensemble de (résidus de) produits phytopharmaceutiques (insecticides, fongicides, herbicides, etc.). Cependant, le comportement des travailleurs et leur manque de perception de la dangerosité des produits phytopharmaceutiques peuvent influencer le niveau d'exposition. La majorité des répondants ont en effet une attitude passive en ce qui concerne l'utilisation des pesticides et le risque encouru, et par conséquent aucune proposition d'amélioration n'a été formulée ou souhaitée de leur part au cours de l'enquête.

L'exposition des travailleurs aux produits phytosanitaires est une réalité, car les résidus de ceux-ci sont encore présents et peuvent persister pendant plusieurs heures, voire plusieurs jours, après l'application. C'est pourquoi le respect d'un « délai de réentrée » est normalement imposé après une application pour garantir la sécurité et protéger la santé des personnes intervenant dans les parcelles traitées. Durant cette période, généralement indiquée sur l'étiquette, il sera normalement interdit aux personnes de pénétrer sur ou dans les lieux où a été appliqué un produit phytopharmaceutique sans utilisation de moyens de protection individuels. Ce délai, qui dépend de la dangerosité du produit, est au moins de 6 heures mais il peut durer jusqu'à 48 heures. Or l'enquête réalisée a montré qu'en Tunisie la majorité des travailleurs (65%) retournent dans les parcelles ou les serres immédiatement ou quelques heures après le traitement ce qui favorise leur exposition aux pesticides.

Les équipements de protection peuvent réduire l'exposition et jouent donc un rôle très important dans la politique de prévention. Ils sont destinés à être portés par les travailleurs en vue de les protéger contre les résidus de pesticides menaçant leur

santé. Malheureusement, il a également été constaté que la majorité des travailleurs observés ne portaient pas d'équipements appropriés tels que : des gants (seulement 8% en portent), des tabliers (15%) ou certains vêtements spéciaux (22%). De plus, les mauvaises attitudes, les habitudes et le manque de règles d'hygiène observés pendant l'enquête contribuent à augmenter le risque d'exposition de ces travailleurs agricoles aux résidus de pesticides.

2.2.2. Transfert des résidus sur les mains des travailleurs tunisiens

L'estimation de la quantité de résidus de pesticides sur les mains des travailleurs constitue souvent la principale mesure de l'exposition cutanée (Bradman *et al.,* 2008; Zhang, 2005). Au début des années 1980, des études non publiées de l'EPA montrent que les résidus recueillis sur les gants de coton portés par les travailleurs pourraient atteindre 60-95% de l'exposition totale aux pesticides externes (Li, 2009). Le port de gants en coton qui servent de collecteurs des dépôts résiduels pour une durée de travail déterminée et l'analyse de ceux-ci, ont permis de conclure que les maraîchers qui réalisent la récolte des piments et des tomates risquaient d'être exposés à des niveaux de résidus de pesticides non négligeables. Les travailleurs qui entrent en contact avec une culture préalablement traitée seront contaminés avec les résidus des pesticides encore disponibles sur la végétation (Krol *et al.,* 2005 ; Dong & Beauvais, 2013). Rappelons qu'une relation linéaire existe entre les niveaux de résidus sur les cultures et l'exposition cutanée des travailleurs pendant les activités de récolte (Nigg *et al.,* 1984; Zweig *et al.,* 1985).

Il en va de même dans notre étude. L'échantillonnage et l'analyse de piments et de tomates ont montré que tous les résidus de pesticides mesurés sur ces légumes ont également été détectés sur les gants portés par les travailleurs agricoles tunisiens pendant la récolte de ces deux produits horticoles. Le contact avec des produits horticoles contaminés a entraîné le transfert des résidus de pesticides présents sur les légumes aux gants portés par les travailleurs. Tous les échantillons de gants analysés semblaient fortement contaminés et par de nombreux résidus de pesticides : au total, 63 et 57 résidus de pesticides (moyenne : 18 résidus de pesticides par échantillon) ont été détectés avec une concentration totale moyenne de 148 ± 285 mg/kg et de 111 ± 193 mg / kg sur les gants des travailleurs qui ont récolté respectivement des piments et des tomates. L'échantillon de gants le plus contaminé a accumulé une teneur totale de résidus allant jusqu'à 622 mg/kg (pour la culture de tomates) et 939 mg/kg (pour la culture de piments) avec une médiane de 30 et 40 mg/kg, respectivement. En outre, selon la classification CLP, la majorité des substances actives détectées sur les gants présentent une toxicité aiguë et/ou chronique.

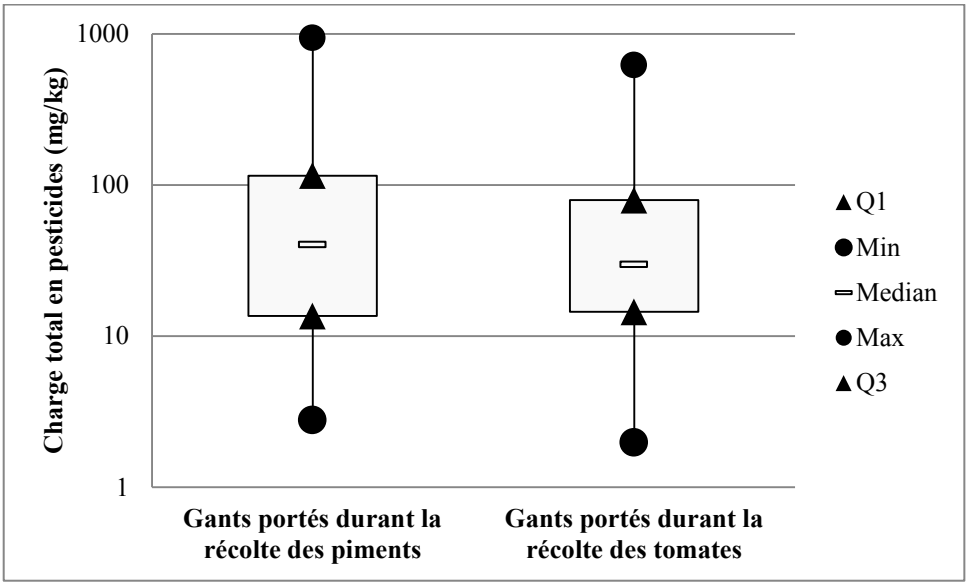

Figure 14: Répartition des charges totales en pesticides (mg/kg) dans les échantillons des gants en coton portés durant la récolte des piments et des tomates cultivés sous serre

2.2.3. Évaluation de l'exposition systémique

Sur base de ces résultats, nous avons pu estimer, pour chaque composé, les expositions systémiques (SE) des travailleurs agricoles correspondant à la concentration maximale en résidus dans les échantillons de gants. L'exposition des travailleurs a dépassé l'AOEL dans le cas de 9 résidus en tomates et 15 résidus de pesticides en piments.

Nos résultats indiquent donc l'existence en Tunisie de situations à risque pour les travailleurs, et pas seulement pour les opérateurs, dont il conviendrait de se préoccuper. L'exposition semblerait être particulièrement critique pour le fongicide chlorothalonil retrouvé dans les gants portés par les travailleurs dans les serres de piments, car les valeurs SE_{MAX} atteignent 113 fois la valeur de l'AOEL (11285%). Nos résultats sont conformes aux valeurs rapportées par Kasiotis *et al.* (2017) qui a mené récemment une étude sur des travailleurs qui réalisent des tâches de réentrée dans des serres de tomates et de piments en Grèce.

Il n'a malheureusement pas été possible de compléter cette évaluation par une mesure de la contamination totale au moyen d'un biomonitoring comme nous avons pu le faire en Belgique pour les fleuristes. Seule cette méthode permettrait de confirmer le risque réel.

Chapitre 8

Conclusion générale, recommandations et perspectives

1. L'exposition des fleuristes belges aux résidus de pesticides

En floriculture, le recours à l'utilisation des produits phytopharmaceutiques (ou « pesticides ») est considéré comme une stratégie très intéressante pour lutter contre les nombreux ravageurs et les diverses maladies qui nuisent non seulement à la production mais aussi à la qualité marchande des fleurs. Il permet aux producteurs de fleurs coupées de rester compétitifs sur des marchés nationaux et internationaux très concurrentiels. Faute de solutions alternatives (non chimiques), que ce soit faute de connaissances et de recherches, de l'absence d'information et de disponibilité d'agents de biocontrôle ou à cause du coût comparativement élevé de ceux-ci par rapport aux produits chimiques, la protection chimique peut souvent être qualifiée d'intensive, spécialement dans les pays exotiques d'où sont importées en Belgique de nombreuses fleurs coupées. Dans ces pays, la règlementation est faible et les préoccupations en matière de santé ou d'environnement ne sont pas prioritaires au regard des enjeux économiques. Par conséquent, les fleurs sont régulièrement pulvérisées durant leur production par des produits insecticides, fongicides, etc. engendrant des dépôts résiduels non négligeables. Les fleuristes, qui manipuleront durant leurs tâches habituelles et quotidiennes ces fleurs traitées, pourraient donc être exposés à de nombreux résidus présents à des niveaux significatifs avec des risques potentiels pour leur santé. De cette constatation découle l'intérêt de procéder à une évaluation de l'exposition par voie cutanée des fleuristes aux résidus de pesticides.

L'analyse des fleurs coupées les plus vendues en Belgique (roses, gerberas et chrysanthèmes) d'une part, et la détermination du transfert potentiel des résidus présents sur les fleurs vers les mains grâce à l'analyse de gants en coton portés par des fleuristes durant leurs activités professionnelles d'autre part, ont permis de conclure que le risque potentiel d'exposition de ces derniers aux résidus de pesticides n'était pas négligeable (nombreuses substances actives et dépôts résiduels élevés). Par la suite, la surveillance biologique (biomonitoring par analyse des urines de groupes exposés et non exposés) s'est avérée être un excellent outil pour confirmer l'exposition et évaluer un niveau d'exposition systémique total réaliste. Cette étude a ainsi permis de confirmer que les fleuristes belges sont exposés à des quantités notablement élevées de résidus de pesticides dont les propriétés toxicologiques, qui ont été recherchées dans diverses bases de données, permettent de penser qu'ils pourraient engendrer des effets possibles sur la santé de ces professionnels.

À la lumière des résultats obtenus, l'exposition des fleuristes semble être un exemple d'une situation professionnelle unique pour plusieurs raisons :

- Les fleuristes sont exposés régulièrement à :
    - Un nombre très élevé de substances actives sur les fleurs : 107 et 111 résidus de pesticides ont été respectivement détectés sur les fleurs

coupées et sur les mains (dépôts mesurés sur les gants) des fleuristes. Septante résidus de pesticides et métabolites ont été identifiés dans leurs urines.

- Des concentrations en résidus très élevées sur les fleurs : la concentration totale cumulée de tous les résidus peut atteindre respectivement 97 mg/kg et 113 mg/kg pour un seul bouquet de 5 roses et un échantillon de gants portés par un fleuriste.
- Des résidus de produits phytopharmaceutiques toxiques : la majorité des substances actives détectées présentent une toxicité aiguë et/ou chronique non négligeable (irritants, corrosifs, sensibilisants, suspectés d'être cancérogènes ou mutagènes, ou d'être toxiques pour la reproduction, etc.)

- Il existe une très bonne corrélation entre les substances détectées sur les fleurs coupées, mesurées sur les gants en coton et retrouvées aussi dans les échantillons d'urine, démontrant le transfert et l'absorption de ces substances, et donc l'exposition.
- La variété et les quantités de résidus de pesticides auxquelles les fleuristes sont exposés sont très élevées si on les compare aux travailleurs réalisant des activités de réentrée dans des serres préalablement traités par des pesticides. En effet, les sources d'approvisionnement de fleurs sont très diversifiées: les fleurs coupées sont importées en Belgique de pays producteurs répartis dans le monde entier où sont utilisés des produits phytopharmaceutiques très variés, contenant souvent des substances actives qui ne sont plus approuvées en Europe, et dont l'emploi répété pour atteindre les standards exigés et des niveaux de production économiquement rentables conduit à des concentrations en résidus très élevées à la récolte. Cependant, à ce niveau, les fleurs supposées d'origine européenne (voire belge) ne se distinguent pas de l'ensemble des origines.
- Un déficit d'information sur la présence et les risques liés à l'exposition répétée aux résidus présents sur les fleurs coupées apparaît clairement durant les interviews réalisées. Les fleuristes représentent donc une catégorie de travailleurs à risque, mais non informée. Ceci est interpellant pour le secteur comme pour les autorités belges (peu ou pas d'encadrement de la profession sur le sujet ou sur les équipements de protection individuels ; pas de recommandations officielles).

En conclusion, cette étude confirme que les fleuristes doivent être considérés (notamment lors de l'évaluation des risques avant la mise sur le marché des produits phytopharmaceutiques destinés à leur emploi sur les fleurs et plantes ornementales) comme des « travailleurs » (workers dans les documents Guidance de l'EFSA), au même titre que les personnes réalisant des activités de réentrée dans un champ ou une serre préalablement traité par des produits phytopharmaceutiques. C'est normalement le cas pour les produits autorisés sur les fleurs ou les plantes ornementales qui sont produites en Belgique (ou ailleurs en Europe) : en effet, une évaluation de l'exposition des travailleurs, notamment lors de la récolte des fleurs,

est réalisée au préalable et le produit phytopharmaceutique ne sera autorisé à être mis sur le marché que si le risque pour le travailleur est acceptable.

L'évaluation qui est réalisée devrait donc être considérée comme couvrant l'estimation du risque des fleuristes car ils sont exposés par les mêmes voies que les autres travailleurs qui effectuent des tâches de réentrée en cultures florales. Toutefois, il faut noter que dans certains cas, l'exposition de ces travailleurs n'est acceptable qu'avec le port de gants et par conséquent, dans ce cas, il faudrait également recommander aux fleuristes d'en porter.

Cependant, l'étude démontre aussi que le problème de l'évaluation est plus complexe qu'il n'y paraît a priori en ce qui concerne les fleuristes du fait de la présence de résidus nombreux et élevés qui trouve essentiellement sa source dans le manque de contrôles effectués dans les pays producteurs hors Europe et, pour les fleuristes qui les manipulent, au manque d'information sur l'origine véritable des fleurs commercialisées en Belgique.

1.1. Perspectives d'études scientifiques complémentaires :

De futurs travaux devraient être réalisés pour mieux documenter la problématique de l'exposition des fleuristes belges aux résidus de pesticides et trouver des solutions pour réduire le niveau de risque. Les études scientifiques complémentaires suivantes demanderaient peu d'efforts et des moyens raisonnables :

- Pour plusieurs substances actives détectées sur les fleurs ou les gants en coton, leurs métabolites urinaires sont non polaires, volatiles, thermostables et de tailles relativement faibles. C'est pourquoi le développement d'une méthode d'analyse des urines des fleuristes par le moyen de la chromatographie en phase gazeuse couplée à la spectrométrie de masse devra être poursuivi. La méthode devra être validée selon les standards internationaux et pourra alors être utilisée pour analyser, dans les échantillons d'urine des fleuristes et des groupes de « référence », ces autres molécules qui n'ont pas pu être déterminées avec la méthode analytique employée (la seule jusqu'ici validée).
- Vu le nombre de résidus différents auxquels les fleuristes belges sont exposés, il serait intéressant d'envisager une évaluation du risque « cumulé » à un groupe constitué de plusieurs substances actives ayant les mêmes modes d'action vis-à-vis des cibles (ex : neurotoxicité, thyroïde, reins, foie, etc.), les mêmes effets phénoménologiques et en prenant en considération leurs effets d'addition et de synergie.
- La majorité des bouquets de fleurs qui sont vendus en Belgique sont constitués généralement, non seulement sur base des trois espèces les plus vendues (roses, gerberas et chrysanthèmes), mais aussi en y intégrant des feuillages (comme le plus souvent : le salal (*Gaultheria shallon*), le Pistachier Lentisque (*Pistacia lentiscus*), l'eucalyptus (*Eucalyptus globulus*), l'aspidistra (*Aspidistra elatior*), les feuilles de cocotier (*Cocos nucifera*), les feuilles d'anthurium (*Anthurium andreanum*), les feuilles de faux philodendron (*Monstera deliciosa*). Même si ces plantes sont en principe moins sensibles aux bioagresseurs et de

moindre valeur commerciale (et donc probablement bien moins traitées), il serait intéressant de procéder également à des analyses des dépôts résiduels de pesticides sur ces feuillages afin d'évaluer les niveaux moyens et la prévalence de la contamination.

- Les équipements de protection individuels constituent en principe une barrière de protection efficace entre les résidus de pesticides présents sur les fleurs coupées et le corps humain. Cependant, plusieurs études ont montré que leur port n'assurait pas toujours pleinement ce rôle. Il faut aussi noter que pour assurer une protection, non seulement la qualité des gants mais également le fait d'en changer régulièrement sont importants. Des études supplémentaires pourraient être effectuées pour évaluer les capacités de protection des gants utilisés (en fonction de la composition du gant, de son épaisseur, de sa résistance au déchirement, etc.) ou de tabliers imperméables (les fleurs sont souvent déposées en paquets sur les bras des fleuristes). De même, l'efficacité de certaines mesures d'hygiène à recommander (lavage des parties du corps en contact avec les fleurs ; lavage et ventilation des locaux ; etc.) pourrait être testée avant de vulgariser ces mesures.

Par contre, les compléments d'étude suivants, qui intéressent les voies d'exposition, les effets sur la santé ou la surveillance biologique, demanderont plus de moyens :

- En ce qui concerne l'exposition des fleuristes belges aux résidus de pesticides, il serait intéressant de poursuivre les travaux d'évaluation en considérant également la voie d'exposition orale (transfert de la main à la bouche) car l'enquête auprès des professionnels révèle qu'ils mangent, boivent et fument au cours du travail (sans nécessairement se laver les mains malgré les recommandations).
- De même, il serait également utile d'envisager la voie d'exposition respiratoire car plusieurs substances actives : diazinon, étridiazole, fenpropidine, ométhoate propamocarbe et triforine sont très volatiles (Annexe 2), et les boutiques des fleuristes représentent un milieu confiné dans lequel les professionnels passent de nombreuses heures quotidiennement. Toutefois, pour conserver la fraîcheur des fleurs, la température des chambres de stockage ou des magasins est en général limitée ce qui peut réduire la volatilisation des substances. Des capteurs passifs ou actifs pourraient être utilisés pour mesurer la concentration des pesticides volatiles dans l'air et voir si des mesures permettant une meilleure ventilation des locaux devraient être proposées.
- Le développement d'un modèle de transfert des résidus de pesticides appliqués sur les fleurs coupées pourrait être envisagé en établissant les DFR, TC et autres facteurs. La validation du modèle développé pourrait ensuite être conduite en utilisant différents pesticides et en étudiant leur transfert des fleurs coupées aux mains des fleuristes durant la manipulation et la préparation des bouquets.
- Enfin, le lien entre l'exposition et les effets sur la santé n'a pas pu être établi. Par conséquent, des études épidémiologiques (en collaboration avec des équipes médicales) seraient nécessaires pour étudier le lien entre les expositions estimées

des fleuristes belges aux résidus de pesticides et la survenue d'effets sur leur santé, à court ou à long terme. Néanmoins, une enquête par questionnaires auprès d'un nombre plus important de fleuristes permettrait d'analyser sides associations existent entre l'exposition des fleuristes aux pesticides et de possibles effets sur leur santé. Mais la limite de cette perspective est la taille de l'effectif belge en fleuristes qui risque d'être insuffisante pour une étude statistiquement valable. Il nous semble, au regard des propriétés toxicologiques des principales substances actives retrouvées, que parmi ces effets, on pourrait notamment investiguer la survenue de :

- Troubles neurologiques (troubles neurocomportementaux, altérations de la mémoire, de l'attention, etc.).
- Problèmes de reproduction (avortement spontané, mort du nouveau-né due à des anomalies congénitales, etc.).
- Certaines maladies chroniques, dont certains cancers, etc.

- La surveillance biologique s'avère être un excellent outil pour évaluer le niveau d'exposition aux résidus de pesticides présents dans les fleurs coupées, notamment parce qu'elle intègre toutes les voies et sources d'exposition. L'approche par la surveillance biologique pourrait être renforcée ou complétée en considérant d'autres matrices que l'urine, même si elles sont plus compliquées à collecter et à traiter (réticence des populations ; préparation et extraction des échantillons ; etc.), telles que :
  - Les cheveux : en tant qu'outils de bio surveillance non invasifs, ils ont été utilisés avec succès pour évaluer l'exposition chronique à différents pesticides. L'analyse d'un cm de cheveux permettrait d'avoir un « historique » de l'exposition sur un mois.
  - Le sang : il pourrait être aussi utilisé comme matrice pour mesurer les résidus de pesticides et leurs métabolites, permettant de surveiller les activités enzymatiques dans les organismes et les perturbations constatées en fonction de l'exposition aux résidus de pesticides , citons à titre d'exemple le dosage de l'acétyl-cholinestérase dans le sang des fleuristes qui sont exposées aux divers insecticides organophosphorées et carbamates (acéphate, diméthoate, pirimiphos-méthyl, profénofos, tolclofos-méthyl, chlorpyrifos-éthyl, iprovalicarbe, carbofuran, pirimicarbe, etc.) retrouvés dans nos analyses.
- Le biomonitoring pourrait être utilisé avec plusieurs marqueurs génétiques. Les tests, les plus souvent utilisés, pour détecter ces biomarqueurs, incluent :
  - Test d'Ames sur urines : il permet de déterminer le pouvoir mutagène d'une substance active en étudiant sa capacité à induire des mutations reverses sur des souches hypersensibilisées de *Salmonella typhimurium*.
  - Test des comètes sur lymphocytes sanguins : il permet la mesure du degré de lésions de l'ADN au sein d'une population cellulaire et non au niveau tissulaire.

- Test des micronoyaux ou la mesure des aberrations chromosomiques sur lymphocytes sanguins : il s'agit de déterminer les anomalies du caryotype sur des cellules eucaryotes, liées à l'exposition à des substances actives génotoxiques entraînant des cassures d'ADN.

En outre, plusieurs études scientifiques en amont ou en aval de la filière seraient également à explorer, même si ces sujets débordent largement du cadre de la présente thèse :

- Au niveau agronomique, une production de fleurs coupées plus durable serait une voie de recherche à développer. Remplacer progressivement les pesticides les plus toxiques par des méthodes alternatives ou des produits de biocontrôle, nécessite de vérifier non seulement leur efficacité mais aussi de s'assurer que les solutions biologiques n'engendrent pas à leur tour un risque inacceptable pour la santé ou pour l'environnement (les produits dits « biologiques » devront être évalués avec les mêmes critères que les composés chimiques). La première étape serait de concevoir des schémas de lutte intégrée en cultures florales pour réduire au strict nécessaire l'usage des produits phytopharmaceutiques. L'intérêt de cette voie de recherche serait non seulement de réduire les coûts de production, mais aussi de minimiser le risque de résistance, le risque pour les producteurs ou l'effet sur les auxiliaires.
- Au niveau environnemental, plusieurs questions pourraient être également abordées. Si les fleurs, feuillages et autres produits végétaux sont autant contaminés, comment s'en débarrasser valablement ? Le compostage est-il une solution acceptable vu la présence des résidus ? Seront-ils dégradés ? De même, les fleurs restent à tremper dans l'eau avant la réalisation des bouquets : ces eaux sont ensuite déversées à l'évier et évacuées par les égouts sans soucis de rejeter un effluent potentiellement contaminé, même si les concentrations dans ce cas seront bien inférieures à celles des fonds de cuve des pulvérisateurs

1.2. Recommandations pratiques :

Afin de limiter le plus possible les niveaux d'exposition aux résidus de pesticides, un certain nombre de règles, souvent simples et peu coûteuses, doivent être respectées. Il est important de s'assurer de ne pas s'exposer inutilement à ces produits, même si les effets ne se font pas ressentir immédiatement. L'habitude conduit souvent à la négligence. Il est donc important de rappeler régulièrement les règles élémentaires d'hygiène. Pour se prémunir du risque d'exposition aux résidus de pesticides, les solutions pratiques suivantes pourraient être recommandées aux fleuristes et/ou aux producteurs de fleurs belges :

- Il est fortement conseillé à l'URFB (Union royale des fleuristes belges) de mener une campagne de sensibilisation et de prévention au niveau national pour éveiller les consciences des fleuristes à ce risque potentiel auquel ils sont exposés.

- Sans attendre les résultats d'une étude plus complète, il faudrait recommander de porter des équipements de protection individuels appropriés (gants, tablier) en fonction de la tâche à accomplir et ventiler régulièrement les locaux.
- L'utilisation de la lutte intégrée contre les ennemis des cultures devrait être encouragée chez les producteurs de fleurs coupées. Plusieurs produits alternatifs efficaces apparaissent sur le marché, et leur nombre va croissant. Citons à titre d'exemple : l'huile essentielle d'orange douce utilisable contre les thrips sur rosiers, sur les cultures florales et sur les plantes vertes ; *Metarhizium anisopliae* s.F52 contre les aleurodes sur gerberas ; *Beauveria bassiana* s.ATCC 74040 contre les aleurodes sur les cultures florales et les plantes vertes ; *Beauveria bassiana* s.GHA contre les aleurodes et les thrips sur les rosiers et contre les aleurodes sur les cultures florales et les plantes vertes. Même dans les pays étrangers producteurs de fleurs, comme le Kenya, des méthodes alternatives sont déjà appliquées avec succès depuis plusieurs années (ex : contre les acariens *Tetranychus urticae*, utilisation de *Metarhizium* 78 ou d'acariens prédateurs tel que *Amblyseius andersoni* produits par Real IPM Company Ltd et utilisés chez Oserian Flowers Ltd au lac Naivasha, Kenya). Malgré leur coût, ces alternatives sont intéressantes étant donné l'apparition des résistances chez les bioagresseurs qui limitent fortement l'efficacité des molécules et appellent à encore plus de traitement par les producteurs !
- Comme pour les autres produits, il est probable qu'il existe une demande pour des fleurs coupées « bio ». Cette perspective devrait être investiguée car aujourd'hui l'offre est quasi-nulle et un marché de niche pourrait se développer.

En outre, il faudrait également s'intéresser à deux aspects particuliers qui cependant demanderont plus d'efforts et de moyens pour être réalisés :

- La mise en place d'un système de traçabilité harmonisé qui permettra le suivi électronique, rapide et efficace, des fleurs coupées entre les différents maillons de la chaîne d'approvisionnement afin :
    - de garantir une production de fleurs coupées dans le respect des Bonnes Pratiques Agricoles (notamment le respect des doses d'application et du nombre maximal d'applications) ;
    - de pouvoir vérifier que les produits phytopharmaceutiques employés sur les fleurs contiennent des substances actives approuvées en Europe ou dont les risques pour la santé sont acceptables.
    - de pouvoir identifier les fleurs coupées jugées non conformes et de pouvoir remonter aux producteurs et/ou aux importateurs en cas de problème (ex : dépassement des niveaux limites maximales admises en résidus).
- Enfin, le contrôle des résidus sur les fleurs coupées devrait être envisagé notamment lors de l'importation en Belgique pour éviter l'exposition des fleuristes à des quantités élevées de substances qui ne sont pas ou plus autorisées en Europe (la problématique d'une fixation d'une « Limite maximale en résidus » pour les fleurs coupées est aussi à soulever).

2. L'exposition des travailleurs horticoles tunisiens aux résidus de pesticides

En Tunisie, les pratiques culturales horticoles impliquent généralement l'utilisation de nombreux pesticides pour faire face aux problèmes créés par les insectes et acariens nuisibles, les maladies fongiques et la prolifération des mauvaises herbes. Il est bien connu que les utilisateurs peuvent être exposés à ces produits lors des activités de préparation de la bouillie ou de l'application et que des moyens de prévention doivent être mis en place pour assurer leur sécurité. Or, d'autres catégories de travailleurs peuvent être exposées lors de l'exécution de certaines tâches manuelles en contact avec les produits horticoles après l'application des produits phytopharmaceutiques. En effet, les travailleurs qui effectuent des activités de sarclage, d'effeuillage ou de récolte peuvent être exposés de façon significative lors d'un contact cutané avec les végétaux et les produits horticoles préalablement traités. L'évaluation de l'exposition cutanée des travailleurs horticoles aux pesticides est donc également nécessaire car les résidus auxquels ils sont exposés peuvent engendrer des effets néfastes pour la santé tels que des allergies cutanées, un risque présumé d'effets graves sur certains organes, ou des effets sur la fertilité ou le développement, suite à des expositions répétées ou à une exposition prolongée. Une exposition par inhalation peut également engendrer des effets sur les voies respiratoires.

Une enquête menée auprès des maraîchers tunisiens a démontré que les expositions conduisant à des intoxications peuvent résulter de mauvaises pratiques, d'un manque d'hygiène ou de négligences au travail. Un manque d'information sur la nature et la dangerosité des résidus présents sur les produits au moment de la récolte apparaît clairement durant les interviews des travailleurs. En cela, la situation des travailleurs horticoles en Tunisie se rapproche de celle des fleuristes en Belgique. Il était donc intéressant de tenter de réaliser une étude parallèle, en utilisant la même approche que pour les fleuristes (analyse des résidus sur les produits, analyse de gants portés durant le travail, calcul de l'exposition potentielle). Seule la bio surveillance n'a pu être réalisée faute de moyens d'analyse sur place.

L'analyse de paires de gants portés par des travailleurs durant les travaux agricoles de récolte de divers légumes ont permis de conclure que les travailleurs du Sahel tunisien semblent être exposés à des niveaux de résidus de produits phytopharmaceutiques (PPP) non négligeables. Il faut rappeler que, si les cas d'intoxications aigües des travailleurs par les PPP sont rarement signalés et documentés, ces produits peuvent avoir par contre des effets à long terme sur leur santé. Il est donc important qu'ils s'assurent de ne pas s'exposer inutilement à ces résidus de PPP, même si les effets ne se font pas ressentir immédiatement.

2.1. Perspectives scientifiques pour évaluer le risque en Tunisie :

De futurs travaux devraient être réalisés pour mieux documenter la problématique d'exposition des travailleurs tunisiens aux résidus de pesticides et de trouver des

solutions à ces problèmes. La réalisation de ces études demandera des efforts et moyens conséquents :

- Il serait intéressant de poursuivre les travaux d'évaluation du risque d'exposition des travailleurs horticoles aux résidus de pesticides, en les répétant (répétition dans l'espace et dans le temps), en les étendant à une plus grande population de travailleurs et en la menant dans d'autres régions agricoles de la Tunisie, pour d'autres tâches et pour d'autres produits récoltés. Ce travail devrait en effet être approfondi car le risque de trouver des résidus n'est pas le même pour tous les produits horticoles (ex : fraises, abricots, agrumes, aubergines, ...).
- Une enquête complémentaire devrait être menée à large échelle pour mesurer les délais réels de réentrée (en champs et en serres), pour observer les pratiques, le port des équipements de sécurité, les règles d'hygiène ainsi que le respect des « Bonnes Pratiques Agricoles » (respect des prescriptions de l'étiquetage).
- Comme pour les fleuristes, il serait intéressant de poursuivre les travaux d'évaluation du risque d'exposition des travailleurs horticoles aux résidus de pesticides en considérant également la voie d'exposition orale (transfert des mains aux bouches), car en Tunisie aussi l'enquête révèle que les travailleurs mangent, boivent et fument au cours du travail. De même que pour les fleuristes, il est aussi important d'envisager la voie d'exposition respiratoire car les serres, comme les magasins de fleuristes, représentent un milieu confiné et certaines substances actives sont très volatiles tel que le propamocarbe et l'ométhoate (Annexe 6). A la différence des magasins, la chaleur dans les serres peut être élevée favorisant la volatilisation des substances dans l'air.
- Plusieurs substances actives détectées sur les mains de travailleurs sont liposolubles (Annexe 6) et peuvent facilement pénétrer dans les organismes humains. Seule une approche par biomonitoring (analyse du sang, des urines, des cheveux, etc.) permettrait d'avoir une idée complète et réaliste de l'exposition systémique des travailleurs horticoles en Tunisie. Au moins, pour des raisons de facilité, la collecte et l'analyse des urines seraient prioritaires. Pour les organophosphorés et les carbamates, la mesure de l'inhibition des acétylcholinestérases (érythrocytaires) serait également aisée car des « kits » existent.
- Une étude épidémiologique permettrait d'évaluer les effets négatifs de ces expositions répétées sur la santé de travailleurs horticoles effectuant des tâches de réentrée. En parallèle il serait utile de tester l'efficacité et l'acceptabilité (ex : confort) par les travailleurs des vêtements et équipements de protection.
- Le développement d'un modèle de transfert des résidus de pesticides appliqués sur les produits horticoles pourrait être envisagé en étudiant la répartition (transfert par le xylème et/ou par le phloème) et le devenir (métabolisme et dégradation) des pesticides sur et dans les fruits et les légumes, afin de connaître les quantités résiduelles disponibles et délogeables. Cette étude pourrait être réalisée en utilisant différents pesticides marqués au carbone 14 sur le noyau phénolique.

- Enfin, vu le niveau des résidus mesurés sur les produits, un programme national de surveillance pourrait être conçu et réalisé afin de contrôler les résidus de pesticides (respect des LMR) sur tous les produits alimentaires consommés localement, en particulier les fruits et légumes.

2.2. Perspectives pratiques et recommandations pour la Tunisie :

En Tunisie, les travailleurs horticoles sont potentiellement exposés de façon non négligeable aux résidus de pesticides, avec des effets possibles sur leur santé. Ce constat soulève la nécessité de faire des efforts pour diminuer les risques d'exposition au maximum en raison des incertitudes liées à l'exposition à long terme à plusieurs produits simultanément. Dans ce sens, plusieurs recommandations peuvent être proposées pour atteindre cet objectif. Toutes les recommandations suivantes sont faisables et réalistes et ne demanderaient pas beaucoup des efforts, ni des moyens, sauf la traçabilité des produits horticoles :

- Une campagne de sensibilisation et de prévention devrait être menée en Tunisie au niveau national à l'instar de celles lancées dans certains pays pour éveiller les consciences des maraîchers, et des travailleurs agricoles en général, aux risques potentiels auquels ils sont exposés. Le respect d'un délai de réentrée entre l'application et le retour à des activités sur le site traité s'est avéré être une des mesures de prévention les plus efficaces pour minimiser les risques d'exposition cutanée des travailleurs aux pesticides. Ainsi, l'accès aux parcelles ayant été traitées avec des PPP devrait être interdit avant l'expiration du délai de réentrée. Des bandes striées, un fanion de couleur ou un panneau avertisseur devraient être mis en place pour éviter l'accès des travailleurs dans la zone récemment traitée.
- Il serait intéressant de renforcer la législation sur la production, la distribution et l'utilisation des PPP, ainsi que la gestion de leurs déchets.
- Comme pour les fleurs coupées, il serait intéressant mettre en place un système de traçabilité harmonisé qui permettrait un suivi des produits horticoles entre les différents maillons de la chaîne d'approvisionnement afin de garantir une production respectant les Bonnes Pratiques Agricoles.
- Le fait d'augmenter la dose n'augmente pas nécessairement l'efficacité du traitement mais accroît plutôt le risque d'exposition des opérateurs, des travailleurs et souvent des consommateurs. Sans oublier les conséquences pour l'environnement et les résistances. Par conséquent, il est important d'utiliser les PPP les moins toxiques, d'appliquer les produits conformément à leur homologation (usage, dose, stade de la culture, etc.) ainsi que les recommandations d'emploi (délai avant récolte, nombre maximum d'applications, réglage des appareils, etc.).
- Les producteurs tunisiens devraient être encouragés par l'Etat à adopter des techniques de lutte alternatives moins nocives et de bannir les PPP les plus nocifs ou toxiques (ex : taxation des PPP les plus toxiques ou interdiction ?). Ils doivent aussi apprendre à comprendre les étiquettes (notamment les

pictogrammes et les mentions de danger) pour pouvoir choisir le traitement le plus sécuritaire possible lorsqu'il n'existe pas d'alternative aux PPP.

- Un plan d'information et de formation aux bonnes pratiques pour les maraîchers, les entreprises et les distributeurs de PPP est fortement recommandé afin d'expliquer les mesures de précaution à prendre et les règles à adopter pour une meilleure gestion des PPP.
- Tous les équipements utilisés pour la préparation et/ou l'application de pesticides (contenant de mesure, balance, pulvérisateur, ...) doivent aussi faire l'objet d'une décontamination après l'utilisation.
- L'équipement de protection individuel sert de barrière contre l'exposition aux pesticides. Afin d'assurer la protection des différentes voies d'exposition (cutanée et respiratoire), il faut toujours prendre certaines mesures de précaution lors des travaux de réentrée en portant des EPI appropriés au degré et à la nature des risques et en fonction de la tâche à accomplir (gants imperméables offrant une bonne protection contre le PPP utilisé, des bottes de caoutchouc, un pantalon long et une chemise à manches longues. Idéalement, le port d'un survêtement préférablement imperméable devrait être préconisé).
- Il faudrait recommander de toujours se laver les mains et le visage après avoir manipulé des produits horticoles traités par des PPP, avant de manger, boire, fumer ou aller aux toilettes. À la fin d'une période de travail, il faudrait pouvoir prendre une douche et (re)mettre des vêtements propres. Pour ce faire l'Etat devrait vérifier que les entreprises mettent à disposition de l'eau pour se laver pendant et après le travail. Idéalement, une douche oculaire devrait même être facilement accessible sur le site de travail.

3. Pour conclure cette étude…

Comme dans de nombreuses situations professionnelles, l'absence d'information et de formation conduit à exposer les travailleurs à des risques non négligeables. Sans la démonstration effective d'un risque d'exposition, la plupart sont peu enclins à changer leurs habitudes car les effets sur la santé sont le plus souvent imperceptibles même si, en Belgique comme en Tunisie, une simple enquête auprès des groupes exposés permet de recueillir rapidement un certain nombre de plaintes sans qu'il soit possible d'établir, par cette méthode, un lien formel de causalité entre « exposition » et « effet sur la santé ». La première étape indispensable est de démontrer l'exposition. Restera ensuite aux toxicologues à déterminer quels effets ces expositions peuvent engendrer sur la santé de ces travailleurs.

Liste des productions scientifiques

Les productions scientifiques suivantes ont été réalisées durant cette thèse.

1. Publications

1) **Toumi K**., Vleminckx C., van Loco J. & Schiffers B., 2016. Pesticide residues on three cut flower species and potential exposure of florists in Belgium. *Int. J. Environ. Res. Public Health* 13(10), 943. **(Article publié)**

2) **Toumi K**., Vleminckx C., Van Loco J. & Schiffers B., 2016. A survey of pesticide residues in cut flowers from various countries. Commun. Agric. Appl. Biol. Sci. 81(3), 493–502. **(Article publié)**

3) **Toumi K**., Joly L., Vleminckx C. & Schiffers B., 2017. Risk assessment of florists exposed to pesticide residues through handling of flowers and preparing bouquets. Int. J. Environ. Res. Public Health 14(5), 526. (**Article publié**)

4) **Toumi K**., Joly L., Vleminckx C. & Schiffers B., 2017. Potential demal exposure of florists to fungicide residues on flowers and risk assessment. *Commun. Agric. Appl. Biol. Sci.* 82(2), 49-60. (**Article publié**)

5) **Toumi K**., Joly L., Tarchoun N., Souabni L., Bouaziz M., Vleminckx C. & Schiffers B., 2018. Risk assessment of Tunisian consumers and farm workers exposed to residues after pesticide application in chili peppers and tomatoes. *Tunis. J. Plant Prot. (in press)*. (**Article accepté**).

6) **Toumi K**., Joly L., Vleminckx C. & Schiffers B., 2018. Exposure of workers to pesticide residues during Re-entry activities: A Review. *Hum. Ecol. Risk Assess. Int. J.* (**Article accepté**).

7) **Toumi K**., Szternfeld P., Schiffers, B. & Joly L., 2018. Multi-residue quantification of pesticides in urine by liquid chromatography coupled to mass spectrometry (LC-MS/MS) for the evaluation of human exposure. *Int. J. Environ. Anal. Chem.* (**Article soumis**).

8) **Toumi K**., Joly L., Vleminckx C. & Schiffers B., 2018. Biological monitoring of exposure to pesticide residues among Belgian florists. *Hum. Ecol. Risk Assess. Int. J.* (**Article soumis**).

9) Toumi K., Joly L., Vleminckx C. & Schiffers B., 2018. Risk assessment of Belgian florists to pesticide residues. *Commun. Agric. Appl. Biol. Sci.* 83 (**Article en rédaction**).

2. Présentations orales

1) **Toumi K**., Vleminckx C., Van Loco J. & Schiffers B., 2016. A survey of pesticide residues in cut flowers from various countries. 68th International

Symposium on Crop Protection, Ghent University, Gand, Belgique, 17 mai 2016.

2) **Toumi K**., Joly L., Vleminckx C. & Schiffers B. Évaluation du risque d'exposition des travailleurs horticoles aux résidus de pesticides en Tunisie. Premier symposium maghrébin sur la protection intégrée des plantes (SYMPIP 2017), Sousse, Tunisie, du 30 octobre au 1er novembre 2017.

3) **Toumi K**., Joly L., Vleminckx C. & Schiffers B. Exposure of Belgian florists to pesticide residues during their professional activities. International Conference on Environmental Pollution, Risk Assesment and Remediation (ICEPRAR 2018), Mahdia, Tunisie, du 18 au 20 Avril 2018.

4) **Toumi K**., Joly L., Vleminckx C. & Schiffers B., 2018. Risk assessment of Belgian florists to pesticide residues. 70th International Symposium on Crop Protection, Ghent University, Gand, Belgique, 22 mai 2018.

3. Posters

1) **Toumi K**., Joly L., Vleminckx C. & Schiffers B. Dermal exposure of Belgian florists to insecticide residues. 22nd National Symposium for Applied Biological Sciences (NSABS), Leuven, Belgique, 07 Février 2017.

2) **Toumi K**., Joly L., Vleminckx C. & Schiffers B. Risk assessment of florists' Exposure to insecticide residues during normal professional tasks. SETAC Europe 27th Annual Meeting in Brussels, Bruxelles, Belgique, du 07 au 11 Mai 2017.

3) **Toumi K**., Joly L., Vleminckx C. & Schiffers B., 2017. Potential demal exposure of florists to fungicide residues on flowers and risk assessment. 69th International Symposium on Crop Protection, Ghent University, Gand, Belgique, 23 mai 2017.

4) **Toumi K**., Joly L., Vleminckx C. & Schiffers B. Évaluation du risque d'exposition des fleuristes belges aux résidus de pesticides. Premier symposium maghrébin sur la protection intégrée des plantes (SYMPIP 2017), Sousse, Tunisie, du 30 octobre au 1er novembre 2017.

5) **Toumi K**., Joly L., Vleminckx C. & Schiffers B. Assessment of exposure of workers to pesticide residues in tomato greenhouses. 23nd National Symposium for Applied Biological Sciences (NSABS), Bruxelles, Belgique, 08 Février 2018.

6) **Toumi K**., Joly L., Vleminckx C. & Schiffers B. Assessment of exposure to pesticide residues in Tunisian crop greenhouses. International Conference on Environmental Pollution, Risk Assesment and Remediation (ICEPRAR 2018), Mahdia, Tunisie, du 18 au 20 Avril 2018.

7) **Toumi K.,** Joly L., Vleminckx C. & Schiffers B. Risk assessment of exposure to pesticide residues with potential negative effects on the Belgian florists' health. 12th European Pesticide Residue Workshop 2018, Munich, Allemagne, du 22 au 25 Mai 2018.

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Annexes

Annexe 1 : Questionnaire confidentiel destiné aux fleuristes belges

Merci de remplir ce questionnaire qui sera traité de façon anonyme et confidentielle

1. Identification de l'enquêté

1.1. Localité……

1.2. Sexe:

□M □F

1.3. Âge _ _ _ ans

1.4. Votre catégorie professionnelle :

- Chef d'entreprise (indépendant) □ Oui □ Non
- Employé □ Oui □ Non

1.5. Depuis combien de temps, travaillez-vous comme / chez un fleuriste : _ _ _ ans

2. Temps de travail

2.1. Nombre d'heures de travail par jour : _ _ _ heures

2.2. Temps estimé de préparation des bouquets et la manipulation des fleurs :

- Période (Saison) : _ _ _ heures/jour
- Période (Hors saison) : _ _ _ heures/jour

2.3. Nombre de jours de travail par semaine : _ _ _ jours/Semaine

2.4. D'autres personnes travaillent-ils avec vous ? Si Oui, donner les informations ci-après, les concernant :

□ Oui □ Non

Si oui, Nombre : _ _ _ personne(s)

3. Protection et problème d'exposition :

3.1. Utilisez-vous des produits phytopharmaceutiques (pesticides) pour protéger ou conserver les fleurs ?

□ Oui □ Non

Si oui, Nom du produit commercial :

……

3.2. Utilisez-vous des équipements de protection individuelle lors de la manipulation des fleurs et la construction des bouquets ?

- Des vêtements spéciaux □ Oui □ Non
- Un tablier □ Oui □ Non
- Des gants □ Oui □ Non

Autres réponses :….....

3.3. Mesures d'hygiène : Après la manipulation des fleurs procédez-vous :

- Au lavage des mains □ Oui □ Non

Si oui, nombre de fois par jour : _ _

- Au lavage des mains et des bras □ Oui □ Non

Si oui, nombre de fois par jour : _ _

- Au lavage des mains, des bras et du visage □ Oui □ Non

Si oui, nombre de fois par jour : _ _

A la toilette complète de tout le corps (douche) □ Oui □ Non

Si oui, nombre de fois par jour : _ _

3.4. Au cours de travail, avez-vous l'habitude de ?

Manger □ Oui □ Non

Boire □ Oui □ Non

Fumer □ Oui □ Non

3.5. Avez-vous vous eu un ennui de santé à la suite de manipulation des fleurs ?

□ Oui □ Non

Si Oui, en quelle(s) année(s): …….

3.6. Cela a-t-il entraîné :

- Aucun □ Oui □ Non
- Une Consultation Médicale □ Oui □ Non
- Un Traitement Médical □ Oui □ Non
- Une Hospitalisation □ Oui □ Non

Description d'état : (dans l'espace qui suit)

……

……

……

3.7. Problèmes de santé aigus : Avez-vous eu des problèmes de santé suite à la manipulation des fleurs ? (Cochez plusieurs cases si nécessaire)

- Problèmes oculaires □ Oui □ Non

- Problèmes respiratoires □ Oui □ Non
- Irritations et démangeaisons □ Oui □ Non
- Nausées □ Oui □ Non
- Maux de tête □ Oui □ Non
- Fatigue répétée □ Oui □ Non
- Fièvres □ Oui □ Non
- Saignements de nez □ Oui □ Non
- Autre □ Oui □ Non

3.8. Souffrez – vous d'une des pathogènes suivantes ? (Cochez plusieurs cases si nécessaire)

- Effets aigus pré-cités qui durent □ Oui □ Non
- Allergies □ Oui □ Non
- Cancer □ Oui □ Non
- Problèmes thyroïdiens □ Oui □ Non
- Maladies neuro-dégénératives □ Oui □ Non
- Problèmes pulmonaires □ Oui □ Non
- Autre □ Oui □ Non

4. Informations concernant les fleurs

4.1. Quelle est votre source d'approvisionnement en fleurs :

- Grossiste □ Oui □ Non
- Détaillant □ Oui □ Non
- Organisme □ Oui □ Non

Autres .....

4.2. Les variétés de fleurs manipulées les plus vendues (ordre de 1 à 3)

Rose

Chrysanthème

Œillet

Gerbera

Lys

Tulipe

Orchidées

Autre :……

4.3. Origine des fleurs

Origine des fleurs	Rose	Œillets	Chrysanthème	Gerbera	Tulipe	Lys	Orchidée
Belgique							
Kenya							
Colombie							
Hollande							
Israël							
Autre :							

5. Connaissance concernant les résidus de pesticides

5.1. Avez-vous reçu des informations sur les résidus de pesticides dans les fleurs ?

- Par les médias (télévision, revues, internet, etc.) □ Oui □ Non
- par un professionnel de santé (médecin, etc.) □ Oui □ Non
- Aucune information reçue □ Oui □ Non

5.2. Pensez-vous être suffisamment informée sur les résidus de pesticides dans les fleurs ?

□ Oui □ Pas suffisamment

6. Vos suggestions/recommandations en rapport avec ce sujet

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……

Pour toute question relative à cette enquête, contactez :
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Annexe 2 : Propriétés physico-chimiques et toxicologiques de substances actives et métabolites détectés sur les échantillons de fleurs coupées et/ou les gants en coton portés par les fleuristes durant la manipulation des fleurs et la préparation des bouquets

Active substances	Flowers	Gloves	Molecular mass (g mol^{-1})*	[2]Vapour pressure at 25°c (mPa)*	[1]Log Kow (Log P) *	CLP classification**
6-benzyladenine	X					-
Acephate	X	X	183.17	0.226	-0.85	H302
Acetamiprid	X	X	222.67	1.73×10^{-04}	0.8	H302
Acrinathrin	X	X	541.44	4.40×10^{-05}	6.3	-
Ametoctradin	X	X	275.39	2.1×10^{-07}	4.4	-
Azadirachtin	X	X				-
Azoxystrobin	X	X	403.4	1.10×10^{-07}	2.5	H331
Benalaxyl	X		325.40	0.572	3.54	-
Benomyl	X	X	290.32	0.005	1.4	H315, H317, H335, H340, H360FD
Bifenazate	X	X	300.35	1.33×10^{-02}	3.4	H317, H373
Bifenthrin	X	X	422.88	0.0178	6.6	H300, H317, H331, H351, H372
Bitertanol	X	X	337.42	1.36×10^{-06}	4.1	-
Boscalid	X	X	343.21	0.00072	2.96	-
Bupirimate	X	X	316.42	0.057	3.68	H317, H351
Buprofezin	X	X	305.44	0.042	4.93	-
Captan		X	300.61	0.0042	2.5	H317, H318, H331, H351
Carbendazim	X	X	191.21	0.09	1.48	H340, H360FD
Carbofuran		X	221.26	0.08	1.8	H300, H330
Carbosulfan	X		380.5	0.0359	7.42	H301, H317, H330
Carboxin	X		235.30	0.02	2.3	-
Chlorantraniliprole	X	X	483.15	6.3×10^{-09}	2.86	-

Active substances	Flowers	Gloves	Molecular mass ($g\ mol^{-1}$)*	[2]Vapour pressure at 25°c (mPa)*	[1]Log Kow (Log P) *	CLP classification**
Chlorfenapyr	X		407.62	9.81×10^{-03}	4.83	H302, H331
Chloridazon	X		221.6	1.0×10^{-06}	1.19	H317
Chlorothalonil	X	X	265.91	0.076	2.94	H317, H318, H330, H335, H351
Chlorpyrifos	X	X	350.58	1.43	4.7	H301
Clofentezine	X	X	303.15	1.40×10^{-03}	3.1	-
Cyflufenamid	X		412.36	0.0354	4.7	-
Cyflumetofen		X	447.45	0.0059	4.3	-
Cyfluthrin	X		434.29	0.0003	6	H300, H331
Cyhalothrin	X	X	449.85	1.00×10^{-09}	6.8	-
Cypermethrin	X	X	416.3	6.78×10^{-03}	5.55	H302, H332, H335
Cyproconazole		X	291.78	0.026	3.09	H301, H360D, H373
Cyprodinil	X	X	225.29	5.10×10^{-01}	4	H317
Deet		X				-
Deltamethrin	X	X	505.2	0.0000124	4.6	H301, H331
Diazinon	X		304.35	11.97	3.69	H302
Dicofol	X	X	370.49	0.25	4.3	H302, H312, H315, H317
Difenoconazole	X	X	406.26	3.33 X 10-05	4.36	-
Diflubenzuron		X	310.68	0.00012	3.89	-
Dimethoate	X	X	229.26	0.247	0.75	H302, H312
Dimethomorph	X	X	387.86	9.85×10^{-04}	2.68	-
Dinotefuran	X		202.21	0.0017	-0.549	-
Diphenylamine		X	169.23	0.852	3.82	H315, H317
Dodemorph	X	X	281.48	0.48	4.6	H314, H317, H361d, H373
Endosulfan		X	406.93	0.83	4.75	H300, H312, H330
Ethirimol	X		209.29	0.267	2.3	H312
Etoxazole	X	X	359.42	0.007	5.52	-

Active substances	Flowers	Gloves	Molecular mass (g mol^{-1})*	[2]Vapour pressure at 25°c (mPa)*	[1]Log Kow (Log P) *	CLP classification**
Etridiazole	X		247.53	1430	3.37	H302, H317, H351
Famoxadone	X	X	374.39	0.00064	4.65	H373
Fenamidone	X	X	311.40	0.00034	2.8	-
Fenamiphos	X		303.36	0.067	3.3	H300, H310, H319, H330
Fenarimol	X		331.20	0.065	3.69	H361fd, H362
Fenazaquin		X	306.40	1.90×10^{-02}	5.51	H301, H332
Fenhexamid	X	X	302.20	4.00×10^{-04}	3.51	-
Fenoxycarb		X	301.34	8.67×10^{-04}	4.07	H351
Fenpropathrin	X		349.42	0.76	6.04	H301, H312, H330
Fenpropidin	X		273.46	17.0	2.6	-
Fenpyroximate		X	421.49	0.01	5.01	H301, H317, H330
Fensulfothion-oxon	X		-	-	-	-
Fenvalerate	X	X	419.90	0.0192	5.01	-
Fipronil	X	X	437.15	0.002	3.75	H301, H311, H331, H372
Flonicamid	X	X	229.16	9.43×10^{-04}	-0.24	H302
Fluazinam		X	465.14	7.5	4.03	H317, H318, H332, H361d
Flubendiamide	X	X	682.39	0.1	4.14	-
Fludioxonil	X	X	248.19	3.90×10^{-04}	4.12	-
Flufenoxuron	X	X	488.77	6.52×10^{-09}	5.11	H362
Fluopicolide	X	X	383.58	3.03×10^{-04}	2.9	-
Fluopyram	X	X	396.76	1.2×10^{-03}	3.3	-
Fluoxastrobin		X	458.83	5.60×10^{-07}	2.86	-
Flusilazole		X	315.39	0.0387	3.87	H302, H351, H360D
Flutolanil		X	323.31	4.10×10^{-04}	3.17	-
Flutriafol		X	301.29	4.0×10^{-04}	2.3	-
Fluxapyroxad		X	381.31	2.7×10^{-06}	3.13	-
Forchlorfenuron	X		247.68	4.60×10^{-05}	3.3	H351

Active substances	Flowers	Gloves	Molecular mass (g mol^{-1})*	[2]Vapour pressure at 25°c (mPa)*	[1]Log Kow (Log P) *	CLP classification**
Fosthiazate	X		283.35	0.56	1.68	H301, H312, H317, H331
Furalaxyl	X		301.34	0.07	2.7	H302
Hexythiazox	X	X	352.88	1.33 X 10^{-03}	2.67	-
Imidacloprid	X	X	255.66	4.0 X 10^{-07}	0.57	H302
Indoxacarb	X	X	527.83	0.006	4.65	H301, H317, H332, H372
Iprodione	X	X	330.17	0.0005	3.0	H351
Iprovalicarb	X	X	320.43	7.90 X 10^{-05}	3.2	-
Isocarbophos	X		289.29	-	2.7	-
Kresoxim-methyl	X	X	313.35	2.30 X 10^{-03}	3.4	H351
Lufenuron	X	X	511.16	4.00 X 10^{-03}	5.12	H317
Malathion		X	330.36	3.1	2.75	H302, H317
Mandipropamid	X	X	411.9	9.40 X 10^{-04}	3.2	-
Mepanipyrim	X	X	223.27	0.0232	3.28	H351
Metalaxyl	X	X	279.33	0.75	1.75	H302, H317
Metalaxyl-M	X	X	279.33	3.3	1.71	H302, H318
Methamidophos	X		141.13	2.3	-0.79	H300, H311, H330
Methiocarb	X	X	225.31	1.50 X 10^{-02}	3.18	H301
Methomyl	X		162.21	0.72	0.09	H300
Methoxyfenozide	X	X	368.47	1.33 X 10^{-02}	3.72	-
Metrafenone	X	X	409.3	0.153	4.3	-
Myclobutanil	X	X	288.78	0.198	2.89	H302, H319, H361d
Nitrothal-isopropyl		X	295.29	0.01	2.04	-
Novaluron	X	X	492.70	1.60 X 10^{-02}	4.3	-
Omethoate	X	X	213.2	19.0	-0.9	H301, H312
Oxadixyl	X		278.3	0.0033	0.65	-
Oxamyl	X		219.26	0.051	-0.44	H300, H312, H330
Oxycarboxin	X	X	267.31	5.60 X 10^{-03}	0.772	H302
Paclobutrazol	X	X	293.8	0.0019	3.11	-

Active substances	Flowers	Gloves	Molecular mass (g mol^{-1})*	[2]Vapour pressure at 25°c (mPa)*	[1]Log Kow (Log P) *	CLP classification**
Penconazole		X	284.18	0.366	3.72	H302, H361d
Permethrin		X	391.3	0.007	6.1	H302, H332, H335
Picoxystrobin	X	X	367.32	0.0055	3.6	-
Piperonyl butoxide	X	X				-
Pirimicarb	X	X	238.39	0.43	1.7	H301, H317, H331, H351
Pirimiphos-methyl		X	305.33	2.00 X 10^{-03}	3.9	H302
Prochloraz	X	X	376.7	0.15	3.5	H302
Procymidone	X	X	284.14	0.023	3.3	-
Profenofos		X	373.63	2.53	1.7	H302, H312, H332
Propamocarb	X	X	188.3	730	0.84	-
Propiconazole		X	342.22	0.056	3.72	H302, H317
Propoxur			209.24	1.3	0.14	H301
Pymetrozine	X	X	217.23	4.20 X 10^{-03}	-0.19	H351
Pyraclostrobin	X	X	387.8	2.60 X 10^{-05}	3.99	H315, H331
Pyridaben	X	X	364.93	0.001	6.37	H301, H331
Pyridalyl	X	X	491.12	6.24 X 10-05	8.1	-
Pyrimethanil	X	X	199.11	1.1	2.84	-
Pyriproxyfen		X	321.37	1.33 X 10-02	5.37	-
Quinalphos	X		298.3	0.346	4.44	H301, H312
Simazine		X	201.66	0.00081	2.3	H351
Spinetoram	X	X	754.0	5.7 X 10^{-02}	4.2	-
Spinosad	X	X	-	-	-	-
Spirodiclofen		X	411.32	3.00 X 10^{-04}	5.83	-
Spiromesifen		X	370.48	7.00 X 10^{-03}	4.55	-
Spirotetramat	X	X	373.48	5.6 X 10^{-06}	2.51	H317, H319, H335, H361fd
Spiroxamine	X	X	297.5	3.5	2.89	H302, H312, H315, H317, H332, H361d, H373

Active substances	Flowers	Gloves	Molecular mass (g mol^{-1})*	[2]Vapour pressure at 25°c (mPa)*	[1]Log Kow (Log P) *	CLP classification**
Tebuconazole	X	X	307.82	1.30 X 10^{-03}	3.7	H302, H361d
Tebufenozide		X	352.47	1.56 X 10^{-04}	4.25	-
Tebufenpyrad		X	333.8	0.0016	4.93	H301, H317, H332, H373
Tetraconazole		X	372.15	0.18	3.56	H302, H332
Tetradifon	X		356.06	3.20 X 10^{-05}	4.61	-
Tetrahydrophtalimide		X	-	-	-	-
Tetramethrin		X	331.41	2.1	4.6	-
Thiabendazole	X	X	201.25	5.30 X 10^{-04}	2.39	-
Thiacloprid	X	X	252.72	3.00 X 10^{-07}	1.26	H301, H332, H336, H351, H360FD
Thiamethoxam	X	X	291.71	6.60 X 10^{-06}	-0.13	H302
Thiodicarb	X		354.47	2.7	1.62	-
Thiophanate methyl	X	X	342.39	9.0 X 10^{-03}	1.40	H317, H332, H341
Tolclofos-methyl	X	X	301.13	0.877	4.56	H317
Triadimenol		X	295.76	0.0005	3.18	H302, H360, H362
Triadimefon		X	293.8	0.02	3.18	H302, H317
Trichlorfon	X		257.4	0.21	0.43	H302, H317
Trifloxystrobin	X	X	408.37	3.40 X 10^{-03}	4.5	H317
Triflumizole	X	X	345.75	0.191	4.77	H302, H317, H360D, H373
Triforine	X		434.96	26	2.4	-

H300: Fatal if swallowed; **H301:** Toxic if swallowed; **H302:** Harmful if swallowed; **H310:** Fatal in contact with skin; **H311:** Toxic in contact with skin; **H312:** Harmful in contact with skin; **H314:** Causes severe skin burns and eye damage; **H315:** Causes skin irritation; **H317:** May cause an allergic skin reaction; **H318:** Causes serious eye damage; **H319:** Causes serious eye irritation; **H330:** Fatal if inhaled; **H331:** Toxic if inhaled, **H332:** Harmful if inhaled; **H335:** May cause respiratory irritation; **H336:** May cause drowsiness or dizziness; **H340:** May cause genetic defects; **H341:** Suspected of causing genetic defects; **H351:** Suspected of causing cancer; **H360:** May damage fertility or the unborn child; **H360D:** May damage the unborn child; **H360FD:** May damage fertility. May damage the unborn child; **H361d:** suspected of damaging the unborn child; **H361fd:** suspected of damaging fertility. Suspected of damaging the unborn child; H362: May cause harm to breast-fed children;

H372: Causes damage to organs through prolonged or repeated exposure; **H373:** May cause damage to organs through prolonged or repeated exposure

* Classification according The PPDB - Pesticides Properties DataBase
** CLP classification according the EU Pesticides database

[1]**Volatility (mPa)** (EFSA, 2014)
< 5.0 mPa = low volatility,
5.0 – 10.0 mPa = moderately volatile,
> 10 mPa = highly volatile

[2] **Octanol-water Partition Coefficient (Log P)** (PPDB - Pesticides Properties DataBase, 2018)
< 2.7 = Low bioaccumulation
2.7 – 3 = Moderate
> 3.0 = High

Annexe 3 : Données relatives aux groupes de référence pour les 3 périodes de vente importantes en Belgique (Concentrations totales en mg/kg et nombre total des résidus (pesticides et métabolites) détectés par échantillon d'urine)

	Valentine's Day		Mother 's Day		All Saints' Day	
Samples	**[C]**	**N**	**[C]**	**N**	**[C]**	**N**
1	11.56	8	0.20	5	0.48	7
2	0.13	3	0.06	1	0.23	4
3	0.02	3	0.50	6	0.29	3
4	0.45	8	0.16	3	0.69	3
5	15.34	6	0.11	4	0.16	1
6	5.54	6	6.66	3	0.19	4
7	0.40	6	0.30	7	0.18	3
8	6.30	5	0.08	1	8.42	4
9	0.14	2	0.63	5	0.29	5
10	0.29	2	0.00	0	0.19	2
11	0.19	6	0.00	0	1.90	3
12	0.85	2	0.16	1	7.59	5
13	12.38	9	0.19	4	1.05	9
14	0.38	3	0.23	2	0.17	4
Mean	**3.86**	**5**	**0.66**	**3**	**1.56**	**4**
Median	**0.42**	**6**	**0.18**	**3**	**0.29**	**4**
Range	**(0.02-15.34)**	**(2-9)**	**(0.00-6.66))**	**(0-7)**	**(0.16-8.42)**	**(1-9)**

Annexe 4 : Résidus (pesticides et métabolites) présents dans les échantillons d'urine des fleuristes et du groupe témoin, et leur fréquence de détection

Pesticide residues and metabolites	Frequency of detection in 42 urine samples of control group	Frequency of detection in 42 urine samples of florists
Pirimicarb	17%	64%
Clofentezin	0%	55%
Acetamiprid-n-desmethyl	29%	33%
Cyproconazol	5%	29%
Azoxystrobin	10%	29%
Tcpy	0%	29%
Pirimicarb-desmethyl	14%	26%
Fenhexamid	7%	24%
Dimethomorph	10%	24%
Metrafenone	0%	21%
Fipronil	17%	19%
Prochloraz	0%	19%
Novaluron	2%	17%
Oxamyl	17%	17%
Flufenoxuron	0%	17%
Diflubenzuron	7%	14%
Fenpyroximate	12%	14%
Boscalid	0%	14%
Bupirimate	0%	14%
Fosthiazate	0%	14%
Spinosad	0%	14%
Cyprodinil	2%	12%
Spiroxamine	2%	12%
Ametoctradin	12%	12%
Buprofezin	0%	12%
Pyridaben	0%	12%
Thiabendazole	2%	10%
Acetamiprid	7%	10%
Mandipropamid	7%	10%
Pyraclostrobin	50%	10%

Pesticide residues and metabolites	Frequency of detection in 42 urine samples of control group	Frequency of detection in 42 urine samples of florists
2ctca	0%	10%
Carbendazim	0%	10%
3-Hydroxy-carbofuran	0%	10%
Fenpropidin	0%	10%
Indoxacarb	0%	10%
Methiocarb sulfoxid	0%	10%
Piperonil-butoxide	0%	10%
Tebufenpyrad	5%	7%
Difenconazole	0%	7%
Fenamiphos sulfone	0%	7%
Fluopyram	0%	7%
Furalaxyl	0%	7%
Methamidophos	0%	7%
Pyrimethanil	0%	7%
Hexythiazox	2%	5%
Metalaxyl	2%	5%
Spirotetramat	2%	5%
Methoxyfenozide	7%	5%
Flutolanil	12%	5%
Fenoxycarb	24%	5%
Carbofuran	0%	5%
Detp	0%	5%
Flutriafol	0%	5%
Isocarbophos	0%	5%
Methiocarb	0%	5%
Methomyl	0%	5%
Spirotetramat-enol-glucoside	0%	5%
Cyflumetofen	10%	2%
Spirotetramat-enol	10%	2%
Fipronil sulfone	40%	2%
Chlorantraniliprole	0%	2%
Dinotefuran	0%	2%
Dmp	0%	2%
Famoxadone	0%	2%
Flonicamid	0%	2%

Pesticide residues and metabolites	Frequency of detection in 42 urine samples of control group	Frequency of detection in 42 urine samples of florists
Flubendiamide	0%	2%
Imidacloprid	0%	2%
Methiocarb sulfon	0%	2%
Quinalphos	0%	2%
Spirodiclofen	0%	2%
Dep	2%	0%
Dodemorphe	2%	0%
Fludioxonil	2%	0%
Iprovalicarb	2%	0%
Mepamipyrim	2%	0%
Paclobutrazole	2%	0%
Spirotetramat-ketohydroxy	2%	0%
Pyriproxyfen	5%	0%
Bitertanol	7%	0%
Thiacloprid	7%	0%
Tebuconazol	10%	0%
Fipronil desulfinyl	12%	0%

Annexe 5 : Questionnaire confidentiel destiné aux maraîchers tunisiens

Merci de remplir ce questionnaire qui sera traité de façon confidentielle

1. Identification de l'enquêté

1.1. Nom et Prénom :….....

1.2. Sexe :

□M □F

1.3. Âge _ _ _ ans

1.4. Catégorie générale des employeurs

- Propriétaire de la parcelle □ Oui □ Non
- Main d'œuvre salarial □ Oui □ Non
- Membre de la famille □ Oui □ Non

1.5. Catégorie professionnelle *selon* les *tâches* effectuées : (Choix multiple)

- Travailleur (worker) □ Oui □ Non

Si oui, merci de remplir ''Partie A''

- Applicateur (operator) □ Oui □ Non

Si oui, merci de remplir ''Partie B''

Si non, qui effectue les traitements ?

- Un membre de la famille □ Oui □ Non
- Un prestataire □ Oui □ Non

1.6. Depuis combien de temps, travaille-t-il comme / chez un horticulteur :

_ _ _ ans

1.7. Niveau d'instruction : Aucun /_ _/ Primaire /_ _/ Secondaire /_ _/Étude supérieur agricole /_ _/ Étude supérieur non agricole /_ _/

2. Temps de travail

2.1. Nombre d'heures de travail par jour : _ _ _ heures

2.2. Temps estimé au contact des cultures : temps estimé de manipulations des produits horticoles traités au moment de la réentrée (récolte, taille, inspection, etc.) ?

Période : _ _ _ heures/jour

2.3. Nombre de jours de travail par semaine : _ _ _ jours/Semaine

2.4. D'autres personnes travaillent-ils avec l'enquêté ?

□ Oui □ Non

Si oui, Nombre : _ _ _ personne(s)

3. Protection d'exposition

Partie A : À l'intention des travailleurs (workers)

3.1. Habitude tabagique :

□ Fumeur □ Non-fumeur

3.2. Au cours de travail (réentrée), a-t-il l'habitude de ?

- Manger □ Oui □ Non
- Boire □ Oui □ Non
- Fumer □ Oui □ Non

3.3. Après un traitement, au bout de combien de temps, retourne-t-il aux parcelles ?

- Immédiatement après le traitement □ Oui □ Non
- Quelques heures après le traitement □ Oui □ Non
- 24h après le traitement □ Oui □ Non
- Semaine après le traitement □ Oui □ Non
- Autre à préciser……..

3.4. Quel type de vêtement porte-t-il pour effectuer ses tâches ?

- Vêtements spéciaux □ Oui □ Non

- Tablier □ Oui □ Non
- Chemise ou chandail à manches longues □ Oui □ Non
- Chemise ou chandail à manches Courtes □ Oui □ Non
- Pantalons à jambes longues □ Oui □ Non
- Pantalons à jambes courtes □ Oui □ Non
- Gants □ Oui □ Non

Types de gants …….

- Autres vêtements de protection individuelle □ Oui □ Non

Lesquels :……...

3.5. Quand enlève-t-il ses vêtements de travail (réentrée) ?

- Sur le site de travail en fin de journée □ Oui □ Non
- Immédiatement en revenant à la maison □ Oui □ Non
- À la maison en fin de soirée □ Oui □ Non

3.6. Mesures d'hygiène prises après la réentrée (taille, récolte, inspection, etc.) :

- Lavage des mains □ Oui □ Non

Si oui, nombre de fois par jour : _ _

- Lavage des mains et des bras □ Oui □ Non

Si oui, nombre de fois par jour : _ _

- Lavage des mains, des bras et du visage □ Oui □ Non

Si oui, nombre de fois par jour : _ _

- Toilette complète de tout le corps (douche) □ Oui □ Non

Si oui, nombre de fois par jour : _ _

Partie B : À l'intention des applicateurs de pesticides (operators)

3.7. Habitude tabagique :

□ Fumeur □ Non-fumeur

3.8. Au cours de travail (application des pesticides), a-t-il l'habitude de ?

- Manger □ Oui □ Non
- Boire □ Oui □ Non
- Fumer □ Oui □ Non

3.9. Au cours de la période de travail (application des pesticides), a-t-il porté les équipements de protection individuelle suivants ? Cochez les cases appropriées

Équipements	Travail Effectué		
	Préparation de la bouillie	Application du produit phytosanitaire	Nettoyage du matériel de pulvérisation
Lunettes de protection			
Masque à poussière			
Masque à filtre			
Combinaison blanche			
Salopette			
Gants			
Bottes			
Autres			

3.10. Quand enlève-t-il ses vêtements de travail (application des pesticides) ?

- Sur le site de travail en fin de journée □ Oui □ Non
- Immédiatement en revenant à la maison □ Oui □ Non
- À la maison en fin de soirée □ Oui □ Non

3.11. Mesures d'hygiène prises après l'application des pesticides :

- Lavage des mains □ Oui □ Non
- Lavage des mains et des bras □ Oui □ Non
- Lavage des mains, des bras et du visage □ Oui □ Non
- Toilette complète de tout le corps (douche) □ Oui □ Non

3.12. Après l'application des pesticides, respecte-t-il le délai avant récolte ?

□ Oui □ Non

Si non, après combien de jours fait-il en moyenne la récolte ? _ _ _ jours

3.10. A-t-il l'habitude de lire les étiquettes sur les emballages de pesticides ?

□ Oui □ Non

3.13. Comprend-t-il les instructions d'utilisation ?

□ Oui □ Non

3.14. Est-ce que l'applicateur connaisse les doses de chaque pesticide qu'il utilise ?

□ Oui □ Non

Si non, comment décide-t-il la dose correcte à utiliser ?

- Conseil d'un fournisseur □ Oui □ Non
- Conseil d'un agent d'agriculture □ Oui □ Non
- Expérience □ Oui □ Non
- Autres fermiers □ Oui □ Non
- Autre à préciser……

4. Gestion des pesticides

4.1. Quelles sont les périodes de l'année où il traite ? (Choix multiple)

Janvier /_ _/ Février /_ _/ Mars/_ _/Avril /_ _/ Mai /_ _/Juin/_ _/ Juillet /_ _/ Aout /_ _/ Septembre/_ _/Octobre/_ _/ Novembre /_ _/ Décembre /_ _/

4.2. Il fait des traitements :

- Préventifs □ Oui □ Non
- Curatifs □ Oui □ Non

4.3. Quels sont les critères de choix des produits à l'achat ?

- Efficacité □ Oui □ Non
- Sélectivité □ Oui □ Non
- Facilité d'emploi □ Oui □ Non

- Prix □ Oui □ Non
- Toxicité □ Oui □ Non
- Risque environnemental □ Oui □ Non

Autres (à préciser)……..

4.4. Pesticides utilisés

Produit(s)utilisé(s) (Nom commercial)	Type de formulation	Dose/ha	Appareillage utilisé (Pulvérisateur à dos /tracté)

4.5. Où sont stockés les produits phytosanitaires ?

- Local spécifique □ Oui □ Non
- Armoire spécifique □ Oui □ Non
- Local technique □ Oui □ Non

Autres (à préciser)……

4.6. Quelle est la source d'approvisionnement ?

- Marchés locaux □ Oui □ Non
- Marchés extérieurs □ Oui □ Non
- Commerçants agréés □ Oui □ Non
- Autres producteurs □ Oui □ Non

- Agent de vulgarisation □ Oui □ Non
- Recherche □ Oui □ Non

Autres (à préciser)……..

5. Production horticole

5.1. Principaux produits horticoles cultivés

1……	4…….	7……
Superficie _ _ _ _	Superficie _ _ _ _	Superficie _ _ _ _
2……	5…….	8……
Superficie _ _ _ _	Superficie _ _ _ _	Superficie _ _ _ _
3……	6…….	9……
Superficie _ _ _ _	Superficie _ _ _ _	Superficie _ _ _ _

5.2. Les produits horticoles sont cultivés :

- Sous abris □ Oui □ Non
- Abri-serres □ Oui □ Non
- Sous serres □ Oui □ Non
- Serres multi-tunnels □ Oui □ Non
- Petits tunnels □ Oui □ Non

Autres (à préciser)…….

6. Problème d'exposition

Partie A : À l'intention des travailleurs (workers)

6.1. A-t-il eu un ennui de santé à la suite de manipulation des produits horticoles ?

□ Oui □ Non

Si Oui, en quelle(s) année(s): ……...

Cela a-t-il entraîné :

- Aucun □ Oui □ Non
- Consultation médicale □ Oui □ Non
- Traitement médical □ Oui □ Non
- Hospitalisation □ Oui □ Non

Description d'état : (dans l'espace qui suit)

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……

6.2. Au cours ou après les travaux (réentrée), a-t-il ressenti certains de ces symptômes ? (Cochez plusieurs cases si nécessaire)

- Problèmes oculaires □ Oui □ Non
- Problèmes respiratoires □ Oui □ Non
- Irritations et démangeaisons □ Oui □ Non
- Irritation de la peau □ Oui □ Non
- Irritation des yeux □ Oui □ Non
- Assèchement de la peau □ Oui □ Non
- Nausées □ Oui □ Non
- Maux de tête □ Oui □ Non
- Fatigue répétée □ Oui □ Non
- Fièvres □ Oui □ Non
- Étourdissements □ Oui □ Non
- Transpiration □ Oui □ Non
- Saignements de nez □ Oui □ Non
- Crampes d'estomac □ Oui □ Non
- Diarrhée □ Oui □ Non
- Perte d'appétit □ Oui □ Non

- Autre □ Oui □ Non

Lesquels:

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6.3. Souffre-t-il d'une des pathologies suivantes ? (Cochez plusieurs cases si nécessaire)

- Effets aigus pré-cités qui durent □ Oui □ Non
- Allergies □ Oui □ Non
- Cancer □ Oui □ Non
- Problèmes thyroïdiens □ Oui □ Non
- Maladies neuro-dégénératives □ Oui □ Non
- Problèmes pulmonaires □ Oui □ Non
- Autre □ Oui □ Non

  Lesquels :

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Partie B : À l'intention des applicateurs de pesticides (operators)

6.4. A-t-il eu un ennui de santé à la suite de l'application des produits phytosanitaires ?

□ Oui □ Non

Cela a-t-il entraîné :

- Aucun □ Oui □ Non

- Consultation médicale □ Oui □ Non
- Traitement médical □ Oui □ Non
- Hospitalisation □ Oui □ Non

Description d'état : (dans l'espace qui suit)

……

……

……

6.5. Au cours ou après les travaux (réentrée), a-t-il ressenti certains de ces symptômes ? (Cochez plusieurs cases si nécessaire)

- Problèmes oculaires □ Oui □ Non
- Problèmes respiratoires □ Oui □ Non
- Irritations et démangeaisons □ Oui □ Non
- Irritation de la peau □ Oui □ Non
- Irritation des yeux □ Oui □ Non
- Assèchement de la peau □ Oui □ Non
- Nausées □ Oui □ Non
- Maux de tête □ Oui □ Non
- Fatigue répétée □ Oui □ Non
- Fièvres □ Oui □ Non
- Étourdissements □ Oui □ Non
- Transpiration □ Oui □ Non
- Saignements de nez □ Oui □ Non
- Crampes d'estomac □ Oui □ Non
- Diarrhée □ Oui □ Non
- Perte d'appétit □ Oui □ Non
- Autre □ Oui □ Non

Lesquels :

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……

……

6.6. Souffre-t-il d'une des pathologies suivantes ? (Cochez plusieurs cases si nécessaire)

- Effets aigus pré-cités qui durent □ Oui □ Non
- Allergies □ Oui □ Non
- Cancer □ Oui □ Non
- Problèmes thyroïdiens □ Oui □ Non
- Maladies neuro-dégénératives □ Oui □ Non
- Problèmes pulmonaires □ Oui □ Non
- Autre □ Oui □ Non

  Lesquels :

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……

7. Connaissance concernant les résidus de pesticides

7.1. A-t-il reçu des informations sur les résidus de pesticides dans les produits horticoles ?

- Par les médias (télévision, revues, internet, etc.) □ Oui □ Non
- Par un professionnel de santé (médecin, etc.) □ Oui □ Non
- Aucune information reçue □ Oui □ Non

7.2. Pense-t-il être suffisamment informé sur les résidus de pesticides dans les produits horticoles ?

□ Oui □ Pas suffisamment

7.3. Contact avec les agents de vulgarisation :

- Aucun □ Oui □ Non
- Visite périodique □ Oui □ Non

- Formation □ Oui □ Non
- Appui-conseil □ Oui □ Non
- Expérimentation □ Oui □ Non

Autres réponses :……..

8. A-t-il des suggestions à faire pour diminuer ses niveaux d'exposition aux pesticides ?

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9. Ses suggestions/recommandations en rapport avec ce sujet

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Pour toute question relative à cette enquête, contactez :
Laboratoire de phytopharmacie Gembloux Agro-Bio-Tech
Adresse : Passage des Déportés, 2 B-5030 Gembloux
Doctorante Khaoula TOUMI
Téléphone : 32(0)498400411
Mail : Khaoula.Toumi@doct.ulg.ac.be

Nous remercions l'enquêté d'avoir participé à cette enquête

Signatures

Signature de l'enquêteur qui a réalisé l'enquête

J'atteste que le présent diagnostic a été réalisé en respectant les règles de l'art et je confirme que les informations recueillies dans ce questionnaire ont été collecté par moi-même auprès du producteur.

Nom et prénom de l'enquêteur ……..

Signature de l'enquêteur

Date : _ _ /_ _ /_ _

Signature de l'exploitant agricole ou de son mandataire

Nom et prénom du producteur ou de son mandataire……

Localité :

Village….....

Ville.....

Région……

Signature du producteur ou de son mandataire

Date : _ _ /_ _ /_ _

Annexe 6 : Propriétés physico-chimiques et toxicologiques des substances actives et des métabolites détectés sur les échantillons de tomate et/ou de piment et /ou sur les gants en coton portés par les travailleurs durant la récolte de ces deux produits horticoles cultivés sous serre

Active substances	Molecular mass ($g\ mol^{-1}$)*	[2]Vapour pressure at 25°c (mPa)*	[1]Log Kow (Log P) *	CLP classification**
2-(1-naphtyl) acetamide	-	-	-	-
Acetamiprid	222.67	1.73×10^{-04}	0.8	H302
Azoxystrobin	403.4	1.10×10^{-07}	2.5	H331
Benalaxyl	325.4	0.572	3.54	-
Benomyl	290.32	0.005	1.4	H315, H317, H335, H340, H360FD
Bifenazate	300.35	1.33×10^{-02}	3.4	H317, H373
Boscalid	343.21	0.00072	2.96	-
Bupirimate	316.42	0.057	3.68	H317, H351
Carbendazim	191.21	0.09	1.48	H340, H360FD
Chlorantraniliprole	483.15	6.3×10^{-09}	2.86	-
Chlorothalonil	265.91	0.076	2.94	H317, H318, H330, H335, H351
Chlorpyrifos	350.58	1.43	4.7	H301
Clofentezine	303.15	1.40×10^{-03}	3.1	-
Cyhalothrin	449.85	1.00×10^{-09}	6.8	-
Cymoxanil	198.18	0.15	0.67	H302, H317, H361fd, H373
Cypermethrin	416.3	6.78×10^{-03}	5.55	H302, H332, H335
Cyproconazole	291.78	0.026	3.09	H301, H360D, H373

Active substances	Molecular mass ($g\ mol^{-1}$)*	[2]Vapour pressure at $25^{o}c$ (mPa)*	[1]Log Kow (Log P) *	CLP classification**
Cyprodinil	225.29	5.10×10^{-01}	4	H317
Deet	-	-	-	-
Diafenthiuron	384.58	0.002	5.76	-
Difenoconazole	406.26	3.33×10^{-05}	4.36	-
Dimethoate	229.26	0.247	0.75	H302, H312
Dimethomorph	387.86	9.85×10^{-04}	2.68	-
Dodine	287.44	5.49×10^{-03}	1.25	H302, H315, H319
Famoxadone	374.39	0.00064	4.65	H373
Fenamidone	311.4	0.00034	2.8	-
Fenpyroximate	421.49	0.01	5.01	H301, H317, H330
Fenvalerate	419.9	0.0192	5.01	-
Fluazinam	465.14	7.5	4.03	H317, H318, H332, H361d
Flubendiamide	682.39	0.1	4.14	-
Fludioxonil	248.19	3.90×10^{-04}	4.12	-
Fluopicolide	383.58	3.03×10^{-04}	2.9	-
Fluopyram	396.76	1.2×10^{-03}	3.3	-
Flusilazole	315.39	0.0387	3.87	H302, H351, H360D
Hexachlorobenzene	284.80	1.45	3.93	H350, H372
Hexaconazole	314.21	0.018	3.9	H302, H317
Hexythiazox	352.88	1.33×10^{-03}	2.67	-
Imidacloprid	255.66	4.0×10^{-07}	0.57	H302
Indoxacarb	527.83	0.006	4.65	H301, H317, H332, H372
Iprodione	330.17	0.0005	3	H351
Iprovalicarb	320.43	7.90×10^{-05}	3.2	-
Kresoxim-methyl	313.35	2.30×10^{-03}	3.4	H351
Linuron	249.09	0.051	3.0	H302, H351,

Active substances	Molecular mass ($g\ mol^{-1}$)*	[2]Vapour pressure at 25°c (mPa)*	[1]Log Kow (Log P) *	CLP classification**
				H360Df, H373
Mandipropamid	411.9	9.40×10^{-04}	3.2	-
Metalaxyl	279.33	0.75	1.75	H302, H317
Metalaxyl-M	279.33	3.3	1.71	H302, H318
Methomyl	162.21	0.72	0.09	H300
Myclobutanil	288.78	0.198	2.89	H302, H319, H361d
Omethoate	213.2	19.0	-0.9	H301, H312
Penconazole	284.18	0.366	3.72	H302, H361d
Permethrin	391.3	0.007	6.1	H302, H332, H335
Pirimicarb	238.39	0.43	1.7	H301, H317, H331, H351
Pirimiphos-methyl	305.33	2.00×10^{-03}	3.9	H302
Propamocarb	188.3	730	0.84	-
Propargite	350.47	0.00404	5.7	H315, H318, H331, H351
Proquinazide	372.2	0.09	5.5	H351
Pyraclostrobin	387.8	2.60×10^{-05}	3.99	H315, H331
Pyrimethanil	199.11	1.1	2.84	-
Spinosad	-	-	-	-
Spiromesifen	370.48	7.00×10^{-03}	4.55	-
Spirotetramat	373.48	5.6×10^{-06}	2.51	H317, H319, H335, H361fd
Spiroxamine	297.5	3.5	2.89	H302, H312, H315, H317, H332, H361d, H373
Tebuconazole	307.82	1.30×10^{-03}	3.7	H302, H361d
Tebufenpyrad	333.8	0.0016	4.93	H301, H317, H332, H373
Thiodicarb	354.47	2.7	1.62	-
Thiophanate methyl	342.39	9.0×10^{-03}	1.4	H317, H332,

Active substances	Molecular mass ($g\ mol^{-1}$)*	[2]Vapour pressure at $25^{o}c$ (mPa)*	[1]Log Kow (Log P) *	CLP classification**
				H341
Triadimefon	293.8	0.02	3.18	H302, H317
Triadimenol	295.76	0.0005	3.18	H302, H360, H362
Trifloxystrobin	408.37	3.40×10^{-03}	4.5	H317
Triflumuron	358.70	0.0002	4.9	-
Zoxamide	336.64	0.013	3.76	H317

H300: Fatal if swallowed; **H301:** Toxic if swallowed; **H302:** Harmful if swallowed; **H312:** Harmful in contact with skin; **H315:** Causes skin irritation; H317: May cause an allergic skin reaction; **H318:** Causes serious eye damage; **H319:** Causes serious eye irritation; **H330:** Fatal if inhaled; **H331:** Toxic if inhaled, **H332:** Harmful if inhaled; **H335:** May cause respiratory irritation; **H340:** May cause genetic defects; **H341:** Suspected of causing genetic defects; **H350:** May cause cancer; **H351:** Suspected of causing cancer; **H360:** May damage fertility or the unborn child; **H360D:** May damage the unborn child; **H360Df:** May damage the unborn child. Suspected of damaging fertility; **H360FD:** May damage fertility. May damage the unborn child; **H361d:** suspected of damaging the unborn child; **H361fd:** suspected of damaging fertility. Suspected of damaging the unborn child; **H362:** May cause harm to breast-fed children; **H372:** Causes damage to organs through prolonged or repeated exposure; **H373:** May cause damage to organs through prolonged or repeated exposure

* Classification according The PPDB - Pesticides Properties DataBase
** CLP classification according the EU Pesticides database

[1]Volatility (mPa) (EFSA, 2014)
< 5.0 mPa = Low volatility,
5.0 – 10.0 mPa = Moderately volatile,
> 10 mPa = Highly volatile

[2] Octanol-water Partition Coefficient (Log P) (PPDB - Pesticides Properties DataBase, 2018)
< 2.7 = Low bioaccumulation
2.7 – 3 = Moderate
> 3.0 = High