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Cette thèse intitulée

Analyse des pesticides dans l'eau de surface, l'eau potable et les produits de consommation par chromatographie liquide couplée à la spectrométrie de masse

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Résumé

L'utilisation intensive de certains pesticides et leur relative persistance vont de pair avec la présence de résidus dans l'eau de surface et l'eau potable mais aussi dans les produits agricoles disponibles pour les consommateurs, y compris les denrées alimentaires. À l'heure actuelle, les effets des pesticides sur la vie aquatique et d'autres organismes non ciblés sont relativement bien connus, et la possibilité des effets sur l'être humain fait débat. Des normes de qualité ont été proposées pour l'eau, que ce soit des critères pour l'eau potable ou des critères de protection de la vie aquatique pour l'eau de surface. Des limites maximales de résidus (MRL) de pesticides ont également été établies pour certains produits, notamment les fruits et légumes. Un des défis pour les chercheurs est la mise en œuvre de nouvelles méthodes analytiques sensibles et robustes pour la quantification ultra-trace de ces composés, afin de déterminer si les différents échantillons sont conformes aux directives ou aux MRL. L'analyse des pesticides modérément polaires dans des matrices complexes repose tout d'abord sur la méthode d'extraction. Plusieurs options sont disponibles, telles que l'extraction liquide-liquide ou en phase solide (SPE, *Solid Phase Extraction*) pour les matrices aqueuses, ou encore dSPE de type QuEChERS (*Quick, Easy, Cheap, Effective, Rugged and Safe*) pour les matrices solides. Actuellement, la chromatographie en phase liquide couplée à la spectrométrie de masse en tandem représente un choix pertinent pour les analyses ultra-traces, mais sa mise en œuvre peut présenter certains défis. Dans ce contexte, les principaux objectifs de ce travail de recherche sont les suivants : i) proposer des méthodes analytiques rapides, sensibles et robustes pour déterminer des pesticides multi-classes aux niveaux d'exposition que l'on retrouve dans différentes matrices comme l'eau potable, les denrées alimentaires et l'urine comme matrice biologique, et ii) évaluer le lien entre les sources de contamination des divers pesticides et leur mobilité afin de documenter la distribution spatiale et temporelle dans l'eau de surface et l'eau potable au Québec. Pour les échantillons aqueux, une méthode SPE en ligne entièrement automatisée couplée à la chromatographie liquide haute performance et spectrométrie de masse en tandem a été développée. La méthode proposée est rapide (8 min par échantillon) avec des limites de détection comprises entre 0.1 et 5 ng L^{-1} pour les pesticides de la famille des néonicotinoïdes et l'atrazine. Pour les produits

alimentaires tels que les fruits et légumes, l'optimisation d'une méthode de type QuEChERS a été réalisée. La méthode permet d'atteindre des niveaux de détection entre 0.05 ng g^{-1} et 2 ng g^{-1} pour une gamme de 22 pesticides couvrant 7 classes différentes, incluant les organophosphorés, les carbamates, les néonicotinoïdes et les triazines, entre autres. La robustesse des diverses méthodes a été démontrée par des expériences de contrôle qualité inter- et intra-journaliers afin de garantir l'exactitude, la précision et l'absence d'effets matriciels pour de longues séquences d'analyse. Les méthodes validées ont été appliquées à des échantillons réels, y compris des échantillons d'eau du robinet couvrant 52 villes de la province du Québec (Canada), 68 échantillons d'eau de surface (fleuve Saint-Laurent et tributaires), et 133 échantillons de laitue, pomme, raisin et tomates achetés sur les marchés locaux. Les résultats indiquent une forte occurrence de l'atrazine, la thiaméthoxame, la clothianidine et l'imidaclopride dans les échantillons d'eau et les quatre produits alimentaires.

Mots-clés : HPLC-MS/MS, néonicotinoïdes, pesticides, eau, QuEChERS, SPE en-ligne

Abstract

The extensive use of certain pesticides and their relative persistence go on par with the presence of residue levels in surface water and drinking water, but also in agricultural products available to consumers (including foodstuffs). There are potential effects on aquatic life and non-target organisms, and the possibility of effects in humans remains a topical issue. Quality standards have been proposed for water, including criteria for drinking water and criteria for the protection of aquatic life (surface water). Maximum residue limits (MRLs) for pesticides have also been established for foodstuff, including fruits and vegetables. One of the challenges for researchers is the implementation of sensitive and robust analytical methods for the ultra-trace quantification of these compounds, with a view to determining whether the samples are compliant with guidelines or MRLs. The analysis of moderately polar pesticides in complex matrices relies notably on the extraction method. Diverse options are available, including liquid-liquid or solid phase extraction (SPE) for aqueous samples, and dSPE approaches such as QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) for solid samples. Liquid chromatography coupled to tandem mass spectrometry is usually selected for separation and detection at the ultra-trace level, but there are some pitfalls. In this context, the main objectives of the present research were as follows: i) to propose fast and robust analytical methods to determine multi-class pesticides at different exposure routes including drinking water and food, and ii) to evaluate the link between the contamination sources of various pesticides and their mobility to document their distribution in surface water and tap water in Quebec. For water samples, a fully automated on-line SPE method coupled to ultra-high-performance liquid chromatography tandem mass spectrometry was developed. The proposed method is rapid (8 min per sample) with detection limits between 0.1 and 5 ng L^{-1} for neonicotinoids and atrazine. For food products (fruits and vegetables), a QuEChERS method was investigated. The optimized procedure shows limits of detection between 0.05 ng g^{-1} and 2 ng g^{-1} for a total of 22 pesticides encompassing 7 different classes, including organophosphorus compounds, carbamates, neonicotinoids and triazines, among others. The robustness of the various methods has been demonstrated by inter-day and intra-day

quality control experiments to ensure suitable accuracy, precision, and the absence of matrix effects in long LC-MS batch sequences.

The validated methods were applied to real samples, including tap water samples from 52 municipalities in the province of Quebec (Canada), 68 surface water samples from the St. Lawrence River and its main tributaries, and 133 fruits and vegetables samples (lettuce samples, apples, grapes and tomatoes) purchased from local markets. The results indicate a high occurrence of atrazine, thiamethoxam, clothianidin, and imidacloprid in the water samples and the four food products.

Keywords: HPLC-MS /MS, pesticides, water, QuEChERS, on-line SPE

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2,4-D : Acide 2,4-dichlorophénoxyacétique

ACN: Acetonitrile

ADI : Acceptable Daily Intake

ADN : Acide désoxyribonucléique

AHAS : Acétohydroxyacide synthétase

ALS : Acétolactate synthétase

ARLA : Agence de Réglementation de la Lutte Antiparasitaire

-AW : Weak Anion Exchange

B : Secteur Magnétique

-CW : Weak Cation Exchange

-CX : Strong Cation Exchange

DDT : Dichlorodiphényltrichloroéthane

DEA : Desethyl atrazine

DEET : N,N-diethyl-m-toluamide

DIA : Desisopropyl atrazine

DL_{50} : Dose létale pour le 50% de la population étudie.

DNOC : Dinitro-ortho-cresol

dSPE : Dispersive Solid Phase Extraction

ECD : Détecteur par capture d'électrons

EEC : European Economic Comission

EPSP : 5-Énolpyruvylshikimate-3-phosphate syntése

ESI : Electro spray ionization

EU : European Union

FAO : Organisation pour l'alimentation et l'agriculture

FT-ICR : Résonance ionique cyclotronique à transformée de Fourier

GC/CPG : Chromatographie en Phase Gazeuse

GFF : Glass Fiber Filter

HCH : β-hexachlorocyclohexane

HLB : Hydrophilic-Lipophilic Balance

HPLC : High Performance Liquid Chromatography

i.a. : Ingrédients actifs

LC50 : Concentration létale pour 50% de la population étudiée

LIT : Trappe ionique – Quadrupôle linéaire

LLE : Liquid – Liquid Extraction

LMR : Limite Maximale de Résidus de pesticides

LOD : Limit of Detection

LOEC : Low Observed Effect Concentration

LOQ : Limit of Quantification

mAChRs : Muscarinic acetylcholine receptors

MAE : Microwave Assisted Extraction

MCPA : acide 2-méthyl-4-chlorophénoxyacétique

MRM : Multiple Reaction Monitoring

MS/MS : Tandem Mass Spectrometry

MS : Mass Spectrometry

NOEC : Non Observed Effect Concentration

NPLC : Normal Phase Liquid Chromatography

PAS : Passive Air Sampler

PMRA : Pest Management Regulatory Agency

POCIS : Polar Organic Chemical Integrative Sampler

POP's : Polluants Organiques Persistants

PPO: Protoporphyrinogèene

PSA : Primary and Secondary Amine

PUF : Polyurethane foam disks

PVDF : Polyvinylidene Difluoride Filter

Q : Quadrupôle

QIT : Trappe ionique - Quadrupôle

QqQ : Triple Quadrupôle

QuEChERS : Quick, Easy, Cheap, Effective, Rugged and Safe

RPLC : Reverse Phase Liquid Chromatography

SPE : Solid Phase Extraction

TOF : Time of flight

TWA : Time-weighted average concentrations

US EPA : United States of America-Environmental Protection Agency

VDC : Voltage direct en continue

VRF : Voltage de radio fréquence en alternatif

WHO : World Health Organization

To Krishna…

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Chapitre 1. Introduction

1. Les pesticides

L'agriculture fait face à des défis majeurs afin de fournir des produits en quantité et qualité suffisantes pour satisfaire les besoins de la population globale en forte croissance depuis le milieu du XXe siècle. Afin d'assurer de meilleurs rendements, les techniques de rotation (jachère) et de contrôle mécanique de mauvaises herbes ont été remplacées par l'emploi massif de produits chimiques (p. ex., herbicides), mais à quel prix pour l'environnement ? C'est là une question qui préoccupe la communauté scientifique et motive la réalisation de nombreuses études d'envergure (suivis environnementaux, tests écotoxicologiques, études épidémiologiques, etc.).

Le terme pesticide fait référence à toute substance ou mélange de substances servant à contrôler, prévenir, détruire, repousser ou atténuer certains organismes qui pourraient causer des pertes ou des dommages aux cultures. Ces nuisibles incluent, par exemple, les insectes, les rongeurs, les nématodes phytophages, les mauvaises herbes, les champignons, etc. Ainsi, selon l'organisme ciblé, les pesticides sont notamment classifiés comme insecticides, rodonticides, nématicides, herbicides, fongicides, etc. Indépendamment du type de nuisible ciblé, les pesticides peuvent aussi être regroupés selon les quatre grandes classifications suivantes (Hough, 2013):

1. Pesticides naturels, tels que des composés provenant d'extraits des plantes (p. ex. la nicotine).
2. Biologiques, dont l'utilisation de microorganismes (p. ex. *Bacillus thuringiensis*) pour le contrôle de nuisibles.
3. Inorganiques, dérivés des minéraux comme le soufre et l'arsenic.
4. Synthétiques, des substances chimiques organiques comme les organochlorés, les organophosphorés, les acides phénoxyacétiques, les carbamates, les pyréthrines synthétiques, etc.

Durant la première moitié du XXe siècle, la production de pesticides reste modeste, mais la fin de la Seconde Guerre mondiale voit une multiplication des pesticides introduits sur le marché (**Tableau 1.1**). Parmi les pesticides de synthèse organochlorés, le dichlorodiphényltrichloroéthane (DDT) représente un exemple emblématique et polémique. Le DDT a été massivement utilisé dans le domaine militaire, dans l'agriculture, par des particuliers (usage résidentiel) ainsi que pour combattre les insectes porteurs de maladies comme le typhus et le paludisme. À titre d'exemple, le DDT a été utilisé pour l'éradication d'une épidémie de typhus à Naples en 1944 (Italie).

Tableau 1.1 Année d'introduction de pesticides (Matthews, 2006).

ANNÉE	TYPE	PESTICIDES
1850	Herbicide	Ferrous sulphate
1882	Fungicide	Bordeaux mixture
1930	Herbicide	DNOC
1931	Fungicide	Thiram
1939	Insecticide	DDT (commercialisé 1944)
1942	Herbicide	2,4-D
1943	Fungicide	Zineb
1944	Insecticide	HCH (lindane)
1946	Insecticide	Parathion
1948	Insecticide	Aldrin, dieldrin
1949	Fungicide	Captan
1952	Insecticide	Diazinon
1953	Herbicide	Mecoprop
1955	Herbicide	Paraquat (commercialisé 1962)
1956	Insecticide	Carbaryl
1965	Nematicide	Aldicarb
1968	Fungicide	Bénomyl

1971	Herbicide	Glyphosate
1972	Insecticide	Diflubenzuron
1973	Insecticide	Permethrin
1990	Insecticide	Imidacloprid
	Fungicide	Azoxystrobin
	Insecticide	Spinosad

La prise de conscience des effets des pesticides organochlorés sur l'environnement, particulièrement sur les oiseaux et leur reproduction, survient notamment grâce à Rachel Carlson et son œuvre *Le Printemps Silencieux* (*Silent Spring*) (Carson, 1962) dont les conclusions soulignent les effets négatifs des pesticides sur l'environnement. Spécifiquement le cas du DDT, qui était par exemple l'une des causes de l'amincissement des coquilles d'œufs, entraînant une diminution de la survie des oisillons et donc de la population des oiseaux. L'œuvre de Rachel Carson contribuera à la création de l'agence américaine de protection de l'environnement en 1970 et à l'interdiction du pesticide DDT en 1972 (U.S. EPA, 2019). La prise de conscience qui en a résulté constitue un des points de départ du mouvement environnementaliste actuel (Chandran et al., 2019).

1.2 Situation actuelle des pesticides dans le monde et au Québec

L'utilisation de pesticides dans le globe est en augmentation continuelle : leur usage est passé de 1.5 kg/ha en 1990 à 2.57 kg/ha en 2016 selon l'Organisation de l'Alimentation et l'Agriculture des Nations Unies (FAO) (Figure 1.1).

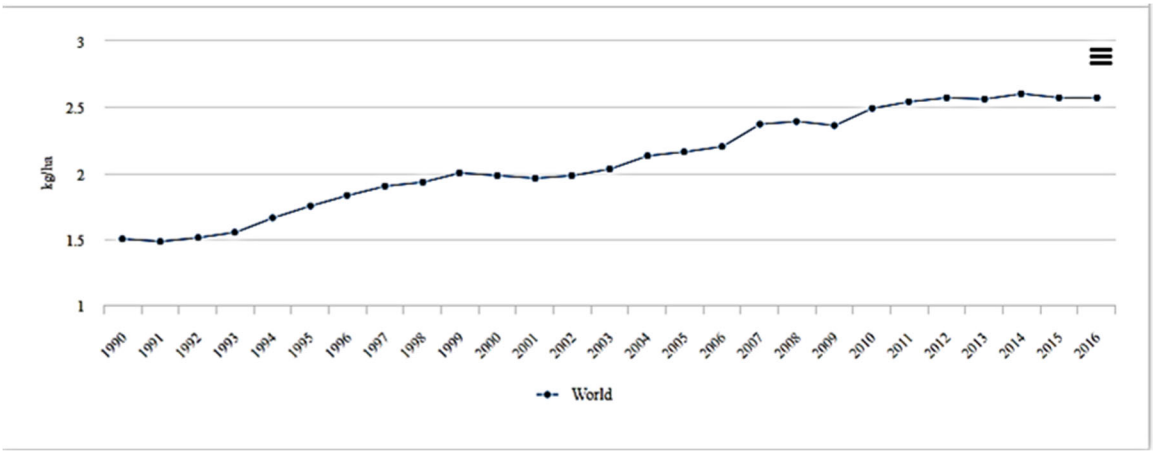


Figure 1.1 Total des pesticides utilisés dans le monde de 1990 à 2016, en kilogrammes d'ingrédient actif/hectare (image tirée de FAOSTAT, 2019).

Parmi les pays qui ont utilisé le plus de pesticides par unité de surface de 1990 à 2016, on retrouve le Japon, la Chine, l'Italie et la Colombie avec une moyenne supérieure à 6 kg d'ingrédient actif par hectare (kg i.a./ha). Dans le cas de l'Amérique du Nord, le Mexique et les États-Unis ont enregistré une utilisation moyenne de ≤ 3.03 kg i.a./ha, suivi du Canada avec ≤ 1.17 kg i.a./ha (Figure 1.2).

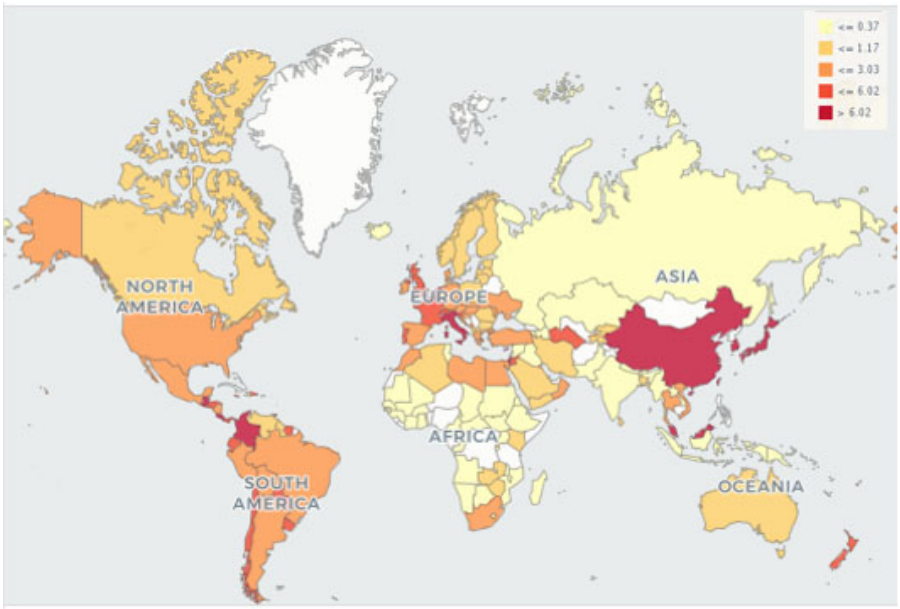


Figure 1.2 Utilisation moyenne de pesticides en kilogrammes d'ingrédients actifs/hectare par pays, de 1990 à 2016 (image tirée de FAOSTAT, 2019).

En 2017 au Québec, 4 170 tonnes d'i.a. de pesticides ont été vendues, dont 81.9% ont été consacrés aux usages propres de fermes (3 415 tonnes). Les ventes d'herbicides (glyphosate et atrazine, notamment) représentaient 68.1% du total. Pour l'année 2016, les ventes de pesticides pour usage domestique représentaient 12.2% avec 507,856 kg i.a., principalement appliqués dans les résidences, les commerces et les terrains de golf. Finalement, 247 145 kg du total vendu ont été utilisés dans l'industrie pour maintenir libres et propres les corridors routiers, ferroviaires et 2014 selon le Bilan de ventes de pesticides au Québec (2016-2017), rapport du Ministère du Développement durable, de l'Environnement et de la Lutte contre les changements climatiques (2017).

Dans le cas spécifique des pesticides les plus vendus et utilisés au Canada, il est possible de citer l'atrazine, laquelle a été bannie dans l'Union Européenne depuis 2005 sous la directive 91/414/EEC (OJEC, 1991). Citons également le glyphosate qui est un des herbicides les plus controversés ces dernières années, et les insecticides néonicotinoïdes considérés comme l'un des facteurs de la chute des populations d'abeilles et autres pollinisateurs naturels.

Les ventes d'atrazine au Canada en 1988 étaient autour de 2 millions de kilogrammes d'ingrédient actif, dont 70% pour l'Ontario. En Ontario les ventes d'atrazine ont présenté une certaine diminution ces dernières décennies : 499,000 kg i.a. en 2003, contre 297,000 kg i.a. en 2013, ce qui représente une diminution de près de 70% de 1983 à 2003 et une diminution de près de 40% de 2003 à 2013 (OMAFRA, 2015).

Le glyphosate dans la dernière mise à jour d'homologation de 2017 faite par Santé Canada (Health Canada, 2017) bénéficie de 15 années supplémentaires d'utilisation permise au Canada. L'utilisation du glyphosate en Ontario se répartit comme suit (données 2013-2014): 1,151,051 kg i.a. utilisés pour les grains (maïs, soya, canola, blé, etc.), 13,194 kg i.a. pour les fruits, et 9,869 kg i.a. pour les légumes. Au Québec, le glyphosate avec toutes ses variantes (forme acide et différents sels) a été classé parmi les pesticides les plus vendus (classe F) avec des ventes de 100,000 à 1,000,000 kg i.a. en 2016.

En 2017 au Québec, 81,6% des ventes totales de pesticides ont été consacrés à l'utilisation agricole et 9,8% pour l'utilisation en milieu urbain. Dans le cas particulier des néonicotinoïdes, l'imidaclopride, le thiaméthoxame et la clothianidine ont été classés avec des ventes entre 1 000 et 10 000 kg i.a. (Classe C).

En 2008, la vente et l'utilisation des néonicotinoïdes représentaient un tiers des insecticides dans le monde, c-à-d., 24% du marché mondial des produits agrochimiques soit un peu plus de 9 milliards de dollars canadiens. En raison des risques potentiels pour l'environnement, l'utilisation des néonicotinoïdes a été limitée dans plusieurs pays de l'Union Européenne en 2013 avec une suspension partielle pendant deux ans (EU 2015/495). En 2018 leur réévaluation conduit à interdire l'utilisation de trois néonicotinoïdes dans l'Union Européenne parmi ceux les plus vendus : l'imidaclopride, la clothianidine et le thiaméthoxame (2018/840).

Au Canada leur emploi fait encore l'objet d'une certaine controverse. Trois des six néonicotinoïdes prioritaires, tels que l'imidaclopride, la clothianidine et la thiaméthoxame ont été réévalués au cours des dernières années. Lors de la réévaluation de 2016, l'utilisation de l'imidaclopride fut sujet d'une proposition d'élimination graduelle sur une période de 3-5 ans pour son utilisation en l'agriculture. Les trois ingrédients actifs ont été réévalués en fonction de leurs effets néfastes sur les abeilles ; la clothianidine et la thiaméthoxame ont par ailleurs été réévalués en relation aux effets sur la vie aquatique. Dans le rapport de la réévaluation de 2019 est publiée la date finale pour prendre une décision sur ces principes actifs. L'imidaclopride est sujet à une étude spéciale axée sur les abeilles tandis que la clothianidine et la thiaméthoxame sont sujettes à une réévaluation générale avec une date de décision finale pour le début de l'année 2020 (ARLA, 2017).

Naturellement, une diminution de ventes de l'un ou l'autre des produits ne veut pas dire qu'il n'y a pas d'autres pesticides qui seront utilisés comme remplacement. En 1981, l'aire traitée par des herbicides en Ontario représentait seulement 16.61% comparée à 20.76% en 2011. Au Québec l'aire traitée de 1981 à 2011 est même passée de 5.64% à 12.78%. Dans le cas des insecticides, pour ces mêmes provinces, l'aire traitée représenterait 2.19% en 1981 et 4.15% en 2011 en Ontario; la proportion au Québec est passée de 0.70% (1981) à 1.26% (2011) (AGR Canada, 2011).

S'il y a une diminution des ventes d'atrazine, mais une augmentation dans les superficies traitées, il est pertinent de se demander quel sont les pesticides qui prennent le relais, et quels sont leurs toxicités et leurs effets sur l'environnement.

La distribution spatiale de ces composés est, bien entendu, liée à la quantité vendue et utilisée, mais aussi à leur grande diversité d'applications. En fin de compte, leur présence peut être envisagée dans l'ensemble des compartiments environnementaux

1.3 Classification et mode d'action

Les pesticides identifiés comme des contaminants nocifs pour l'environnement ont été regroupés avec d'autres composés chimiques lors d'un accord international qui pourrait agir comme organisme régulateur. Notamment, la Convention de Stockholm signée en 2001 fait référence à l'interdiction de certains produits polluants. Au total, 180 pays en sont membres, mais seulement 152 ont signé ledit accord. Les pesticides organochlorés tels que le DDT (**Figure 1.3**) ainsi que certains autres composés (composés industriels, sous-produits de combustion, etc.) y ont été désignés comme « polluants organiques persistants » (POPs). Ces composés peuvent être retrouvés dans tous les compartiments de l'environnement, être bioaccumulables dans les organismes vivants et ils sont toxiques pour l'homme et la faune (POP's, 2008).

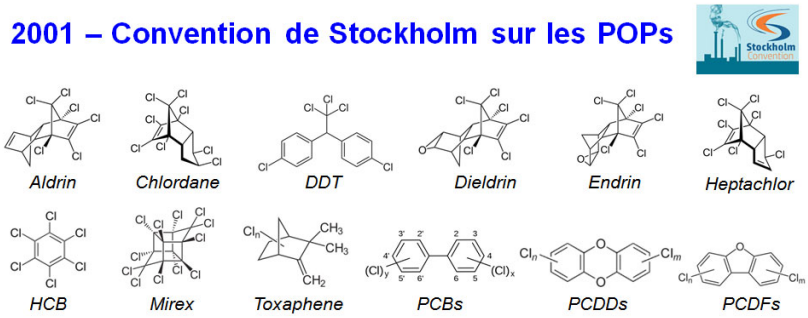


Figure 1.3. Les 12 substances initialement inscrites à la Convention de Stockholm sur les polluants organiques persistants (POPs) (POP's, 2008).

À l'époque il semblait que la solution à la problématique soulevée par ce type de pesticides consisterait à développer d'autres pesticides plus spécifiques, qui cibleraient les insectes ou les herbes nuisibles tout en ayant peu ou pas d'impact sur les oiseaux et les mammifères et bien évidemment, l'être humain. Une façon de créer cette spécificité, a consisté à développer des composés chimiques capables d'interagir avec certains neurotransmetteurs

propres aux invertébrés, ce qui donna lieu à la synthèse de nouveaux insecticides systémiques.

Les pesticides systémiques, qui agissent sur le système nerveux, représentent d'ailleurs l'un des quatre modes d'action possibles des pesticides (Rathore et al., 2012):

i) Par empoisonnement **physique**, tel que la silice et le charbon en poudre, ceux qui interfèrent dans le processus de passage d'air en s'accumulant dans les voies respiratoires et en causant de la suffocation.
ii) L'empoisonnement **nerveux** est un mode d'action par lequel les pesticides vont initier une excitation nerveuse extrême, donnée par la libération excessive de substances rétroactives à cause d'un agoniste ou un antagoniste des récepteurs nerveux. Des exemples de pesticides avec ce genre d'empoisonnement incluent les néonicotinoïdes, le DDT, le malathion, le parathion, etc.
iii) L'empoisonnement **protoplasmique**, dont l'effet sera une précipitation protéinique dans le corps du nuisible entraînant des dommages au foie et finalement la mort.
iv) Finalement, l'empoisonnement **respiratoire** est corrélé à l'inactivation d'enzymes comme les oxydases, peroxydases et réductases, pour provoquer finalement un blocage des voies respiratoires et une suffocation aiguë.

Parmi les pesticides avec un mode d'action par empoisonnement nerveux, on retrouve les néonicotinoïdes, les triazines, les urées substituées, les organophosphorés, les carbamates, les benzimidazoles et les inhibiteurs de la synthèse d'acides aminés.

Dans les années 1970, la société Shell a proposé la molécule de base pour de nouveaux insecticides systémiques nommés néonicotinoïdes. Les néonicotinoïdes sont une famille de substances actives agissant sur le système nerveux central, liées au récepteur nicotinique de l'acétylcholine. Contrairement aux insecticides organochlorés hautement hydrophobes et lipophiles, cette nouvelle génération d'insecticides se caractérise par un faible coefficient

de partage octanol-eau, ce qui les rend principalement hydrosolubles et peu bioaccumulables (Bonmatin et al., 2015).

Insecticides qui agissent sur le système nerveux chez les insectes

Les néonicotinoïdes et le fipronil

Les néonicotinoïdes sont classifiés comme insecticides systémiques car ils peuvent agir sur le système nerveux des insectes comme agonistes dans l'ouverture des canaux de cations des récepteurs nicotiniques de l'acétylcholine (nAChRs) et dans les ponts de voltage des canaux de calcium (Yamamoto et al., 1998; Ishaaya et al., 2001; Tomizawa et al., 2001). Ils peuvent remplacer l'acétylcholine, produisant dans l'insecte une paralysie, des convulsions, jusqu'à la mort par crampe généralisée.

L'archétype néonicotinoïde est l'imidaclopride, formé par l'addition d'un groupe 3-pyridylméthyle à un groupe hétérocyclique nitrométhylène. À partir du 6-chloro-3-pyridyl, les générations suivantes ont été développées telles que l'acétamipride, le clothianidine, le dinotefuran, le nitenpyram, le thiaclopride et la thiaméthoxame (**Figure 1.4**).

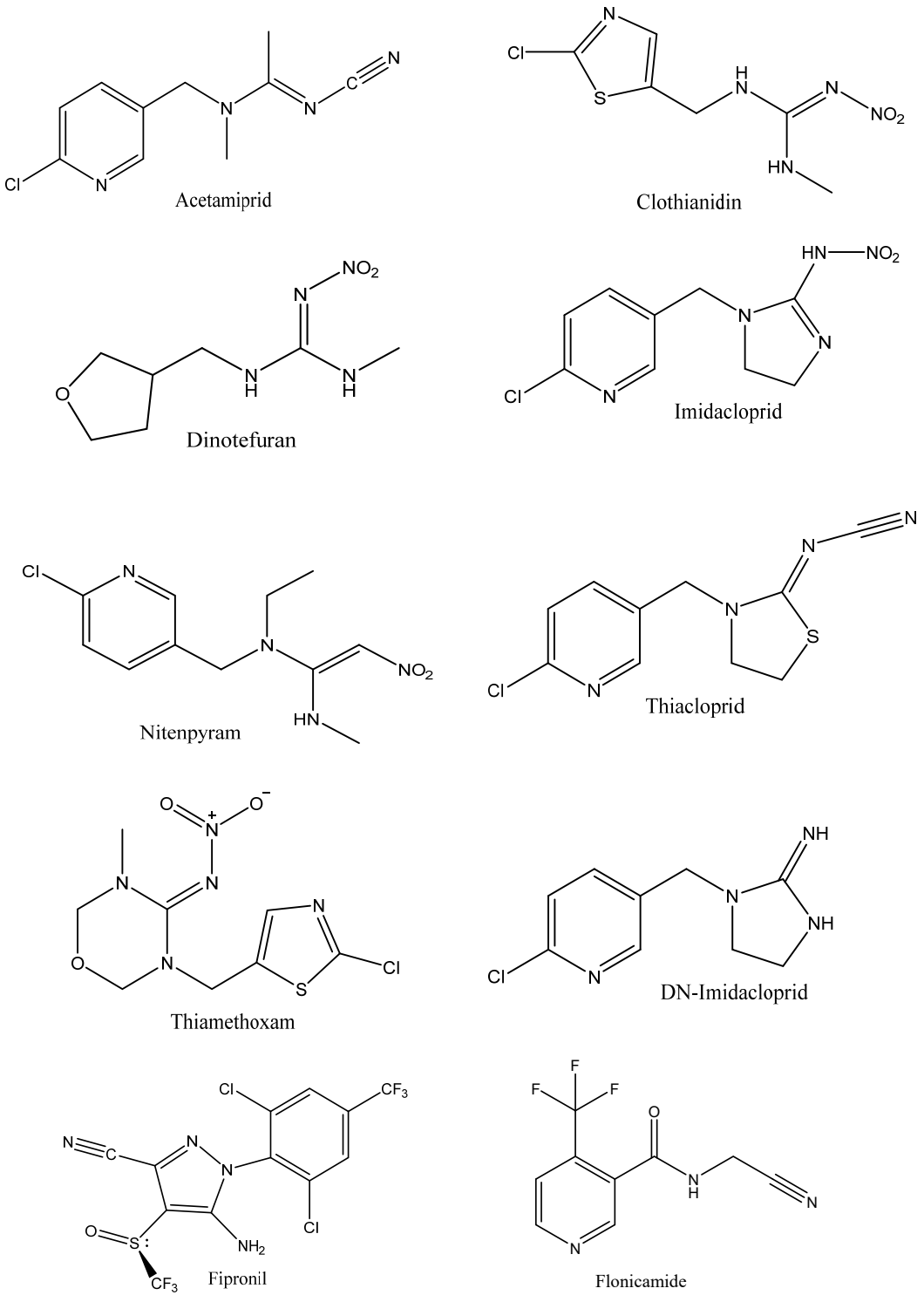
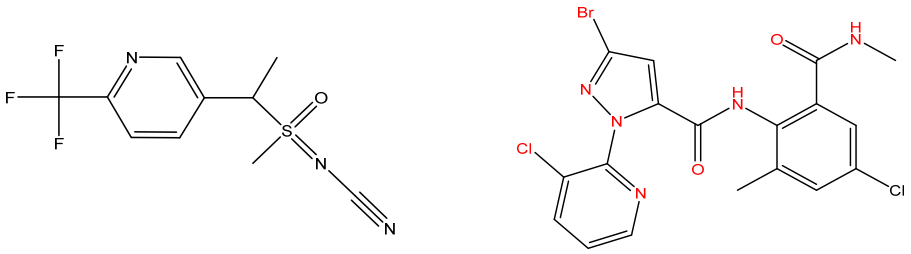


Figure 1.4 Structures moléculaires des 8 principaux néonicotinoïdes ainsi que du fipronil et du flonicamide.

La haute sélectivité des néonicotinoïdes envers les arthropodes et leur faible toxicité pour les mammifères est donnée par les différences en propriétés et conformations des subunités des nAChRs (Matsuda et al., 2001; Tomizawa et al., 2000). Les néonicotinoïdes peuvent former une liaison avec les membranes du cerveau des insectes et comme résultat, des excitations en continu, c'est ce qui produit des décharges en provoquant paralysie et épuisement de l'énergie cellulaire.

La première génération de néonicotinoïdes comprend le nitenpyram, l'imidaclopride, l'acétamipride et le thiaclopride. Parmi ces derniers, l'imidaclopride a présenté une haute toxicité pour les abeilles (Suchail et al., 2001). Il agit ainsi comme agoniste partiel des nAChRs nicotiniques dans les cellules Kenyon du corps de l'abeille domestique (*Apis mellifera*), lesquelles sont responsables des hauts processus neuronaux dans le cerveau comme l'apprentissage olfactif (Déglise et al., 2002). Le thiaméthoxame et son métabolite, la clothianidine, sont des néonicotinoïdes de la deuxième génération. Ils agissent différemment de la première génération, présentent très peu d'effet sur les nAChRs mais agissent comme agonistes dans l'interneurone synapses « cercal afferent/giant » produisant une forte dépolarisation, c'est à dire, un passage de potentiel de membrane d'une valeur négative (en repos) vers une valeur positive (excité) (Thany et al., 2011).

Dans la troisième génération de néonicotinoïdes, le dinotefuran fait son apparition. Le dinotefuran montre une activité nerveuse excitatrice inférieure à celle de l'imidaclopride mais comparable à celle de la clothianidine, ainsi qu'une activité de blocage des nerfs comparable à celle de l'imidaclopride et légèrement supérieure à celle de la clothianidine (Wakita et al., 2003). Le sulfoxaflor s'apparente à un « néonicotinoïde » de quatrième génération qui présente une forte activité insecticide contre un large éventail d'insectes (Longhurst et al., 2013; Babcock et al., 2011). Il peut également agir sur les nAChR et donc pourrait être considéré comme un néonicotinoïde. Au cours des dernières années, des insecticides de remplacement aux néonicotinoïdes ont fait leur apparition. Tel est le cas des insecticides avec une structure anthranilique de diamide comme la chlorantraniliprole (**Figure 1.5**) qui interrompt la contraction musculaire normale chez les insectes (Brugger et al., 2010).



Sulfoxaflor Chlorantraniliprole

Figure 1.5 Structures moléculaires du sulfoxaflor et de la chlorantraniliprole.

Les organophosphorés et carbamates

Parmi les autres insecticides capables de perturber le système nerveux, on trouve les organophosphorés et les carbamates comme le carbaryl et le carbendazime (voir **Figure 1.6**). Ces deux familles d'insecticides inhibent l'enzyme acétylcholine estérase. Dans ce cas spécifique, l'interaction entre les enzymes avec les insecticides s'opère par l'interaction d'un groupe hydroxyle d'un acide aminé sérine dans le site actif. L'oxygène de cette partie fait une attaque nucléophile sur un atome électro-déficient des insecticides. Ce type d'effet est produit dans l'atome de phosphore des organophosphorés et dans l'atome de carbone dans les carbamates. L'altération des chaînes latérales des insecticides est une façon d'augmenter le pouvoir d'inhibition enzymatique en renforçant les interactions intermoléculaires avec le reste du site actif (Madariaga-Mazon et al., 2019).

Le carbaryl est aussi considéré comme insecticide systémique car il perturbe le fonctionnement du système nerveux en assemblant son fragment carbamyle avec le site actif de l'enzyme acétylcholinestérase et l'empêche d'interagir avec l'acétylcholine. Lorsque cette enzyme est inhibée, l'acétylcholine en excès s'accumule, ce qui entraîne une stimulation excessive du système nerveux (U.S. EPA, 2019).

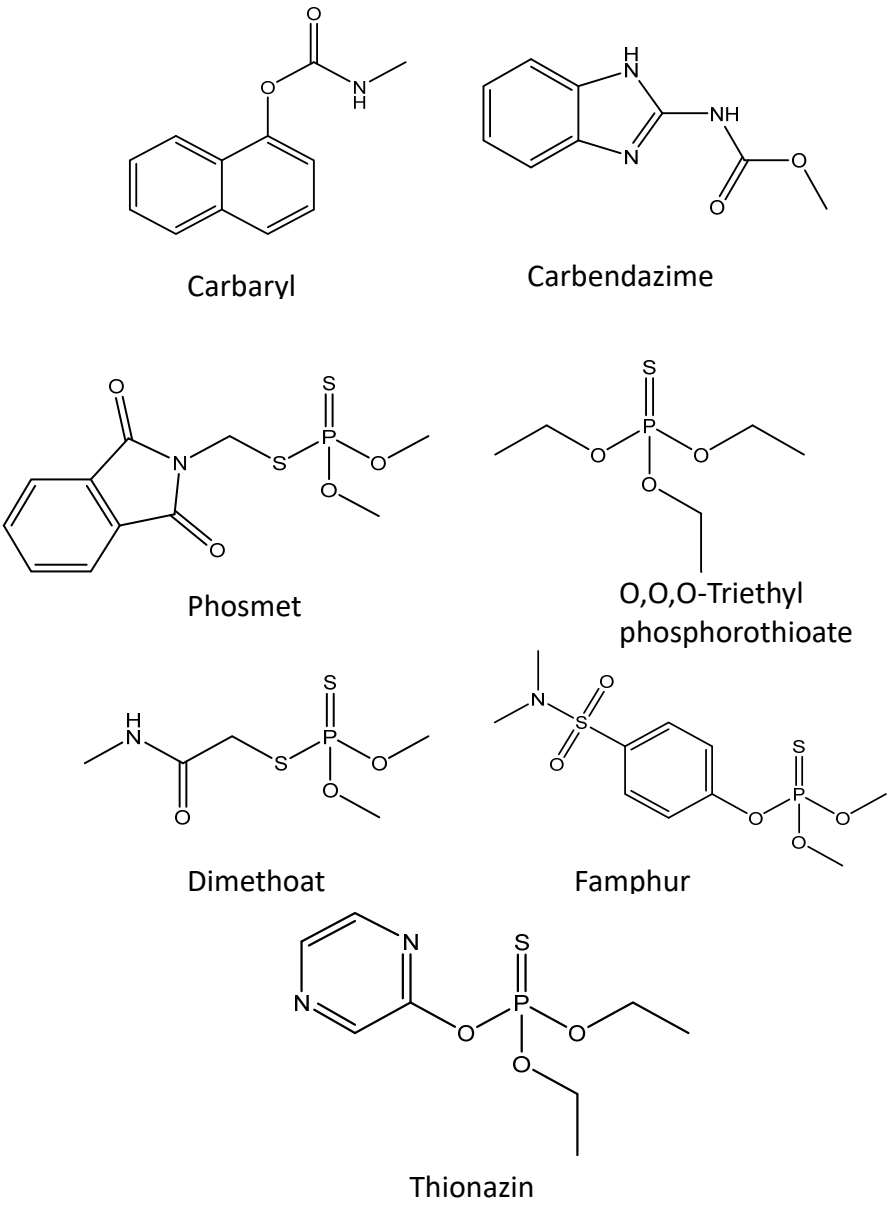


Figure 1.6 Structures moléculaires des organophosphorés et des carbamates sélectionnés.

Herbicides inhibiteurs de photosynthèse

Les triazines

Le mode d'action des inhibiteurs de photosynthèse tel que les triazines (atrazine, DEA, DIA, simazine, cyanazine, la hexazinone, le métribuzine et la prométryne, **Figure 1.7**) est relativement bien connu. Les triazines peuvent interagir par l'inhibition de la photosynthèse dans le site A des cellules végétales. Plus précisément, en empêchant le transfert d'électrons au site réducteur du complexe de photosynthèse II dans les chloroplastes et le transfert de l'énergie lumineuse (OMAFRA, 2000).

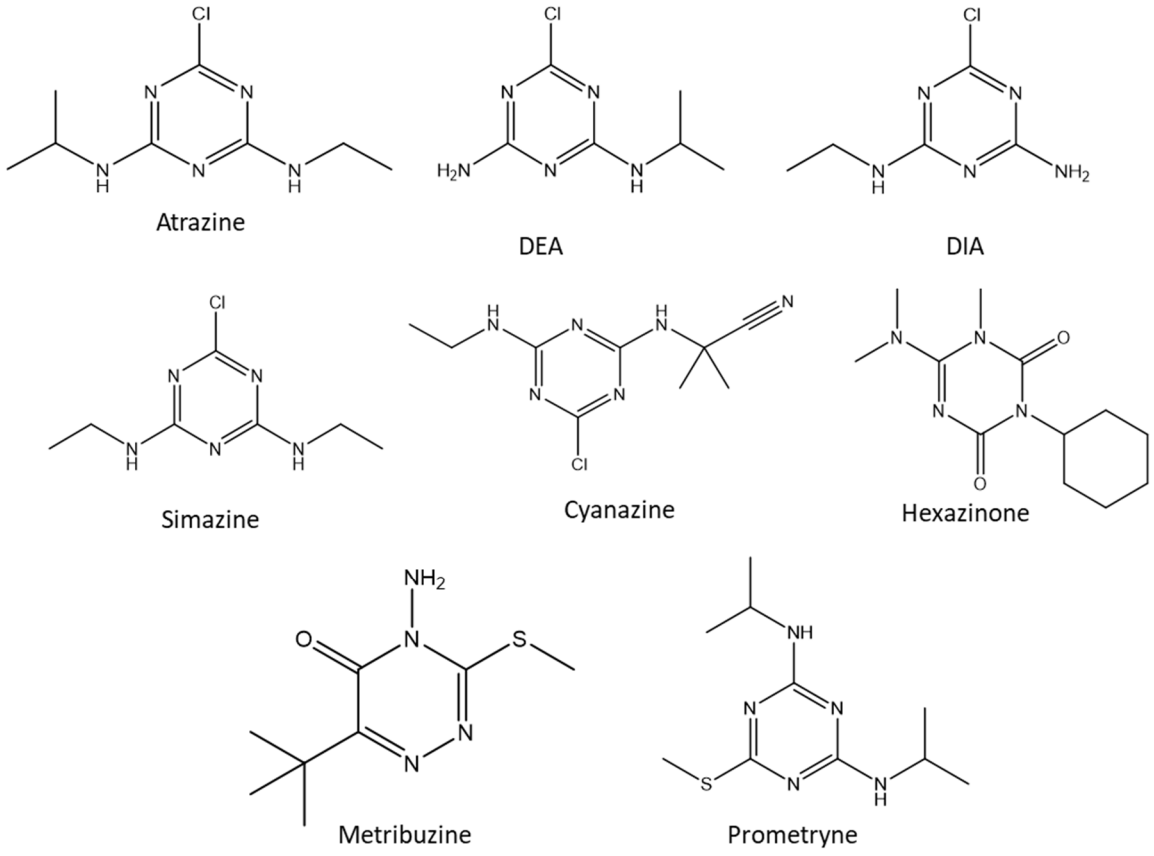


Figure 1.7 Structure moléculaires des triazines sélectionnés.

Les urées substituées

Les pesticides de type urées substituées telles que le linuron (**Figure 1.8**) appartiennent aussi au groupe des herbicides inhibiteurs de la photosynthèse. Ils agissent en bloquant le transport d'électrons et le transfert de l'énergie lumineuse au niveau du photosystème II dans le site B (OMAFRA, 2000).

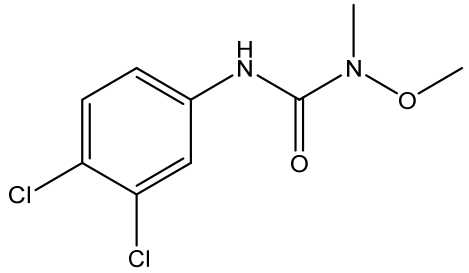


Figure 1.8 Structure moléculaire du linuron.

Le glyphosate

Le glyphosate (**Figure 1.9**) appartient à un groupe d'herbicides inhibiteurs de la synthèse d'acides aminés aromatiques (EPSP synthèse) mobiles dans le phloème. Spécifiquement, le glyphosate inhibe la 5-enolpyruvylshikimimate-3-phosphate synthèse (EPSP). Le feuillage des plantes commence par jaunir et s'ensuit un virage au brun et la mort du végétal dans les 10 à 14 jours après l'application de l'herbicide (OMAFRA, 2000; Franz et al., 1997; Duke et al., 2008).

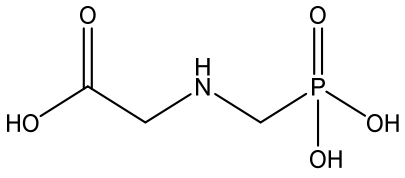


Figure 1.9 Structure moléculaire du glyphosate.

1.4 Pesticides comme contaminants d'intérêt émergent

La nuance entre la définition d'un contaminant historique et un contaminant d'intérêt émergent repose sur la quantité d'informations disponibles sur les effets toxicologiques et environnementaux de certains composés qui viennent d'être commercialisés, ou sur le fait que leurs effets commencent à intéresser la population et la communauté scientifique. Il existe ainsi des pesticides qui sont utilisés depuis longtemps, mais les informations disponibles ne sont que parcellaires et leurs effets sur l'environnement commencent à être suspectés.

Comme pesticides en tant que contaminants d'intérêt émergent, on peut notamment citer les insecticides néonicotinoïdes et le glyphosate. L'atrazine quant à elle n'est pas *stricto sensu* un contaminant d'intérêt émergent. Elle est largement étudiée depuis plusieurs décennies, mais reste en usage en Amérique du Nord avec de possibles impacts sur la santé et l'environnement, ce qui justifie la poursuite des recherches sur le sujet. Dans le cas des néonicotinoïdes, on peut citer l'hypothèse de leur contribution à l'effondrement de colonies entières d'abeilles; dans le cas du glyphosate et de l'atrazine, des effets de perturbation endocrinienne et de carcinogénicité pour de nombreux modèles biologiques (Bonmatin et al., 2015; Pisa et al., 2015; van der Sluijs et al., 2015; Health Canada, 1993; Hayes et al., 2011; Gasnier et al., 2009; Thongprakaisang et al., 2013).

Ces problèmes ont attiré l'attention sur ces pesticides et leur devenir dans l'environnement. Des analyses de néonicotinoïdes ont été réalisées dans les abeilles, le miel, le pollen (Chen et al., 2014; Sanchez-Hernandez et al., 2016) avec l'objectif de vérifier les niveaux de contamination. Par ailleurs, compte tenu de leur hydrosolubilité et de leur relative persistance, les êtres humains et d'autres organismes non ciblés (p.ex., faune aquatique) peuvent être exposés à ces composés par de multiples voies (eau de surface et sols, produits de consommation humaine comme l'eau potable et la nourriture, etc.). Caractériser les niveaux de contamination constitue une première étape pour déterminer l'exposition et les risques possibles sur la santé et l'environnement.

Après application, les néonicotinoïdes sont distribués dans les plantes et le sol et finissent par contaminer les eaux de surface; un très grand nombre d'espèces non ciblées peuvent ainsi être exposées dans l'ensemble des écosystèmes. En termes de toxicité aiguë, la dose létale pour 50% de la population étudiée (DL50) des néonicotinoïdes chez les mammifères est inférieure ou égale à celle du DDT, mais ce n'est pas nécessairement le cas chez les insectes. Par exemple, l'imidaclopride est 7300 fois plus toxique pour les abeilles que le DDT, alors que les quantités par hectare ne sont que deux à six fois plus faibles. La DL50 varie largement en fonction du modèle biologique considéré, rendant difficile l'établissement de normes de qualité environnementale. Par exemple, chez les vertébrés aquatiques, les poissons, les petits oiseaux et les petits mammifères, les effets sont observés à des doses plus élevées ou sur des expositions plus longues (Simon-Delso et al., 2015; Morrissey et al., 2015).

Compte tenu de toutes les informations antérieures relatives à l'ubiquité des néonicotinoïdes, des problèmes de santé humaine pourraient être anticipés. L'être humain en tant que consommateur pourrait être exposé à des doses faibles, mais chroniques, à cause des produits alimentaires contaminés par les pesticides.

À titre d'exemple, le Japon, qui est un producteur et consommateur important de néonicotinoïdes, a réalisé des études sur des populations humaines exposées (expositions liées à l'activité professionnelle ou à l'alimentation), dans lesquelles 90% de la population ont montré des résidus pour au moins quatre néonicotinoïdes (Taira et al., 2014). Huit métabolites sur 27 ont été rapportés chez 3 patients dans une étude réalisée par Taira et al., (2013). Récemment, les néonicotinoïdes ont été classés comme probablement génotoxiques, cytotoxiques, neurotoxiques et cancérigènes (Chen et al., 2014). Un problème de santé publique lié à l'omniprésence des néonicotinoïdes pourrait éventuellement se poser. Actuellement, les néonicotinoïdes ont été évalués dans plusieurs pays pour déterminer les concentrations restrictives et maximales admissibles dans différents produits de consommation.

1.4.1 Occurrence des pesticides

1.4.1.1. Néonicotinoïdes

L'occurrence environnementale de pesticides d'intérêt émergent tels que les néonicotinoïdes n'est documentée que depuis relativement récemment. Malgré leur commercialisation dans les années 1990, il faudra attendre une dizaine d'années pour voir les premières études environnementales sur le sujet (Denning et al. 2014 in CCME 2007; Sanchez-Bayo et al. 2014; Starner and Goh, 2012; Main et al. 2014; Samson-Robert, 2014). Dû à leur mode d'application spécifique par enrobement de semences, environ 20% des néonicotinoïdes sont assimilés par la plante et les 80% restants migrent vers le sol et l'eau environnante. On retrouve ainsi ces contaminants dans l'eau souterraine, l'eau de surface et même dans l'eau potable à des niveaux faibles mais détectables (typiquement de l'ordre du ng L^{-1} ou de la dizaine de ng L^{-1}) (Seccia et al. 2005; Dujakovic et al. 2010; Hao et al. 2015; Hladik and Calhoun, 2012).

Diverses revues de littérature ont été réalisées sur l'occurrence et la distribution des insecticides néonicotinoïdes (Klarich et al. 2017; Bonmatin et al. 2015; Anderson et al. 2015; Wood et al. 2017; Cimino et al. 2017; Gibbons et al. 2015). Plutôt qu'une présentation exhaustive des données d'occurrence, nous nous appuierons sur quelques études à titre d'exemple (voir aussi information résumée dans le **Tableau 1.2**).

La revue de littérature de Morrissey et al. (2015) présente notamment un histogramme des niveaux de contamination des néonicotinoïdes dans les eaux de surface à travers le monde (**Figure 1.10**). La section (**a**) de la **Figure 1.10** présente les moyennes des concentrations de chaque étude, et la section (**b**) présente les concentrations maximales; dans les deux cas, une échelle logarithmique est utilisée (µg L^{-1}). La courbe rouge représente la probabilité de distribution cumulée en utilisant toutes les données disponibles de concentrations des néonicotinoïdes dans l'eau de surface. Ainsi, sur le panel (**a**), la médiane des concentrations moyennes à niveau mondial est estimée à 0.07 µg L^{-1}. Les traits noirs verticaux représentent quant à eux les critères de qualité d'eau des divers organismes gouvernementaux, considérant l'imidaclopride en tant que représentant modèle des néonicotinoïdes : Institut

national néerlandais pour la santé publique et l'environnement (RIVM), 2014: 0.0083 µg L^{-1}, Conseil canadien des ministres de l'environnement (CCME), 2007: 0.23 µg L^{-1}, Agence de protection de l'environnement des États-Unis (U.S. EPA): 1.05 µg L^{-1}, et Autorité européenne de sécurité des aliments (EFSA), 2008: 0.2 µg L^{-1}).

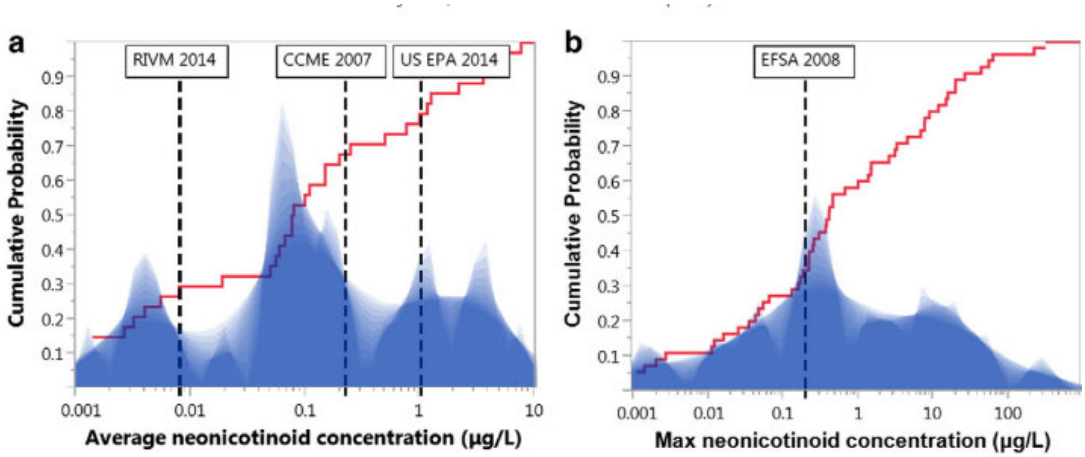


Figure 1.10 Distribution des concentrations moyenne et maximale des néonicotinoïdes dans l'eau de surface. Histogramme extrait de la publication de Morrissey et al. (2015).

La concentration maximale médiane se trouve autour de 0.3 µg L^{-1} (**Figure 1.10**, panel (**b**)). Cette concentration est supérieure au critère des Pays-Bas ainsi qu'à celui adopté au Canada en 2018, fixé à 0.0083 µg L^{-1}. Quelques études ont également rapporté des concentrations supérieures à la centaine de µg L^{-1} dans l'eau de surface. Par exemple, l'imidaclopride a été détecté dans les eaux de surface agricoles aux Pays-Bas avec une concentration maximale de 320 µg L^{-1} (Van Dijk et al. 2013). La thiaméthoxame et l'acétamipride ont été quantifiés à une concentration maximale de 225 µg L^{-1} dans certaines zones humides au Texas (Anderson et al. 2013).

Les fréquences de détection des néonicotinoïdes dans les eaux environnementales varient entre les sites d'étude et selon les performances des méthodes analytiques. Dans des études spécifiques aux États-Unis et au Canada, leur occurrence dans les eaux de surface a été rapportée entre 53% et 100% (Hladik et al. 2016; Schaafschma et al. 2015) avec des concentrations entre 0.001 µg L^{-1} et 0.043 µg L^{-1}. Dans une étude menée par Klarich et al. (2017), les néonicotinoïdes ont été retrouvés à des concentrations de 0.24 à 57.3 ng L^{-1}

dans des échantillons d'eau potable collectés aux États-Unis. Au Québec, l'imidaclopride a été quantifié dans l'eau de surface des zones agricoles entre 10 et 41 µg L^{-1} avec 61% occurrence entre 2008 et 2009 (Anderson et al. 2015). Dans le cadre du suivi de la qualité de l'eau de surface du Fleuve St-Laurent et ses tributaires, une étude réalisée par Giroux, Hébert et Berryman (2016), la clothianidine et la thiaméthoxame ont dépassé le critère de qualité pour la protection de la vie aquatique dans 9-100% et 18-100% des échantillons de 17 rivières tributaires du fleuve St-Laurent. L'imidaclopride a dépassé ce critère dans 9-80% des échantillons pour les 5 rivières où il a été analysé.

1.4.1.2. Atrazine

Bien qu'ayant été interdite dans l'Union Européenne, l'atrazine reste encore très utilisée en Amérique du Nord. Toutefois, l'atrazine peut encore être retrouvée dans l'environnement en Europe même 14 ans après son interdiction. Cotton et al. ont ainsi rapporté une occurrence de 100% dans les eaux de surface en France, avec des concentrations entre 0.5 et 10 ng L^{-1} (Cotton et al. 2016). En Espagne, une étude de Postigo et al. (2010) a montré des fréquences d'occurrence entre 76-100% dans les eaux de surface et souterraine, à des niveaux plus élevés qu'en France. Dans une vue plus globale de l'Union Européenne, l'atrazine a été retrouvée dans 20% des échantillons analysés entre 2012 et 2017, avec des concentrations >0.5 µg L^{-1}. Comparativement, des niveaux supérieurs ont été relevés en 2013 aux États-Unis, entre 0.04 et 120 µg L^{-1} dans 54% des échantillons analysés (Mahler et al., 2017). Au Québec, l'atrazine a été quantifiée dans l'eau potable avec une concentration maximale de 1 µg L^{-1} entre 2005 et 2009 et 0.3 µg L^{-1} entre 2010 et 2014 selon le Bilan de la Qualité de l'eau potable au Québec (2010-2014), rapport du *Ministère du Développement durable, de l'Environnement et de la Lutte contre les changements climatiques* (2016). L'atrazine a été détectée dans 98% des échantillons couvrant 4 rivières entre 2011-2014 dans le sud du Québec, avec des concentrations maximales de 9.7-13 µg L^{-1} (Giroux, 2015).

1.4.1.3. Glyphosate et AMPA

Le glyphosate a aussi fait l'objet de suivis au Québec par le *Ministère du Développement durable de l'Environnement et de la Lutte contre les changements climatiques* (2016), lequel a relevé des concentrations maximales dans l'eau potable de l'ordre de 1.5 µg L^{-1} au cours de la période 2010-2014, et de l'ordre de 2.1 µg L^{-1} pour la période de surveillance antérieure (2005-2009). Le glyphosate a été également retrouvé dans 88% des échantillons de rivières collectés entre 2011 et 2014, avec une concentration maximale de 18 µg L^{-1} (Giroux, 2015). La présence de cet herbicide varie amplement entre pays et en fonction de l'activité agricole. Aux États-Unis, dans une étude exhaustive de Battaglin et al. (2014) couvrant un large nombre d'échantillons (n= 3 732) entre 2001 et 2010, le glyphosate fut détecté dans 53%, 5.8% et 70% des échantillons d'eau de surface, d'eau souterraine et de précipitations, respectivement. Les concentrations maximales étaient de l'ordre de 2–3 µg L^{-1} (Battaglin et al. 2014). Un de ses produits de dégradation, l'AMPA, a été détecté à des concentrations maximales de l'ordre de 4 µg L^{-1} dans les eaux de surface et souterraine de cette même étude (Battaglin et al. 2014). Dans une autre étude réalisée aux États-Unis, Mahler et al. (2018) ont retrouvé le glyphosate dans 45% des échantillons d'eau de surface à des niveaux compris entre 0.2 et 27.8 µg L^{-1}. Dans une étude d'Aparicio et al. (2013), le glyphosate a été détecté dans 35% des échantillons d'eau de surface en Argentine avec des concentrations entre 0.5 et 4 µg L^{-1}, tandis que l'AMPA était également retrouvé dans 33% des échantillons (gamme de concentration des échantillons positifs: 0.5 à 2.3 µg L^{-1}).

Tableau 1.2 Occurrence globale des pesticides

Auteur	Analytes	Matrice	LD	Occurrence %	Gamme de concentration	Année	Pays
Sanchez-Bayo et al. 2016	Néonicotinoïdes	Eau de surface et eau agricole	0.01 µg L^{-1}	13-57	0.08-320 µg L^{-1}	2009-2016	Mondial
Klarich et al. 2017	Néonicotinoïdes	Eau potable	0.1 ng L^{-1}	100	0.24-57.3 ng L^{-1}	2016	USA
Anderson et al. 2015	Imidacloprid	Eau de puits	0.1 µg L^{-1}	61	0.1-6.1 µg L^{-1}	2008-2009	Quebec, Ca
Morrissey et al. 2015	Néonicotinoïdes	Eau de surface et eau agricole	0.1 µg L^{-1}	27-93	0.13-0.63 µg L^{-1}	1998-2013	Mondial
Shaafschma et al. 2015	Néonicotinoïdes	Eau de surface	0.1 ng L^{-1}	100	0.001-0.043 µg L^{-1}	2013	Ontario, Ca
Hladik et al 2016	Néonicotinoïdes	Eau de surface	2 ngL^{-1}	53	0.030 µg L^{-1}	2012-2014	USA
Székács et al. 2015	Néonicotinoïdes	Cours d'eau	3 µg L^{-1}	8	10-41 µg L^{-1}	1990-2015	Hongrie
Cotton et al. 2016	Atrazine	Eau surface	1 µg L^{-1}	100	0.5 – 10 ng L^{-1}	2016	France

Mahler et al. 2017	Atrazine	Eau de surface	0.02 µg L^{-1}	54	0.04 - 120 µg L^{-1}	2013	USA
Mahler et al. 2018	Glyphosate	Eau de surface	0.2 µg L^{-1}	45	0.2 µg L^{-1} - 27.8 µg L^{-1}	2013	USA
Sousa et al. 2018	Atrazine	Eau de surface	0.5 µg L^{-1}	20	> 0.5 µg L^{-1}	2012-2017	UE
Postigo et al. 2010	Atrazine	Eau de surface Eau souterraine	0.53 ng L^{-1}	76-100	< 39 ng L^{-1} < 756 ng L^{-1}	2008-2014	Spain
Aparicio et al. 2013	Glyphosate AMPA	Eau de surface	0.5 µg L^{-1}	35 33	0.5 - 4 µg L^{-1} 0.5 - 2.3 µg L^{-1}	2011-2012	Argentine
Battaglin	Glyphosate/ AMPA	Eau de surface Eau souterraine Précipitation	0.1 µg L^{-1}	53/89 5.8/14 70/71	2.03 - 3.08 µg L^{-1} 0.48 – 4.88 µg L^{-1}	2001-2010	USA
Struger et al. 2008	Glyphosate	Eau de surface	5 µg L^{-1}	2-5	17-40.8 µg L^{-1}	2004-2005	Ontario, Canada

1.4.2 Règlementation et contrôle : mise en contexte

Le marché des pesticides a beaucoup changé depuis l'implémentation du « International Code of Conduct on Pesticide management ». À l'époque, seulement 15 compagnies européennes et américaines dominaient les ventes comparativement aux 6 multinationales - Syngenta, BASF, Bayer, Dow, Du Pont et Monsanto - qui ont pris le contrôle de la majorité du marché des composés phytosanitaires.

Même si la politique de réglementation des pesticides fut établie il y a longtemps, on constate que la plupart des substances ne respectent pas les sept normes internationales (Organisation mondiale de la santé (OMS), Organisation de l'agriculture et l'alimentation (FAO)) à propos des pesticides (Hough, 2013; FAO-WHO, 2016):

- Nous devons nous efforcer d'atteindre des rendements alimentaires optimaux.
- Les maladies et les dommages causés par les ravageurs devraient être limités.
- L'abus de pesticides menant à l'intoxication humaine devrait être empêché.
- Le commerce international des pesticides devrait être réglementé.
- Les pesticides ne doivent pas être surexploités.
- La pollution de l'environnement par les pesticides devrait être limitée.
- La contamination des aliments par les pesticides devrait être limitée.

1.4.3 Exposition humaine et ses implications

Dans le cadre de cette section, divers aspects sur l'exposition humaine comme conséquence de la contamination dans l'eau et la nourriture seront abordés. Une brève comparaison des différentes valeurs seuils dans l'eau et la nourriture, ainsi que le degré d'exposition humaine via la présence des pesticides dans l'urine comme marqueur de contamination, seront aussi présentées.

1.4.4 Eau

Dans le contexte des valeurs de référence de la qualité d'eau, que ce soit pour l'eau potable ou pour la protection de la vie aquatique, les lignes directrices varient par pays (**Tableau 1.4**).

L'imidaclopride par exemple a été pris comme valeur de référence pour tous les composés de sa classe, les néonicotinoïdes, et les valeurs de références sont dérivées des valeurs disponibles de toxicité aiguë et chronique: Concentrations létales (LC_{50}), Concentrations avec effets (EC_{50}), Concentration sans effets observés (NOEC), et Concentration la plus petite à laquelle on peut observer des effets (LOEC) issues de différentes études de toxicologie. En se basant sur plusieurs études et chiffres officiels, Morrisey et al. (2015) ont proposé des valeurs limites pour la somme des néonicotinoïdes dans l'eau de surface pour la protection de la vie aquatique : 0.2 $\mu g\ L^{-1}$ comme concentration maximale à court terme et 0.035 $\mu g\ L^{-1}$ pour la moyenne à long terme (Morrissey et al., 2015). Les Pays-Bas ont proposé en 2014 des valeurs plus protectrices. En 2014 après une révision sur la toxicité des néonicotinoïdes, les Pays-Bas ont abaissé la valeur pour la protection de la vie aquatique de 63 à 8.3 $ng\ L^{-1}$ (RIVM, 2014). En 2018, le Québec a adopté la même valeur comme mesure de protection en passant de 230 $ng\ L^{-1}$ à 8.3 $ng\ L^{-1}$ (CCME, 2007-2018).

Dans le cas de d'atrazine, les valeurs limites pour l'eau potable sont variables en fonction du pays et de l'organisation qui les établit. Au Canada, l'atrazine est utilisée comme herbicide et Santé Canada a établi une valeur de 5000 $ng\ L^{-1}$ comme concentration maximale acceptable pour l'eau potable (Health Canada, 1993). Aux États-Unis, le seuil a été placé à 3000 $ng\ L^{-1}$ (U.S. EPA, 2007) et à 2000 $ng\ L^{-1}$ par l'Organisation Mondiale de la Santé (WHO, 2011). L'Union Européenne a fixé cette valeur à 100 $ng\ L^{-1}$ et l'interdiction de son utilisation avec effet en 2005 (2004/248/EC).

Dans le cas du glyphosate, le Canada a établi un critère de 800 $\mu g\ L^{-1}$ pour la protection de la vie aquatique (effet chroniques) (CCME, 2012) tandis que l'U.S. E.P.A. a établi une valeur de 700 $\mu g\ L^{-1}$ pour l'eau potable (U.S.E.P.A., 2016) et au Canada 280 $\mu g\ L^{-1}$ (Health Canada, 2008).

Tableau 1.4. Critères internationaux de qualité d'eau pour la vie aquatique.

Compound	Guideline (µg L^{-1})	Organization	Reference
Imidacloprid	0.23	Canadian Council of Ministers of the Environment	CCME, 2007
ΣNeonicotinoids	0.0083	Quebec Ministry of the Environment	RIVM, 2014
Atrazine	1.8	Canadian Council of Ministers of the Environment	CCME, 2012
Atrazine	10	U.S. Environmental Protection Agency	USEPA, 2006
Atrazine	2	European Union Directive	2008/105/EC
Glyphosate	800	Canadian Council of Ministers of the Environment	CCME, 2012

1.4.5 Nourriture

Les néonicotinoïdes sont appliqués dans un grand nombre de produits agricoles dans plus de 120 pays (Jaschke et al., 2008). L'addition de variables telles que les grands nombres de cultures, de pesticides et de pays complique l'établissement de limites maximales de résidus de pesticides (MRL) dans la nourriture à niveau mondial, voir **Tableau Annexe B** pour les 4 matrices ciblées dans cette thèse (pomme, laitue, raisin et tomate).

En termes d'effets toxiques, les chercheurs utilisent les valeurs de prise acceptable (ADI, Acceptable Daily Intake), c.-à-d., les concentrations en dessous desquelles les produits ne présenteraient pas d'effets néfastes sur la santé en cas d'ingestion chronique, soit par consommation d'eau ou de nourriture. Par exemple, pour l'imidaclopride, qui a été pris comme modèle pour les néonicotinoïdes, la valeur moyenne de référence de prise acceptable est de 0.06 mg/kg de poids corporel par jour.

Les valeurs des ADI's en vigueur pour le Canada sont régulées par la ARLP (Agence de réglementation de la lutte antiparasitaire (Health Canada, 2019)) qui indique que ces valeurs sont similaires à celles recommandées par l'US EPA et l'OMS (« the ADI set by the ARLP are similar to those recommended by the United States-Environmental Protection Agency (U.S. EPA) and the World Health Organization (WHO) »). Cependant, il existe certaines divergences entre les valeurs proposées par ces deux organismes de réglementation (**Tableau 1.3**).

Tableau 1.3. Valeurs des ADI's publiées par l'U.E. et l'OMS pour divers pesticides, exprimées en mg/kg de poids corporel.

Pesticides	Union Européenne	FAO/WHO
Acétamipride	0.025	0-0.07
Clothianidine	0.097	0-0.1
Dinotefuran	NTI	0-0.2
Fipronil	0.0002	0.0002
Imidaclopride	0.06	0-0.06
Nitenpyram	NTI	NF
Thiaclopride	0.01	0-0.01
Thiaméthoxame	0.026	0-0.08
Atrazine	0.02	NF
Cyanazine	NTI	NF
Simazine	NTI	NF
Carbendazim	0.02	0-0.03
Carbaryl	0.0075	0-0.008
Linuron	0.003	NF
Phosmet	0.01	0-0.01
O,O,O-Triethyl phosphorothioate	NF	NF
Dimethoate	0.001	0.002
Famphur	NF	NF

NTI: No Toxicological Information

Il existe un manque d'information important pour plusieurs pesticides, et pour certains composés qui ont un ADI enregistré, il s'agit de valeurs datées de 1986 même si la dernière mise à jour de la base de données du Canada a été réalisée en 2008.

Le présent travail n'a pas pour objectif de conduire une étude de toxicologie détaillée mais plutôt de fournir des données préliminaires sur le niveau d'exposition des consommateurs par les pesticides à travers différentes voies. Dans les chapitres 3 à 6 nous montrerons la présence de divers pesticides retrouvés dans l'eau potable, l'eau de surface (qui peut servir à la production d'eau potable), ainsi que dans la nourriture.

Dans le cas de la nourriture, plusieurs études ont été réalisées pour analyser une grande diversité de pesticides dans différents produits de consommation (Anastassiades et al. 2003, Zhang et al.2013; Badoud et al. 2018; Chamkasem et al. 2013; Chen et al. 2014; Mac Loughlin et al. 2018). L'acétamipride est l'un des néonicotinoïdes le plus récurrents dans les fruits et légumes. Ainsi que l'ont montré Golge et Kabak (2015), l'acétamipride a été retrouvé dans les tomates avec une gamme de concentrations entre 15 et 370 μg kg^{-1}. La concentration moyenne de l'acétamipride dans le même produit était de 4.3 μg kg^{-1} dans une base de données aux É-U. publiée par Cradock et al. (2019). Des herbicides comme l'atrazine ont aussi été retrouvés dans les fruits et légumes disponibles en Argentine et en Chine, dans une gamme de concentrations entre 35 et 310 μg kg^{-1} (Mac Loughlin et al. 2018; Tian et al. 2014).

1.4.6 Analyse dans l'urine comme traceur d'exposition humaine

Pour mieux comprendre les effets des métabolites des néonicotinoïdes sur la santé humaine, Taira (2014) a réalisé des études sur des populations exposées, dans lesquelles 90% de la population testée (Japon) ont répondu positivement à au moins un des quatre néonicotinoïdes. Les effets montrés sur les patients avec une exposition aigüe et subaigüe sont : tremblements des doigts, troubles de la mémoire à court terme, fièvre, fatigue générale, mal de tête, douleurs abdominales et musculaires en générale ainsi que faiblesse musculaire et spasmes musculaires. L'analyse des métabolites responsables de ces symptômes pour mieux comprendre la dégradation des insecticides dans le corps humain est encore sous étude. De même Taira et al. (2014) a fait l'analyse d'échantillons d'urine prévenants de trois individus soupçonnés d'une exposition subaiguë aux pesticides néonicotinoïdes, dont 7 métabolites sur 57 connus ont été retrouvés.

Il existe un manque d'information en relation entre l'apport quotidien de pesticides et son élimination urinaire (Chen et al. 2015). Pour mieux comprendre ce processus, Harada et al. (2016) a réalisé une étude sur 9 adultes japonais qui ont accepté d'ingérer une quantité de néonicotinoïdes à faible dose pour faire un suivi dans le processus de dégradation. Les résultats d'Harada montrent que la clothianidine ne se dégrade pas pendant 3 jours, le dinotefuran

pendant 1 jour et dans le cas de l'imidaclopride seulement 10% de la quantité fournie a été retrouvée sans dégradation. A contrario, l'acétamipride a été métabolisée complètement dans sa forme déméthylée.

L'analyse d'urine pour quantifier les néonicotinoïdes et ses métabolites reste limitée aux pays comme le Japon, et à la connaissance des auteurs de la présente thèse de recherche, il n'existe pas de données publiées pour le Canada concernant les niveaux d'exposition de la population.

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Chapitre 2. Méthodes pour l'analyse de pesticides

Le choix d'une méthode analytique adaptée dépend notamment de la nature du composé, de la complexité de la matrice et du niveau de performance souhaité. Dans le contexte de cette thèse, l'accent sera mis sur les pesticides organiques modérément polaires présents à l'état de traces dans les matrices environnementales et certains produits alimentaires. Idéalement, la méthode analytique doit être robuste et versatile, c.-à-d. applicable pour une large gamme de pesticides et une grande variété de matrices, bien que chacune puisse présenter des défis spécifiques lors des étapes d'extraction, de purification et d'analyse instrumentale. En général, les méthodes d'analyse comportent les étapes suivantes :

- Extraction du composé d'intérêt de la matrice.
- Purification après l'extraction, suivie si nécessaire d'une étape de préconcentration.
- Introduction de l'extrait dans l'appareil d'analyse instrumentale.
- Séparation analytique, réalisée pour la plupart de pesticides modérément polaires par chromatographie liquide.
- Détection, généralement réalisée par spectrométrie de masse.
- Détermination quantitative et/ou qualitative de résidus de pesticides et leurs métabolites.

2.1 Traitement de l'échantillon

Les techniques de traitement de l'échantillon dépendent de la nature des composés ciblés (polarité, volatilité, stabilité etc.) et de la nature de la matrice. Certaines étapes préliminaires peuvent être utiles avant de procéder à l'extraction. Par exemple, une étape de broyage peut s'avérer utile afin d'homogénéiser le matériau préalablement à l'extraction. C'est notamment le cas pour les matrices solides comme les sols et sédiments,

les fruits et légumes, ou encore les tissus d'origine animale. L'objectif est à la fois d'obtenir une poudre ou une matrice homogène et d'augmenter la surface de contact entre la matrice et le solvant d'extraction.

S'agissant des échantillons aqueux (p.ex., eau de rivière), une étape de prétraitement usuelle consiste à faire passer l'échantillon à travers une membrane de filtration. L'objectif de la filtration est d'enlever les matières en suspension/particulaires. Cependant, cette étape ne doit pas occasionner une perte de l'analyte dissous par rétention sur la membrane du filtre. Ainsi, le choix de la nature de cette membrane est dépendant des propriétés des analytes ciblés. Dans la littérature, la membrane en fibre de verre ou GFF (Glass fiber filter) est la plus couramment utilisée pour les pesticides organiques modérément polaires tels que les néonicotinoïdes, triazines, carbamates et organophosphorés (**Tableau 2.1**) (Dujakovic et al. 2010; Hladik et al. 2012; Qi et al. 2014; Heeb et al. 2012; Peruzzo et al. 2008). Dans certains cas spécifiques comme pour le glyphosate, Peruzzo et al. ont rapporté l'utilisation d'autres types de membranes telles que l'acétate de cellulose (Peruzzo et al. 2008). Une étude portant sur l'influence de la nature du filtre sur la récupération des néonicotinoïdes est abordée dans le Chapitre 4.

Tableau 2.1. Différents filtres utilisés pour le pré-traitement d'échantillons pour l'analyse de pesticides.

Auteur	Type d'échantillon	Analytes	Traitement de l'échantillon	Filtre
Dujakovic et al. 2005	Surface and Groundwater	Various pesticides*	**SPE	***PVDF
Hladik et al. 2015	Surface water	Neonics	Filtration/SPE	GFF-0.7µm
Qi et al. 2014	Surface water	Various pesticides*	Filtration/SPE	GFF-0.7µm
Heeb et al. 2012	Surface water	Various pesticides*	Filtration/SPE	GFF-0.7µm
Peruzzo et al. 2008	Surface water	Glyphosate	Filtration/Derivatization	Cellulose acetate-0.45µm

* Organophosphates, néonicotinoïdes, carbamates, diacylhydrazines, benzimidazoles, triazines et phenylureas.
** Solid Phase Extraction (extraction sur phase solide)
***Polyfluorure de vinylidène

2.1.1 Extraction Liquide-solide

Ce type d'extraction représente une technique souvent utilisée pour l'analyse de pesticides dans les matrices solides (p.ex., fruits et légumes, sédiments, sols, etc.). Elle repose sur une simple extraction avec un solvant dans un homogénéiseur, ainsi que l'ont montré Watanabe et al. (2014) en procédant à l'extraction d'insecticides néonicotinoïdes dans le concombre et l'aubergine. Dans cette étude, les auteurs ont testé une extraction avec de l'eau avec l'assistance des ultrasons ou des micro-ondes. Dans une étude de Chen et al. (2015) pour l'extraction de néonicotinoïdes et certains organophosphorés dans les sédiments, les auteurs ont utilisé une procédure en deux étapes se basant sur une prise d'essai de 2g d'échantillon de sédiments. La première étape d'extraction a été réalisée avec 2x 10 mL d'ACN et buffer de McIlvaine (pH 4.0) (1:1, v/v) et dans un second temps les sédiments ont suivi une deuxième extraction avec $Mg(NO_3)_2$-$NH_3 \cdot H_2O$ (96 :4, v/v) avec assistance des ultrasons durant 15 min.

Lors de l'extraction assistée par micro-ondes, la matrice est placée dans une cellule d'extraction revêtue de téflon. La température normale d'extraction est 50 à 100 °C plus élevée que la température d'ébullition des solvants à pression atmosphérique; ceci permet que les pesticides soient extraits plus efficacement. L'efficacité de l'extraction dépend de variables comme la température, la puissance du micro-ondes, le temps de cycle et le solvant (nature et volume). Des solvants comme l'acétate d'éthyle et l'acétonitrile sont couramment utilisés (**Tableau 2.2**).

Tableau 2.2. Exemples d'études utilisant l'extraction assistée par micro-onde (MAE) pour l'analyse de pesticides dans diverses matrices.

Auteur	Analytes	Type d'échantillon	Quantité initiale de matrice	Volume du solvant	Nature du solvant
Haroune et al. 2015	Néonics	Insectes	NA	5 mL	Acetate d'ethyle
Zheng et al. 2015	Néonics	Herbes médicinales	0.5g	25 mL	Acetonitrile
Su et al. 2017	Triazines	Jus de fruits	5 mL	180 µL	*C_6MIM([PF_6) - **(C_4MIM)(BF_4)

*1-hexyl-3-methylimidazolium hexafluorophosphate

**1-butyl-3-methylimidazolium tetrafluoroborate

2.1.2 Extraction Liquide-Liquide

L'extraction de partitionnement liquide-liquide ou LLE (Liquid-liquid extraction en anglais) permet de transférer les pesticides d'une phase aqueuse vers une phase organique non-miscible comme l'acétate d'éthyle. Par exemple, Caballero-Diaz et al. ont utilisé la LLE pour l'extraction de l'atrazine dans 20 mL d'eau de surface avec 4 mL d'acétate d'éthyle dans un seul cycle avec agitation à la main (Caballero-Diaz et al. 2013).

2.1.3 Extraction en Phase Solide

Pour les échantillons aqueux, l'extraction liquide-liquide a été progressivement remplacée par l'extraction en phase solide (SPE) qui nécessite de moins grandes quantités de solvant organique. L'extraction de l'analyte d'intérêt est réalisée par une petite quantité (typiquement 100-500 mg) de phase stationnaire solide contenue dans une cartouche support. La nature du l'absorbant dans les cartouches est variable. Il en existe des polymériques avec une partie hydrophile et une partie lipophile pour la rétention d'une large gamme de composés (p.ex., Thermo HyperSep Retain PEP, Oasis HLB, Phenomenex Strata X), d'autres encore sont conçus pour l'exchange de cations ou d'anions (p.ex., Oasis

WAX, Strata X-AW, Strata X-CW, HyperSep Retain-CX). Ce type d'extraction présente comme avantage de réduire la quantité de solvant utilisée par rapport à la LLE, et peut également contribuer à réduire les interférents présents dans la matrice.

La SPE se déroule généralement en cinq étapes: conditionnement, charge de l'échantillon sur la phase stationnaire, lavage, séchage et élution (**Figure 2.1**). Lors de la première étape, le conditionnement est réalisé en deux temps : i) rinçage des cartouches avec le solvant qui sera utilisé pour éluer les pesticides ciblés (p.ex., méthanol, acétonitrile, dichlorométhane, acétone, etc.) (Dujakovic et al. 2010; Hao et al. 2015; Hladik et al. 2012) et ii) conditionnement des cartouches avec un solvant qui dépend du milieu de l'échantillon à charger; il est possible de rincer avec de l'eau ultra-pure, de l'eau à pH modifié ou encore avec des tampons. Par la suite, l'étape de chargement de l'échantillon aqueux (typiquement 100-1000 mL) doit être réalisée à un débit adéquat pour garantir une bonne interaction des pesticides ciblés avec la phase stationnaire; une charge trop lente ou trop rapide peut induire des pertes d'analyte. L'étape de lavage après le chargement est optionnelle mais peut s'avérer décisive afin de réduire la charge en sels (p.ex., échantillons d'eau salée) ou en matière organique et autres interférents (p.ex., effluents de station de traitement des eaux usées). De l'eau ultra-pure ou des mélanges eau - solvant organique peuvent être utilisés pour cette étape, en éluant sélectivement les interférents sans affecter les composés d'intérêt. Une autre étape optionnelle est le séchage, normalement utilisé pour enlever l'eau résiduelle dans l'adsorbant et ainsi faciliter les étapes postérieures d'élution et d'évaporation.

Après l'étape de séchage, l'étape critique est l'élution dont le but est de décrocher les pesticides de l'adsorbant avec une efficacité maximale. Idéalement, l'élution devrait sélectivement décrocher les pesticides d'intérêt tout en évitant d'éluer les interférents matriciels restants mais ce n'est pas toujours le cas. Cette étape est réalisée avec le même solvant utilisé pour le conditionnement, le méthanol ou l'acétonitrile étant les plus souvent utilisés (Dujakovic et al. 2010; Hladik et al. 2012; Hao et al. 2015; Seccia et al. 2005) en raison de leur pouvoir éluant et du compromis analytes/interférents.

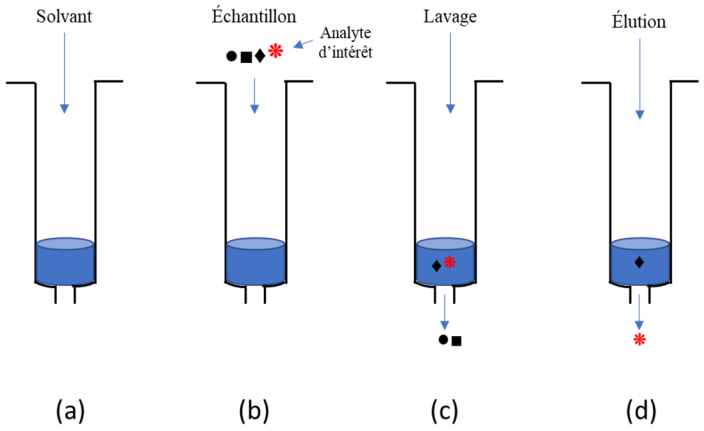

Figure 2.1 Représentation générale des étapes à suivre lors de la SPE, (a) Conditionnement par le choix de solvants, (b) charge de l'échantillon, (c) lavage avec un solvant faible pour éliminer les interférents, suivi par une étape optionnelle de séchage pour enlever les traces d'eau si nécessaire et (d) élution de l'analyte d'intérêt via l'interaction avec un solvant organique adapté.

Ce type d'extraction peut également être appliqué en mode « en-ligne » (*on-line SPE*, en anglais) en suivant les étapes auparavant présentées mais directement couplées au système instrumental LC-MS. L'extraction, la séparation analytique et la détection sont ainsi réalisées dans une même séquence ce qui réduit l'intervention de l'opérateur et le temps total d'analyse.

En mode d'analyse SPE en ligne, une pompe sert dans un premier temps à conditionner la colonne de charge (SPE) tandis qu'en parallèle la pompe analytique conditionne la colonne de séparation chromatographique. Une fois la colonne conditionnée, un volume d'échantillon est chargé sur la colonne SPE au débit préalablement optimisé (**Figure 2.2A**). Après une éventuelle phase de lavage additionnelle (avec un objectif similaire à la SPE hors ligne), dans un deuxième temps, la valve bascule pour laisser passer la phase mobile analytique à travers la colonne SPE (dans le sens contraire de la charge précédente) et ainsi éluer les analytes. Après élution de la colonne SPE, les analytes sont séparés sur la colonne chromatographique (**Figure 2.2B**) et détectés par spectrométrie de masse après leur ionisation.

Les méthodes en-ligne ont été appliquées avec succès pour divers contaminants organiques comme les hormones et les herbicides (Garcia-Ac et al. 2009; Fayad et al. 2013). Divers

paramètres doivent être optimisés afin de réduire le temps total d'analyse y compris l'extraction SPE et l'analyse LC-MS en tant que telle. Les facteurs critiques incluent notamment le volume de charge sur la colonne SPE et le débit (vitesse) de charge, lesquels peuvent affecter le taux de récupération et la sensibilité de la méthode (**Tableau 2.3**).

L'optimisation d'une méthode et de ses paramètres critiques peut être réalisée séquentiellement, une variable à la fois. Il existe plusieurs désavantages à cette approche, tels que l'augmentation du nombre total d'expériences selon le nombre des variables et selon les niveaux testés pour chacune d'entre elles. Autre inconvénient, optimiser la méthode un facteur à la fois n'est pas toujours pertinent car certains facteurs peuvent présenter des interactions (Miller and Miller, 2010).

Dans le cas de la SPE par exemple, des variables critiques comme le volume de charge et la vitesse de charge peuvent présenter des interactions, et l'effet de cette interaction peut différer selon l'analyte considéré ce qui complique l'optimisation dans le cadre d'une méthode multirésidus. Deux options sont possibles: 1) une optimisation « une variable à la fois » (Rodrigues et al. 2016) et 2) une optimisation via un plan d'expérience factoriel complet ou fractionnaire (Zhang et al. 2017). Un plan d'expérience présente l'avantage de minimiser le nombre total d'expériences tout en considérant les interactions potentielles entre facteurs étudiés. Divers types de plan d'expériences fractionnaires peuvent être appliqués, incluant les approches de type *Plackett Burman*, *Taguchi* ou encore *Box-Behnken*. Après la réalisation du plan d'expérience, qui consiste à faire varier les valeurs des facteurs f_1,f_2,f_3… à optimiser selon un nombre n de combinaisons, le choix de la combinaison optimale peut être réalisé selon un critère de choix d (par exemple, aire du pic chromatographique) ou, dans des approches multicritères, selon plusieurs critères de choix d_1,d_2,d_3… (par exemple, aire du pic chromatographique, précision de la mesure, forme du pic chromatographique, etc.). Il est possible d'utiliser la fonction de désirabilité de Derringer (**Eq. 1**) (D'Hondt et al. 2014), par laquelle on peut d'ailleurs assigner un facteur d'importance à chaque critère d_i, afin de guider le choix de la meilleure combinaison parmi celles testées. Pour chaque combinaison testée, la désirabilité globale est calculée, la combinaison/méthode optimale étant celle présentant la valeur maximale de D.

Eq. 1 $$D = \sqrt[n]{\prod_{i=1}^{n} d_i}$$

Tableau 2.3. Exemples de méthodes dans la littérature avec les principaux paramètres qui peuvent affecter la sensibilité et le taux de récupération lors d'une extraction par SPE en-ligne. Analyse de différentes pesticides et contaminants organiques dans une matrice d'eau (e.g. eau de surface, eau potable, eau souterraine et eau de mer).

Auteur	Analytes	Matrice	Colonne SPE	Volume de charge	Débit de charge	% Récupération	LOD [ng L^{-1}]
Cotton et al. 2016	Divers pesticides	Eau de surface	HLB, Waters	5 mL	0.5 mL/min	-	<5
Garcia-Ac. et al. 2009	Divers pesticides	Eau potable	Strata X, Phenomenex	10 mL	1.5mL/min	60-109	<18
Poiger et al. 2017	Glyphosate	Eau de surface	Two stacked Gemini-NX C18 cartouches, Phenomenex	1 mL	1mL/min	91-103	5
Rodriguez-Gonzalez et al. 2015	Atrazine	Eau de mer	HLB, Waters	5 mL	1mL/min	80.3-99.8	7-180
Huntscha et al. 2012	Divers pesticides	Eau de surface, souterraine	10 mg HLB + 10 mg Strata X-AW/Strata X-CW/Isolute ENV+ (1/1/1.5, m/m)	20 mL	2mL/min	80-120	< 3

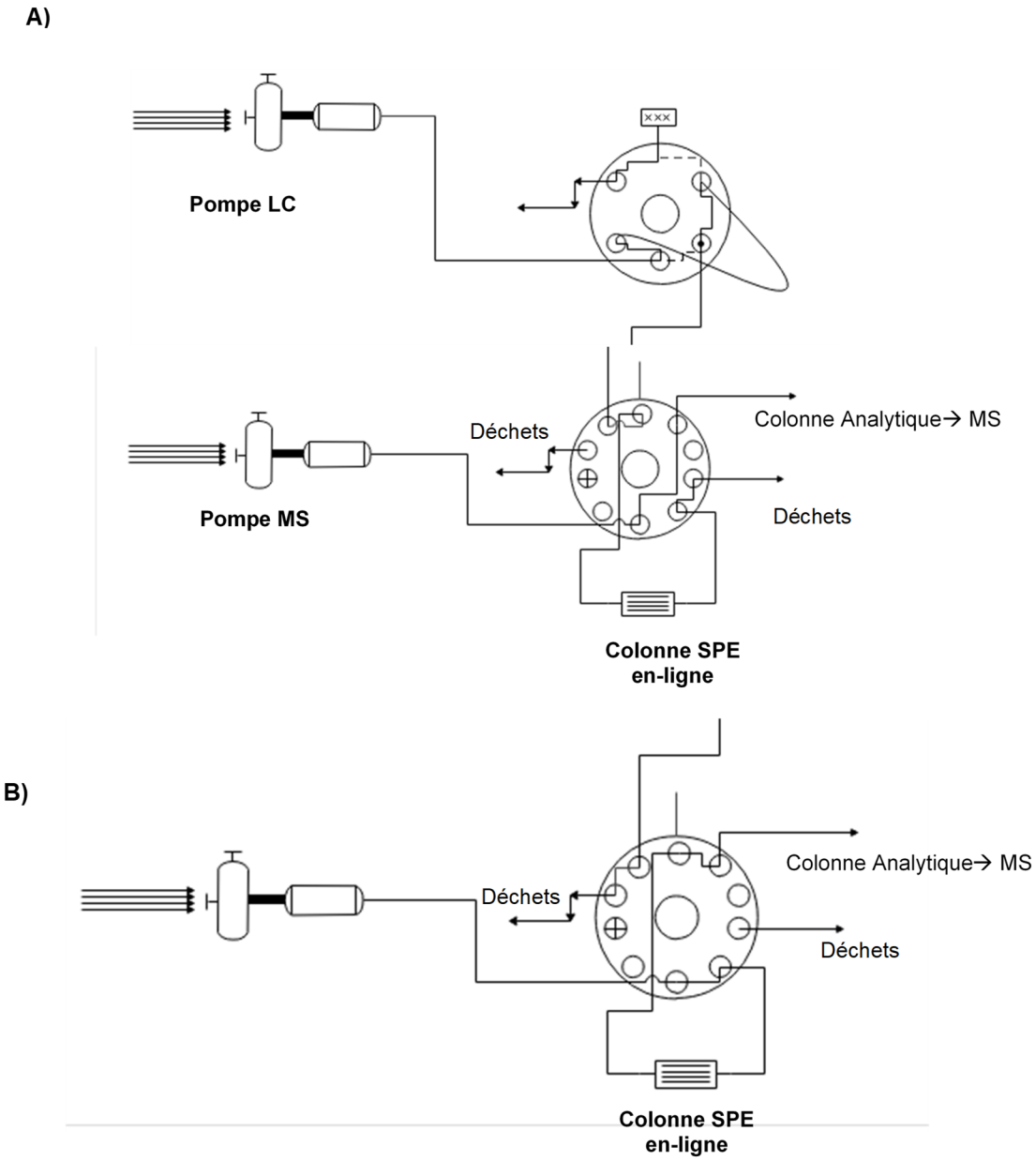

Figure 2.2. SPE en ligne - Configuration du système UHPLC-MS / MS. A) Étape de chargement réalisée par la pompe SPE; B) Étape d'élution effectuée par la pompe LC-MS.

2.1.4 Extraction en Phase Solide dispersive QuEChERS

Dans les variations possibles de l'extraction en phase solide, la méthode qui s'est imposée dans la dernière décennie pour les matrices solides est celle développée par Anastassiades et al. (2003), nommée QuEChERS (Quick Easy Cheap Effective Rugged Safe). Cette méthode devait répondre à la problématique de la complexité des matrices alimentaires et la grande variabilité de polarité des pesticides ciblés.

L'ajout du solvant d'extraction comme l'acétonitrile ou le méthanol est la première variable qui joue un rôle crucial afin de permettre un bon taux de récupération grâce à l'affinité des analytes ciblés pour le solvant. Pour favoriser cette interaction, on procède typiquement à un 'salting-out' : l'utilisation de sels tels que le sulfate de magnésium ou le chlorure de sodium, parmi d'autres, permet la diminution de molécules d'eau disponibles pour la solvatation des composés qui sont, de fait, partitionnés vers la phase organique (solvant d'extraction). Après l'extraction, il est possible de procéder à une étape de nettoyage par l'ajout de PSA (amines primaires et secondaires, pour leur sigle en anglais) ou de florisil, selon la nature de l'analyte. Dans cette méthode QuEChERS, la réduction de la quantité de solvants d'extraction, de la quantité d'échantillon et aussi du temps de manipulation sont des facteurs avantageux par rapport aux méthodes traditionnelles d'extraction.

Après la première publication de la méthode QuEChERS, une grande diversité d'applications dans différentes matrices ont vu le jour, que ce soit pour la quantification des pesticides dans les fruits, les légumes, les céréales, ou le miel (**Tableau 2.4**). Un développement QuEChERS pour l'analyse de pesticides multi-classes dans les fruits et légumes sera détaillé au Chapitre 7.

Tableau 2.4. Exemples d'études pour l'analyse d'insecticides néonicotinoïdes dans les fruits, légumes, miel et céréales par la méthode QuEChERS.

Auteur	Néonics (nb de composés)	Matrice	Quantité initiale de matrice	Volume de solvant	Nature du solvant
Chen et al. 2014	8	Fruits-Légumes/Miel	10 g Fruits-Légumes 5g Miel	10 mL	Acétonitrile
Proietto et al. 2013	6	Miel	5 g	10 mL 10 mL	H_2O Acétonitrile
Tanner et al. 2011	10	Miel	1 g	10 mL 20 mL	H_2O Méthanol
Wang et al. 2012	7	Grains	10 g	10 mL	Acétonitrile
Zhang et al. 2012	6	Fruits-Légumes	10 g	10 mL	Acétonitrile
Zhang et al. 2013	4	Céréale, Fruits, Légumes	10g	5 mL 10 mL	H_2O Acétonitrile

2.2 Technique de séparation : La chromatographie liquide à haute performance (HPLC)

Les techniques de séparation analytique comme étape antérieure à la détection sont par prédilection la HPLC, la GC et l'électrophorèse capillaire, dépendamment de la nature de l'analyte. La HPLC reste cependant la technique la plus utilisée pour les pesticides modérément polaires.

La chromatographie liquide a une variante qui permet de réaliser la séparation plus rapidement et efficacement : la chromatographie liquide à ultra-haute performance ou UHPLC (Ultra-High Performance Liquid Chromatography). En général, les méthodes UHPLC utilisent un garnissage (*packing*) de particules de plus petits diamètres comparativement à la HPLC (typiquement <2 μm pour la UHPLC), ce qui a pour incidence de plus hauts débits et pressions de travail ainsi que des pics chromatographiques plus fins (Holler et al. 2003).

Le principe de la rétention chromatographique repose sur l'équilibre du soluté entre les phases stationnaire et mobile. Selon l'équation de Van Deemter, la hauteur du plateau théorique H est notamment reliée à la vitesse de la phase mobile (Ux), une hauteur plus petite de plateau donnant une meilleure résolution :

$$H \approx A + B/Ux + CUx \qquad \text{eq.2}$$

La hauteur théorique est contrôlée par la diffusion turbulente (A, dans l'eq. 1), la diffusion longitudinale du soluté dans la colonne (B) et la résistance au transfert de masse (C), tout en prenant en compte le débit de la phase mobile (Ux). Notamment, la phase mobile peut emprunter différents trajets possibles à cause de la taille et la forme de particules remplies à l'intérieur de la colonne. Ces paramètres peuvent affecter la largeur des pics.

La taille de particule est un facteur d'efficacité important en conjonction avec la longueur de la colonne. Une taille de particule plus petite donnera une haute efficacité de séparation, mais une augmentation de la pression est prévisible (ainsi qu'une diminution du temps de rétention), donc le rôle de la longueur de la colonne sera de stabiliser cette pression ou retenir la même résolution (Harris, 2007; Holler et al. 2003).

Pour aider la résolution et la séparation des solutés, la phase mobile joue un rôle important. Le choix de la nature de la phase mobile et la phase stationnaire est basé sur la nature des analytes. En chromatographie en phase normale NPLC (Normal Phase Liquid Chromatography) la phase stationnaire est polaire et la phase mobile apolaire. La chromatographie en phase inverse RPLC (Reversed Phase Liquid Chromatography) utilise une phase stationnaire apolaire et une phase mobile polaire. En RPLC, la problématique de pics soumis au phénomène de *peak tailing* (pics traînants) est diminuée car il y a moins de sites où le soluté pourrait interagir fortement avec la phase stationnaire.

L'élution du soluté par la phase mobile est possible à l'aide d'un gradient dans le cas où les solutés ont des propriétés physicochimiques semblables et donc, sont plus difficiles à séparer. En RPLC, le gradient débute traditionnellement avec des conditions aqueuses (voie A), en augmentant graduellement le pourcentage de solvant organique (voie B) au cours du temps. Les composés polaires/hydrophiles sont élués en premier et les

composés les plus apolaires/hydrophobes en dernier. A noter que l'élution peut également être réalisée par un seul solvant ou mélange fixe de solvants pour créer une élution isocratique (Harris, 2007; Holler et al. 2003).

En général, les publications portant sur les pesticides modérément polaires font appel à la chromatographie liquide en phase inverse, des colonnes de type C18 étant le plus fréquemment utilisées (**Tableau 2.5**), avec quelques variations selon le fournisseur (p.ex., Waters ACQUITY UPLC HSS T3 C18, Thermo Hypersil Gold C18, Agilent ZORBAX C18, etc). Sur les 17 publications répertoriées dans le **Tableau 2.5**, on recense à la fois des colonnes de type UHPLC (d_p = 1.7–1.9 μm) et des colonnes de type HPLC (d_p = 2.1–5 μm). Les auteurs utilisent fréquemment des phases mobiles organiques de type acétonitrile ou méthanol (**Tableau 2.5**). Divers modificateurs de phase mobile peuvent être employés afin de favoriser la séparation et/ou l'ionisation des composés. Par exemple, les modificateurs utilisés pour l'analyse des néonicotinoïdes incluent l'acide formique, le formate d'ammonium, et l'acétate d'ammonium (**Tableau 2.5**).

Tableau 2.5. Exemples de conditions analytiques pour l'analyse de divers pesticides.

Auteur	Analytes	Matrice	Colonne analytique	Chimie de la colonne	Dimensions	Volume d'injection	Phases mobiles	Analyseur
Cotton et al. 2016	Divers pesticides	Eau de surface	ACQUITY UPLC HSS T3 par Waters	C18	100Å, 1.8 µm, 2.1 mm i.d. × 75 mm	5 mL	(A) ACN + 0.08% HCOOH (B) H_2O + 0.08% HCOOH	Q-Exactive parThermo
Garcia-Ac. et al. 2009	Divers pesticides	Eau potable	SynergiFusionRP par Phenomenex	C18	50 mm×2mm i.d., 4µm	10 mL	A) H_2O + 0.1% A.F. B) MeOH + 0.1% A.F. C) ACN + 0.1% A.F.	LC-MS/MS Quantum Ultra QqQ par Thermo
Poiger et al. 2017	Glyphosate	Eau de surface	Colonne Gemini-NX C18 par Phenomenex	C18	150 mm × 2.0 mm i.d, 5 µm	1mL	A) H_2O + buffer carbonate d'ammonium B) MeOH	API 4000 QqQ par Sciex
Rodriguez-Gonzalez et al. 2015	Atrazine	Eau de mer	ACQUITY UPLC BEH C18	C18	2.1 mm × 100 mm, 1.7 µm particle size	10 mL	A) H_2O + 5 mM acétate d'ammonium B) MeOH + 5 mM acétate d'ammonium	Acquity triple-quadrupole mass spectrometer TQD par Waters
Huntscha et al. 2012	Divers contaminants organiques	Eau souterraine et eaux usées	Colonne Atlantis T3 par Waters	C18	3.0 mm i.d. × 150 mm, 3 µm	20 mL	MeOH+0.1% A.F., H_2O HPLC 5mM acétate d'ammonium	TSQ Quantum Ultra triple quadrupole MS par Thermo
Proietto et al. 2013	Néonicotinoïdes	Miel	Colonne Hypersil gold par Thermo	C18	50mm× 2.1 mm i.d., 1.9 µm	5 µL	A) H_2O + 0.05% HCOOH et 2mM $HCOONH_4$ B) MeOH + 0.05% HCOOH et 2mM $HCOONH_4$	TSQ Quantum Access Max par Thermo

Tanner et al. 2011	Néonicotinoïdes	Miel	Colonne Synergi Fusion RP par Phenomenex	C18	50 mm× 2 mm i.d., 4 µm	25 µL	A) H_2O + 5 mM formate d'ammonium B) MeOH + 5mM formate d'ammonium	PE SCIEX API 2000 QqQ MS par Sciex
Wang et al. 2012	Néonicotinoïdes	Semences	Colonne Agilent-C18 par Agilent	C18	4.6 × 250 mm i.d., 5.0 µm	20 µL	Mélange d'acetonitrile + 0.3% (v/v) d'acide formique dans l'eau (20:80; v/v)	Agilent Technology 1100 - DAD
Zhang F. et al. 2012	Néonicotinoïdes	Légumes	Colonne ZORBAX C18 par Agilent	C18	50 mm× 2.1 mm i.d., 1.8 µm	5 µL	MeOH et H_2O (v/v = 25:75)	Agilent 6410 Triple Quadrupole
Zhang Y. et al. 2013	Néonicotinoïdes	Fruits	Colonne ACQUITY UPLC BEH C18 par Waters	C18	100 mm × 2.1 mm, 1.7 µm	5 µL	A) H_2O B) MeOH	QqQ par Waters
Chen M. et al. 2015	Various contaminants organiques	Sédiments	Colonne Kinetex C18 par Phenomenex	C18	100 mm × 3.0 mm i.d., 2.6 µm	10 µL	A) H_2O + 0.1% A.F., B) ACN + 0.1% A.F.	Agilent 6490 triple quadrupole mass spectrometer (MS/MS) par Agilent
Haroune et al. 2015	Pesticides	Insectes	Colonne Acquity UPLC HSS-T3 par Waters	C18	100 mm × 2.1 mm i.d.,1.8 µm	5 µL	A) H_2O + 0.20% A.F. B) MeOH/ACN (80:20,v/v) + 0.20% A.F.	Acquity UPLC XEVO TQmass spectrometer par Waters
Zheng et al. 2015	Néonicotinoïdes	Plantes	Colonne Luna C18 par Phenomenex	C18	2.0 mm i.d. × 150 mm, 5 µm	5 µL	A) MeOH 100% B) H_2O + 2 mM d'acétate d'ammonium avec 0.1% A.F.	TSQ Quantum Discovery mass spectrometer system par Thermo

Su et al. 2017	Triazines	Jus	Colonne Zorbax Eclipse XDB-C18 par Agilent	C18	3.5 µm, 4.6 mm i.d.× 150 mm	20 µL	A) ACN B) H_2O	1100 series LC - DAD par Agilent
Dujakovic et al 2005	Pesticides	Eau de surface et souterrain	Colonne Zorbax Eclipse® XDB-C18 par Agilent	C18	75mm×4.6mm i.d., 3.5 µm	10 µL	A) H_2O B) MeOH C) H_2O + 10% acide acétique	LCQ Advantage quadrupole ion trap mass spectrometer par Thermo
Hladik et al 2015	Néonicotinoïdes	Eau de surface	Colonne Zorbax Eclipse XDB-C18 par Agilent	C18	2.1 mm i.d. × 150 mm, 3.5 µm	10 µL	A) ACN B) H_2O + 5 mM A.F.	6430 tandem MS system par Agilent
Main et al. 2014	Néonicotinoïdes	Zones humides	XTerra MS-C8 par Waters	C8	100 mm × 2.1 mm. i.d., 3.5 µm	20 µL	A) H_2O + 0.1% A.F. B) ACN/H_2O (90:10, v/v) + 0.1% A.F.	Micromass Quattro Premier triple quadrupole mass spectrometer par Waters
Schaafsma et al 2015	Néonicotinoïdes	Eau de surface et sols	Colonne Gemini C18 phase inverse par Phenomenex	C18	150 mm × 2 mm i.d, 5 µm	50 µL	A) MeOH+ 5 mM A.F. B) H_2O + 5 mM A.F.	Ionics EP 10+ modified API 365 triple quadruple mass spectrometer par AB SCIEX

2.2.2 Performance des méthodes analytiques

Le développement de nouvelles méthodes pour l'analyse de pesticides passe par une étape critique d'évaluation des performances analytiques. Les critères d'acceptabilité de ces performances sont établis par différents organismes de normalisation afin de contrôler la qualité des méthodes. Ces variables sont, notamment, le taux de récupération qui doit idéalement être autour de 60-140% (SANTE/11813/2017), des limites de détection et de quantification (LODs et LOQs) suffisamment faibles, une gamme linéaire de concentrations (corrélation entre le signal instrumental et la quantité d'analyte), une précision évaluée comme coefficient de variation CV <20%, et une exactitude (accord entre la valeur mesurée et la valeur attendue) comprise entre 70 et 130% selon l'US EPA. En cas de non-conformité à ces critères, il est conseillé de procéder à une étape de réoptimisation et/ou d'ajustement de la méthode analytique.

Dans le **Tableau 2.6**, on illustre la performance de diverses méthodes de la littérature. Pour les méthodes développées notamment via une extraction en phase solide (SPE) pour les néonicotinoïdes ou les triazines dans l'eau potable, de surface ou même souterraine, les limites de détection sont généralement de l'ordre du ng L^{-1}. S'agissant de matrices solides, la performance des méthodes telles que les limites de détection de l'ordre du ng g^{-1} et le pourcentage de récupération vont dépendre de la méthode d'extraction. Pour les insecticides néonicotinoïdes, les pourcentages de récupération rapportés par les auteurs sont globalement entre 70 et 120% (**Tableau 2.6**).

Tableau 2.6. Performance de diverses méthodes analytiques de séparation pour les néonicotinoïdes et triazines.

Méthodes	Auteurs	Matrice	Analytes	% Récupération	LOD [ppt]	LOQ [ppt]
MAE	Haroune et al. 2015	Insectes	Néonics	49-106	100-3000	400-7000
	Zheng et al. 2015	Herbes médicinales	Néonics	70.4–113.7	870-1920	2610-5760
	Su et al. 2017	Jus de fruits	Triazines	76.7–105.7	1010-1570	3370-5240
U-S AE	Chen et al. 2015	Sédiment	Antibiotiques/Pesticides	56-107	10-450	30-1350
QuEChERS	Chen et al. 2014	Fruits-Légumes/Miel	Néonicotinoïdes	95.6-110.4	33-166	100-500
	Proietto et al. 2013	Miel	Néonicotinoïdes	89-114	33-1333	100-4000
	Tanner et al. 2011	Miel	Néonicotinoïdes	70-120	600-2000	20-10000
	Wang et al. 2012	Grains	Néonicotinoïdes	76-123	2000-4000	7000-14000
	Zhang et al. 2012	Fruits-Légumes	Néonicotinoïdes	73.7-103.8	200-850	660-2840
	Zhang et al. 2013	Céréales, Fruits, Légumes	Néonicotinoïdes	70-120	280-680	930-2620
SPE	Seccia et al. 2005	Eau potable	Néonicotinoïdes	96.1-102	10	30
	Dujakovic et al. 2010	Eau de surface	Pesticides	72-121	0.4-5.5	1.1-18.2
	Hladik et al. 2012	Eau de surface	Diuron et néonics	75-99	3.2-6.2	9.6-18.6
	Schaafsma et al. 2015	Eau de surface	Néonicotinoïdes	74.5-106.2	4-17	11-37
	Hao et al. 2015	Eau de surface	Néonicotinoïdes	78-110	2-7	6-21

2.3 Détection par Spectrométrie de Masse

La spectrométrie de masse est une technique d'analyse qui permet d'avoir de meilleures sensibilités, limites de détection et vitesse d'analyse, avec une grande diversité d'applications comparée à d'autres méthodes de détection traditionnelles. La spectrométrie de masse a évolué rapidement depuis la fin des années 1990, même si les premières notions et découvertes remontent à la fin du XIXe siècle.

La spectrométrie de masse se base notamment sur les principes généraux suivants :

1. Le composé à analyser est ionisé (cation ou anion) en phase gazeuse.
2. La molécule sous sa forme ionique va être soumise à des fragmentations. Ces fragments auront de propriétés chimiques différentes et chaque ion dérivé du produit primaire de l'ion moléculaire peut aussi suivre des fragmentations.

Les ions produits sont séparés à l'intérieur du spectromètre de masse par rapport à leur ratio de masse sur charge (m/z) et détectés en proportion de leur abondance.

2.3.1 Sources d'ionisation

Les techniques d'ionisation peuvent être très énergétiques en causant une fragmentation extensive, comme le cas de l'ionisation par impact électronique. D'autres techniques plus douces peuvent majoritairement produire des ions pseudomoléculaires, comme l'ionisation chimique et l'ionisation de champ. Ces techniques fonctionnent uniquement pour une ionisation en phase gazeuse, c'est à dire, pour des composés suffisamment volatils et thermiquement stables.

Étant donné que la plupart des composés sont thermiquement labiles ou ne sont pas assez volatils, il faut les transférer directement de la phase condensée à la phase gazeuse. Pour ces sources d'ionisation directe, on peut compter la source d'ionisation en phase liquide, dans laquelle l'analyte est en solution. Cette solution est nébulisée et l'ion est formé est introduit dans le spectromètre à travers quelques étapes de vide. Les sources qui fonctionnent avec ce type d'ionisation sont l'ionisation par électro nébulisation (ESI en

anglais), l'ionisation chimique à pression atmosphérique et la photo-ionisation à pression atmosphérique.

2.3.1.1 L'ionisation par électro nébulisation (ESI)

Les sources d'ionisation par électro nébulisation (ESI) ont commencé à gagner du terrain quand il fut démontré qu'elles pouvaient produire des ions à charges multiples en permettant la détection de molécules de faible poids moléculaire. La technique d'ESI a été appliquée avec succès aux polymères, biopolymères, et aux petites molécules polaires comme les pesticides et les médicaments.

Le principe de la source ESI est basé sur l'application d'un fort champ électrique sous pression atmosphérique à la solution qui passe à travers un tube capillaire à flux faible. Le champ électrique de l'ordre de 106 V m^{-1} est produit par l'application d'une différence de potentiel (3-6 kV) entre le tube capillaire et la contre électrode ceux qui sont séparés d'environ 0.3-2 cm (**Figure 2.3**). L'application de ce champ provoque l'accumulation de charges à la surface de la solution, laquelle va former des gouttelettes hautement chargées. Afin de limiter la nébulisation dans l'espace, un gaz est injecté coaxialement à faible débit. Finalement, les gouttelettes passent à travers un rideau de gaz inerte chauffé (N_2) ou un capillaire chauffé dans le but d'enlever les molécules de solvant restantes avant l'entrée dans l'analyseur.

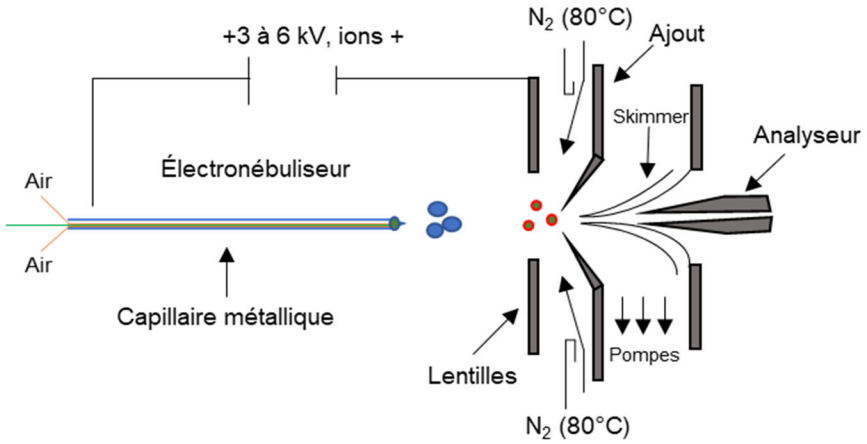

Figure 2.3 Diagramme d'une source par Électronébulisation (ESI), schéma basé de l'ouvrage *Mass Spectrommetry : Principles and Applications* (Hofmann and Stroobant, 2007.

2.3.1.2 Analyseurs

Le cœur de la technique spectrométrie de masse est l'analyseur, dont il existe plusieurs types (**Tableau 2.7**). Afin de se focaliser sur les analyseurs utilisés dans le présent travail de recherche, nous détaillerons plus particulièrement les analyseurs de type Quadripôle et Orbitrap.

Tableau 2.7 Les types d'analyseurs les plus communs dans la spectrométrie de masse (Gross, 2011).

Type	**Acronyme (Anglais)**	**Principe**
Temps de vol	TOF	Dispersion temporelle d'un faisceau ionique pulsé ; séparation par temps de vol
Secteur magnétique	B	Dispersion d'un faisceau ionique en continu ; séparation selon la quantité de mouvement des ions dans un champ magnétique par les Forces de Lorentz
Quadrupôle linéaire	Q	Faisceau ionique continu dans un champ quadripolaire linéaire de radiofréquence ; séparation selon la stabilité des trajectoires.
Trappe ionique-Quadrupôle linéaire	LIT	Faisceau ionique continu et ions piégés ; accumulation et séparation dans un champ linéaire de radiofréquence quadripolaire
Trappe ionique-quadrupôlaire	QIT	Ions piégés ; séparation selon la trajectoire stable d'ions dans un champ quadripolaire tridimensionnelle de radiofréquence
Résonance ionique	FT-ICR	Ions piégés ; séparation selon la fréquence cyclotronique (Force de Lorentz) dans un champ magnétique.

cyclotronique à transformée de Fourier		
Orbitrap	orbitrap	Oscillation axiale dans un champ électrique non homogène ; détection de fréquence après transformation de Fourier de transitoire signal

2.3.1.3 Le Quadrupôle

Le quadrupôle est un ensemble de quatre électrodes en forme hyperbolique ou cylindrique disposées dans la direction z et montées dans une configuration x-y. Un voltage positif (+) est appliqué à une des paires et un voltage négatif à l'autre paire avec un combination de voltage direct en continue (VDC) et alternatif (VRF) de fréquence ω (voir **Figure 2.4**).

De cette manière, les ions vont osciller entre les électrodes grâce aux forces électriques alternatives, c'est à dire, d'attraction et répulsion. Les ions seront filtrés selon leur rapport m/z : les électrodes avec un voltage positif filtrent ceux avec une masse élevée tandis que les ions de faible masse seront filtrés par les électrodes avec un voltage négatif.

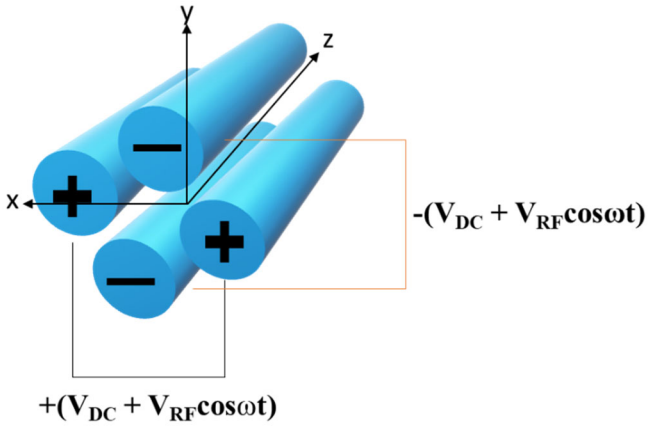

Figure 2.4 Diagrame d'un Quadrupôle, schéma basé de l'ouvrage *Mass Spectrometry : A textbook* (Gross, J.H., 2011).

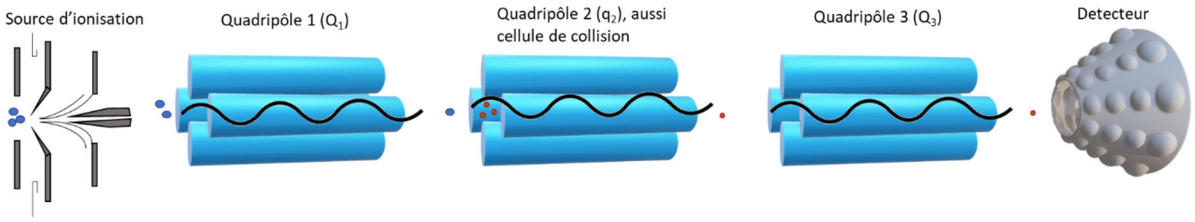

Figure 2.5 Configuration d'un analyseur de type triple quadrupôle

Après que les ions sortent de la source ils sont focalisés à l'aide des lentilles. Les ions entrent dans le premier quadrupôle (Q1) où ils seront filtrés selon la masse d'intérêt. Les ions parents sélectionnés entreront dans le deuxième quadrupôle (q2) qui sert de cellule de collision afin de créer une fragmentation de l'ion parent. Les ions produits (ions fils) seront amenés vers le troisième quadrupôle (Q3) pour faire un deuxième filtrage ou sélection d'ions d'intérêt et finalement entrer dans le détecteur. Par exemple, la molécule d'atrazine a une masse molaire de 215.09 g/mol. L'atrazine lors d'une ionisation par ESI en mode positif ($M+H^+$) aura un rapport de masse sur charge m/z de 216 au moment d'entrer dans le premier quadrupôle (Q1). Après la fragmentation dans le q2, les deux ions fils les plus intenses, 174 m/z et 104 m/z, seront successivement sélectionnés à travers le Q3 (on parle de balayage des transitions). Les analyseurs de type triple quadrupôle présentent généralement une gamme de masses possible à analyser entre 50 et 1250 m/z avec une gamme dynamique de 10^6, une précision de masse de ~0.01 % et une résolution de ~1500.

Il est possible de suivre deux ou plusieurs fragmentations dans une même analyse avec le balayage à multiples fragmentations sélectives (en anglais Multiple Reaction Monitoring, MRM) (**Figure 2.6**). Ce type de balayage MRM a été utilisé pour l'analyse de pesticides comme les néonicotinoïdes, les triazines et d'autres composés dans les chapitres 4, 5, 6 et 7.

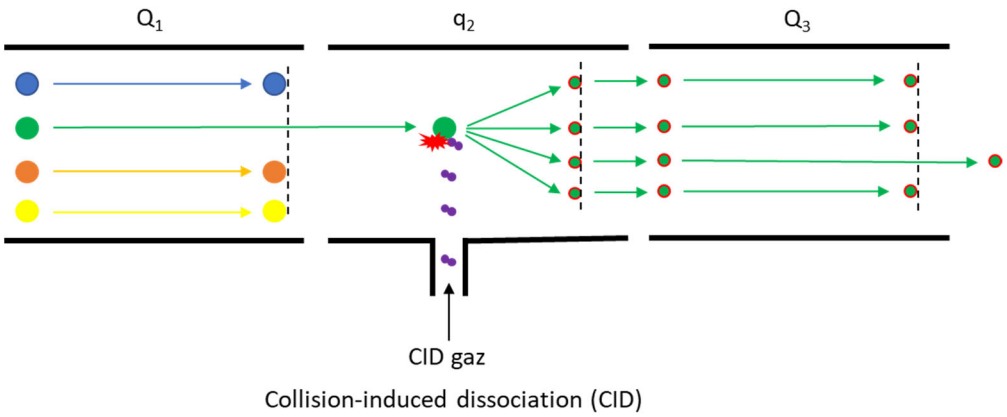

Figure 2.6 Représentation d'un balayage en mode « Multiple Reaction Monitoring » (MRM).

Prenons l'exemple de l'imidaclopride et sa fragmentation suivie lors d'un balayage par MS/MS, laquelle est représentée en **Figure 2.7**. L'imidaclopride après ionisation par ESI présente une m/z de 256. Dans un analyseur de type QqQ, cet ion sera filtré dans le premier quadrupôle (Q_1) et par la suite sera fragmenté dans la cellule de collision (q_2). Dans cette fragmentation il y aura une multiplicité d'ions fils, dont deux sont plus abondants : le fragment de m/z 209, correspondant à la perte de NO_2, et le fragment de m/z 175, correspondant à la perte supplémentaire du chlore. Le troisième quadripôle (Q_3) permettra successivement le passage de ces deux fragments vers le détecteur.

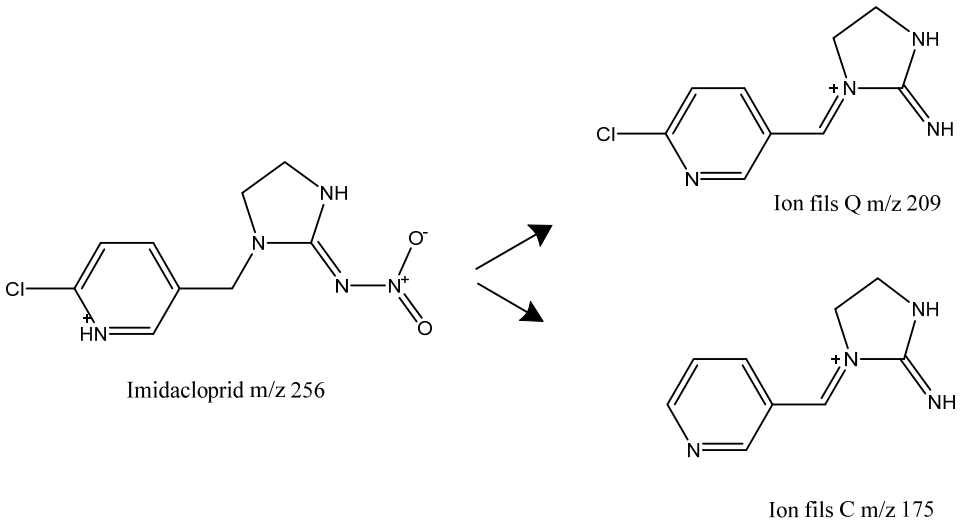

Figure 2.7 Fragmentation de l'imidaclopride via MS/MS dans un analyseur de type QqQ.

2.3.1.4 Orbitrap

Un autre type d'analyseur est l'Orbitrap, qui permet de piéger les ions dans un champ électrostatique en les faisant osciller (**Figure 2.8**). Les ions sont enfermés à l'aide d'une barrière de potentiel donné par des électrodes afin d'implanter une orbite stable autour d'un fil chargé. Il existe trois fréquences d'oscillation : la fréquence de rotation ω_φ, la fréquence d'oscillation radiale ω_r et la fréquence d'oscillation axiale ω_z. La fréquence d'oscillation peut être contrôlée en variant la forme des électrodes (Makarov, 2000). Les principaux avantages de la trappe orbitale sont entre autres une résolution supérieure à 60,000, une précision de masse <5 ppm, une gamme de masses entre 50-2,000 ou 200-4,000, et sa sensibilité inférieure aux femtomoles.

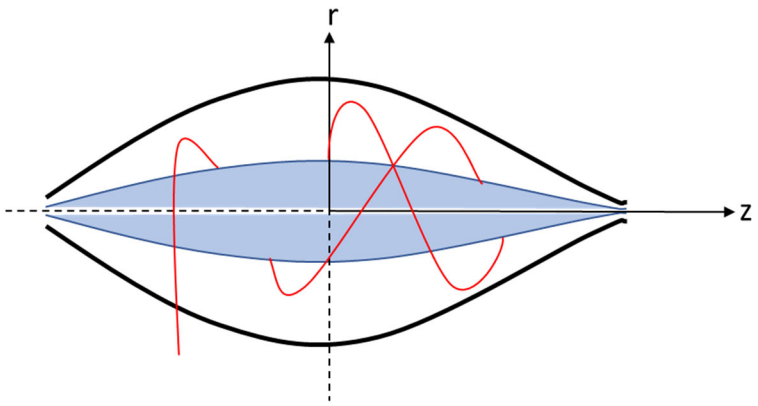

Figure 2.8 Schema representatif du movement ionique à l'interieur d'une trappe orbitale (Orbitrap),

L'analyseur Orbitrap peut être couplé à d'autres analyseurs afin d'augmenter leur puissance et performance. Les analyseurs hybrides, avec une versatilité remarquable, permettent la sélection préalable d'ions précurseurs par le système quadripolaire et une détection d'ions haute résolution de masse exacte donnée par l'Orbitrap (voir **Figure 2.9**).

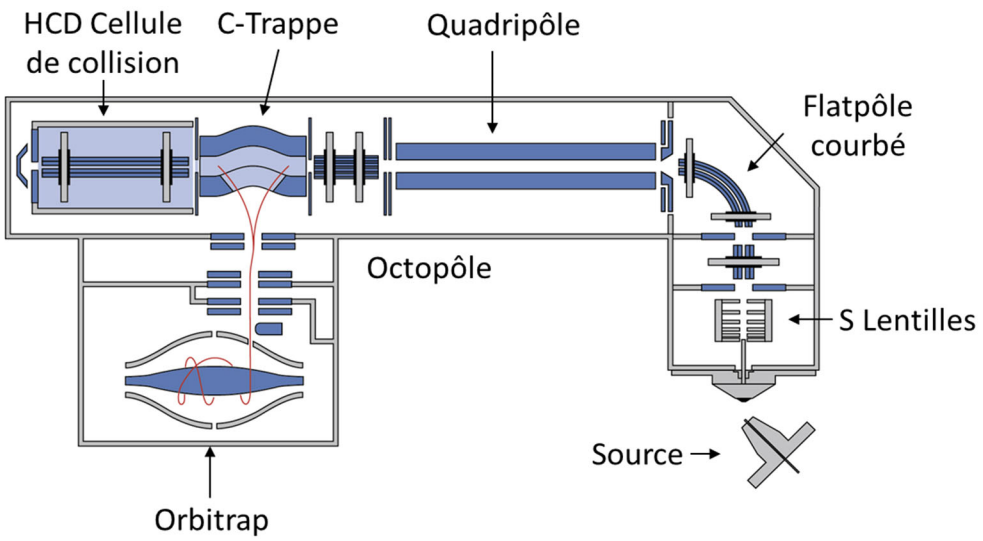

Figure 2.9 Schéma du système hybride Q-Orbitrap avec quadripôle (http://planetorbitrap.com/q-exactive#tab:schematic), Thermo Scientific.

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Chapitre 3. Problématiques, hypothèses de recherche et structure de la thèse

3.1 Problématique

Au moment de commencer cette thèse de recherche, il n'y avait pas de méthode publiée dans la littérature rapportant l'analyse des insecticides néonicotinoïdes par SPE en ligne. Certaines méthodes analytiques préexistantes, comme la méthode de Hao et al. (2015) pour la quantification des néonicotinoïdes dans les eaux naturelles par injection directe, montraient de bonne performance de quantification avec une exactitude entre 70 – 120%. Cependant, étant donné l'absence d'une étape de préconcentration de la méthode d'injection directe (Hao et al. 2015), les limites de quantification (LOQs) rapportées entre 150 – 600 ng L^{-1} étaient entre 20 et 80 fois plus élevés que le critère des effets chroniques pour la protection de la vie aquatique fixé à 8.3 ng L^{-1} (CCME 2018). Afin d'améliorer ces LOQs, certains auteurs ont développé et validé des méthodes en ayant recours à la SPE hors ligne (p.ex., Schaafsma et al. 2015) avec des facteurs de concentration entre 250 et 1000x. Cependant, ces méthodes comportent des limitations, telles que le nombre de néonicotinoïdes ciblés et, même si les limites de détection étaient meilleures, celles-ci n'étaient pas toujours compatibles avec l'évaluation du critère d'eau de surface (8.3 ng L^{-1}). Par ailleurs, de nombreuses étapes de traitement et de manipulation des échantillons étaient requises, ce qui augmentait le temps total d'analyse par rapport à l'injection directe. Les travaux de recherche de la présente thèse se sont attachés à améliorer et/ou à complémenter ces méthodes initiales, via des approches de type SPE en ligne (**chapitre 4**). Bien que la SPE en ligne ait été validée pour l'analyse d'autres types de contaminants tels que les produits pharmaceutiques, hormones et pesticides (Fayad et al. 2013; Garcia-Ac et al. 2009), il nous a semblé utile de proposer une méthode basée sur la SPE en-ligne pour analyser 8 néonicotinoïdes dans l'eau de surface et l'eau potable.

Dans le contexte d'analyse de contaminants dans l'eau potable, des suivis de la qualité de l'eau au Québec ont été réalisés par le *Ministère de l'Environnement et de la Lutte contre les changements climatiques* (p. ex., Giroux, 2015; Giroux et al. 2016). Ces

suivis indiquent que, concernant la zone échantillonnée couvrant certaines régions agricoles du Québec méridional, l'atrazine peut être retrouvée dans l'eau potable, et l'atrazine et les néonicotinoides sont fréquemment retrouvés dans les eaux de tributaires du St. Laurent avec parfois des dépassements pour le cas des néonicotinoïdes. À niveau mondial, les pesticides ont fait l'objet de nombreux suivis environnementaux. Cependant, certains points mériteraient des études plus approfondies. Par exemple, il existait relativement peu d'études sur les variations intra-annuelles à haute résolution des pesticides dans l'eau potable. Il y avait également un manque d'information sur la distribution spatiale des néonicotinoides au sein des systèmes fluviaux complexes, certains pouvant comporter des masses d'eau d'origines distinctes qui s'écoulent sans se mélanger sur de grandes distances (Amazone, St. Laurent). Une forte hétérogénéité spatiale des contaminants au sein d'un système fluvial complexe, pourrait également présenter des implications en termes d'exposition humaine, selon que la prise d'eau pour la production d'eau potable se situe ou non dans la masse d'eau contaminée. Les présents travaux de recherche visent à répondre à certaines de ces questions via une stratégie d'échantillonnage élargie temporellement et spatialement, pour l'atrazine dans l'eau potable au Québec méridional (**chapitre 5**), et pour l'atrazine, le glyphosate et les néonicotinoïdes dans l'eau de surface du fleuve St-Laurent et ses tributaires (**chapitre 6**).

Étant donné la distribution globale des pesticides, une des conséquences de leur surutilisation est que ces derniers se sont retrouvés dans tous les sphères de l'environnement, y compris certains produits destinés à la consommation comme les fruits et légumes. Si la problématique de l'analyse de pesticides dans la nourriture n'est pas en soi un sujet nouveau (Anastassiades et al. 2003), la documentation des niveaux de contamination dans les produits de consommation des Canadiens reste un enjeu important et relativement peu documenté. L'Agence de réglementation de la lutte antiparasitaire (ARLA) suit régulièrement la conformité aux limites maximales de résidus de pesticides (MRL) mais l'occurrence et les niveaux de concentration rapportés restent liés aux performances des méthodes analytiques. Pour répondre à cette problématique, le travail de recherche discuté dans le **chapitre 7** s'attache à développer une méthode d'analyse sensible de pesticides dans divers produits de consommation, qu'ils soient produits via agriculture conventionnelle ou biologique.

3.2 Objectifs et hypothèses de recherche

Ces travaux de recherche visaient à améliorer les connaissances sur l'occurrence de certains pesticides d'intérêt émergent (notamment les néonicotinoïdes) dans l'environnement et dans certains produits susceptibles d'être consommés par la population (eau potable, fruits et légumes). À cet effet, de nouvelles méthodes d'analyse pour la quantification de pesticides par chromatographie liquide couplée à la spectrométrie de masse ont été développées. Les objectifs spécifiques et hypothèses associées sont présentés ci-dessous.

Objectif 1 : Développer une nouvelle méthode d'analyse rapide des insecticides systémiques (néonicotinoïdes et fipronil) dans l'eau potable et de surface, faisant appel à une extraction de type SPE en-ligne couplée à la UHPLC-MS/MS. Les hypothèses de recherche sont les suivantes :

i) Les principaux paramètres pouvant influencer sur le recouvrement et la sensibilité de la méthode pourraient être la nature de la membrane de préfiltration, la phase mobile analytique, et le volume d'échantillon chargé par SPE en-ligne ;

ii) Un plan d'expériences pour l'optimisation des paramètres de la SPE en-ligne permettrait de mieux couvrir le domaine expérimental par rapport à une méthode traditionnelle uni-variable ;

iii) Selon le volume d'échantillon considéré, la méthode pourrait atteindre des niveaux de détection compétitifs par rapport aux méthodes de SPE hors ligne;

iv) Les éventuels effets matrice pourraient être compensés par une approche de quantification ajustée, par exemple ajouts dosés (*standard additions*) ou étalonnage avec adaptation matricielle (*matrix-matched calibration*).

Objectif 2. Documenter la présence d'atrazine et son métabolite de dégradation la désethylatrazine (DEA) dans l'eau potable au Québec méridional pour mieux comprendre sa distribution spatiale et ses variations temporelles. Les hypothèses de recherche sont les suivantes :

i) Les variations saisonnières de l'atrazine dans l'eau potable pourraient refléter les tendances d'utilisation en milieu agricole (notamment, pic en fin de printemps en lien avec l'application dans les champs de culture afin d'éliminer les mauvaises herbes et valeurs minimales en fin d'hiver). Un échantillonnage intensif d'eau potable sera réalisé sur une période de 18 mois afin de vérifier cette hypothèse ;

ii) Les tendances spatiales de l'atrazine dans l'eau potable des municipalités au Québec devraient être liées à la source d'eau pour produire l'eau potable. Les villes qui puisent l'eau de surface du fleuve St-Laurent pour produire l'eau potable seraient susceptibles de présenter des résidus d'herbicides à de plus fortes concentrations à cause de la prépondérance des sources en amont (Lac Ontario). En accord avec le gradient d'occupation agricole, les villes qui puisent l'eau de surface dans certains tributaires de la Rive Sud (rivières Yamaska, Saint-François) seraient également plus susceptibles de contenir des résidus d'atrazine comparativement à d'autres bassins versants moins impactés des Rives Sud (Chaudière) et Nord (Yamachiche, Maskinongé). Un large échantillonnage couvrant quelques-unes des principales municipalités (n = 52) du Québec méridional sera réalisé à cet effet.

Objectif 3. Déterminer l'occurrence et la distribution spatiale des néonicotinoïdes, de l'atrazine et du glyphosate sur un tronçon de 200 km du fleuve St-Laurent et ses tributaires. Les hypothèses de recherche sont les suivantes :

i) Les variations des concentrations de pesticides devraient refléter les différents types d'occupation des sols au sein du bassin versant de chaque tributaires (majoritairement agricole *vs.* majoritairement forestier) ;

ii) L'hydrologie particulière du fleuve Saint-Laurent, avec un mélange transversal limité des masses d'eau (eaux brunes de la rivière des Outaouais qui s'écoulent en parallèle aux eaux bleu-vert des Grands Lacs laurentiens, sans se mélanger), devrait permettre de distinguer des profils spécifiques de pesticides au sein du fleuve, en lien avec les différentes

sources. A cet effet, plusieurs transects orthogonaux seront réalisés le long du fleuve afin de caractériser les masses d'eau qui s'écoulent près de la rive Nord, au centre du fleuve, et près de la rive Sud.

Objectif 4. Évaluer l'occurrence de pesticides de diverses classes (insecticides, herbicides et fongicides) dans les fruits et légumes issus de la culture biologique et conventionnelle. À cet égard une méthode d'extraction par QuEChERS et UHPLC-MS/MS a été validée. Les hypothèses de recherche sont les suivantes :

i) La robustesse de la méthode d'analyse pour 22 pesticides multi-classes pourrait être assurée en réalisant une optimisation détaillée des différents paramètres d'extraction par QuEChERS;

ii) Les produits issus de l'agriculture biologique devraient présenter des niveaux de contamination faibles comparativement aux produits de l'agriculture conventionnelle.

3.3 Structure de la thèse

Le **Chapitre 1** présente une mise en contexte générale sur les pesticides : classification, modes d'action, et occurrence dans les différents compartiments environnementaux.

Le **Chapitre 2** présente un aperçu des méthodes analytiques couramment utilisées pour l'analyse de pesticides dans les différentes matrices d'intérêt.

Le **Chapitre 3** présente les objectifs, les hypothèses de recherche et la structure générale de la thèse.

Le **Chapitre 4** aborde l'objectif 1 via le développement et l'optimisation d'une nouvelle méthode d'analyse des insecticides néonicotinoïdes dans l'eau potable et l'eau de surface. L'extraction est optimisée par la méthodologie des surfaces de réponse et des fonctions de désirabilité de Derringer. Les pesticides ont été traités par extraction SPE en-ligne couplée

à la chromatographie liquide et à la spectrométrie de masse en tandem. La référence de l'article associé est indiquée ci-après.

Montiel-León, J. M., S. V. Duy, G. Munoz, M. Amyot and S. Sauvé (2018). "Evaluation of on-line concentration coupled to liquid chromatography tandem mass spectrometry for the quantification of neonicotinoids and fipronil in surface water and tap water." Anal Bioanal Chem **410**(11): 2765-2779.

Le **Chapitre 5** correspond à l'objectif 2 avec l'analyse d'une série temporelle d'échantillons d'eau potable (robinet) de la région de Montréal entre 2015-2016 (n = 450) et d'une série spatiale d'échantillons d'eau potable (robinet) couvrant 52 municipalités du sud-ouest du Québec. La référence de l'article associé est indiquée ci-après.

Montiel-León, J. M., S. Vo Duy, G. Munoz, M. F. Bouchard, M. Amyot and S. Sauvé (2019). "Quality survey and spatiotemporal variations of atrazine and desethylatrazine in drinking water in Quebec, Canada." Sci Total Environ **671**: 578-585.

Le **Chapitre 6** aborde l'objectif 3 via la détermination de pesticides dans 68 échantillons d'eau provenant du Fleuve Saint-Laurent, dont une série de transects en plusieurs points kilométriques le long du fleuve (mission Lampsilis 2017) et quelques-uns des principaux tributaires. Le glyphosate, l'atrazine et les néonicotinoïdes montrent des profils différents selon la masse d'eau considérée au sein du fleuve. La référence de l'article associé est indiquée ci-après.

Montiel-León, J. M., G. Munoz, S. Vo Duy, D. T. Do, M. A. Vaudreuil, K. Goeury, F. Guillemette, M. Amyot and S. Sauvé (2019). "Widespread occurrence and spatial distribution of glyphosate, atrazine, and neonicotinoids pesticides in the St. Lawrence and tributary rivers." Environ Pollut **250**: 29-39.

Le **Chapitre 7** aborde l'objectif 4, et met en œuvre une méthode QuEChERS – UHPLC-MS/MS pour 22 pesticides de différentes classes. L'application de cette méthode permet de documenter l'occurrence de pesticides dans 133 échantillons de fruits et légumes (pomme, laitue, raisin et tomate) issues de l'agriculture biologique et conventionnelle. La référence de l'article associé est indiquée ci-après.

Montiel-León, J. M., S. V. Duy, G. Munoz, M.-A. Verner, M. Y. Hendawi, H. Moya, M. Amyot and S. Sauvé (2019). "Occurrence of pesticides in fruits and vegetables from organic and conventional agriculture by QuEChERS extraction liquid chromatography tandem mass spectrometry." Food Control **104**: 74-82.

Le **Chapitre 8** présente les principales conclusions de ces travaux et propose quelques perspectives de recherche pour de futures études.

Chapitre 4. Évaluation d'une méthode de préconcentration en-ligne couplée à la chromatographie liquide et à la spectrométrie de masse en tandem pour la quantification des néonicotinoïdes et du fipronil dans l'eau de surface et du robinet

Article publié dans *Analytical and Bioanalytical Chemistry* (2018) 410: 2765-2779.
"**Evaluation of on-line concentration coupled to liquid chromatography tandem mass spectrometry for the quantification of neonicotinoids and fipronil in surface water and tap water".** Auteurs: **Montiel-León, J. M., S. V. Duy, G. Munoz, M. Amyot and S. Sauvé**

Description: Cet article décrit l'optimisation et le développement d'une méthode pour l'analyse d'insecticides néonicotinoïdes dans l'eau de surface et l'eau potable via extraction en phase solide couplée en ligne à la chromatographie liquide et la spectrométrie de masse en tandem.

Contributions: J'ai effectué la conception du projet, la collecte des échantillons sur le terrain, la réalisation des manipulations, le traitement de données et la rédaction de l'article.
Co-auteurs: Sung Vo Duy et Gabriel Munoz m'ont aidé avec une partie des manipulations et à réviser l'article.
Co-directeur: Marc Amyot m'a aidé à réviser l'article.
Directeur: Sébastien Sauvé m'a aidé à la conception du projet et à réviser l'article.

Abstract

A study was initiated to investigate a fast and reliable method for the determination of selected systemic insecticides in water matrixes, and to evaluate potential sources of bias in their analysis. Acetamiprid, clothianidin, desnitro-imidacloprid, dinotefuran, fipronil, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam were amenable to analysis via on-line sample enrichment hyphenated to ultra-high performance liquid chromatography tandem mass spectrometry. The selection of on-line solid phase extraction parameters was dictated by a multi-criteria desirability approach. A 2-mL on-line injection volume with 1500 $\mu L \cdot min^{-1}$ loading flow rate met the objectives sought in terms of chromatographic requirements, extraction efficiency, sensitivity, and precision. A total analysis time of 8 min per sample was obtained with method limits of detection in the range of 0.1–5 ng L^{-1} for the scope of targeted analytes. Automation at the sample concentration step yielded intra-day and inter-day precisions in the range of 1–23 and 2–26%, respectively. Factors that could affect the whole method accuracy were further evaluated in matrix-specific experiments. The impact of the initial filtration step on analyte recovery was evaluated in ultrapure water, tap water, and surface water. Out of the 9 membranes tested, glass fiber filters and polyester filters appeared as the most appropriate materials. Sample storage stability was also investigated across the three matrix types; the targeted analytes displayed suitable stability during 28 days at either 4°C or -20°C, with little deviations (±10%) with respect to the initial T_0 concentration. Method applicability was demonstrated in a range of tap water and surface water samples from the province of Québec, Canada. Results from the present survey indicated a predominance of thiamethoxam (<0.5–10 ng L^{-1} and 3–61 ng L^{-1} in tap water and river water, respectively), clothianidin (<0.5–6 ng L^{-1} and 2–88 ng L^{-1} in tap water and river water, respectively), and imidacloprid (<0.1–1 ng L^{-1} and 0.8–38 ng L^{-1} in tap water and river water, respectively) among the targeted analytes.

4.1. Introduction

Pesticides have been widely used for disease control, household pest eradication, and agricultural pest management. Nitromethylene heterocyclic compounds were developed in the 1970s, and their specific modes of action immediately aroused the interest of the agricultural industry, being imidacloprid the first synthesized neonicotinoid (Matsuda et al., 2008; Tomizawa et al., 2001). Neonicotinoid insecticides share a common mode of action, affecting the central nervous system playing the role of acetylcholine nicotinic receptor agonist (nAChR) (Matsuda et al., 2008). Another systemic insecticide of concern is Fipronil, a GABA-gated chloride channel blocker pertaining to group 2B (phenylpyrazoles) of the latter classification (Simon-Delso et al., 2015). Non-target organisms may be affected by such insecticides, including domesticated insect pollinators such as Western honey bees (*Apis mellifera*) and wild pollinators such as bumblebees (e.g., *Bombus impatiens*) (David et al., 2015; Tison et al., 2015). Neonicotinoid exposure may affect the health status of apiaries, contributing to a decline in brood production and total adult numbers —a syndrome otherwise known as Colony Collapse Disorder (Scholer et al., 2014; Cabrera et al., 2016). Despite their alleged specific mode of action on insects, neonicotinoid insecticides may also exert direct or indirect effects to vertebrate wildlife (Gibbons et al., 2015). In recent years, neonicotinoid residues were reported in surface water (Hladik et al., 2014; Wang et al., 2015; Morrissey et al., 2015), wetlands (Main et al., 2015), and soils (Schaafsma et al., 2015). Trace levels of neonicotinoid insecticides were found in consumer products, including fruit and vegetables, fish, and livestock products (Chen et al., 2014; Ferrer et al., 2015; Xiao et al., 2013; Seccia et al., 2008; Xiao et al., 2011). Neonicotinoid residues were also evidenced in both human urine and serum (Taira et al., 2013; Yamamuro et al., 2014).

In light of these concerns, a task force was created to further investigate the environmental impacts of systemic insecticides, published into a World Integrated Assessment (Bonmatin et al., 2015; van der Sluijs et al., 2013). A number of recommendations were proposed to bridge current knowledge gaps including exposure routes, environmental fate, and further assessment of the ecotoxicological impacts to non-target organisms, effects on general ecosystems, and implications for food safety. Designing robust analytical procedures to

determine neonicotinoids in a variety of matrixes will certainly contribute to improving the study of their fate in ecosystems.

For the ultra-trace analysis of systemic insecticides, liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) through electrospray ionization is the current method of choice (Schaafsma et al., 2015; Dujakovic et al., 2010; Hladik et al., 2012; Hao et al., 2015). In the case of water samples, it is often preceded by a concentration step [e.g., via off-line solid phase extraction (SPE)] to eliminate possible interfering matrix components and reach a suitable concentration factor (Schaafsma et al., 2015; Dujakovic et al., 2010; Hladik et al., 2012; Hao et al., 2015; Seccia et al., 2005). Thanks to the high concentration factor (typically >250, depending on the initial sample intake and the final extract volume), excellent detection limits were previously reported with off-line SPE – HPLC-MS/MS workflows (Dujakovic et al., 2010; Hladik et al., 2012; Hao et al., 2015). For instance, Hladik and Calhoun (Hladik et al., 2012) loaded 1L water samples through hydrophilic-lipophilic balance cartridges (Oasis HLB) prior to reducing the organic extracts and final HPLC-MS/MS analysis, with method detection limits in the range of 4–6 ng L^{-1}.

Off-line preparation methods may, however, present some disadvantages such as requiring resource-intensive workflows and necessitating a relatively large sample intake. This not only increases the total analysis time but also enhances the risk of unintended contamination (false positives) or recovery losses (false negatives) through sample manipulation. Hao et al. (Hao et al., 2015) did examine a direct injection approach wherein SPE was bypassed, although in the latter case the absence of a preconcentration step led to higher method LODs**.** One possible solution to mitigate these issues would be to integrate the concentration and instrumental analysis stages within a single procedure. For aqueous samples, this can be achieved using an on-line solid phase extraction method that involves steps not dissimilar to those of the off-line approach, but incorporates them into a fully-automated procedure. In recent years, this on-line pre-concentration approach has gained popularity and is being successfully implemented for the analysis of contaminants of emerging concern (Garcia-Ac et al., 2009; Fayad et al., 2013; Valsecchi et al., 2015). Combined with ultra-high performance liquid chromatography tandem mass spectrometry

(UHPLC-MS/MS), these workflows can also yield fast method turnaround times and improved reproducibility.

Some critical knowledge gaps remain to be addressed in order to ensure the accuracy of ultra-trace measurements in aqueous samples. For instance, the initial filtration step can be a source of bias; therefore, the filtration membrane should be adequately selected to avoid undesirable artifacts (e.g., recovery losses). Another critical aspect is the storage stability of aqueous samples. Given that it may not be always feasible to readily analyze large series of samples upon arrival at the laboratory, the samples may be typically filtered and stored for variable durations at 4°C or frozen, before further processing. Hence, in order to ensure the accuracy of the analytical measurements, we need to verify that this wait time does not affect the integrity of analyte concentrations. At the sample processing step, the sample concentration —be it performed off-line or on-line— could entail further pitfalls. While loading an increasing amount of sample could be expected to improve the sensitivity, interfering matrix components may also become prevalent and hamper analyte retention at the SPE step. Co-eluting matrix components may also modify the analyte instrumental response, for instance through the alteration of its ionization efficiency. If such matrix effects are not mitigated, or if the quantification approach is not well suited, this could greatly impact the reliability of the analytical results thus generated. Another robustness factor often overlooked in off-line or on-line developments is the selection of a suitable loading speed. Although the loading speed and loading volume are unlikely independent (Munoz et al., 2017), one-factor-at-a-time methods are traditionally applied for their optimization. Therefore, the optimization of the enrichment step needs to be more specifically addressed to ensure suitable method accuracy and precision, for instance through a statistical multivariate approach.

In the present study, a group of 9 systemic insecticides were analyzed in water by automated on-line enrichment coupled to UHPLC-MS/MS, for the first time. The overall challenge was to maximize recovery and sensitivity through a simple and rapid analytical procedure, all the while using a minimal amount of sample. To this end, method operating parameters were investigated using experimental designs and Derringer's desirability functions were constructed in order to ascertain optimal on-line enrichment conditions. Factors that may affect the analytical accuracy, including the impact of filtration and

sample storage stability, were examined in matrix-specific experiments. Validation endpoints such as linearity range, determination coefficients (R^2), limits of detection (LOD), accuracy, and precision were also determined in HPLC-water, tap water, and surface water. A particular attention was devoted to the instrumental matrix effect, evaluated for tap water samples and surface water samples from several locations. The developed analytical method was subsequently applied to a selection of tap water, river water, and agricultural flood water samples collected from the province of Québec, Canada.

4.2 Experimental

4.2.1 Chemicals

Desnitro-imidacloprid hydrochloride (purity ≥ 99.8%), dinotefuran (purity ≥ 98.6%), nitenpyram (purity ≥ 99.9%), thiacloprid (purity ≥ 99.9%), imidacloprid (purity ≥ 99.9%), acetamiprid (purity ≥ 99.9%), thiamethoxam (purity ≥ 99.6%), clothianidin (purity ≥ 99.9%), and fipronil (purity ≥ 97.9%) were purchased from Sigma Aldrich (St. Louis, MO, USA). The chemical structures of the selected compounds are provided in the SI (**Figure S3.1**). Isotope-labelled internal standards (ISs) imidacloprid-d_4 (purity ≥ 99.9%), acetamiprid-d_3 (purity ≥ 98%), clothianidin-d_3 (purity ≥ 99.9%), and thiamethoxam-d_3 (purity ≥ 99.9%) were also obtained from Sigma Aldrich (St. Louis, MO, USA), while fipronil-$^{13}C_4$ (purity ≥ 98%) was obtained from Santa Cruz Biotechnology (Dallas, TX, USA). Solvents were all of HPLC grade quality and were purchased from Fisher Scientific (Whitby, ON, Canada). Formic acid (purity ≥ 95%) was acquired from Sigma-Aldrich (St. Louis, MO, USA).

4.2.2 Sample collection

At each site, amber glass bottles were rinsed three times with the site tap water or surface water. The bottles were then filled to the brim and sealed. Upon collection, samples were

kept in an ice-box (4–8 °C) and shipped to the laboratory. River water samples (n = 24) were collected in early December 2016 before winter freeze and in the 2017 summer season after harvesting at 4 monitoring locations: Saint-Régis River, Des Hurons River, Chibouet River, and Saint-Zéphirin River (Québec, Canada). These sampling sites were selected based on the 2011–2014 report of Québec's Ministry of Sustainable Development, Environment and the Fight against Climate Change (Giroux et al., 2015), signaling a high occurrence of neonicotinoid pesticides in surface waters affected by soy and corn cultures in the corresponding watersheds. Samples were collected from agricultural floodplain sites (n = 54) at five different locations in the early spring 2015 just after snowmelt, near Lake Saint-Pierre (Québec, Canada). Tap water samples (n = 12) were collected from domestic homes in Montreal, Laval, Chicoutimi, and Saint-Hyacinthe (Québec, Canada).

4.2.3 Sample preparation and analysis

Samples were filtered using a polyester membrane filter (0.2 µm x 25 mm) (Sterlitech Corporation, Kent, WA, USA) fitted into a Swinnex filter holder by MilliPore (Etobicoke, ON, Canada). After the initial filtration step, the samples were spiked with an isotope-labelled IS solution for a final concentration of 100 ng L^{-1} each. Samples were then analyzed by on-line enrichment – UHPLC-MS/MS.

Analyses were performed using a sample delivery system with a dual switching-column array. A HTC thermopal autosampler (CTC analytics AG, Zwingen, Switzerland) was used for in-loop sample injection. An Accela 600 quaternary pump (Thermo Fisher, San Jose, CA, USA) was used to transfer the sample from the loop to the on-line enrichment column. The column switching system was composed of two-position six-port and ten-port valves (VICIs Valco Instruments Co. Inc., Houston, TX); a quaternary pump Accela 1250 (Thermo Finnigan, San Jose, CA, USA) was used for sample elution from the enrichment column and subsequent separation on the analytical column.

A schematic diagram of the on-line analysis is shown in the SI (**Figure S4.2**). The on-line enrichment column and analytical column were both from Thermo Fisher Scientific (San Jose, CA, USA). The first step consisted in loading 2 mL of sample at 1500 µL·min^{-1} into

a Thermo Hypersep Retain PEP column (20 mm x 2.1 mm, 40–60 µm particle size). After this step, target analytes were back-flushed at 350 $\mu L \cdot min^{-1}$ by a H_2O:acetonitrile mixture (A_1:B_1), prior to separation on a Thermo Hypersil Gold column (50 mm x 2.1 mm, 1.9 µm particle size) thermostated at 30°C. The aqueous on-line mobile phase (A_2) was HPLC-water with 0.1% HCOOH and the organic on-line mobile phase (B_2) was MeOH with 0.1% HCOOH. Full details regarding the mobile phase gradient programs are provided in the SI (**Table S4.1**).

The TSQ Quantiva triple quadrupole mass spectrometer from Thermo Scientific (Waltham, MA, USA) was coupled to a heated electrospray ionisation (HESI) source. The optimized source parameters were as follows: sheath gas was set at 60 A.U. (arbitrary units), auxiliary gas at 20 A.U., sweep gas at 0 A.U., ion transfer tube temperature at 350°C, and vaporizer temperature at 400°C. The ion spray voltage was +3000V for neonicotinoids and -2900V for Fipronil. The analyzer was operated in selected reaction monitoring (SRM) mode. The first (Q1) and third (Q3) quadrupoles were operated at a resolution of 0.7 Da FWHM. The collision gas (CID) pressure in the second quadrupole (q2) was set at 1.5 mTorr. Compound-dependent parameters were optimized by direct infusion of neonicotinoids at 5 $\mu g \cdot mL^{-1}$ to obtain collision energies, quantification and confirmation transitions, and RF Lens values, as summarized in the SI (**Table S4.2**).

4.2.4 Method optimization

Preliminary experiments were carried out to optimize the UHPLC-MS/MS settings. Three analytical mobile phase conditions (A_1:B_1) were examined, namely: H_2O:methanol, H_2O:acetonitrile, and H_2O:methanol with 5 mM of ammonium formate. Following the selection of suitable UHPLC-MS/MS conditions, combinations of on-line sorbent nature and mobile phase composition were jointly investigated. Two SPE columns were then examined (Betabasic-C18, and Hypersep Retain PEP), in combination with different on-line mobile phases (A_2:B_2): H_2O:methanol, H_2O:acetonitrile, and H_2O:methanol with variable percentages of formic acid or ammonium hydroxide (0, 0.1, 0.5 and 1% v/v).

The classical one-variable-at-a-time approach has been previously used in analytical developments, yet may not be always adequate since only a limited portion of the experimental domain is explored and potential interactions between factors are typically overlooked. In order to simultaneously optimize potentially interacting factors, multifactorial designs based on response surface methodology may be implemented (Bezerra et al., 2008; Munoz et al., 2015). In the present work, this approach was undertaken for two on-line SPE variables for which interactions were suspected, namely loading volume and flow rate. These variables were jointly investigated at 4 levels each. The experimental domain explored for loading volume (1–10 mL) and flow rate (1000–2500 $\mu L \cdot min^{-1}$) was set according to the on-line SPE literature for other organic contaminants (Garcia-Ac et al., 2009; Fayad et al., 2013). We used a response surface methodology approach with a full factorial design (16 conditions, n = 3). The selection of optimal parameters was then operated through Derringer's functions as a multi-criteria optimization tool for multiple responses (Bezerra et al., 2008; Munoz et al., 2015; Bekele et al., 2014).

In order to derive the overall desirability (D), four conditional criteria (d_i) were first defined in order to ensure maximal efficiency in terms of analyte recovery (d_r), precision (d_p), chromatographic performances by assessing the asymmetric factor (AF) (d_{af}), and sensitivity (d_s):

i. $\boldsymbol{d_r}$: derived from the number of compounds with normalized area/volume ≥ 75% (for each analyte, absolute area to loading volume ratios were normalized to the maximum value observed across all 16 conditions), in order to guarantee on-line recovery performances;
ii. $\boldsymbol{d_p}$: derived from the number of compounds with relative standard variation (RSD) (n = 3, RSD based on the absolute area) ≤ 5% in order to guarantee method precision;
iii. $\boldsymbol{d_{af}}$: based on the fulfillment of the criterion for acceptability of chromatographic peak symmetry (0.9<AF<1.5, where AF is the asymmetric factor), in order to guarantee chromatographic performances;

iv. d_s: derived from the mean normalized absolute area (for each analyte, the absolute area was normalized to the maximum value observed across all 16 conditions) in order to yield the lowest LODs.

These definitions were used to construct linear increasing functions for the determination of d_r, d_p, and d_s. For each of the 16 investigated conditions, d_i scores can theoretically range from 0 (lowest desirability) to 1 (highest desirability). Note that for the d_{af} criterion, the function was constructed depending on whether the criterion for acceptability of chromatographic peak symmetry was fulfilled, based on a quantitative measure of the asymmetric factor (AF) which is an empirical but accepted method (Bonino et al., 1996; Papai et al., 2002). In order to penalize combinations that would yield unacceptable chromatographic performances (i.e., significant peak tailing or peak fronting), d_{af} was set to 0 whenever an analyte displayed an AF >1.5 or <0.9 (Bonino et al., 1996; Papai et al., 2002; Dolan et al., 2003); d_{af} was assigned the maximum value otherwise.
The overall desirability was then derived as follows (**Equation 1**):

Equation 1

$$D = \sqrt[n]{\prod_{i=1}^{n} d_i}$$

Response surfaces were generated with SigmaPlot™ 11.0 (Systat software). Statistical significance was set as $p<0.05$.

4.2.5 Method validation and quality control

Positive identification of the targeted analytes was based on matching retention times, detectable signals (S/N > 3) for both quantification (Q) and confirmation (C) transitions, and compliance of Q/C ratios. Peak integration was carried out with the XCalibur 2.2 version software from Thermo Fisher Scientific.
Procedural blanks consisted of HPLC-water passed through polyester filters, spiked at 100 ng L^{-1} with isotope-labelled ISs, and subjected to the on-line SPE – UHPLC-MS/MS

analysis as described in Section 2.3. Field/trip blanks consisted of amber glass bottles carried to a subset of the sampling sites, filled on site with HPLC-water, and further processed in parallel to the other samples. None of the targeted analytes was detected in any of these blanks.

A stability test was carried out to evaluate the influence of storage time on three test water matrixes (HPLC-water, tap water, and surface water). For this purpose, ultrapure water, tap water, and pre-filtered surface water samples were spiked at 100 ng L^{-1} on the day of reception at the laboratory (T_0) and stored for variable periods of time (3, 7, 14, and 28 days) at 4°C. For each time point and each matrix, three replicates were considered. In order to perform a preliminary assessment of the impact of temperature, an additional set of bottles was stored at -20°C for a duration of 28 days. At the end of each time point, an aliquot of the stored bottles was retrieved and isotope-labelled ISs were added prior to analysis (for a final concentration of 100 ng L^{-1} each). The on-line SPE – UHPLC-MS/MS analyses were performed at days 0, 3, 7, 14, and 28 for the corresponding samples, using freshly prepared matrix-matched calibration curves for each matrix type.

Linearity range and determination coefficients (R^2), method limits of detection (LOD) and quantification (LOQ), accuracy, precision, recovery, and matrix effects were the parameters examined to further validate the analytical method.

Linearity was evaluated for up to ten calibration levels (native analyte concentrations tested: 0.1, 0.5, 1, 5, 10, 50, 100, 200, 500, and 1000 ng L^{-1}). In all cases, the isotope-labelled ISs were spiked for a final concentration of 100 ng L^{-1} (see also **Table S4.2** for the correspondence between target analytes and isotope-labelled ISs). Each calibration level was analyzed in triplicate. The calibration curve was plotted using the native analyte to IS peak area ratio (y-axis) versus native analyte concentration (x-axis), fitted with an inverse-weighted (1/x) linear regression.

In accordance with the IUPAC (Thompson et al., 2002)**,** the limit of detection (LOD) was defined as the smallest analyte concentration in the test sample that could be distinguished from the background with a signal-to-noise ratio (S/N) = 3. The use of the most abundant MS/MS ion product could yield lower LODs, but at these levels, the possible absence of the second transition could preclude the confirmation. In the present study, since two MS/MS transitions were monitored for analyte identification, the transition with the lowest

S/N was therefore chosen for LOD calculation. Note that the LOD can be alternatively determined based on the calibration curve (Munoz et al., 2017; Munoz et al., 2016); the values derived from the latter method were in close agreement to those reported based on the S/N method. The quantification limit (LOQ) was defined as the smallest concentration that could be accurately determined with a S/N ≥ 10.

Accuracy was assessed at two quality control levels (n = 5 each) not previously included in the calibration curve regression, and was expressed as percentage of the expected value. The first level (QC_1) was set at 4 ng L^{-1}, tantamount to ~ 8 x LOD for most of the investigated compounds, while the second level (QC_2) was set at 75% (i.e., 750 ng L^{-1}) of the highest calibration level of the tested linear range. Likewise, intermediate precision was evaluated for the two aforementioned quality control levels. Intraday precision corresponded to the RSD of accuracy values of 5 preparations analyzed within a single work day. The process was repeated on a second (n = 5) and third (n = 5) work day, and the interday precision derived from the overall RSD (n = 15).

The extraction efficiency was evaluated in ultrapure water at two concentration levels, by comparing the analyte absolute peak area from on-line large volume injection (2000 µL, using a 2000-µL loop) analysis versus on-line small volume analysis (50 µL, using a 50-µL loop) of an equivalent amount into the UHPLC column. Accordingly, the two QC levels tested were 4 ng L^{-1} and 750 ng L^{-1} for the large volume on-line SPE mode, and 160 ng L^{-1} and 30 µg L^{-1} for the small volume on-line SPE mode. Each of the aforementioned conditions was tested in triplicate.

The quantification strategy relied on an internal standard calibration based on a matrix-matched approach. For each matrix type (i.e., tap water and surface water), a composite matrix was made by pooling a subset of each sample. Additions of native analytes and isotope-labelled internal standards were then performed in each composite matrix prior to on-line SPE UHPLC-MS/MS analysis of the calibration curve levels. Tap water samples were therefore quantified based on the matrix-matched (composite tap water) calibration curve, while surface water samples were quantified based on the matrix-matched (composite surface water) calibration curve. The concentrations in the water samples were derived based on the native analyte to IS response ratio in the particular sample, divided by the slope of the corresponding matrix-matched calibration curve [$A_{native}/A_{IS} = f(C_{native})$].

Within a given matrix type (i.e., tap water or surface water), the possibility of residual matrix effects occurring with sample variation was evaluated on a subset of the samples by comparing the standard additions calibration curve slope ($m_{standard\ additions}$) in each particular sample to that of the matrix-matched (composite) calibration curve ($m_{matrix\text{-}matched}$), as described hereafter (**Equation 2**).

Equation 2

$$Matrix\ effect\ (\%) = 100 * \left(\frac{m_{standard\ additions}}{m_{matrix-matched}} - 1 \right)$$

4.3. Results and discussion

4.3.1 Influence of filter type

Performing an initial filtration step could be critical for particulate-laden aqueous samples to ensure suitable method robustness at the later instrumental stage (e.g., avoid back-pressure increase). However, the impact of this step on analyte loss should be considered so that the filtrates analyzed remain representative of the original water that was sampled (Fayad et al., 2013). If overlooked, the filtration artifact due to undesirable analyte sorption onto the filter membrane could translate into the underestimation of true concentrations. Even in a situation wherein the reported concentrations would take into account filtration losses, the selection of a poorly-suited filtration material could nevertheless result in false negatives, especially at ultra-trace levels.

A filtration experiment was carried out in HPLC-water spiked with a mix of target compounds at 100 ng L^{-1} and passed through 9 different filtration materials (porosity range: 0.2–0.3 µm). Filtration recoveries of the targeted analytes ranged between 68 and 90% for polyester, 52–98% for glass fiber, 0–22% for mixed cellulose ester, 25–64% for polytetrafluoroethylene, 0–57% for Nylon, 0–61% for nitrocellulose, 0–55% for cellulose acetate, 23–71% for polycarbonate, and 0–54% for polypropylene (SI **Table S4.3**).

Accordingly, the GFF and polyester filters were further evaluated in matrix-specific filtration experiments (i.e., tap water and surface water). In the particular case of the surface water matrix, note that water samples were first filtered through GFF to eliminate all suspended particles. The pre-filtered surface water was then spiked with a mix of the

targeted compounds at 100 ng L^{-1} and subsequently aliquoted; six aliquots were subjected to filtration through either GFF or polyester, while three aliquots were left non-filtered to serve as a matrix-matched reference to determine the recovery (Darwano et al., 2014).

Filtration recoveries in tap water and surface water are summarized in **Table 4.1**. To facilitate the comparison with the matrix-free reference, the results previously obtained for HPLC-water were aggregated into the table. Regardless of filter type, filtration recoveries in the tap water matrix were in overall agreement with those previously obtained for HPLC-water. Interestingly, the surface water matrix filtration recoveries were somewhat higher than those observed in HPLC-water and drinking water. This is unlikely the result of an instrumental matrix effect, because the filtration recoveries for each of the three matrixes relied upon a comparison with the corresponding matrix-matched reference. Albeit the filtration test was limited to a surface water sample from one specific location (thereby precluding definite conclusions), we can surmise that diverse natural organic matter components of the surface water matrix, present at much higher concentration than the targeted analytes, could somehow shield the analytes of interest from filtration losses.

According to the available literature on neonicotinoids, the most commonly used membrane for water-based samples is glass fiber. In the present study, the latter filter performed satisfactorily overall in tap water (82–95%) except for acetamiprid and imidacloprid (filtration recoveries = 67–70%). Polyester exhibited slightly higher recoveries than GFF in tap water. Both filters performed satisfactorily for the surface water sample examined. We finally opted for polyester for the equivalent or higher recoveries for all target analytes, in agreement with the SANCO 2015 guideline reference (SANTE/11945/2015).

Table 4.1. Filtration recovery (%) (mean ± SD, n = 3) of selected systemic insecticides on glass fiber filters (GFF) and polyester filters (PETE). The experiment was carried out in HPLC-water, tap water, and surface water spiked at 100 ng L^{-1} with the targeted analytes.

	HPLC water				Tap water				Surface water			
	GFF		PETE		GFF		PETE		GFF		PETE	
Acetamiprid	60	± 1	77	± 3	69	± 1	87	± 2	106	± 1	98	± 1
Clothianidin	71	± 3	80	± 2	82	± 1	93	± 1	99	± 5	98	± 2
Desnitro-Imidacloprid	52	± 1	87	± 3	94	± 0	93	± 1	90	± 5	94	± 11
Dinotefuran	71	± 3	77	± 1	93	± 2	95	± 2	100	± 10	97	± 3
Fipronil	92	± 1	90	± 0	89	± 2	85	± 1	91	± 1	86	± 1
Imidacloprid	63	± 2	80	± 1	67	± 0	86	± 4	102	± 2	93	± 1
Nitenpyram	76	± 3	85	± 2	89	± 2	91	± 1	101	± 3	105	± 1
Thiacloprid	70	± 4	68	± 12	87	± 1	89	± 2	94	± 1	97	± 4
Thiamethoxam	75	± 3	78	± 4	95	± 2	97	± 1	107	± 2	100	± 3

4.3.2 Influence of storage time and temperature

A matrix-specific storage stability experiment was conducted at a spike level of 100 ng L^{-1} in ultrapure water, tap water, and surface water; note that surface water was filtered prior to spiking (see also Section 2.5 for other experimental details). **Figure 4.1** illustrates the temporal follow-up of normalized concentrations (% with respect to T_0) of imidacloprid throughout the 28-day monitoring period. Analyte concentrations (ng L^{-1}) quantified at each of the 5 time points (0, 3, 7, 14, and 28 days) are further provided in the Supplemental Material for the HPLC-water (SI **Table S4.4**), tap water (SI **Table S4.5**), and surface water (SI **Table S4.6**) matrixes. Analyte concentrations a few hours after spiking (T_0) averaged 98 ng L^{-1}, 104 ng L^{-1}, and 105 ng L^{-1} in the HPLC-water, tap water, and surface water matrixes, respectively, suggesting no immediate sorption losses or degradation (**Tables S4.4-S4.6**). After 28 days of storage at 4°C (T_0+28), relative concentrations (T_0 = 100%) averaged 98% (range: 93–110% for the 9 analytes), 99% (range: 94–113%), and 97% (range: 87–104%) for the HPLC-water, tap water, and surface water matrixes, respectively. Under the investigated conditions and for the sample types considered, the integrity of initial concentrations did not seem to be impacted by storage time for up to 28 days. A preliminary assessment of the effect of temperature was also conducted by comparing the concentrations determined at T_0 and after 28 days of storage either at 4°C or at -20°C, suggesting no particular differences (SI **Tables S4.4–S4.6**).

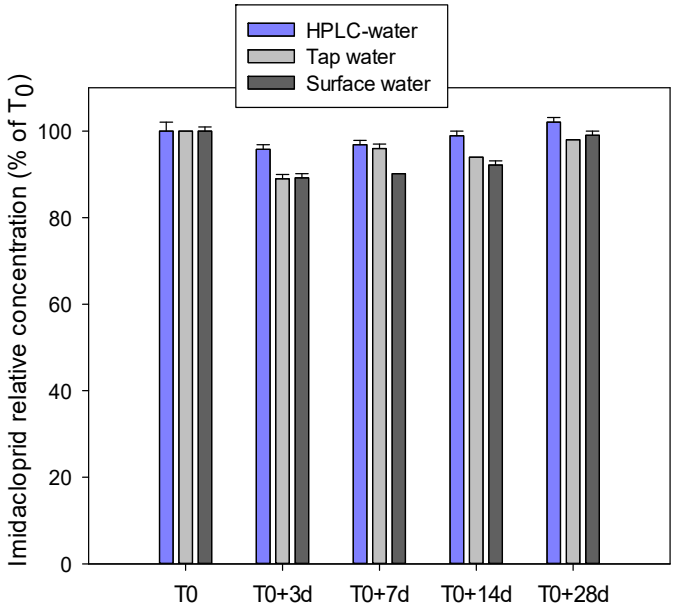

Figure 4.1. Analyte storage stability over 28 days at 4ºC illustrated for imidacloprid across the three investigated matrixes (surface water was pre-filtered prior to spiking). For the sake of clarity, relative concentrations (%) are shown normalized to the initial concentrations (i.e., T_0 = 100%). Full details on non-normalized concentrations (ng L^{-1}) for the 9 targeted analytes are further provided in the SI (**Tables S4.4–S4.6**).

4.3.3 Optimization of on-line enrichment – UHPLC-MS/MS

With the objective of ensuring chromatographic performances and improving ionization efficiency, analytical mobile phases should be carefully selected. In a preliminary experiment, three analytical mobile phase combinations for UHPLC-MS/MS were examined. Analytical mobile phases amended with ammonium formate have been previously used for the analysis of neonicotinoids similar to the target list from the present survey (except desnitro-imidacloprid) (Chen et al., 2014; Kasiotis et al., 2014; Masia et al., 2013). In agreement with the literature, H_2O:MeOH containing 5 mM of ammonium formate yielded suitable signal intensities (SI **Figure S4.3**) and chromatographic peak shapes; a noteworthy exception was desnitro-imidacloprid, for which skewed peaks were observed. The H_2O:methanol (A_1:B_1) combination generally produced the highest slope

intensities among the three tested conditions (SI **Figure S4.3**). This notwithstanding, asymmetric peaks (AF>2) were still observed in the case of desnitro-imidacloprid. Taking into account chromatographic requirements, the analytical mobile phase composition with the second-best intensity performance was the H_2O:acetonitrile (A_1:B_1) combination, which was therefore selected for subsequent optimization experiments.

Consecutively, it was necessary to determine an adequate combination of on-line SPE mobile phase composition and on-line SPE sorbent nature. The mobile phase combinations examined were H_2O:methanol and H_2O:acetonitrile, supplemented or not with a modifier (formic acid or ammonium hydroxide at variable concentrations). These tests were carried out for different types of on-line SPE sorbents (Betabasic-C18 and Hypersep Retain PEP) that show greater affinity toward polar compounds than the sorbents traditionally used in reversed-phase chromatography (i.e., C18). Even though the highest intensities were obtained with the Betabasic-C18 sorbent for all analytes (unpublished data), the latter sorbent presented the disadvantage of a higher back-pressure (Naldi et al., 2016) that could later affect the method robustness when analyzing large series of samples. In order to maintain an acceptable compromise for all compounds, the Hypersep Retain PEP (i.e., hydrophilic-lipophilic balance, consisting of polystyrene divinylbenzene modified with urea-containing functional groups) on-line column was finally selected for its low back-pressure, short equilibration time, and suitable signal intensity for the targeted analytes. The influence of on-line SPE mobile phase nature on analyte signal with this column is illustrated in **Figure 4.2**. The use of a NH_4OH-amended on-line aqueous mobile phase generally provided the highest intensities compared to the other two conditions (**Figure. 4.2**). However, it also entailed the disadvantage of a deformed peak shape for desnitro-imidacloprid. The HCOOH-amended on-line aqueous mobile phase yielded significantly higher ($p<0.05$) absolute areas for 7 out of 9 analytes when compared with the non-amended one (i.e., HPLC-water) (**Figure 4.2**). Among the tested HCOOH concentrations, HPLC-water containing 0.1% HCOOH generally provided a suitable compromise between signal intensity (absolute area or signal height) and chromatographic peak shape (unpublished data), which led to its selection. The on-line SPE mobile phase settings finally retained were HPLC-water with 0.1% HCOOH as the aqueous (A_2) and methanol with 0.1% HCOOH as the organic (B_2) mobile phase.

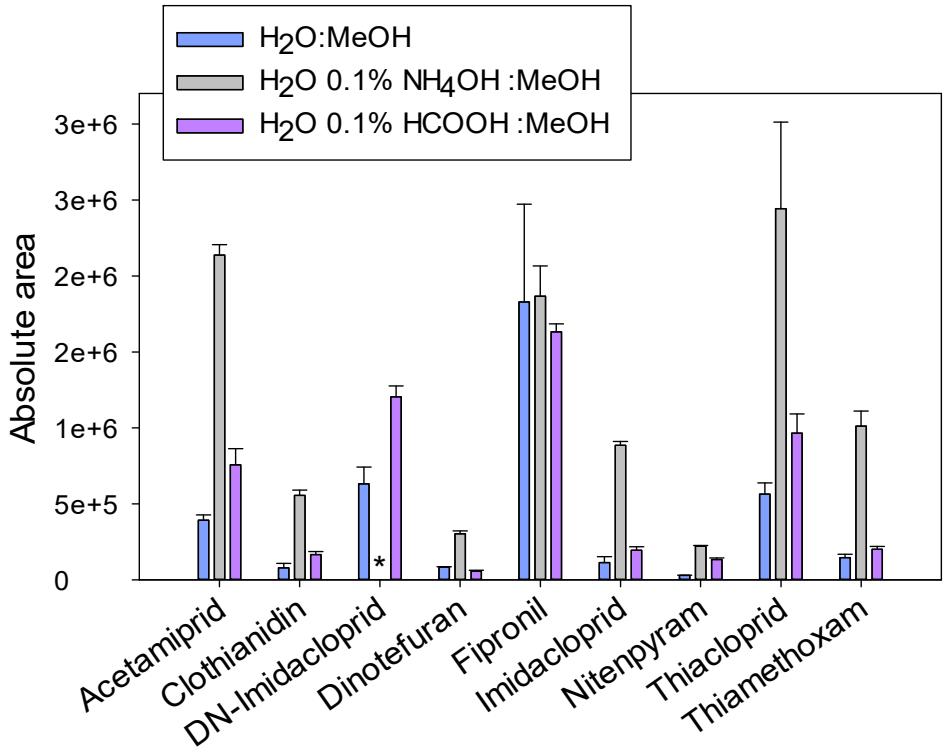

Figure 4.2. Influence of on-line SPE mobile phase nature (with a Hypersep Retain PEP on-line SPE column) on analyte absolute area, investigated at a spike level of 100 ng L^{-1}. *In the case of desnitro-imidacloprid, the deformed peak shape observed with the NH_4OH-based loading mobile phase did not allow for a reliable integration.

Upon the selection of an appropriate combination of on-line SPE column and mobile phases, some factors still required optimization in order to improve the method sensitivity without compromising recovery. As such, the on-line SPE loading volume and loading flow rate are critical factors to optimize because they are also related to total analysis time, whole-method detection limits, and extraction efficiency. Increasing sample loading volumes may result in increased sensitivity performance, yet should require a careful optimization of loading flow rate to avoid analyte breakthrough and excessive analysis time. Additionally, sample loading speed and loading volumes may not be independent, hence the necessity to design an optimization scheme considering possible interactions between these factors. A one-variable-at-a-time optimization approach would typically overlook such interactions. An alternative approach could rely on multivariate statistical

techniques, such as those involving a response surface methodology (RSM) (Bezerra et al., 2008). In the present study, a full factorial design was applied, the on-line SPE loading volume and loading flow rate being jointly optimized (Munoz et al., 2017) (see also Section 2.4 for details).

An illustration of the response surfaces generated after execution of the experimental design is shown in the SI (**Figure S4.4-S4.5**). Fipronil presented a rather ideal case, wherein absolute areas increased almost linearly with increasing sample loading volume, regardless of the sample loading speed (**Figure S4.4**). This would allow for considerable flexibility in the choice of on-line SPE operating conditions. The case of clothianidin is also noteworthy: while loading speed had little or no influence on analyte absolute area when sample loading volume was varied in the range 1–5 mL (**Figure S4.4**), this did not hold true at the highest loading volume (10 mL). This observation is also apparent in the clothianidin absolute area to sample volume ratios (**Figure S4.5**) that dropped by a factor of ~2 between the lowest and the highest flow rates at 10 mL, reflecting decreased retention efficiency.

With the overarching goal to obtain highest sensitivity, maximum recovery, adequate precision, and suitable chromatographic performance, it was difficult to select a method just from the observation of the response surface graphics. For this purpose, four desirability functions were built to refine the selection process (see also Section 2.4 for details). As shown in **Figure 4.3** presenting the cumulative d_i, Methods M#15 and M#16 were readily discarded due to the loss of appropriate chromatographic performances for desnitro-imidacloprid. Based on the overall desirability (SI **Table S4.7**), Method M#6, consisting in a 2-mL loading volume and 1500 $\mu L \cdot min^{-1}$ flow rate, yielded the best balance of d_i criteria (D = 0.66). On-line concentration – UHPLC-MS/MS chromatograms generated with the final optimal settings are shown in the SI (**Figure S4.6**). Note that the other 2 mL-based methods presented D values close to that of M#6 (range = 0.59–0.62), indicating the robustness of the 2-mL method over the range of loading speeds investigated (SI **Table S4.7**).

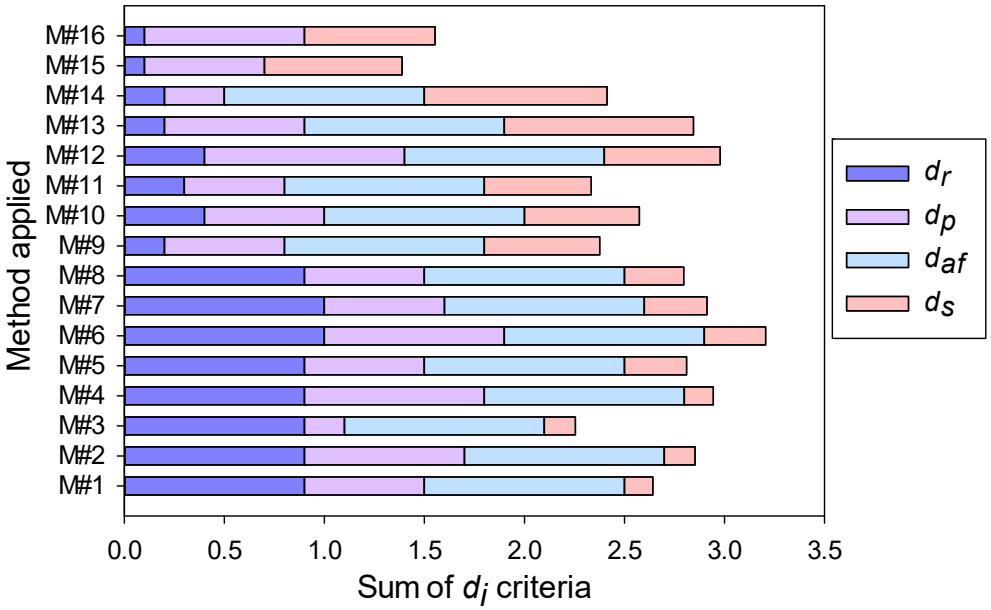

Figure 4.3. Optimization of on-line SPE loading speed and flow rate through a desirability approach: cumulative d_i for the selection of on-line SPE loading conditions. Methods M#1 through M#16 reflect the 16 combinations of sample loading volumes and flow rates considered (see also SI **Table S4.7** for the correspondence). The d_i criteria (d_r: recovery; d_p: precision; d_{af}: chromatography; d_s: sensitivity) are fully defined in Section 2.4.

4.3.4 Method performance

Coefficients of merit of the matrix-free (i.e., HPLC-water) and matrix-matched calibration curves are shown in **Table 4.2**. Matrix-free calibration curves were produced with suitable linearity over 3–4 orders of magnitude, with determination coefficients (R^2) in the range 0.9977–0.9999. The bias for individual calibration levels remained between ± 10% of the calculated trend lines. In the matrix-matched approach, the linearity range also spanned 3–4 orders of magnitude, with R^2 in the range 0.9975–0.9999 and 0.9958–0.9993 in the case of the tap water and surface water matrix-matched calibration curves, respectively.

Limits of detection were compound-specific yet remained little affected by the matrix overall (**Table 4.2**). Method LODs were generally between 0.1–1 ng L^{-1}, and LOQs between 0.3–3 ng L^{-1}. Of note, the on-line SPE method described herein yielded method

LODs in the same order of magnitude or lower than off-line SPE workflows, all the while requiring a reduced sample size (2 mL *Vs* 100–1000 mL) (**Table 4.3**). Automation of the concentration step and the implementation of ultra-high performance LC-MS/MS (particle size = 1.9 μm) substantially reduced total analysis time (8 min per sample). This also represents a noteworthy improvement compared to earlier methods (**Table 4.3**). The latter were typically based on off-line workflows and instrumental analysis involving HPLC chromatographic columns (2.6–5 μm) that required longer run times (18–40 min) (Schaafsma et al., 2015; Dujakovic et al., 2010; Hladik et al., 2012; Hao et al., 2015; Seccia et al., 2005).

Absolute recovery of the on-line SPE sorbent was examined at two fortification levels, following the procedure described in Section 2.5. On-line SPE absolute extraction efficiencies were acceptable, remaining generally in the range 80–100%. A notable exception was desnitro-imidacloprid, which presented substantially lower absolute recoveries on the SPE sorbent (~ 35–40%) (SI **Table S4.8**). However, despite this fact, the recovery bias did not affect the *accuracy* of the method as a whole (see also **Table S4.9** and the next paragraph), because the calibration curve levels were also submitted to the on-line SPE concentration step (hence integrating the recovery losses into the quantification procedure). The adequate extraction efficiencies for 8 out of the 9 targeted compounds — and relatively low variability observed at this stage even without IS correction— further consolidate the choices made at the earlier optimization step, when these criteria (i.e., d_r and d_p) were included into the desirability approach.

Accuracy and intermediate precision were also evaluated at two fortification levels (4 ng L^{-1} and 750 ng L^{-1}) in the three tested aqueous media (SI **Table S4.9**). The quantification approach performed satisfactorily, with accuracies generally in the range 90–110% (SI **Table S4.9**). Regardless of the spike level examined, intraday precision (n=5) remained between 1–23% for the targeted systemic insecticides. Interday precision averaged 9.3%, 9.4%, and 10.4% for HPLC-water, drinking water, and surface water spiked samples, respectively (SI **Table S4.9**).

Although matrix-matched calibration curves were used for sample quantification, it cannot be entirely discounted that particular samples could deviate from the composite matrix-matched sample used for this purpose. For instance, a particular surface water location

could contain interfering matrix components at substantially higher concentrations —or lower, for that matter— than the composite surface water sample used for generating the calibration curve. The examination of absolute areas of surrogate standards or internal standards among individual samples is often proposed as a means of evaluating such effects (Munoz et al., 2017). The latter approach may not, however, entirely reflect the actual impacts on the method accuracy (since area ratios, rather than absolute areas, are typically considered at the quantification stage). In the present study, the so-called residual matrix effect (Munoz et al., 2017) was assessed by comparing the slopes of standard additions (with IS correction) to several samples of each type of water (i.e., tap water and surface water) with those yielded by the corresponding matrix-matched calibration (also with IS correction), as described in Section 2.5. Standard additions in the tap water samples from distinct sampling locations yielded slopes generally within ± 7% (range: -7.4% to +6.6%) of the composite matrix-matched curve, except for nytenpyram at one particular location (see also **Table S4.10**). In the case of surface water, 8 out of the 9 targeted analytes showed acceptable deviations from the matrix-matched curve, in the range of -5.1% to +2.2%, -6.3% to +9.8%, and -4.1% to +3.0% for standard additions to L'Assomption River, St-Lawrence River, and Yamaska River samples, respectively (see also **Table S4.11**). Desnitro-imidacloprid showed larger and more variable deviations (-17% to +33%) from the matrix-matched calibration curve in surface water. Since this particular analyte was not actually detected in any of the surface water samples from the present survey, it was still deemed acceptable to retain a matrix-matched calibration curve approach for the quantification of the other targeted analytes detected in the present study.

Table 4.2. Analytical figures of merit of the solvent-based and matrix-matched calibration curves (linearity range and determination coefficient) and corresponding method limits of detection (LOD, ng L^{-1}) of the 9 systemic insecticides in HPLC-water, tap water, and surface water.

	Linearity range over tested range (ng L^{-1})			R^2			Method limit of detection (LOD, ng L^{-1})		
	HPLC-water	Tap water	Surface water	HPLC-water	Tap water	Surface water	HPLC-water	Tap water	Surface water
Acetamiprid	0.1–1000	0.5–1000	0.5–1000	0.9995	0.9996	0.9993	0.1	0.5	0.5
Clothianidin	0.5–1000	0.5–1000	1–1000	0.9997	0.9998	0.9987	0.5	0.5	1
Desnitro-Imidacloprid	0.1–1000	0.1–1000	0.5–1000	0.9986	0.9998	0.9977	0.1	0.1	0.5
Dinotefuran	1–1000	1–1000	5–1000	0.9983	0.9997	0.9991	1	1	5
Fipronil	0.1–1000	0.1–1000	0.5–1000	0.9992	0.9988	0.9983	0.1	0.1	0.5
Imidacloprid	0.5–1000	0.5–1000	0.5–1000	0.9997	0.9999	0.9992	0.5	0.5	0.5
Nitenpyram	1–1000	1–1000	1–1000	0.9992	0.9998	0.9982	1	1	1
Thiacloprid	0.1–1000	0.5–1000	1–1000	0.9977	0.9995	0.9976	0.1	0.5	1
Thiamethoxam	0.1–1000	0.5–1000	1–1000	0.9999	0.9999	0.9992	0.1	0.5	1

Table 4.3. Operating settings and performance of the present workflow compared to previously reported LC-MS methods for neonicotinoid analysis.

	Seccia et al., 2005	**Dujakovic et al., 2010**	**Hladik et al., 2012**	**Schaasfsma et al., 2015**	**Hao et al., 2015**	**Hao et al., 2015**	**Present work**
Pesticides analyzed	4	14	10	2	8	8	9
Neonicotinoids analyzed	4	2	6	2	8	8	8
Matrix type	Drinking water	Groundwater, Surface water	Surface water	Surface water	Drinking water, Groundwater, Surface water	Drinking water, Groundwater, Surface water	Drinking water, Surface water
Sample size	100 - 1000 mL	250 mL	1000 mL	10 mL	100 mL	0.8 mL	2 mL
SPE mode	Off-line (disposable cartridges)	Off-line (disposable cartridges)	Off-line (disposable cartdriges)	dSPE	Off-line (disposable cartridges)	- (direct analysis)	On-line (renewable column)
SPE sorbent	LiChrolut EN cartridges (200 mg)	Oasis HLB (200 mg/6 mL)	Oasis HLB (500 mg/6 mL)	QuEChERS	Phenomenex Strata-X (60 mg/3 mL)	-	Thermo Hypersep Retain PEP
Final extract volume	0.5 mL	1 mL	0.2 mL	1 mL	5 mL (methanol:water 20:80)	1 mL	10 mL (for triplicate injection)
Analysis	LC-MS	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS	UHPLC-MS/MS
Injection volume	20 µL	10 µL	10 µL	50 µL	NA	NA	2 mL
LC column	LiChroCart 125-4 LiChrospher (5 µm p.s.)	Zorbax Eclipse XDB-C18 (3.5 µm p.s.)	Zorbax Eclipse XDB-C18 (3.5 µm p.s.)	Phenomenex Gemini C18 (5 µm p.s.)	Kinetex Biphenyl (2.6 µm p.s.)	Kinetex Biphenyl (2.6 µm p.s.)	Thermo Hypersil Gold C18 (1.9 µm p.s.)
Ionization	ESI (positive mode)	ESI (positive mode)	ESI (positive mode)	ESI (positive mode)	ESI (positive mode)	ESI (positive mode)	ESI (positive and negative modes)
MS analyzer	Thermo Finnigan Navigator	Thermo Finnigan LCQ Advantage quadrupole ion trap	Agilent 6430 QqQ	Sciex EP 10+ API 365 QqQ	Sciex Qtrap 4000	Sciex Qtrap 4000	Thermo TSQ Quantiva QqQ

Analysis time	27 min + SPE + evaporation	40 min + SPE + evaporation	12 min + SPE + evaporation	25 min + dSPE + evaporation	18 min + SPE	18 min	8 min
Quantification	External calibration	External matrix-matched calibration	Matrix-matched calibration, with internal standardization	Matrix-matched calibration	Solvent-based curves, with internal standardization	Solvent-based curves, with internal standardization	Matrix-matched calibration, with internal standardization
LOD of neonicotinoids (ng L^{-1})	10	3.2 – 5.0	3.6 – 6.2	4 – 17	2.0 – 7.0	50 – 190	0.1 – 5.0

4.3.5 Method application to surface water and tap water samples

Method applicability was assessed through the analysis of agricultural flood plain water, river water, and drinking water samples, in order to perform a preliminary screening of systemic insecticides "from source to tap".

In agricultural flood plain water samples (**Table 4.4**), imidacloprid was reported in 12/54 samples (concentration range = <lod–4 ng L^{-1}). Clothianidin was reported with the highest concentration (33 ng L^{-1}) and thiamethoxam was the most recurrent (detection frequency = 100%). Field replicates labelled as samples #1–3 were expected to show relatively high concentrations, owing to their location in the immediate vicinity to agricultural fields where neonicotinoids could be used. This hypothesis was further supported by the relatively high levels of clothianidin (33 ng L^{-1}, 29 ng L^{-1}, and 10 ng L^{-1}), the concentrations of which mirrored those of its parent compound thiamethoxam (27 ng L^{-1}, 24 ng L^{-1}, and 9 ng L^{-1}). Further away from the primarily impacted area, sampling sites #4–7 showed substantially lower concentrations of the two aforementioned neonicotinoids.

In river water samples collected in the late autumn 2016 (**Table 4.5**), imidacloprid, thiamethoxam, and clothianidin were the most recurrent (detection frequency = 100%). The latter compounds were in the range of 0.8–4, 3–6, and 2–11 ng L^{-1}, respectively, with limited variations within replicates (<9% on average). The occurrence of second-generation neonicotinoids is in line with Québec's 2011–2014 report (*Présence de pesticides dans l'eau au Québec*), pinpointing the use of such agrochemicals in corn and soya cultures (Giroux et al., 2015). Based on the available literature, neonicotinoids may be recalcitrant to natural attenuation and are expected to remain almost unaffected by wastewater treatment plant processes (Lu et al., 2015; Sadaria et al., 2016)**.** This could explain the occurrence of these compounds in river water, even if the sample collection was conducted several months after the agricultural season. Clothianidin, imicacloprid, and thiamethoxam also presented high frequencies of occurrence (detection frequency = 100%) in samples collected in the summer 2017, and as could be anticipated, the values post-harvest were relatively higher (range: 5–88 ng L^{-1}) suggesting the presence of a seasonal pulse concentration that would require further monitoring and characterization. Acetamiprid was detected in two rivers, St Regis and Des

Hurons, at 0.6 and 2.5 ng L^{-1}, in contrast to the corresponding late autumn samples from the same sites where it was not detected (**Table 4.5**).

Systemic insecticides were screened in tap water samples from 4 municipalities (Montreal, Laval, Chicoutimi, and Saint-Hyacinthe) in the province of Québec, Canada (**Table 4.6**). A positive detection of imidacloprid was confirmed in 9/12 samples (concentration range = 0.1–1 ng L^{-1}). The drinking water sample from Saint-Hyacinthe municipality displayed a total (Σ_9 Insecticides) of 17 ng L^{-1}. At this location, the composition profile comprised mainly thiamethoxam and clothianidin (10 ng L^{-1} and 6 ng L^{-1}, respectively). Although it is difficult to speculate without a specific knowledge of the drinking water source and treatment procedures, this observation seems in reasonable agreement with the fact that Saint-Hyacinthe is situated amidst agricultural areas dominated by corn culture. Fipronil was only detected in the tap water sample from Montreal (5 ng L^{-1}). The latter compound has been reportedly used in antiparasitic treatments for domestic animals. Major urban centers could therefore be anticipated as a plausible source of fipronil to the environment and the St-Lawrence River does receive inputs from many municipal areas across its large watershed.

Table 4.4. Neonicotinoids and fipronil concentrations (ng L^{-1}) in agricultural flood plain water samples collected in April-May 2015 across the province of Québec, Canada, using automated enrichment coupled on-line to UHPLC-ESI-MS/MS.

Sample Code	Replicates	Sampling date	Acetamiprid	Clothianidin	Desnitro-Imidacloprid	Dinotefuran	Fipronil	Imidacloprid	Nitenpyram	Thiacloprid	Thiamethoxam	**Σ_9 Insecticides**
4a	3	23-apr-2015	<lod	<lod	<lod	<lod	<lod	<lod	<lod	<lod	3 ± 1	**3**
7a	3	23-apr-2015	<lod	<lod	<lod	<lod	<lod	<lod	<lod	<lod	4 ± 0.3	**4**
1a	3	23-apr-2015	<lod	33 ± 12	<lod	<lod	<lod	<lod	<lod	<lod	27 ± 9	**60**
2a	3	23-apr-2015	<lod	2 ± 1	<lod	<lod	<lod	<lod	<lod	<lod	4 ± 1	**6**
5a	3	23-apr-2015	<lod	<lod	<lod	<lod	<lod	<lod	<lod	<lod	4 ± 0.3	**4**
4b	3	7-may-2015	<lod	7 ± 2	<lod	<lod	<lod	2 ± 1	<lod	<lod	7 ± 2	**16**
4d	3	30-apr-2015	<lod	4 ± 1	<lod	<lod	<lod	3 ± 1	<lod	<lod	10 ± 0.4	**17**
3a	3	7-may-2015	<lod	<lod	<lod	<lod	<lod	<lod	<lod	<lod	0.7 ± 0.3	**0.7**
2b	3	23-apr-2015	<lod	29 ± 6	<lod	<lod	<lod	<lod	<lod	<lod	24 ± 4	**53**
4c	3	30-apr-2015	<lod	7 ± 1	<lod	<lod	<lod	4 ± 0.3	<lod	<lod	8 ± 1	**19**
5c	3	23-apr-2015	<lod	6 ± 1	<lod	<lod	<lod	<lod	<lod	<lod	4 ± 1	**10**
5b	3	30-apr-2015	<lod	<lod	<lod	<lod	<lod	<lod	<lod	<lod	3 ± 0.4	**3**
1b	3	23-apr-2015	<lod	4 ± 1	<lod	<lod	<lod	<lod	<lod	<lod	6 ± 1	**10**
1c	3	30-apr-2015	<lod	3 ± 1	<lod	<lod	<lod	<lod	<lod	<lod	7 ± 0.1	**10**
3b	3	30-apr-2015	<lod	10 ± 0	<lod	<lod	<lod	1 ± 1	<lod	<lod	9 ± 0.4	**20**
6a	3	23-apr-2015	<lod	1 ± 0	<lod	<lod	<lod	<lod	<lod	<lod	3 ± 0.2	**4**
4c	3	23-apr-2015	<lod	<lod	<lod	<lod	<lod	<lod	<lod	<lod	3 ± 0.4	**3**
7b	3	30-apr-2015	<lod	<lod	<lod	<lod	<lod	<lod	<lod	<lod	8 ± 1	**8**
LOD (ng L^{-1})			0.5	1	0.5	5	0.5	0.5	1	1	1	

Table 4.5: Neonicotinoids and fipronil concentrations (ng L^{-1}) at 4 river monitoring locations sampled in the late autumn 2016 and Summer 2017 (n = 3 replicates per site) from the province of Québec, Canada, using automated enrichment coupled on-line to UHPLC-ESI-MS/MS.

		Saint-Régis		**Des Hurons**		**Chibouet**		**Saint-Zéphirin**	
	LOD (ng L^{-1})	Autumn 2016	Summer 2017	Autumn 2016	Summer 2017	Autumn 2016	Summer 2017	Autumn 2016	Summer 2017
Acetamiprid	0.5	<lod	0.6 ± 0.02	<lod	2.5 ± 0.2	<lod	<lod	<lod	<lod
Clothianidin	0.5	6 ± 2	15 ± 2	11 ± 0.3	88 ± 4	7 ± 0.2	22 ± 0.6	2 ± 0.2	15 ± 0.6
Desnitro-Imidacloprid	5	<lod	<lod	<lod	<lod	<lod	<lod	<lod	<lod
Dinotefuran	5	<lod	<lod	<lod	<lod	<lod	<lod	<lod	<lod
Fipronil	0.1	0.3 ± 0.1	<lod	<lod	<lod	<lod	<lod	<lod	<lod
Imidacloprid	0.5	4 ± 0.2	38 ± 0.7	2 ± 0.1	10 ± 0.2	0.8 ± 0.3	6 ± 0.1	0.8 ± 0.04	1.2 ± 0.3
Nitenpyram	1	<lod	<lod	<lod	<lod	<lod	<lod	<lod	<lod
Thiacloprid	0.5	1 ± 0.05	<lod	1 ± 0.06	<lod	<lod	<lod	<lod	<lod
Thiamethoxam	0.5	6 ± 0.1	5 ± 0.5	4 ± 0.1	61 ± 2	3 ± 0.04	11 ± 0.6	5 ± 0.05	6 ± 1.4
Σ_9 Insecticides		**16**	**59**	**18**	**161**	**11**	**39**	**8**	**22**

Table 4.6. Neonicotinoids and Fipronil concentrations (ng L^{-1}) in tap water samples collected from 4 municipalities in the province of Québec, Canada, using automated enrichment coupled on-line to UHPLC-ESI-MS/MS.

	LOD (ng L^{-1})	**Montréal**	**Laval**	**Chicoutimi**	**Saint-Hyacinthe**
Acetamiprid	0.5	<lod	<lod	<lod	<lod
Clothianidin	0.5	<lod	<lod	<lod	6 ± 0.4
Desnitro-Imidacloprid	1	<lod	<lod	<lod	<lod
Dinotefuran	5	<lod	<lod	<lod	<lod
Fipronil	0.1	5 ± 2	<lod	<lod	<lod
Imidacloprid	0.1	0.2 ± 0.01	0.1 ± 0.04	<lod	1 ± 0.1
Nitenpyram	1	<lod	<lod	<lod	<lod
Thiacloprid	0.5	<lod	<lod	<lod	<lod
Thiamethoxam	0.5	<lod	<lod	<lod	10 ± 1
Σ_9 Insecticides		**5.2**	**0.1**	**<lod**	**17**

4.4 Conclusions

A rapid and sensitive analytical method has been developed to determine 9 systemic insecticides in drinking water and surface water by on-line solid phase extraction – UHPLC-MS/MS. Given the various analyte properties, a particular challenge was the delineation of common conditions satisfying suitable sensitivity and chromatographic requirements for all targeted compounds. Under the optimized conditions, a Hypersep Retain PEP on-line SPE column was used in conjunction with HCOOH-amended on-line mobile phases. Instead of a heuristic one-factor-at-a-time approach, an alternative optimization procedure was carried out to explore potentially cross-linked sample loading parameters. The selection of optimal settings was subsequently dictated by a multicriteria desirability approach (Derringer's functions). A 2-mL on-line injection volume with 1500 $\mu L \cdot min^{-1}$ loading flow rate was finally retained, and a sample run time of 8 min was achieved. Method limits of detection were little or not affected by the presence of a matrix, and remained at or below ng L^{-1} levels for the scope of targeted analytes (LOD range = 0.1–5 ng L^{-1}). This represents a noteworthy advance compared to previously published methods for neonicotinoids in water that generally employed an off-line concentration step prior to analysis. Factors that could jeopardize the analytical accuracy, such

as the initial filtration step, sample storage time and temperature, or matrix-dependent phenomena at the instrumental stage, were evaluated. Glass fiber filters and polyester filters yielded the lowest filtration artifacts out of the 9 filtration membranes tested. Preliminary assessment of sample storage stability on three matrix types suggested suitable stability within a range of 28 days at either 4°C or -20°C. The possibility of matrix effects was mitigated through the quantification procedure that involved a matrix-matched calibration with isotope-labelled internal standard correction. Within a given matrix type, typical deviations of standard additions (to individual samples) from the composite matrix-matched reference generally remained within ±10%. Such performances suggest that a reliable quantification of samples from various locations could be attained, which could legitimate a rigorous comparison between samples (e.g., inter-site differences) in future surveys. The proposed method yields fast data generation and presents a suitable robustness, making it a relevant option for large-scale monitoring surveys to document the occurrence and fate of systemic insecticides in aquatic environments.

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Compliance with ethical standards

The authors declare they have no conflict of interest other that the financial and technical support above.

4.5 Supplementary Material

Table S4.1. Valve program, on-line SPE (loading pump) and UHPLC (analytical pump) gradient conditions used for concentration and separation of selected systemic insecticides.

Loading pump					**Analytical pump**				
	Time (min)	A_2 (%)	B_2 (%)	Flow rate (µL/min)	Time (min)	A_1 (%)	B_1 (%)	Flow rate (µL/min)	
On-line SPE loading step	0	100	0	1500	0	80	20	350	Column re-equilibration
	3.45	100	0	1500	3.45	80	20	350	Elution and chromatographic separation
Loop wash	7.15	0	100	2000	7.35	5	95	350	
SPE column conditioning	7.25	100	0	1500	9.35	5	95	350	
	10	100	0	1500	9.45	80	20	350	Column re-equilibration
					10	80	20	350	

Table S4.2. Chromatographic retention time and mass spectrometry compound-dependent parameters of selected systemic insecticides. The MS/MS transition type is also specified: Q (quantification transition), C (confirmation transition), or IS (isotope-labelled internal standard).

Compound	RT (min)	Precursor Ion (m/z)	Product Ion (m/z)	MS/MS Transition (type)	RF Lens (V)	Collision Energy (V)	IS
Acetamiprid	6.12	223.2	125.9	**Q**	51	23	Acetamiprid-d3
			99.0	C	51	39	
Clothianidin	5.86	250.1	169.0	**Q**	36	13	Clothianidin-d3
			131.9	C	36	17	
DN-Imidacloprid	4.79	211.1	126.0	**Q**	75	25	Thiamethoxam-d3
			175.0	C	75	17	
Dinotefuran	4.93	203.2	157.0	**Q**	30	10	Thiamethoxam-d3
			129.1	C	30	10	
Fipronil	7.26	435.0	329.8	**Q**	71	16	Fipronil-$^{13}C_4$
			249.9	C	71	28	
Imidacloprid	5.98	256.1	209.0	**Q**	44	17	Imidacloprid-d4
			175.0	C	44	19	
Nitenpyram	5.07	271.2	237.0	**Q**	48	19	Thiamethoxam-d3
			225.1	C	48	16	
Thiacloprid	6.27	253.1	126.0	**Q**	56	22	Acetamiprid-d3
			185.9	C	56	15	
Thiamethoxam	5.58	292.1	211.0	**Q**	38	12	Thiamethoxam-d3
			180.9	C	38	22	
Acetamiprid-d3	6.11	226.2	126	IS	51	22	
Clothianidin-d3	5.85	253.1	172	IS	36	14	

Fipronil-$^{13}C_4$	7.26	439.0	333.9	IS	67	16
Imidacloprid-d4	5.97	260.2	213	IS	44	18
Thiamethoxam-d3	5.57	295.1	214	IS	38	13

Table S4.3. Filtration recovery (%) (mean ± SD, n = 3) of selected systemic insecticides on different membrane materials. The experiment was carried out in HPLC-water spiked at 100 ng L^{-1} with target analytes. The following filters were evaluated: glass fiber filter (GFF), mixed cellulose-ester (MCE), teflon (PTFE), nylon, nitrocellulose, cellulose acetate, polycarbonate, polypropylene, and polyester (PETE).

	GFF	MCE	PTFE	Nylon	Nitrocellulose	Cellulose Acetate	Polycarbonate	Polypropylene	PETE
Acetamiprid	60 ± 1	9 ± 2	38 ± 7	8 ± 5	28 ± 4	40 ± 2	44 ± 6	53 ± 0	77 ± 3
Clothianidin	71 ± 3	17 ± 2	48 ± 5	7 ± 6	39 ± 5	38 ± 1	45 ± 6	50 ± 1	80 ± 2
Desnitro-Imidacloprid	52 ± 1	NA*	25 ± 4	NA	NA	NA	23 ± 2	NA	87 ± 3
Dinotefuran	71 ± 3	NA	36 ± 5	12 ± 2	30 ± 4	9 ± 2	64 ± 17	48 ± 1	77 ± 1
Fipronil	92 ± 1	NA	29 ± 12	5 ± 2	1 ± 0	NA	35 ± 8	NA	90 ± 1
Imidacloprid	63 ± 2	9 ± 2	48 ± 5	11 ± 6	34 ± 4	43 ± 1	50 ± 6	51 ± 2	80 ± 1
Nitenpyram	76 ± 3	NA	30 ± 4	57 ± 8	NA	NA	39 ± 4	54 ± 1	85 ± 2
Thiacloprid	70 ± 4	2 ± 1	54 ± 5	18 ± 3	22 ± 5	36 ± 1	46 ± 6	51 ± 1	68 ± 12
Thiamethoxam	75 ± 3	7 ± 0	36 ± 5	3 ± 1	23 ± 1	27 ± 2	35 ± 3	41 ± 2	78 ± 4

*NA: not available (<LOD).

Table S4.4. Time/temperature storage stability. Temporal follow-up of concentrations (ng L^{-1}) (mean, n=3, SD) in **HPLC water** throughout 28 days of storage. The spiked initial concentration was 100 ng L^{-1} (day 0).

	T_0		**T_0 + 3 days (4°C)**		**T_0 + 7 days (4°C)**		**T_0 + 14 days (4°C)**		**T_0 + 28 days (4°C)**		**T_0 + 28 days (-20°C)**	
	[ng L^{-1}]	SD	[ng L^{-1}]	SD	[ng L^{-1}]	SD	[ng L^{-1}]	SD	[ng L^{-1}]	SD	[ng L^{-1}]	SD
Acetamiprid	95	1	93	1	92	0	94	1	97	1	96	1
Clothianidin	96	1	92	2	96	1	92	2	93	2	93	1
DN-Imi	103	4	100	2	112	9	98	6	110	3	102	3
Dinotefuran	98	7	85	4	105	5	100	6	100	1	80	3
Fipronil	94	1	91	2	87	1	93	1	95	3	87	0
Imidacloprid	95	2	91	1	92	1	94	1	97	1	96	1
Nintenpyram	99	2	94	2	99	2	91	5	98	1	88	2
Thiacloprid	104	4	86	1	93	1	79	1	94	2	91	2
Thiamethoxam	96	0	95	1	97	1	97	1	97	1	97	2

Table S4.5. Time/temperature storage stability. Temporal follow-up of concentrations (ng L^{-1}) (mean, n=3, SD) in **tap water** throughout 28 days of storage. The spiked initial concentration was 100 ng L^{-1} (day 0).

	T_0		**T_0 + 3 days (4°C)**		**T_0 + 7 days (4°C)**		**T_0 + 14 days (4°C)**		**T_0 + 28 days (4°C)**		**T_0 + 28 days (-20°C)**	
	[ng L^{-1}]	SD	[ng L^{-1}]	SD	[ng L^{-1}]	SD	[ng L^{-1}]	SD	[ng L^{-1}]	SD	[ng L^{-1}]	SD
Acetamiprid	101	1	94	6	94	0	93	0	98	1	99	0
Clothianidin	98	1	88	1	93	1	91	0	96	1	97	1
DN-Imi	113	14	101	5	104	5	93	4	113	6	117	2
Dinotefuran	113	7	91	4	99	1	91	3	99	2	99	2
Fipronil	98	4	89	1	91	4	92	4	94	2	89	1
Imidacloprid	100	0	89	1	96	1	94	0	98	0	96	2
Nintenpyram	110	4	98	12	98	3	92	2	98	1	99	1
Thiacloprid	101	1	100	8	94	3	94	1	97	5	99	0
Thiamethoxam	99	2	89	1	93	1	89	0	94	1	97	1

Table S4.6. Time/temperature storage stability. Temporal follow-up of concentrations (ng L^{-1}) (mean, n=3, SD) in **surface water** throughout 28 days of storage. The spiked initial concentration was 100 ng L^{-1} (day 0).

	T_0		**T_0 + 3 days (4°C)**		**T_0 + 7 days (4°C)**		**T_0 + 14 days (4°C)**		**T_0 + 28 days (4°C)**		**T_0 + 28 days (-20°C)**	
	[ng L^{-1}]	SD	[ng L^{-1}]	SD	[ng L^{-1}]	SD	[ng L^{-1}]	SD	[ng L^{-1}]	SD	[ng L^{-1}]	SD
Acetamiprid	98	2	91	1	91	1	93	0	102	1	101	2
Clothianidin	107	2	109	1	85	4	96	1	99	2	96	1
DN-Imi	117	3	91	3	97	1	98	10	104	1	112	5
Dinotefuran	118	6	82	12	101	2	86	1	102	3	97	5
Fipronil	95	1	87	2	89	2	90	3	96	4	87	1
Imidacloprid	102	1	91	1	92	0	94	1	101	1	101	4
Nintenpyram	122	3	87	3	91	4	84	2	87	4	94	6
Thiacloprid	87	1	82	2	85	2	86	0	89	1	88	2
Thiamethoxam	102	2	93	1	93	1	90	2	97	0	95	1

Table S4.7. Overall desirability (Derringer desirability) for the sixteen investigated methods (simultaneous optimization of on-line SPE loading volume and flow rate), showing the highest global desirability with method 6 (2 mL loaded at 1500 µL·min^{-1}) with an overall D value of 0.66.

	Replicates	Volume (mL)	Speed (µL min^{-1})	Global desirability D
Method 1	3	1	1000	0.46
Method 2	3	1	1500	0.50
Method 3	3	1	2000	0.40
Method 4	3	1	2500	0.50
Method 5	3	2	1000	0.60
Method 6	3	**2**	**1500**	**0.66**
Method 7	3	2	2000	0.62
Method 8	3	2	2500	0.59
Method 9	3	5	1000	0.45
Method 10	3	5	1500	0.56
Method 11	3	5	2000	0.48
Method 12	3	5	2500	0.61
Method 13	3	10	1000	0.54
Method 14	3	10	1500	0.46
Method 15	3	10	2000	0.00
Method 16	3	10	2500	0.00

Table S4.8. On-line enrichment absolute recovery for the neonicotinoids and fipronil investigated, at two spike levels in HPLC-water. QC_1 and QC_2 corresponded to injected amounts of 8 pg and 1,500 pg of each analyte, respectively, submitted to large volume on-line injection *versus* small volume on-line injection (**Section 4.2.5**).

	Absolute extraction efficiency (%)	
	QC_1	QC_2
Acetamiprid	86 ± 1	84 ± 4
Clothianidin	82 ± 1	76 ± 5
Desnitro-Imidacloprid	41 ± 2	35 ± 8
Dinotefuran	92 ± 4	75 ± 4
Fipronil	104 ± 10	91 ± 7
Imidacloprid	99 ± 9	92 ± 9
Nitenpyram	91 ± 3	84 ± 2
Thiacloprid	91 ± 1	80 ± 5
Thiamethoxam	96 ± 2	83 ± 8

Table S4.9. Method validation. Mean accuracy (n=5), intraday (n=5) and interday (n=15) precision for the 9 systemic insecticides at two quality control levels (4 ng L^{-1} and 750 ng L^{-1}) in the three tested matrices (HPLC-water, tap water, and surface water).

Compound	Intra-day precision (%RSD) (n=5)						Inter-day precision (%RSD) (n=15)						Accuracy (%) (n=5)					
	HPLC water		Tap water		Surface water		HPLC water		Tap water		Surface water		HPLC water		Tap water		Surface water	
	QC_1	QC_2	QC_1	QC_2	QC_1	QC_2	QC_1	QC_2	QC_1	QC_2	QC_1	QC_2	QC_1	QC_2	QC_1	QC_2	QC_1	QC_2
Acetamiprid	5	1	4	5	3	2	7	6	5	4	3	3	92	104	88	90	94	114
Clothianidin	4	3	4	4	5	1	9	4	8	3	4	8	90	105	90	89	78	116
Desnitro-Imidacloprid	23	3	14	6	6	5	26	9	25	9	14	8	107	101	104	70	89	94
Dinotefuran	7	2	16	5	<LOQ	3	25	2	10	6	<LOQ	26	102	112	69	71	<LOQ	107
Fipronil	3	2	2	4	2	1	7	3	5	3	3	2	105	104	109	93	111	112
Imidacloprid	5	2	4	5	6	1	9	4	6	4	6	3	95	103	86	88	92	111
Nitenpyram	4	2	1	5	5	2	12	10	24	23	24	26	100	107	88	81	94	110
Thiacloprid	8	1	3	6	6	1	6	12	11	15	22	15	83	108	82	85	117	121
Thiamethoxam	6	3	5	5	4	1	8	4	6	3	6	2	92	104	89	89	103	112

*QC_1: 4 ng L^{-1}. *QC_2: 750 ng L^{-1}.

Table S4.10. Method validation. Residual matrix effect (%) in the three tested **tap water** samples from different locations, upon comparison with the matrix-matched reference (composite sample made from the tap water samples received).

	Matrix 1 (Montréal Tap water)			Matrix 2 (Québec Tap water)			Matrix 3 (Bécancour Tap water)			Matrix-matched reference	
	R^2	Slope	Matrix effect (%)	R^2	Slope	Matrix effect (%)	R^2	Slope	Matrix effect (%)	R^2	Slope
Acetamiprid	0.9961	10.37	+0.89	0.9967	10.27	0	0.9965	10.53	+2.5	0.9951	10.27
Clothianidin	0.9990	22.4	-1.8	0.9998	22.81	0	0.9997	23.35	+2.4	0.9981	22.82
DN-Imidacloprid	0.9981	29.83	-7.4	0.997	31.52	-2.1	0.9999	34.34	+6.6	0.9987	32.21
Dinotefuran	0.9995	2.2	-2.6	0.9997	2.2	-2.8	0.9997	2.27	0	0.9997	2.27
Fipronil	0.9947	7.72	-2	0.9953	7.63	+0.88	0.9958	7.76	+2.7	0.9934	7.56
Imidacloprid	0.9999	13.82	+3.4	0.9988	13.2	-1.2	0.9996	13.84	+3.6	0.9966	13.37
Nitenpyram	0.9906	1.65	-29	0.9993	2.36	+1	0.9993	2.34	+0.39	0.9996	2.33
Thiacloprid	0.9990	1.24	+3.4	0.9972	1.2	0	0.9997	1.24	+3.3	0.9964	1.2
Thiamethoxam	0.9999	13.6	+2.8	0.999	13.05	-1.3	0.9994	13.69	+3.6	0.9969	13.22

Table S4.11. Method validation. Residual matrix effect (%) in the three tested **surface water** samples from different locations, upon comparison with the matrix-matched reference (composite sample made from the surface water samples received).

	Matrix 1 (L'Assomption River, Repentigny)			Matrix 2 (St Lawrence River, Québec City)			Matrix 3 (Yamaska River, Yamaska)			Matrix-matched reference	
	R^2	Slope	Matrix effect (%)	R^2	Slope	Matrix effect (%)	R^2	Slope	Matrix effect (%)	R^2	Slope
Acetamiprid	0.9992	10.38	-0.80	0.9994	10.11	-3.4	0.9992	10.52	+0.52	0.9982	10.46
Clothianidin	0.9992	19.50	-2.5	0.9980	21.91	+9.6	0.9966	20.59	+3.0	0.9972	20.00
DN-Imidacloprid	0.9995	28.98	-33	0.9985	55.86	+30	0.9976	35.64	-17	0.9899	43.00
Dinotefuran	0.9991	1.82	-4.7	0.9984	1.87	-2.1	0.9991	1.83	-4.1	0.9948	1.91
Fipronil	0.9964	7.63	-5.1	0.9951	7.66	-4.8	0.9953	7.77	-3.4	0.9962	8.05
Imidacloprid	0.9997	13.04	-1.1	0.9996	13.40	+1.7	0.9993	13.17	-0.062	0.9982	13.18
Nitenpyram	0.9994	2.45	+2.2	0.9991	2.36	-1.8	0.9996	2.36	-1.5	0.9991	2.40
Thiacloprid	0.9995	0.97	+1.3	0.9989	0.89	-6.3	0.9989	0.94	-1.6	0.9987	0.95
Thiamethoxam	0.9995	12.23	-1.6	0.9994	12.88	+3.7	0.9997	12.56	+1.1	0.9987	12.43

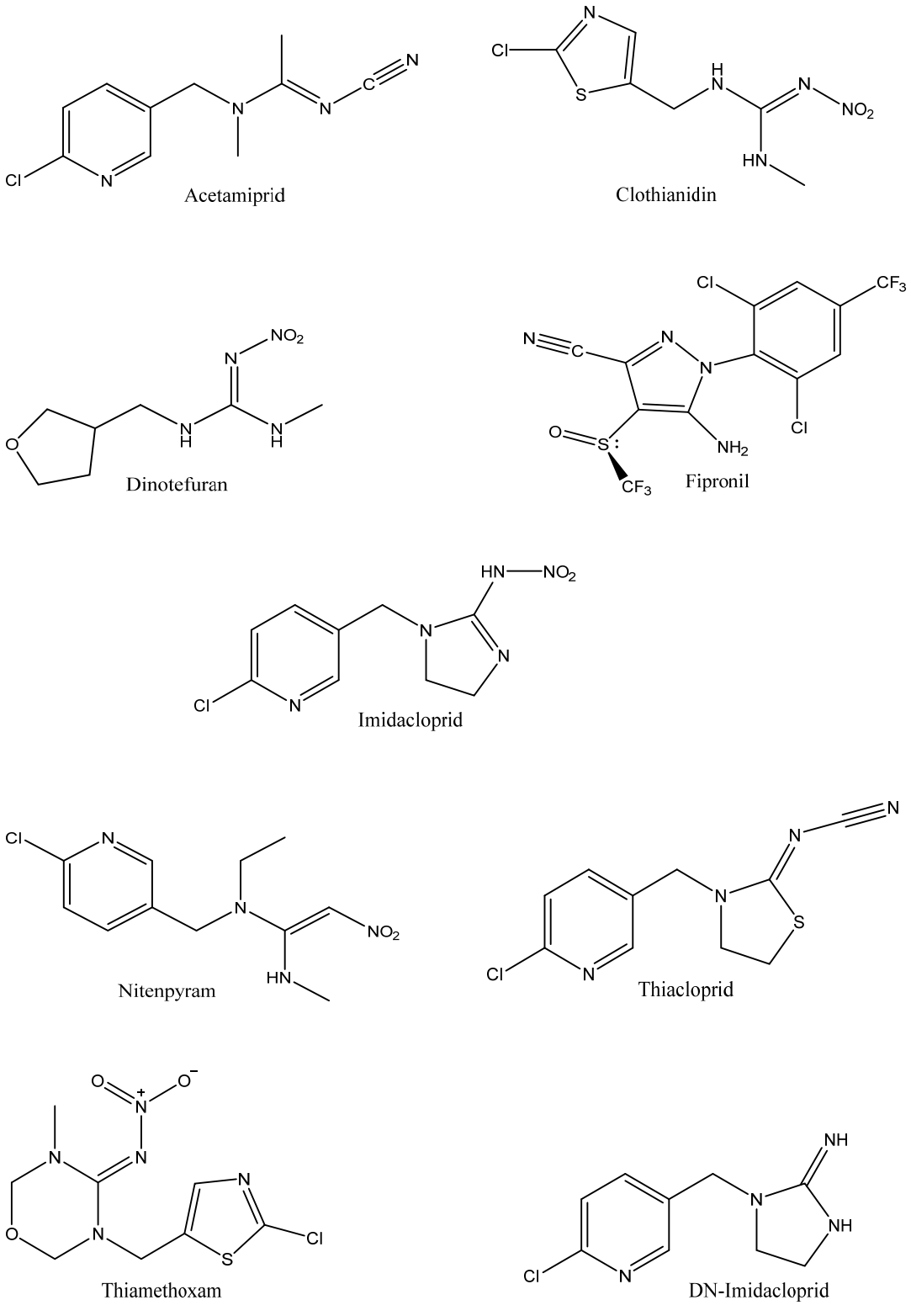

Figure S4.1. Chemical structures of the 9 systemic insecticides investigated.

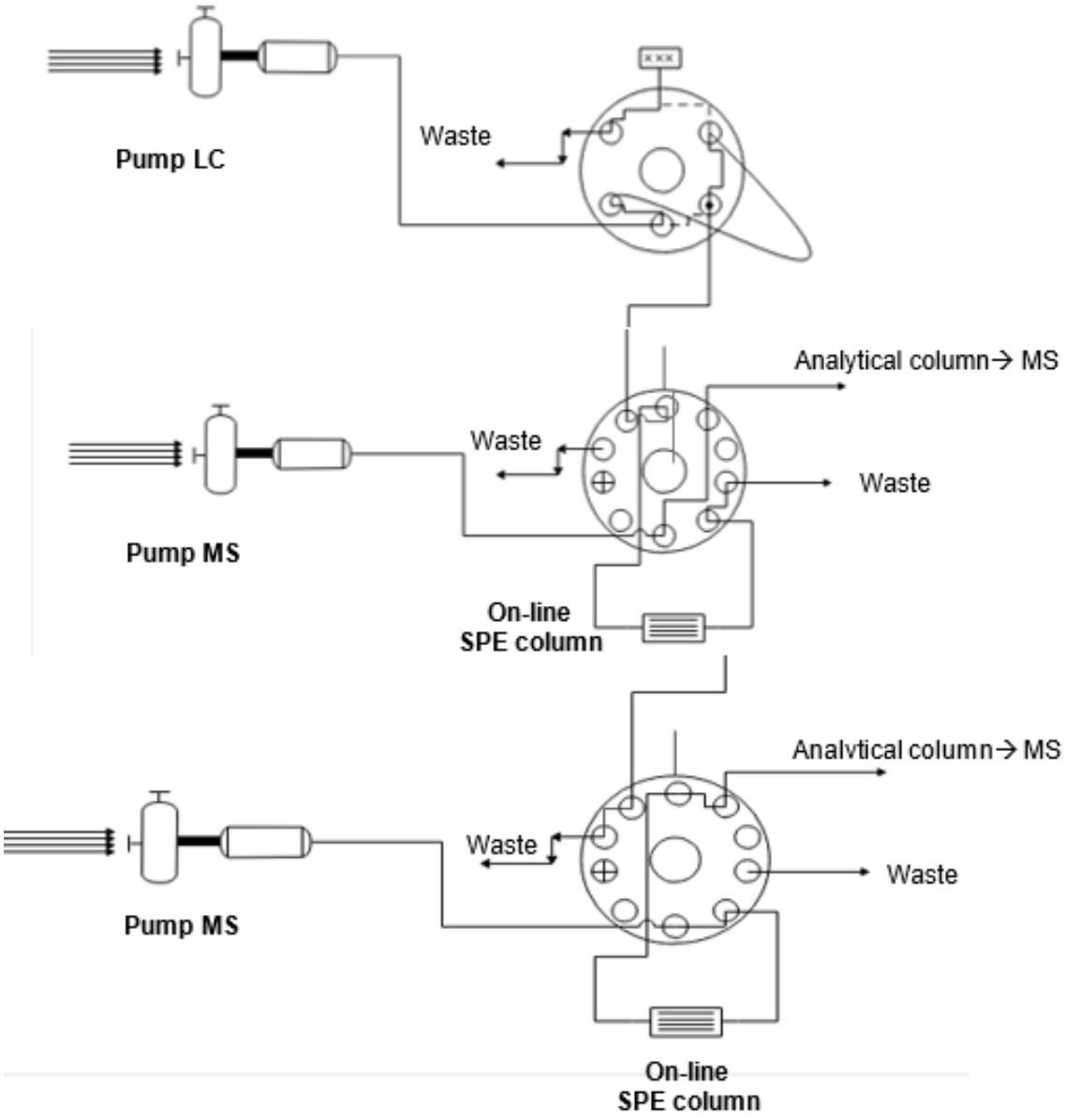

Figure S4.2. On-line SPE – UHPLC-MS/MS system configuration. A) Loading step performed by the Accela 600 pump, using a 2-mL injection loop and 1500 µL min^{-1} loading flow rate with on-line SPE aqueous mobile phase (HPLC-water with 0.1 % Formic Acid); B) Back-flush elution step performed by the Accela 1250 pump with the UHPLC mobile phase mixture (HPLC-water : Acetonitrile) at a flow rate of 400 µL min^{-1}.

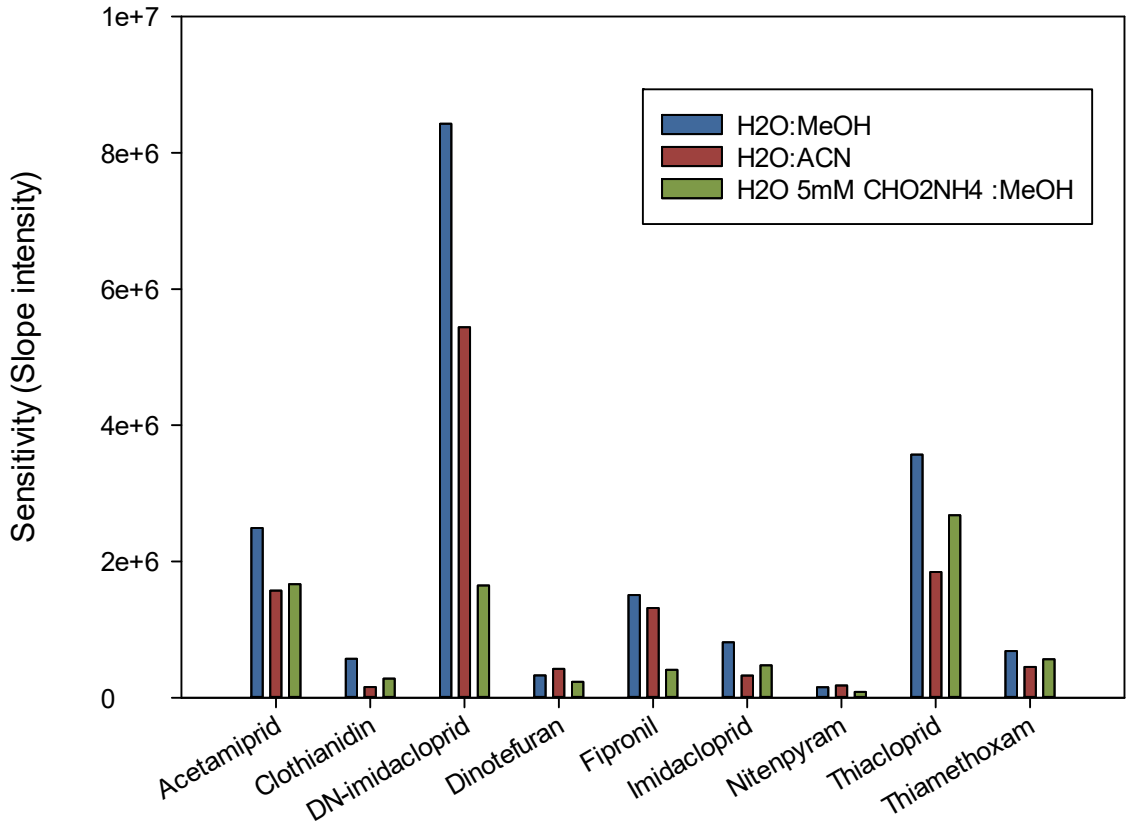

Figure S4.3. Effect of analytical mobile phase composition on analyte slope intensity. The analytical mobile phase investigated were H_2O:methanol, H_2O:acetonitrile, and H_2O:methanol with 5 mM CHO_2NH_4. For estimation of the slope intensity, calibration curves were prepared based on four calibration levels in ultra-pure HPLC water fortified at 5 ng L^{-1}, 10 ng L^{-1}, 25 ng L^{-1} and 50 ng L^{-1} with target native analytes.

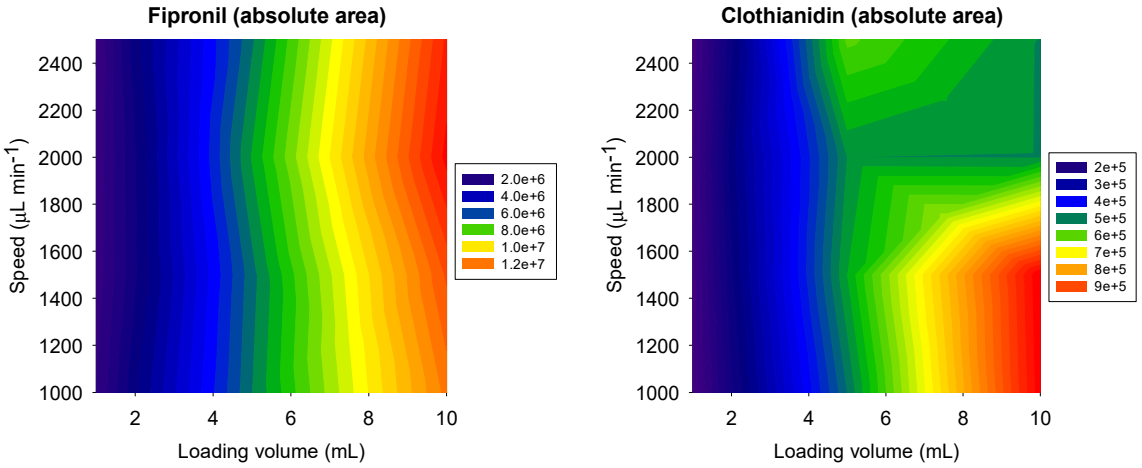

Figure S4.4. Response surfaces generated upon experimental design variation of on-line sample loading volume and loading flow rate, illustrated for fipronil and clothianidin absolute areas.

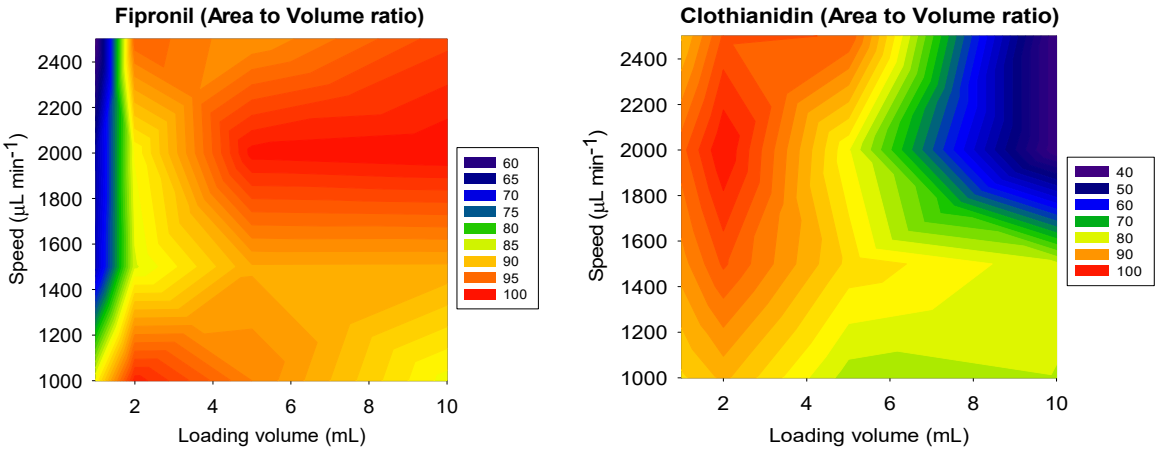

Figure S4.5. Influence of on-line sample loading volume and loading flow rate on area to volume ratios, illustrated for fipronil and clothianidin. For each compound, area to volume ratios were normalized to the maximum observed across the 16 investigated conditions.

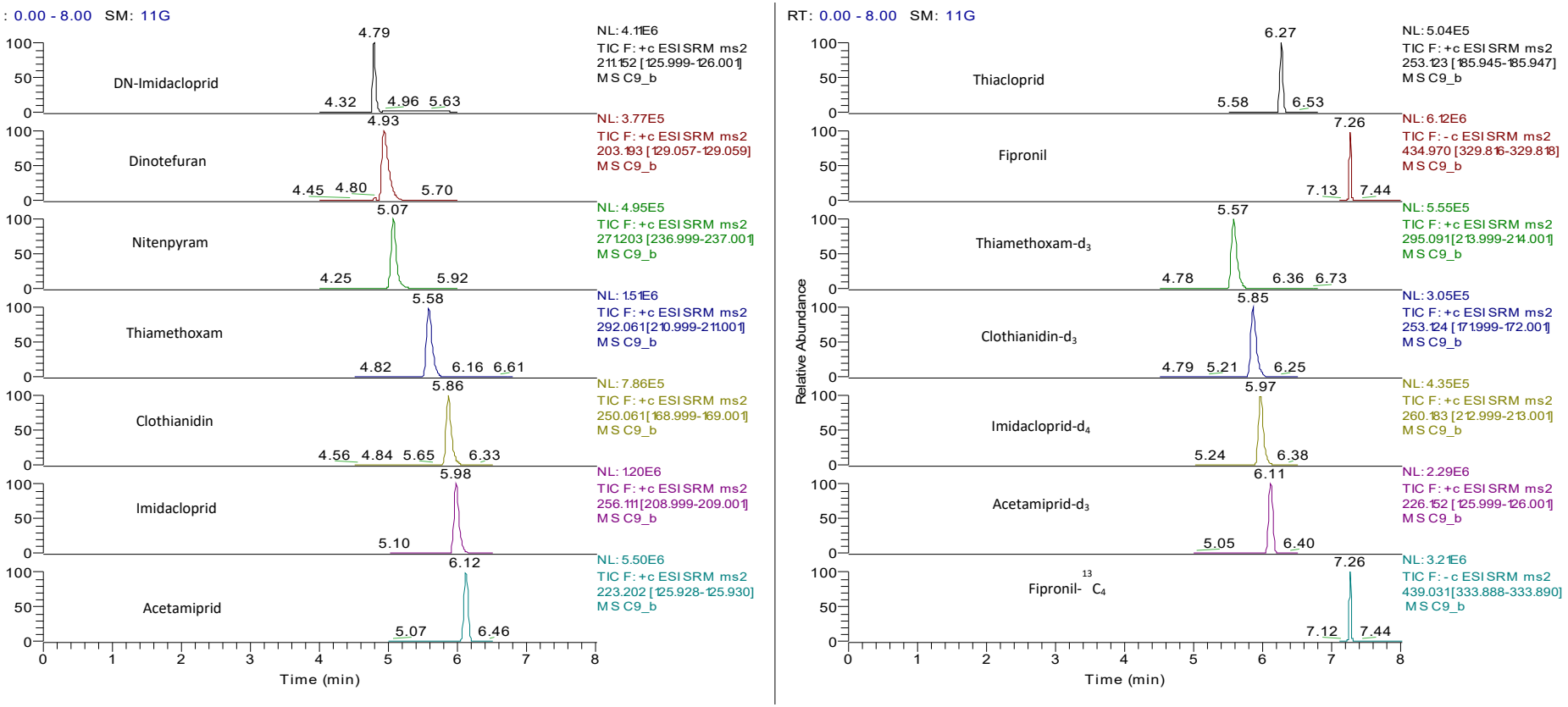

Figure S4.6. On-line SPE UHPLC-MS/MS chromatograms of the 9 systemic insecticides (quantification transition) and the five isotope-labelled ISs, when applying the method optimal settings with respect to analytical mobile phase composition (H_2O:acetonitrile), on-line SPE loading mobile phase (H_2O HPLC with 0.1% HCOOH), on-line SPE loading flow rate (1500 µL min^{-1}), and sample volume (2 mL).

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Chapitre 5. Variations spatiotemporelles de l'atrazine et de la déséthylatrazine dans l'eau potable au Québec (Canada)

Article publié dans le journal *Science of the Total Environment* 671 (2019) 578-585: "**Quality survey and spatiotemporal variations of atrazine and desethylatrazine in drinking water in Quebec, Canada**". Auteurs**: Montiel-León, J. M., S. Vo Duy, G. Munoz, M. F. Bouchard, M. Amyot and S. Sauvé.**

Description: Cet article étudie la variabilité spatiale et temporelle de l'atrazine dans un ensemble d'échantillons d'eau potable (robinet) collectés dans 52 villes au Québec. Nous avons montré un lien entre la source d'eau utilisée pour la production d'eau potable et les niveaux de contamination, notamment pour les municipalités qui puisent leur eau dans le fleuve Saint-Laurent.

Contributions: J'ai participé à la conception du projet ainsi que la collecte d'échantillons sur le terrain, la préparation des échantillons au laboratoire, le traitement et l'exploitation des données et rédigé l'article.
Co-auteurs: Sung Vo Duy m'a aidé avec une partie des manipulations et à la rédaction. Gabriel Munoz m'a aidé avec la collecte des échantillons sur le terrain et la révision de l'article. Maryse F. Bouchard m'a aidé à une partie de la rédaction de l'article.
Co-directeur: Marc Amyot m'a aidé à l'amélioration de la rédaction.

Directeur: Sébastien Sauvé m'a aidé à la conception du projet et à la rédaction de l'article.

Abstract

The herbicide atrazine remains in use in Canada, the United States, and several other countries, while being banned since 2003 in the European Union. A comprehensive quality survey of atrazine (ATZ) and one of its metabolites, desethylatrazine (DEA), was conducted in 2015-2018 in drinking water available to consumers in Quebec, Canada. Temporal variations of ATZ and DEA were monitored in tap water from the Montreal area for 18 consecutive months (Temporal survey 2015-2016). Within this time window, the sum of ATZ and DEA in tap water samples (n = 450) varied from 40 to 250 ng L^{-1} (median: 98 ng L^{-1}). ATZ was systematically detected (100%), with a concentration range of 30–195 ng L^{-1} (median: 49 ng L^{-1}) while DEA was in the range of 10–187 ng L^{-1} (median: 36 ng L^{-1}). Maximum ATZ concentrations remained about 25X lower than the Canadian drinking water quality guideline (5000 ng L^{-1}), but 48% of the samples were above that of the European Union (100 ng L^{-1}) regarding the sum of ATZ and DEA. Trends of ATZ and DEA in drinking water were also examined across southwestern Quebec (Spatial survey 2017-2018). The sum of the two triazines in this second set of samples varied from below the method detection limit (for 33 out of the 52 surveyed municipalities) to 104 ng L^{-1}. Apart from Montreal, locations in the southern shore of the St. Lawrence showed generally higher levels of atrazine and DEA. The highest concentrations clustered in the Montérégie region, along the St. Lawrence River (e.g., Brossard, Longueuil, Saint-Constant) and/or downstream from agricultural areas. The ATZ concentrations are suggested to have decreased compared to previous surveys, which is consistent with the decrease in the sales of active ingredients in Ontario (upstream sources) and Quebec.

Keywords

Atrazine; Desethylatrazine; Drinking water; Tap water; Temporal variations; Water quality guidelines

5.1 Introduction

Reports on harmful chemicals in raw and treated drinking water raise obvious concerns for human health. The exposure to naturally-occurring and/or anthropogenic contaminants may occur intermittently or chronically, including for those persistent compounds that are no longer used or produced (Focazio et al., 2008; Lapworth et al., 2012; Loos et al., 2010; Stuart et al., 2012; Stuart et al., 2014). A noteworthy example is the case of atrazine that has been banned for >15 years in the European Union, but is still regularly detected across its surface waters and groundwaters (Barchanska et al., 2017; Caquet et al., 2013; Masia et al., 2015; Pascual Aguilar et al., 2017; Rodriguez-Gonzalez et al., 2016). Atrazine (ATZ) and one of its degradation products, desethylatrazine (DEA), were also found in bottled drinking water samples in France, one decade after the ban came into effect (Le Coadou et al., 2017).

Atrazine is a triazine herbicide used for weed control and is relatively persistent in the environment, with a half-life of ~1 to ~4 months. Environmental degradation of ATZ may be mediated by photolysis, hydrolysis of the chloro- substituent, and/or N-dealkylation by microorganisms to form dealkylated (e.g., DEA) and hydroxylated (e.g., ATZ-OH) metabolites (WHO, 2011). Atrazine is not classified as bioaccumulative, but its mobility and relative persistence can lead to the contamination of drinking water sources (Villanueva et al., 2005). It can exert negative effects at different levels including acute and chronic toxicity, genotoxicity, and reproductive toxicity, and was classified in Group III as a possible carcinogenic to humans (Health Canada, 1993). Studies have examined the effects of chronic exposure to triazine-contaminated drinking water (Flynn et al., 2013; Jowa and Howd, 2011; Ochoa-Acuña et al., 2009), with possible links to birth defects (Almberg et al., 2018; Markel et al., 2015; Mattix et al., 2007; Stayner et al., 2017; Villanueva et al., 2005; Winchester et al., 2009). Exposure to ATZ is associated with endocrine disruption and could relate to androgen decrease (Tavera-Mendoza et al., 2002; USEPA, 2007) and estrogen increase (Hayes et al., 2011) in different biological models and also in human cultured cells (Sanderson et al., 2000). Note that similar toxicity and

modes of action were reported for chlorinated ATZ metabolites including DEA (WHO, 2011).

Early concerns led the European Union to ban the use of atrazine since 2003, with a maximum concentration limit in drinking water of 100 ng L^{-1}. Health Canada has established an interim maximum acceptable concentration (IMAC) in drinking water of 5000 ng L^{-1} (Health Canada, 1993), while the U.S. Environmental Protection Agency (EPA) and World Health Organization (WHO) have established restrictive maximum concentration limits (MCL) of 3000 ng L^{-1} (EPA, 2007) and 2000 ng L^{-1} (WHO, 2011) in drinking water.

As ATZ remains of continued use in Canada, monitoring data remain of high relevance, including for surface and drinking water. Giroux et al. reported relatively high levels of ATZ in hydrosystems near to agricultural zones (Giroux, 2015), with concentrations sometimes above the part-per-billion levels in surface water. In another study conducted in 2012-2014 (Giroux et al., 2016), maximal concentrations of ATZ in Quebec surface waters were 3300 ng L^{-1} in 17 tributaries and 1800 ng L^{-1} in Lake Saint-Pierre (St. Lawrence River). Analyses of drinking water samples collected in the Montreal area (QC, Canada) reported triazine concentrations of ~20 ng L^{-1} for ATZ and 310 ng L^{-1} for DEA in winter 2008 (Garcia-Ac et al., 2009), and ~50 ng L^{-1} for ATZ in spring 2010 (Segura et al., 2011). Robert and Bolduc (2012) conducted an extensive drinking water survey between 2005 and 2008 (Robert and Bolduc, 2012), indicating concentrations up to 1000 ng L^{-1} for ATZ and its metabolites.

While concentrations of ATZ found in drinking water from Montreal and other surrounding municipalities are influenced by its continued use in the province of Quebec, these levels may also reflect upstream sources, e.g., those from the Laurentian Great Lakes (Pham et al., 2000). Since atrazine use has been decreasing in Ontario and Quebec in the last decades, and water treatment technologies may evolve, a new monitoring survey conducted in Montreal is of interest to examine long-term trends in drinking water levels. Another research question to address is to what extent ATZ and DEA drinking water concentrations may fluctuate at a smaller timescale. In the context of a drinking water quality survey, the existence and magnitude of peak concentrations not accounted for by a low-intensity sampling regime may, indeed, preclude an accurate prediction.

We pursued two main objectives. First, a high-intensity sampling of drinking water concentrations of ATZ and DEA was performed to gain a better understanding of temporal variations in peak exposures. This survey was conducted in the Montreal area (QC, Canada) and will be referred to as "Temporal survey (2015-2016)". For this purpose, a fast and sensitive analytical method was employed, involving automated solid phase extraction coupled on-line to ultra-high-performance liquid chromatography tandem mass spectrometry (on-line SPE – UHPLC-MS/MS). This comprehensive database (n = 450 samples) allowed to identify a median concentration for this period and verify the compliance with drinking water quality criteria. We also compared the data from the present survey with historical ones (e.g., (Robert and Bolduc, 2012)), to corroborate our hypothesis of decreasing trends in relation with active ingredient sales in Ontario and Quebec (Farm and Food Care Ontario, 2015; Ministère du développement durable, 2011). The second major goal of this study was to investigate trends in ATZ concentrations by carrying out a large spatial scale survey in Southwestern Quebec. This survey is referred as "Spatial survey (2017-2018)". Sampling sites were selected along a highly populated 300-km reach of the St. Lawrence River, targeting municipalities either close to the St-Lawrence or to one of its tributaries.

5.2 Materials and methods

5.2.1 Chemicals and materials

Standards of atrazine (ATZ) and desethylatrazine (DEA) (purity ≥97%) were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). The isotopically-labelled internal standard ATZ-$^{13}C_3$ (purity ≥99%) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, U.S.A.). Solvents were all of HPLC-grade quality and were purchased from Fisher Scientific (Whitby, ON, Canada). Formic acid (purity ≥95%) was acquired from Sigma-Aldrich (St. Louis, MO, U.S.A.), while glass fiber filters (0.3 µm) were obtained from the Sterlitech Corporation (Kent, WA, U.S.A.).

5.2.2 Sample collection and preparation

For the Temporal survey (2015-2016), drinking water samples were collected twice per week by qualified University personnel from a public tap (with no filtration device) in the downtown Montreal area (QC, Canada) from March 2015 to September 2016. The sampling site was the same all along the study and was chosen by convenience for proximity with our facilities but was no farther than 10 km from the Drinking Water Treatment Plant (DWTP). Before the collection of replicate samples for each sampling date (overall n = 450), the tap water was left to flow for 5 min prior to rinsing amber-glass bottles three times with the tap water, after which the bottle was filled to the brim with tap water, capped and stored at 4°C until analysis (USGS, 2006).

For the Spatial survey (2017-2018), ATZ and DEA were also measured in public tap water from Montreal and selected municipalities in Southern Quebec (Canada), covering 10 administrative regions (Capitale-Nationale, Centre-du-Québec, Chaudière-Appalaches, Estrie, Lanaudière, Laurentides, Laval, Mauricie, Montérégie, and Montréal). The sampling campaign was conducted in late spring 2017 (12 municipalities) and late spring – early summer 2018 (52 municipalities). These 52 municipalities were selected based on population. They are located along a highly populated axis between Salaberry-de-Valleyfield and Quebec City grouping some of the largest municipalities in the province of Quebec (e.g., Laval, Montreal, Quebec City, Sherbrooke). The 52 surveyed municipalities (SI **Table S5.1**) represent a total of *circa* 4.7 million people (more than half of the total population of the province as of 2018) and are situated either in proximity to the St. Lawrence River or to its tributaries. The water source type and treatment technologies from the corresponding DWTPs can be found in SI (**Table S5.1**). Samples were collected during field trips organized and performed by qualified University personnel, following a common sampling procedure and pre-cleaned amber-glass bottles for all samples. At each sample location, the tap water was left to flow for 5 min and the 125-mL amber glass collection bottle was rinsed three times with the site tap water, filled to the brim, sealed, and stored in an ice box (4 °C to 8 °C) until arrival at the laboratory facilities.

Once in the laboratory, tap water samples were filtered with a glass fiber membrane filter (0.3 µm), the filtrate being recovered into a 10-mL amber glass vial. The samples were acidified with formic acid for a final concentration of 0.5% (v/v), spiked with the internal standard (Atrazine- $^{13}C_3$) for a final concentration of 50 ng L^{-1}, and analyzed by on-line SPE – UHPLC-MS/MS (Section 4.2.3).

5.2.3 Quantitative analyses

ATZ and DEA were quantitatively targeted in drinking water samples based on a previous on-line SPE – UHPLC-MS/MS method (Morissette et al., 2015), with some modifications. The analyses were performed using a sample delivery system with a dual switching column array. An HTC thermopal autosampler (CTC analytics AG, Zwingen, Switzerland) was used for 5 mL in-loop sample injection. The sample was then transferred from the loop to the on-line preconcentration column (Hypersil Gold aQ C18 column, 20 mm × 2.1 mm, 12 µm particle size, Thermo Fisher) by an Accela 600 quaternary pump (Thermo Fischer, San Jose, CA, U.S.A.). The sample was then eluted from the on-line SPE column (using an Accela 1250 quaternary pump, Thermo Fisher) to the analytical column (Hypersil Gold C18 column, 100 mm × 2.1 mm, 1.9 µm particle size, Thermo Fisher) for chromatographic separation. Analyte detection was performed in selected reaction monitoring mode (SRM) with a TSQ Quantiva triple quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, U.S.A.) with a heated electrospray ionization (heated-ESI) source operated in positive ionization mode. Further details regarding the on-line SPE – UHPLC-MS/MS analysis are provided in the Supporting Information (SI **Tables S5.2-S5.3**).

The identification of targeted analytes in the samples was based on matching retention times with certified standards and detectable signals for both quantification and confirmation MS/MS transitions. The quantification strategy applied to determine the concentrations of ATZ and DEA in the samples relied on a matrix-matched calibration (Montiel-Leon et al., 2018). A blank tap water sample was used for fortification of native analytes at six calibration levels (1, 5, 10, 50, 100, 200 ng L^{-1}), the isotope-labeled internal standard (IS) being kept constant (50 ng L^{-1}) prior to on-line SPE – UHPLC-MS/MS

analysis. Linear regressions were constructed by plotting the native standard to Internal Standard (IS) area ratio (A_N/A_{IS}) as a function of native analyte concentration (C_N).

5.2.4 Quality assurance and quality control

Injection blanks consisted of HPLC water directly subjected to the on-line SPE – UHPLC-MS/MS workflow. They were systematically injected at the beginning of the LC-MS sequence and after quality control spikes to evaluate possible carryover. Laboratory procedural blanks consisted of HPLC water passed through GFF filters, spiked at 50 ng L^{-1} with the internal standard, and subjected to on-line SPE – UHPLC-MS/MS. Low levels of atrazine were occasionally detected in such blanks (<2 ng L^{-1}). For each batch, the blank was therefore subtracted from the corresponding samples value to avoid false positives.
Matrix-matched calibration curves were run for each on-line SPE – UHPLC-MS/MS analytical sequence with suitable linearity for both ATZ and DEA (multi-batch replicates, R^2 >0.995). After running the initial calibration, continued calibration verification (CCV) standards were run regularly along the on-line SPE – UHPLC-MS/MS sequence to control accuracy and precision. Accuracy remained within the acceptable range of 80-120% for both ATZ and DEA. The relative standard deviations of area ratios were typically <10%, corresponding to suitable precision performance as per SANCO guidelines (SANTE/11945/2015, 2015). The method reporting limits (RLs) were 2 ng L^{-1} and 6 ng L^{-1} for ATZ and DEA, respectively. Consistent sensitivity performances were obtained across the various on-line SPE – UHPLC-MS/MS sequences.

5.2.5 Qualitative screening of other atrazine degradation products

Using High-Resolution Mass Spectrometry (HRMS), we conducted a prospective screening of additional atrazine degradation products, including dealkylated and/or hydroxylated species. The developed on-line SPE – UHPLC-MS/MS method allowed for a fast-quantitative screening of atrazine and a major degradation product in these samples.

The method may not, however, allow to capture the traces of less abundant ATZ degradation products, given the low levels expected in drinking water. For this purpose, a 500-mL tap water sample from the Montreal area was extracted by off-line SPE as per the procedure described in SI.

Parent ions ($[M+H]^+$) of desisopropylatrazine (DIA), desethyldesisopropylatrazine (DEDIA), hydroxyatrazine (ATZ-OH), desethyl-hydroxyatrazine (DEHA), desisopropyl-hydroxyatrazine (DIHA), and desethyldesisopropyl-hydroxyatrazine (DEDIHA) were qualitatively scouted by liquid chromatography positive electrospray ionization high-resolution mass spectrometry (Q-Exactive Orbitrap). When their exact mass was detected within a ± 5 ppm mass accuracy window in full scan MS (Kabore et al., 2018), the samples were submitted to a second acquisition using the high-resolution parallel reaction monitoring (PRM) mode of the Q-Exactive Orbitrap.

5.3 Results

5.3.1 Temporal survey (2015-2016) in drinking water from the Montreal area

ATZ and DEA displayed frequencies of detection of 100% (n = 450) in the Temporal survey (2015-2016) conducted in the Montreal area. The variations of their concentrations are shown in **Figure 5.1**. Atrazine displayed a concentration range between 30 and 195 ng L^{-1} and DEA between 10 and 187 ng L^{-1}. The average concentration during this period was 69 and 46 ng L^{-1} for ATZ and DEA, respectively (median of 49 ng L^{-1} for ATZ and 36 ng L^{-1} for DEA). The sum of ATZ and DEA ranged from 51 to 242 ng L^{-1}, with an average concentration of 115 ng L^{-1} (slightly above the European Union guideline limit of 100 ng L^{-1}) and a median value at 98 ng L^{-1}.

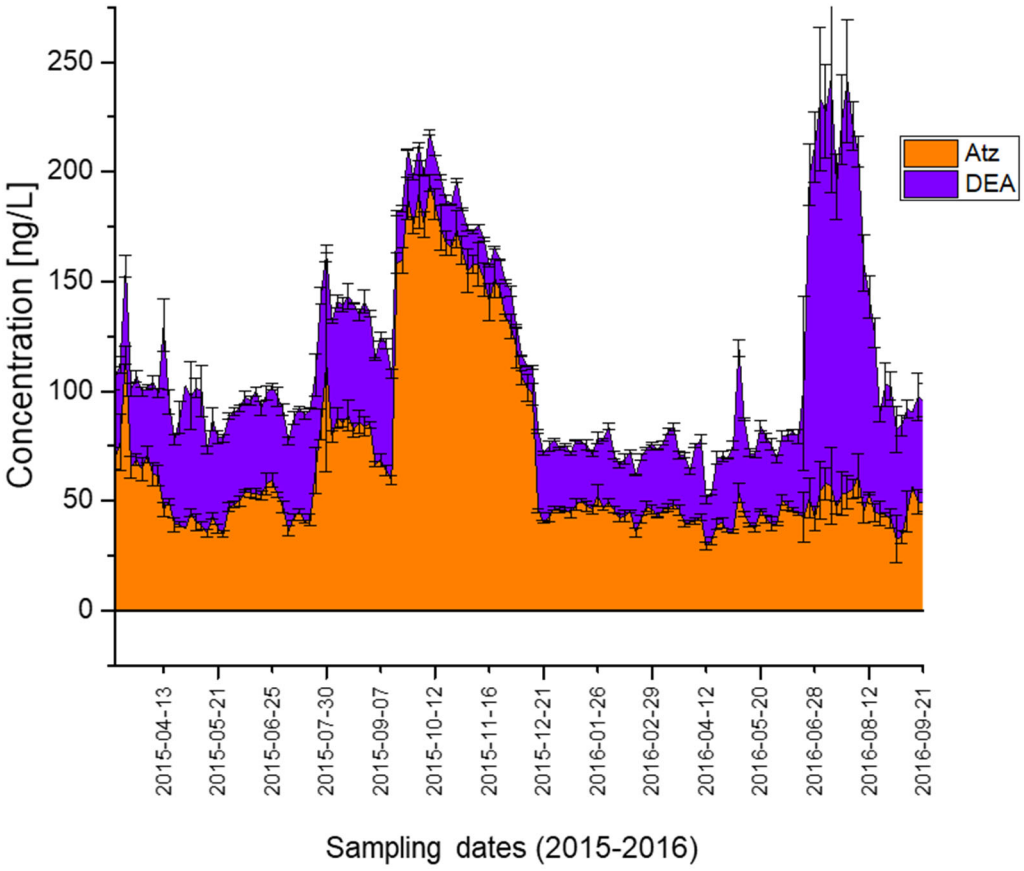

Figure 5.1. Variations in the concentration (ng L^{-1}; stacked values) of atrazine (ATZ) and desethylatrazine (DEA) in drinking water samples (n = 450) from the Montreal area (QC, Canada). Tap water samples were collected between March 2015 and September 2016 and analyzed by on-line SPE – UHPLC-MS/MS.

It is noteworthy that ATZ showed a quasi-stable behavior through both years except for a specific period of 88 days between mid-September and mid-December 2015 with a high concentration peak, while the DEA concentration pulse was observed from 15th June to 17th August 2016 (see **Figure 5.1**). These results could be compared with those obtained by Stayner et al. (2017) or Winchester et al. (2009) in the U.S.A., who observed concentration pulses of ATZ in drinking water generally between May-July.

Figure 5.2 presents the distribution per concentration class for the 450 positive samples from downtown Montreal (Temporal survey 2015-2016). For ATZ, 234 samples were found at concentrations lower than 50 ng L^{-1}, 135 samples between 50 ng L^{-1} and 100 ng

L^{-1}, and 81 higher than 100 ng L^{-1}. When considering ATZ and DEA altogether, our survey showed 234 positive samples between 50 ng L^{-1} and 100 ng L^{-1} and 216 higher than 100 ng L^{-1}. This implies that about half of the 450 drinking water samples from Montreal were found at concentrations higher than the European Union guideline (100 ng L^{-1}). However, no sample was found to surpass the Canadian, WHO, or U.S. EPA drinking water guidelines.

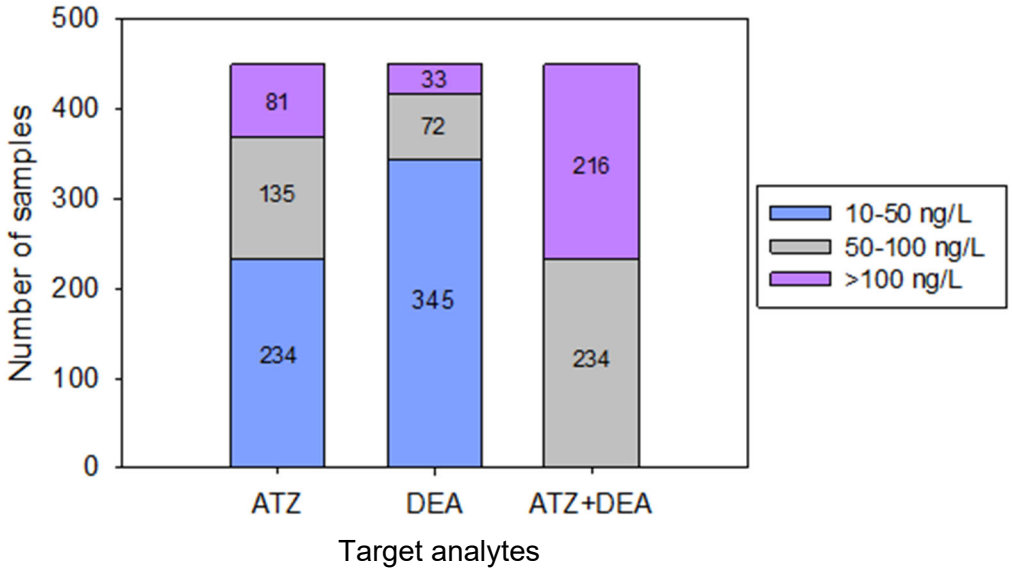

Figure 5.2. Number of drinking water samples from the temporal survey in the Montreal area (2015-2016), arranged per concentration class of ATZ, DEA, and the sum of the two.

- ATZ and DEA were systematically detected in a public tap water supply over 18 consecutive months (2015-2016)
- ATZ concentrations were compliant with Canadian, WHO, and U.S. EPA drinking water guidelines
- About 50% of samples exceeded the E.U. guideline of 100 ng L^{-1}

5.3.2 Spatial survey (2017-2018) in Southern Quebec

With the aim to investigate spatial trends of ATZ and DEA in tap water in Southern Quebec, two additional sampling campaigns were conducted. In the late spring 2017 (**Table 5.1**), the ATZ concentration was ~30 ng L^{-1} in downtown Montreal ($\Sigma_{ATZ+DEA}$ = 58 ng L^{-1}). These relatively low levels agree with our Temporal survey (2015-2016) at the same monitoring location (Section 3.1). Out of the 14 targeted municipalities, 6 were positive to either ATZ or DEA. It is noteworthy that ATZ and DEA remained below the method reporting limits in municipalities located on the northern shore of the St. Lawrence (Repentigny, Trois-Rivières, Donnacona, Quebec). This may be related to the low agricultural pressures in the northern bank, except perhaps for the L'Assomption River watershed (Pham et al., 2000). In contrast, detections of both ATZ and DEA were reported for Montreal, Brossard, and Longueuil (**Table 5.1**). Brossard and Longueuil are situated on the southern bank just opposite the Montreal Island. The neighboring sites in fact share the similar drinking water source (St. Lawrence River) and water treatment technology involving chlorination and filtration (SI **Table S5.1**), which could explain their similar ATZ patterns (http://www.environnement.gouv.qc.ca/eau/potable/production/).

Table 5.1. Concentrations (ng L^{-1}) of atrazine (ATZ) and desethylatrazine (DEA) in tap water samples (n=28) collected across 14 municipalities in southwestern Quebec (Canada) in the late spring 2017. Individual concentrations are given as average ± standard deviation, and the sum of ATZ and DEA concentrations is also indicated ($\Sigma_{ATZ+DEA}$). The LOD was 2 ng L^{-1} for ATZ and 6 ng L^{-1} for DEA.

	ATZ [ng L^{-1}]	**DEA [ng L^{-1}]**	**$\Sigma_{ATZ+DEA}$ [ng L^{-1}]**
Bécancour	<LOD	<LOD	<LOD
Brossard	41 ± 1.7	33 ± 2.3	74
Donnacona	<LOD	<LOD	<LOD
Joliette	<LOD	<LOD	<LOD

Laval	<LOD	<LOD	<LOD
Lévis	8.4 ± 0.9	8.8 ± 0.9	17
Longueuil	32 ± 1.5	35 ± 1.2	67
Montréal	30 ± 1.3	28 ± 2	58
Nicolet	<LOD	<LOD	<LOD
Québec	<LOD	<LOD	<LOD
Repentigny	<LOD	<LOD	<LOD
Sorel-Tracy	12 ± 0.7	<LOD	12
Trois-Rivières	<LOD	<LOD	<LOD
Yamaska	7.1 ± 0.2	<LOD	7.1
LOD	2	6	

In order to confirm these results, we repeated the sampling exercise in 2018 targeting a higher number of municipalities (52 in total). The detailed concentrations of ATZ and DEA from this sampling campaign are shown in **Table 5.2**. The majority of the investigated municipalities, 33 out of 52 (63%), showed no detections of ATZ nor DEA in the tap water samples. Out of the 52 surveyed municipalities, 19 (37%) were positive (range of $\Sigma_{ATZ+DEA}$ = 7.9 to 104 ng L^{-1}). The maximum concentration ($\Sigma_{ATZ+DEA}$) at 104 ng L^{-1} is still about 50X lower than the Canadian IMAC in drinking water (Health Canada, 1993).

Table 5.2. Concentrations (ng L^{-1}) of atrazine (ATZ) and desethylatrazine (DEA) in tap water samples (n=104) from 52 municipalities in the province of Quebec (Canada). Results are given as average ± standard deviation. The sampling campaign was conducted in the late spring – early summer 2018 (May-June). The LOD was 2 ng L^{-1} for ATZ and 6 ng L^{-1} for DEA.

	ATZ [ng L^{-1}]	**DEA [ng L^{-1}]**	**$\Sigma_{ATZ+DEA}$ [ng L^{-1}]**
Acton Vale	15 ± 0.9	<LOD	15
Asbestos	<LOD	<LOD	<LOD
Ayers' Cliff	<LOD	<LOD	<LOD
Bécancour	10 ± 1.4	37 ± 2.6	47
Blainville	<LOD	<LOD	<LOD
Boucherville	<LOD	21.4	21.42

Brossard	41 ± 0.3	63 ± 1.3	104
Chambly	8.2 ± 0.5	14 ± 1.2	22
Châteauguay	<LOD	<LOD	<LOD
Coaticook	<LOD	<LOD	<LOD
Cookshire-Eaton	<LOD	<LOD	<LOD
Cowansville	<LOD	<LOD	<LOD
Dollard-des-Ormeaux	<LOD	<LOD	<LOD
Donnacona	<LOD	<LOD	<LOD
Drummondville	<LOD	<LOD	<LOD
East Angus	<LOD	<LOD	<LOD
Granby	<LOD	<LOD	<LOD
Joliette	<LOD	<LOD	<LOD
L'Assomption	<LOD	<LOD	<LOD
Lac-Brome	<LOD	<LOD	<LOD
Lac-Mégantic	<LOD	<LOD	<LOD
Laval	<LOD	<LOD	<LOD
Lévis	9.9 ± 0.6	21 ± 0	31
Longueuil	31 ± 2.3	30 ± 1	61
Magog	<LOD	<LOD	<LOD
Mirabel	<LOD	7.9 ± 0.1	7.9
Montréal	38 ± 0.1	49 ± 0.7	87
Mont-Saint-Hilaire	<LOD	<LOD	<LOD
Nicolet	<LOD	<LOD	<LOD
Pierreville	<LOD	<LOD	<LOD
Québec	<LOD	<LOD	<LOD
Richelieu	8.1 ± 3.6	<LOD	8.1
Richmond	<LOD	<LOD	<LOD
Saint-Bruno-de-Montarville	27 ± 1.7	20 ± 1	47
Saint-Césaire	<LOD	<LOD	<LOD
Saint-Constant	17 ± 0.3	59 ± 2.2	76
Sainte-Marie	<LOD	<LOD	<LOD
Saint-Georges	<LOD	<LOD	<LOD
Saint-Hyacinthe	<LOD	7.6 ± 0.07	7.6
Saint-Jean-sur-Richelieu	<LOD	<LOD	<LOD
Saint-Jérôme	<LOD	7.4 ± 1	7.4
Salaberry-de-Valleyfield	21 ± 0.6	40 ± 1.3	61
Shawinigan	<LOD	<LOD	<LOD

Sherbrooke	<LOD	<LOD	<LOD
Sorel-Tracy	6.2 ± 0.2	7.1 ± 0.8	13
Terrebonne	<LOD	<LOD	<LOD
Thetford Mines	<LOD	<LOD	<LOD
Trois-Rivières	<LOD	<LOD	<LOD
Varennes	9.7 ± 0.2	35 ± 1	45
Vaudreuil-Dorion	<LOD	<LOD	<LOD
Victoriaville	<LOD	25 ± 18	25
Yamaska	3.6 ± 0.2	11 ± 2.4	15
LOD	2	6	

The spatial distribution of the sum of ATZ and DEA was plotted and color-coded according to concentration class (**Figure 5.3**). The 10 municipalities that used groundwater as a source for drinking water production (**SI Table S5.1**) had non-detectable levels of ATZ and DEA, while 42% of municipalities using surface water as a source were positive to ATZ and/or DEA. Consistent with the 2017 campaign, municipalities located close to the St. Lawrence River but on the northern bank (#48,14,31) did not show detectable levels of ATZ nor DEA in tap water, while those close to the St. Lawrence and on the southern bank showed near-systematic detections of triazine herbicides (see also the hierarchical cluster analysis in SI **Figure S5.1**). This could reflect, in part, persistent contamination from upstream St. Lawrence sources (Ontario), for those municipalities that use the St. Lawrence River as a source for drinking water production.

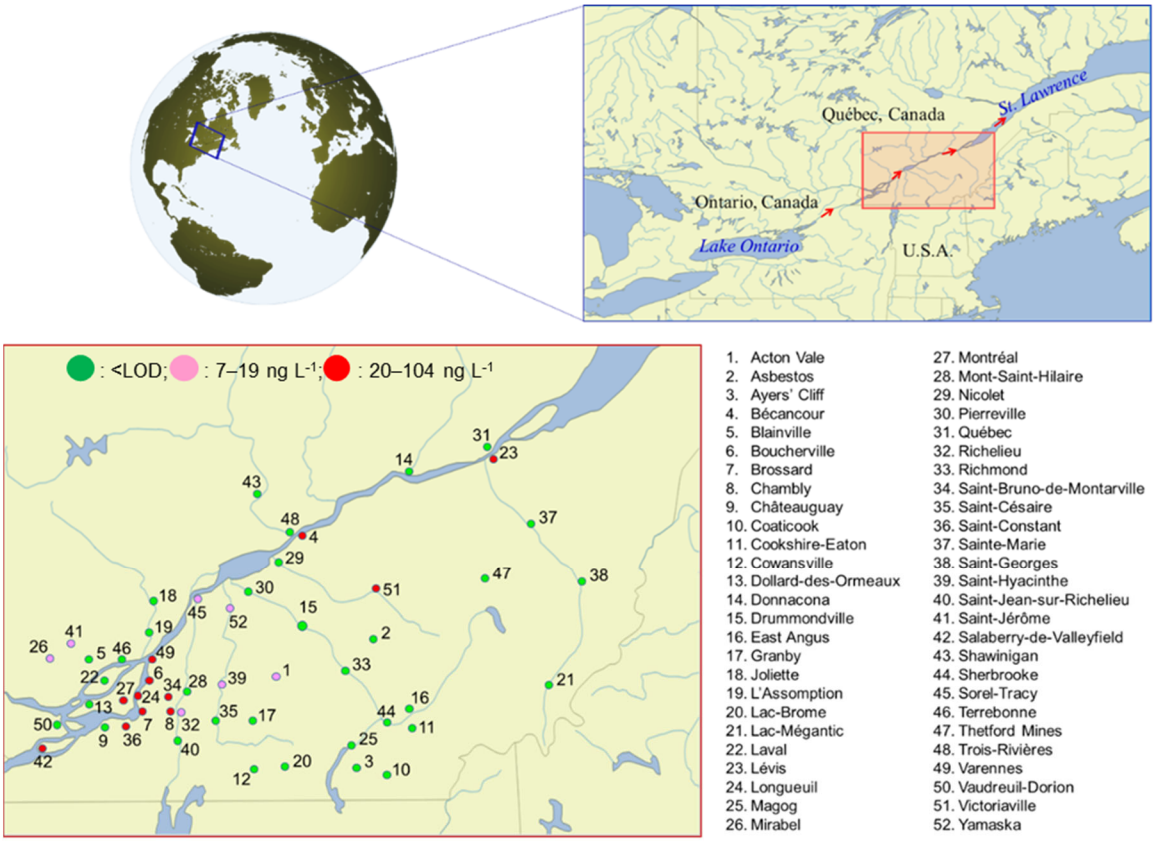

Figure 5.3. General location of the sampling area and detailed map of tap water samples collected in 2018 across 52 municipalities in Southern Quebec, Canada (Spatial survey). Each sampling site was color-coded according to the sum of atrazine + desethylatrazine (green: <LOD; pink: 7–19 ng L^{-1}; red: 20–104 ng L^{-1}). The maximum value observed during the present survey was for location #7 ($\Sigma_{ATZ+DEA}$ = 104 ng L^{-1}). In the top panel, red arrows indicate the path of the St. Lawrence River, which flows in a north-easterly direction across Ontario and Quebec provinces.

Out of the 11 municipalities that are using the St. Lawrence as a source of drinking water, 9 showed positive detections of both ATZ and DEA. Additionally, when considering the 10 highest observed concentrations in the 2018 survey, 9 corresponded to sites served by DWTPs using the St. Lawrence River as a source (SI **Table S5.1**): Lévis (31 ng L^{-1}), Varennes (45 ng L^{-1}), Bécancour (47 ng L^{-1}), Saint-Bruno-de-Montarville (47 ng L^{-1}), Longueuil (61 ng L^{-1}), Salaberry-de-Valleyfield (61 ng L^{-1}), Saint-Constant (76 ng L^{-1}), Montréal (87 ng L^{-1}), and Brossard (104 ng L^{-1}). Comparatively, sites located north of the

Montreal Island and served by DWTPs using the Des Prairies River, Mille Iles River, or Des Deux Montagnes Lake (e.g., Blainville, Laval) typically showed non-detectable levels of ATZ and DEA. This could reflect the lower ATZ inputs from the Ottawa River (which discharges into Des Deux Montagnes Lake) compared to the water mass originating from Lake Ontario (which flows south of the Montreal Island). A similar trend is apparent upon analysis of the tap water data by Segura et al. (2011), with relatively low ATZ concentrations for Ottawa and Laval (~1.5-2.0 ng L^{-1}), compared to that of Montreal (53 ng L^{-1}).

South bank municipalities situated farther from the Saint-Lawrence, but close enough to tributaries within or downstream from agricultural areas, were also linked to detections of ATZ and DEA. Such is the case of Chambly, Richelieu and Yamaska municipalities ($\Sigma_{ATZ+DEA}$ = 8–22 ng L^{-1}) that use the Richelieu river as source for drinking water production, while Saint-Hyacinthe ($\Sigma_{ATZ+DEA}$ = 7.6 ng L^{-1}) uses the Yamaska River. The Richelieu and Yamaska watersheds are characterized by higher agricultural pressures, compared to north shore tributaries where forestry occupies a large proportion of land use (Pham et al., 2000). Interestingly, ATZ and DEA were not found in tap water samples collected from municipalities south of a line between Saint-Jean-sur-Richelieu (Montérégie) and Sainte-Marie (Chaudière-Appalaches). This would correspond to locations in the upstream watershed of the tributaries, where lower impacts are expected compared to downstream locations.

- 52 municipalities representing in total 4.7 million people were screened
- Only 37% (19/52) of surveyed municipalities showed levels above the LOD
- Tap water derived from groundwater had non-detectable level of ATZ/DEA
- Montreal and south shore municipalities using the St. Lawrence River as a source for drinking water production showed higher ATZ levels (21-104 ng L^{-1})

5.3.3 Other atrazine degradation products identified in drinking water by HRMS

Other atrazine degradation products were screened in a tap water sample from the Montreal area concentrated through large volume off-line SPE and submitted to UHPLC-HRMS analysis (Orbitrap Q-Exactive). An illustration of the full scan and MS/MS chromatographic peaks for ATZ-OH is given in the SI (**Figure S5.2**), along with the interpreted MS/MS spectrum. Using a normalized collision energy of +50%, we observed the ATZ-OH parent ion at 2.00 min at 198.13551 m/z (Δ(ppm) = 0.1), along with 5 characteristic fragment ions of nominal m/z 156, 128, 114, 86, and 71, in agreement with the literature (Abián et al., 1993; Di Corcia et al., 1997). Similarly, DEA and DIA were detected with high mass accuracy (Δ(ppm) = 0.4 and 1.1, respectively) and conclusive high-resolution MS/MS confirmation (see also SI **Table S5.4**). Since non-negligible signal intensities were reported for ATZ-OH and DIA (~ 0.35 × and 0.15 × that of ATZ, respectively), it would seem relevant to include them in follow-up quality surveys of drinking water in Quebec, Canada.

- ATZ and DEA were confirmed through Q-Exactive Orbitrap HRMS and MS/MS
- ATZ-OH and DIA were also detected in tap water from the Montreal area

5.4 Discussion

Concentration ranges of atrazine observed during the Temporal survey (2015-2016) varied by about one order of magnitude. Seasonal concentrations pulses may be explained by ATZ transport from agricultural fields, either via surface runoff into ditches and adjacent brooks/rivers, or via leaching into groundwater. Atrazine does not bind strongly to soil/sediment particles (Meakins et al., 1995) and upon release can be transported with slow degradation. Major raining events in Eastern Canada are not uncommon at the end of the summer, which could support the ATZ peak observed in the present study in 2015 (**Figure 5.1**). In cold climates such as that of Canada, it is also possible that secondary peaks may occur in Spring (March-April) due to snowmelt and remobilization of ATZ after a long stabilization at low temperature. The ATZ mass budget would increase during snowmelt all the while experiencing little photodegradation and biodegradation. DEA is presumed to appear as a result of the degradation of ATZ (EPA, 2006). In the meantime, DEA would not be increasing considerably until ATZ degradation is favored in the summer months. This hypothesis is corroborated by the results obtained in the present study, with a peak in the DEA concentration occurring in July-August 2016. The fluctuations in the concentration of ATZ may also be related to its application in agricultural fields, which is normally carried out in May and June. Variations in concentrations may not be observed until the rain facilitates the transport of ATZ into groundwater, tributary rivers and other water bodies that may serve as a source for drinking water production. Some of the pesticide load probably comes from agricultural flood water, and also partly from slow release from accumulated pesticides in interstitial water within the soils, which will presumably be very slow to decrease. This is further illustrated by atrazine peaks observed in rivers affected by agricultural activities in France, many years after it was banned (Caquet et al., 2013; Dubois and Lacouture, 2011).

In addition to intra-annual variations, long-term trends are also critical to assess the status of contaminants in drinking water. Robert and Bolduc (2012) assessed the drinking water quality for the province of Quebec between 2005-2009 (Robert and Bolduc, 2012). Their results indicated a maximum of triazines concentration in drinking water of 1000 ng L^{-1},

about an order of magnitude higher than those from the present study. The decrease in ATZ concentrations between the two surveys (2005-2009 *Vs.* 2015-2016) could be related to the concomitant decrease in the quantity of ATZ active ingredients used in the province of Ontario (upstream sources) and Quebec. For instance, the use of atrazine has decreased considerably in Ontario through the last two decades from 1983 to 2013 (Farm and Food Care Ontario, 2015). These herbicides may be transported from the Great Lakes into the St. Lawrence River that serves as a major source for the production of tap water in Montreal and many other municipalities in the Quebec province (SI **Table S5.1**).

A comparative assessment of atrazine occurrence and concentration levels in tap water from the present survey, and other locations around the world is provided in SI (**Table S5.5**). Low concentrations of atrazine were reported in recent drinking water surveys from the European Union (Barbosa et al., 2016; Cotton et al., 2016), compliant with the current E.U. guideline at 100 ng L^{-1}. In the U.S.A., ATZ concentrations are occasionally found above the EPA guideline at 3000 ng L^{-1} when considering either pre-2010 or post-2010 samples (Stayner et al., 2017; Strosnider, 2017). Mean annual concentrations of certain U.S. community water systems may remain above the ATZ maximum contaminant level with implications for consumers (Strosnider, 2017). In Canada, Segura et al. (2011) surveyed 9 cities from various provinces, with concentration ranges in the same order of magnitude as results from the present study.

The concentrations of ATZ and DEA measured in the present survey can be considered low when compared with most current regulatory levels for drinking water. However, the maximum concentration of atrazine in drinking water considered safe varies widely between the various regulatory bodies or institutions promulgating guidelines. For instance, the Canadian guideline is 2.5 times higher than the WHO guideline, and the latter is 20 times higher than the E.U. standard. These guidelines represent threshold levels at which daily exposure should have no adverse health effect over a 70 years lifetime. Atrazine is a recognized endocrine disruptor, and can disrupt the hypothalamic-pituitary-gonadal axis (USEPA, 2007). The wide discrepancy in guidelines for drinking water reflects the high level of scientific uncertainty in the quantification of atrazine toxic risk, as well as different attitudes towards this uncertainty. Unlike other drinking water quality

guidelines for atrazine, the E.U. guideline is based on the precautionary principle. The guideline concentration of 100 ng L^{-1} represents the limit of quantification for atrazine at the time when it was promulgated, reflecting the position that no measurable amount of this pesticide should be tolerated in drinking water (Li and Jennings, 2018). This standard being set at a very low concentration, it should be more protective of human health than other standards that are set at much higher levels.

Standards other than that of the E.U. are based on risk assessments relying on animal data for atrazine toxicity, where the critical effect to determine the toxic dose was a reduction in the weight of offspring of female rodents exposed to atrazine. Uncertainties remain on the residual risk that could result from exposure to atrazine and its metabolites, even in the low range of concentration observed in the present study. Most data on atrazine toxicity comes from animal model studies, which are usually conducted at much higher level of exposure but shorter duration of exposure than what is experienced by human populations. Several epidemiological studies have investigated the association between adverse reproductive outcomes, such as preterm birth and low birth weight in babies, and exposure to atrazine (Almberg et al., 2018; Chevrier et al., 2011; Ochoa-Acuña, 2009; Stayner et al., 2017). Human studies are consistent with those from animal models showing reduced pup weight after in utero exposure to atrazine. For instance, a large study including 14,445 newborns from Ohio reported increased risk of low birth weight with higher atrazine levels in water, and this association was even present when restricting the analysis study participants exposed to levels below the current U.S. EPA maximum contaminant level of 3000 ng L^{-1} (Almberg et al., 2018). This study suggests that this concentration might not be sufficiently protective of human health, but the threshold concentration without significant risk has not been identified. Hence, continuing efforts are necessary to determine the threshold for safe level of exposure to atrazine, and to ensure that new scientific evidence are taken into account when updating drinking quality guidelines to ensure that they are protective.

5.5 Conclusions

A temporal survey conducted over 18 consecutive months (2015-2016) in the Montreal area (QC, Canada) revealed limited variations of atrazine (ATZ) in a public tap water supply. ATZ was detected in all 450 samples and varied by less than one order of magnitude over the studied period (30-195 ng L^{-1}). The ATZ concentrations are suggested to have decreased compared to earlier surveys, which is consistent with the decrease in the sales of active ingredient in Ontario (upstream sources) and Quebec. We also conducted a spatial survey of public tap water for 52 municipalities in southern Quebec (Canada). Municipalities other than Montreal generally showed low or non-detectable levels of ATZ and DEA. In addition to Montreal, higher concentrations of atrazine in drinking water were observed for municipalities on the southern bank that use the St. Lawrence River as a source for drinking water production, and those that use tributary rivers downstream from agriculturally-exposed areas. Current research efforts are underway to characterize the spatial-temporal trends of ATZ, DEA, and other pesticides in the St. Lawrence and its major tributary rivers, which may help further interpret these results. The ATZ concentrations observed in the present tap water survey were well below the acceptable limits for Canada (5000 ng L^{-1}), although in some instances the concentrations were above the European Union drinking water guideline at 100 ng L^{-1}. The comprehensive database gathered in the present study, together with past and future large-scale monitoring surveys of contaminants in drinking water, could be useful for future analyses aiming to evaluate the link between exposure and health status of human populations.

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Compliance with ethical standards

The authors declare that they have no conflict of interest.

5.6 Supportin Information

Table S5.1. Additional information on water source and treatment technology of DWTPs in Quebec.

Province	Administrative region	Municipality	Sampling		Water source	Water treatment
			2017	2018		
Quebec	Montérégie	Acton Vale		June	Noire River	A,B
Quebec	Estrie	Asbestos		May	Nicolet-sud River	A,B,C
Quebec	Estrie	Ayers' Cliff		June	Groundwater	A
Quebec	Centre-du-Québec	Bécancour	May	May	St. Lawrence River	A,B,D
Quebec	Laurentides	Blainville		May	Mille-Îles River	A,B,C,D
Quebec	Montérégie	Boucherville		May	St. Lawrence River	A,B,C
Quebec	Montréal	Brossard	May	May	St. Lawrence River	A,B,C
Quebec	Montréal	Chambly		June	Richelieu River	A,B
Quebec	Montérégie	Châteauguay		June	Mixed sources, includ. groundwater	A,D,E
Quebec	Estrie	Coaticook		June	Groundwater	G
Quebec	Estrie	Cookshire-Eaton		June	Groundwater	A
Quebec	Montérégie	Cowansville		June	Davgnon Lake	A,B,C
Quebec	Montréal	Dollard-des-Ormeaux		June	St. Lawrence River/des Prairies River	A,B,D
Quebec	Capitale-Nationale	Donnacona	May	May	Jacques-Cartier River	A,B

Quebec	Centre-du-Québec	Drummondville		May	St. François River	A,B,C
Quebec	Estrie	East Angus		May	Groundwater	A
Quebec	Montérégie	Granby		May	Haute-Yamaska River	A,B,C
Quebec	Lanaudière	Joliette	May	May	L'Assomption River	A,B,D
Quebec	Lanaudière	L’Assomption		May	L'Assomption River	A,B,C,D
Quebec	Montérégie	Lac-Brome		June	Groundwater	A
Quebec	Estrie	Lac-Mégantic		June	Groundwater	A,B,F,G
Quebec	Laval	Laval	May	May	des Prairies River/Mille-Îles River	A,B,D
Quebec	Chaudière-Appalaches	Lévis	May	May	St. Lawrence River	A,B,D,E
Quebec	Montérégie	Longueuil	May	May	St. Lawrence River	A,B,C
Quebec	Estrie	Magog		June	Lac Memphrémagog	A
Quebec	Laurentides	Mirabel		May	Mille-Îles River	A,B,C,D
Quebec	Montréal	Montréal	May	May	St. Lawrence River	A,B
Quebec	Montérégie	Mont-Saint-Hilaire		June	Richelieu River	A,B,D
Quebec	Centre-du-Québec	Nicolet	May	May	Nicolet River	A,B
Quebec	Centre-du-Québec	Pierreville		June	St. François River	A,B,D
Quebec	Capitale-Nationale	Québec	May	May	St. Charles River	A,B,D
Quebec	Lanaudière	Repentigny	May	May	L'Assomption River	A,B,C,D
Quebec	Montérégie	Richelieu		June	Richelieu River	A,B
Quebec	Estrie	Richmond		June	Groundwater	A,B,F
Quebec	Montérégie	Saint-Bruno-de-Montarville		June	St. Lawrence River	A,B,C
Quebec	Montérégie	Saint-Césaire		June	Groundwater	A
Quebec	Montérégie	Saint-Constant		June	St. Lawrence River	A,B,D
Quebec	Chaudière-Appalaches	Sainte-Marie		June	Chaudière River	A,B,D
Quebec	Chaudière-Appalaches	Saint-Georges		June	Chaudière River	A,B,C,D,E

Quebec	Montérégie	Saint-Hyacinthe		June	Yamaska River	A,B,C,D
Quebec	Montérégie	Saint-Jean-sur-Richelieu		May	Richelieu River	A,B,C
Quebec	Laurentides	Saint-Jérôme		May	Du Nord River	A,B,E
Quebec	Montérégie	Salaberry-de-Valleyfield		May	St. Lawrence River	A,B,D
Quebec	Mauricie	Shawinigan		May	Lac-à-la-Pêche/Lac-des-Piles	A
Quebec	Estrie	Sherbrooke		May	Lac Memphrémagog	A,B,D
Quebec	Montérégie	Sorel-Tracy	May	May	Richelieu River	A,B,D
Quebec	Lanaudière	Terrebonne		May	Mille-Îles River	A,B,D,E
Quebec	Chaudière-Appalaches	Thetford Mines		May	Bécancour River	A,B
Quebec	Mauricie	Trois-Rivières	May	May	St. Maurice River/Groundwater	A,B
Quebec	Montérégie	Varennes		June	St. Lawrence River	A,B,D
Quebec	Montérégie	Vaudreuil-Dorion		June	Lac des Deux Montagnes	A,B,D
Quebec	Centre-du-Québec	Victoriaville		June	Réservoir Beaudet	A,B,C,D
Quebec	Montérégie	Yamaska	May	May	Richelieu River	A,B,D

A: Chlorination

B: Filtration

C: Activated carbon

D: Ozonation

E: Ultraviolet

F: Iron and manganese removal (groundwater)

G: Other

http://www.environnement.gouv.qc.ca/eau/potable/production/resultats.asp

Table S5.2. Valve program, on-line SPE (loading pump) and UHPLC (analytical pump) gradient conditions used for pre-concentration and separation of the quantitatively targeted compounds.

Loading pump					**Analytical pump**				
	Time (min)	A_2 (%)	B_2 (%)	Flow rate (µL/min)	Time (min)	A_1 (%)	B_1 (%)	Flow rate (µL/min)	
On-line SPE loading	0	100	0	1500	0	80	20	350	Column re-equilibration
step	1.5	100	0	1500	1.5	80	20	350	Elution and chromatographic
Loop wash	5.40	0	100	2000	5.40	5	95	350	separation
SPE column wash	7.40	0	100	1500	7.40	5	95	350	
and conditioning	7.50	100	0	1500	7.50	80	20	350	Column re-equilibration
	10	100	0	1500	10	80	20	350	

A_1: Aqueous mobile phase for analytical pump.

B_1: Organic mobile phase for analytical pump.

A_2: Aqueous mobile phase for loading pump.

B_2: Organic mobile phase for loading pump.

Table S5.3. Mass spectrometry compound-dependent parameters of the quantitatively targeted compounds. Q.T.: quantification transition; C.T.: confirmation transition. Details on the corresponding isotope-labelled internal standard (IS) are also provided.

Compound	**Molecular formula**	**Precursor Ion (m/z)**	**Product Ion (m/z)**	**MS/MS Transition**	**RF Lens (V)**	**Collision Energy (V)**	**IS**
Atrazine	$C_8H_{14}ClN_5$	216.2	174.0	**Q.T.**	62	17	Atrazine-$^{13}C_3$
			104.0	C.T.	62	29	
DEA	$C_6H_{10}ClN_5$	188.1	146.0	**Q.T.**	57	17	Atrazine-$^{13}C_3$
			103.9	C.T.	57	26	
Atrazine-$^{13}C_3$	$C_5{}^{13}C_3H_{14}ClN_5$	219.2	176	IS	60	18	

Table S5.4. Summary of the exact mass accuracy of parent ions of atrazine (ATZ), desethylatrazine (DEA), desisopropylatrazine (DIA), and hydroxyatrazine (ATZ-OH), and their major fragment ions, when submitting a Montréal tap water extract to UHPLC–HRMS and MS/MS confirmatory analysis. The Thermo Orbitrap Q-Exactive was operated in full scan MS and parallel reaction monitoring (PRM) modes.

	Ion formula	Type	$m/z_{theoretical}$	$m/z_{observed}$	Δppm
ATZ	**(C8H15N5Cl)+**	**Parent**	**216.10193**	**216.1016**	**-1.5**
	(C5H9N5Cl)+	Daughter ion	174.0541	174.05482	4.1
	(C3H5N5Cl)+	Daughter ion	146.02279	146.02341	4.2
	(C5H8N5)+	Daughter ion	138.07742	138.07809	4.8
	(C4H7N3Cl)+	Daughter ion	132.0323	132.03297	5.1
	(C2H3N3Cl)+	Daughter ion	104.001	104.00188	8.4
	(C4H6N3)+	Daughter ion	96.05562	96.05654	9.5
	(C3H7N2)+	Daughter ion	71.0603748	71.06141	14.6
DEA	**(C6H11N5Cl)+**	**Parent**	**188.0703**	**188.07037**	**0.4**
	(C5H5N5Cl)+	Daughter ion	146.02279	146.02342	4.3
	(C3H4N5)+	Daughter ion	110.046122	110.0469	7.1
	(C2H3N3Cl)+	Daughter ion	104.001001	104.00187	8.4
	(C2H2N3)+	Daughter ion	68.0243237	68.02544	16.4
DIA	**(C5H9N5Cl)+**	**Parent**	**174.05465**	**174.05485**	**1.1**
	(C5H7N5Cl)+	Daughter ion	172.03845	172.03938	5.4
	(C3H5N5Cl)+	Daughter ion	146.022799	146.02341	4.2
	(C5H8N5)+	Daughter ion	138.077422	138.07802	4.3
	(C4H7N3Cl)+	Daughter ion	132.032302	132.03297	5.1
	(C2H3N3Cl)+	Daughter ion	104.001001	104.00184	8.1
	(C4H6N3)+	Daughter ion	96.0556238	96.05654	9.5
	(C3H7N2)+	Daughter ion	71.0603748	71.06143	14.8
	(C2H2N3)+	Daughter ion	68.0243237	68.0254	15.8
ATZ-OH	**(C8H16N5O)+**	**Parent**	**198.13549**	**198.13551**	**0.1**
	(C5H10N5O)+	Daughter ion	156.087987	156.08845	3
	(C5H10N3O)+	Daughter ion	128.08183	128.08241	4.5
	(C4H8N3O)+	Daughter ion	114.066188	114.06688	6.1

(C2H4N3O)+	Daughter ion	86.0348883	86.03578	10.4
(C3H7N2)+	Daughter ion	71.0603748	71.0614	14.4

Table S5.5. Comparative data of atrazine in drinking water samples reported worldwide and in this study, presenting the geographical area and sampling year surveyed, method reporting limit (RL; ng L^{-1}), occurrence frequency (proportion of samples ≥RL; %), and concentration range of positive samples (min–max; ng L^{-1}).

Reference	Geographical area	Sampling year	RL [ng L^{-1}]	Occurrence %	Range [ng L^{-1}]
This study	**Montreal, Canada**	**2015–2016**	**2**	**100**	**30–195**
This study	**QC, Canada**	**2017–2018**	**2**	**36**	**3–41**
(Robert and Bolduc, 2012)	QC, Canada	2005–2009	100	86	500–1000
(Garcia-Ac et al., 2009)	Montreal, Canada	2009	0.7	100	15–21
(Segura et al., 2011)	Canada	2010	0.03	55	0.3–52.6
(Munger, 1997)	U.S.A.	1984–1990	200	na*	700–2200
(Rinsky, 2012)	U.S.A.	2000–2008	3	na	9–8900
(Stayner et al., 2017)	U.S.A.	2004–2008	3	na	410–1300
(Glassmeyer et al., 2017)	U.S.A.	2007, 2010–2012	1	16	1–270
(Bradley et al., 2018)	U.S.A.	2016-2017	2.3	38	3-100
(Machado et al., 2016)	Brazil	2011–2012	1	75	2–24
(Villanueva et al., 2005)	Spain (E.U.)	1990–1998	15	13-58	29–1000
(Barbosa et al., 2016)	Portugal (E.U.)	2016	0.12	46	1.14–2.24
(Cotton et al., 2016)	France (E.U.)	2016	0.1	100	0.5–10
(Kruawal et al., 2005)	Thailand	2002–2003	1.6	100	58–106
(Chen et al., 2015)	China	2015	6	100	8.8–10.2

*na: Not available.

Hierarchical cluster analysis based on ATZ and DEA compositions grouped samples from Salaberry-de-Valleyfield (#42), Montreal (#27), and those municipalities located along the southern shore of the St. Lawrence river between Saint-Constant and Varennes (#36,7,24,6,49) (SI **Figure S5.1**). Such samples showed summed concentrations of ATZ and DEA above 50 ng L^{-1} (see also Main text **Figure 5.3**).

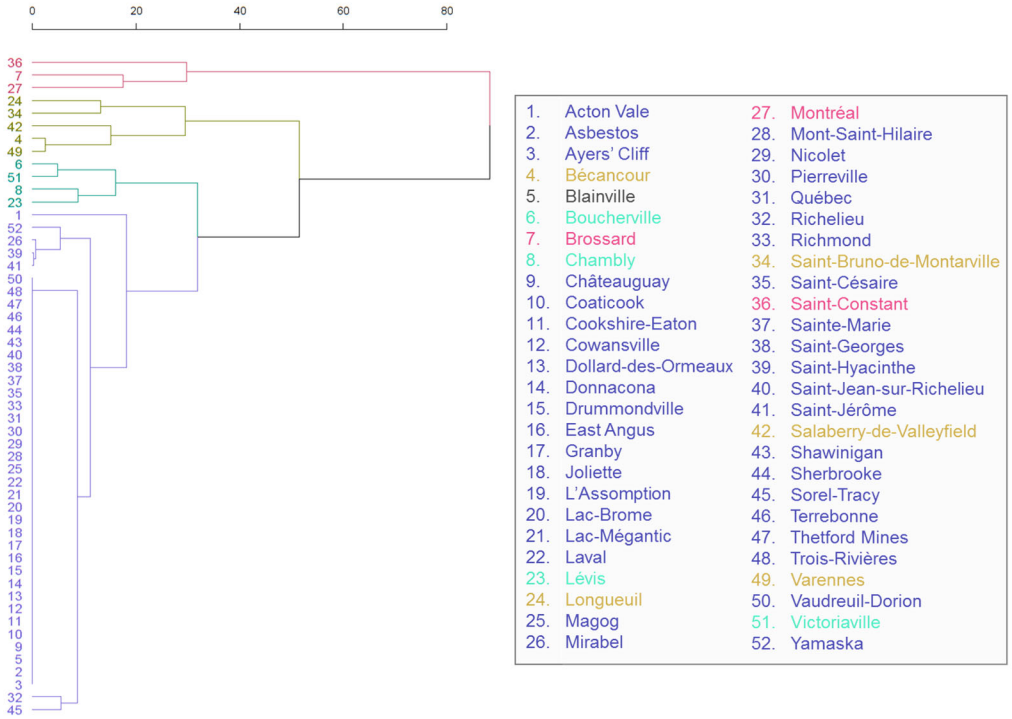

Figure S5.1. Hierarchical cluster analysis of ATZ and DEA compositions in drinking water across 52 municipalities in southwestern Quebec, Canada. The pink, brown and light blue clusters group sites with relatively higher ATZ and DEA

concentrations, and were systematically located on the southwestern shore of the St. Lawrence River (except the islands of Montreal and Valleyfield).

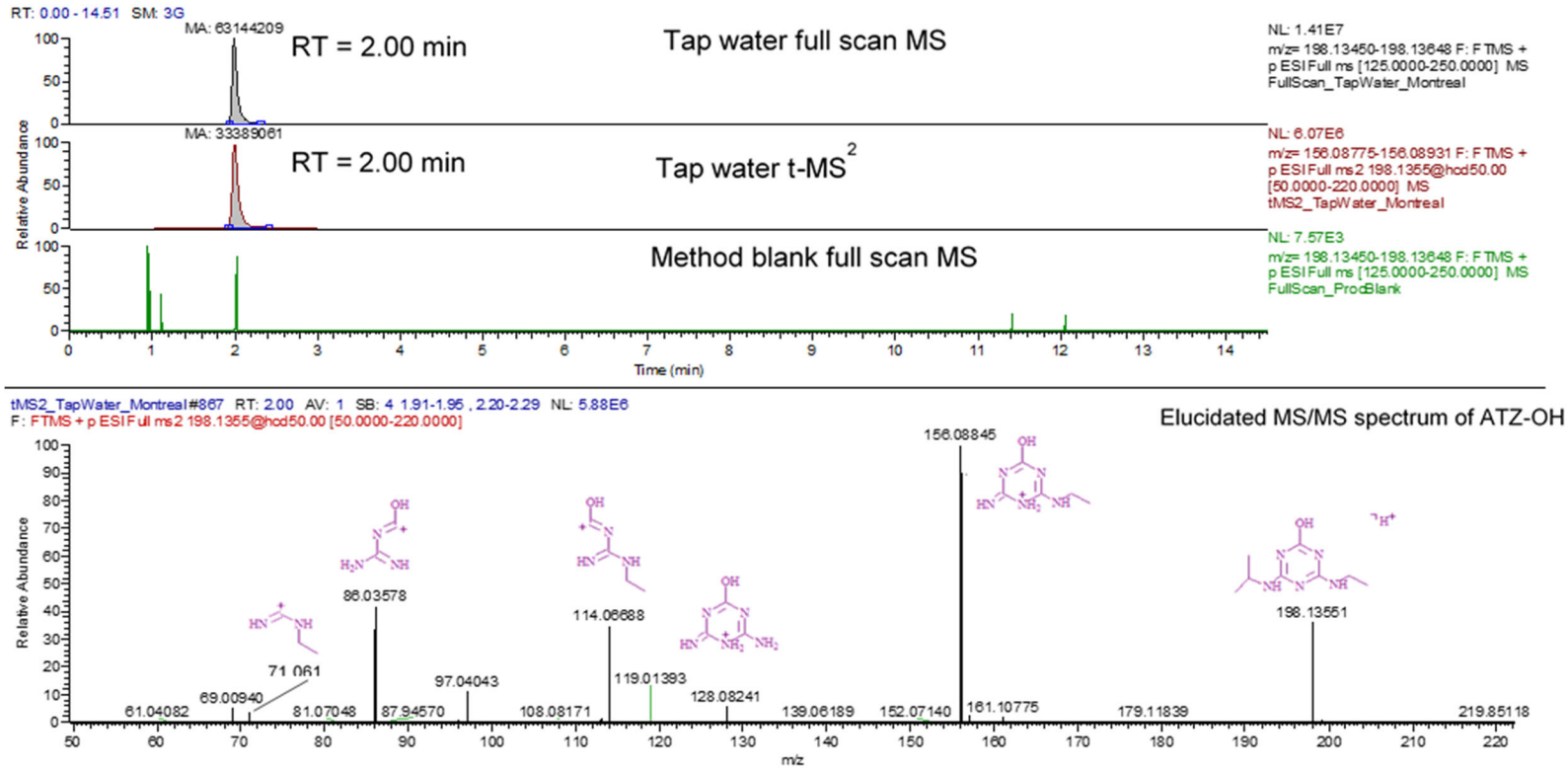

Figure S5.2. Identification of hydroxyatrazine in tap water from downtown Montréal, using the full scan MS and parallel reaction monitoring (t-MS^2) acquisition modes of the Orbitrap Q-Exactive.

Analytical procedure for the prospective screening of other ATZ degradation products. In view of the anticipated low concentrations of atrazine degradation products (other than DEA) in drinking water, we used off-line pre-concentration. Briefly, Thermo Hypersep Retain PEP cartridges (200 mg) (hydrophilic lipophilic balance) were mounted on a SPE manifold and conditioned with 2x4 mL of methanol and 2x4 mL of HPLC-water. After this step, the cartridge was loaded either with 500 mL of tap water sample from downtown Montréal, or with 500 mL of ultrapure water (method blank), at a flow rate of ~10 mL/min. The cartridges were then dried under vacuum, and analytes were eluted with 2x4 mL of methanol. The eluates were evaporated to near-dryness and reconstituted in a 90:10 Water:MeOH mixture prior to analysis by ultra-high-performance liquid chromatography (UHPLC) coupled to a high-resolution Q-Exactive Orbitrap mass spectrometer (Thermo Scientific, Waltham, MA, U.S.A.) operated in positive ionization mode. The first injection was conducted using full scan MS mode, with the parameters as follows. The mass scan range was set at 100-250 m/z, resolution at 70,000 (m/z 200), AGC target at 3e6, and IT at 100 ms. Further confirmation was then achieved by using the parallel reaction monitoring (PRM) mode of the Q-Exactive Orbitrap, with normalized collision energies assayed at different levels (NCE = 25, 35, and 50%). For these MS/MS analyses, resolution was set at 17,500 (m/z 200), AGC target at 2e5, and IT at 100 ms. Interpretation of the generated spectra was performed based on the literature and the proposed fragment structures using the Mass Frontier 7.0 software (Thermo Fisher).

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Chapitre 6. Occurrence et distribution spatiale de pesticides tel que le glyphosate, l'atrazine et les néonicotinoïdes dans le fleuve Saint-Laurent et ses cours d'eau tributaires

Article publié dans le journal *Environmental Pollution* 250 (2019) 29-39 :
"**Widespread occurrence and spatial distribution of glyphosate, atrazine, and neonicotinoids pesticides in the St. Lawrence and tributary rivers".** Auteurs: **Montiel-León, J. M., G. Munoz, S. Vo Duy, D. T. Do, M. A. Vaudreuil, K. Goeury, F. Guillemette, M. Amyot and S. Sauvé.**

Description: Cet article étudie la distribution du glyphosate, de l'artrazine et des néonicotinoïdes sur un tronçon de 200 km du fleuve St-Laurent. Les profils de ces pesticides ainsi que leurs métabolites ont été établis sur une série de transects, permettant de discriminer différentes sources amont (Grands Lacs laurentiens *vs.* Rivière des Outaouais).

Contributions: J'ai participé à la conception du projet, la collecte des échantillons sur le navire de recherche Lampsilis, la réalisation des manipulations, le traitement de données et la rédaction de l'article.
Co-auteurs: Sung Vo Duy m'a aidé avec une partie des manipulations et à la rédaction. Gabriel Munoz m'a aidé avec une partie des manipulations ainsi que la rédaction de l'article. Dat Tien Do m'a aidé avec une partie des manipulations. Marc-Antoine Vaudreuil et Ken Goeury m'ont aidé avec la collecte d'échantillons sur le terrain. Francois Guillemette a aidé à la collection des échantillons sur le terrain et à réviser l'article. Co-directeur: Marc Amyot a aidé à la révision de l'article. Directeur : Sébastien Sauvé m'a aidé à la conception du projet et à la rédaction de l'article.

Abstract

The occurrence and spatial distribution of selected pesticides were investigated along a 200-km reach of the St. Lawrence River (SLR) and tributaries in Quebec, Canada. Surface water samples (n = 68) were collected in the summer 2017 and analyzed for glyphosate, atrazine (ATZ), 8 systemic insecticides (acetamiprid, clothianidin, dinotefuran, fipronil, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam) and some metabolites. Overall, 99% of the surface water samples were positive to at least one of the targeted pesticides. The most recurrent compounds were glyphosate (detection frequency: 84%), ATZ (82%), thiamethoxam (59%), desethylatrazine (DEA: 47%), and clothianidin (46%). Glyphosate displayed variable levels (4–3,000 ng L^{-1}), with higher concentrations in south tributaries (e.g., Nicolet and Yamaska). In positive samples, the sum of ATZ and DEA varied between 5 and 860 ng L^{-1}, and the sum of 6 priority neonicotinoids between 1.5 and 115 ng L^{-1}. From Repentigny to the Sorel Islands, the spatial distribution of pesticides within the St. Lawrence River was governed by the different upstream sources (i.e., Great Lakes *vs.* Ottawa River) due to the limited mixing of the different water masses. Cross-sectional patterns revealed higher concentrations of glyphosate and neonicotinoids in the north portions of transects, while the middle and south portions showed higher levels of atrazine. In Lake St. Pierre and further downstream, cross-sections revealed higher levels of the targeted pesticides near the southern portions of the SLR. This may be due to the higher contributions from south shore tributaries impacted by major agricultural areas, compared to north shore tributaries with forest land and less cropland use. Surface water samples were compliant to guidelines for the protection of aquatic life (chronic effects) for glyphosate and atrazine. However, 31% of the samples were found to surpass the guideline value of 8.3 ng L^{-1} for the sum of six priority neonicotinoids.

Capsule

Glyphosate, atrazine, and neonicotinoid pesticides showed systematic spatial patterns along a 200 km reach of the St. Lawrence River and tributaries, in relation with their agricultural sources.

Keywords

Pesticides survey – Surface water – St. Lawrence River – Glyphosate – Atrazine – Neonicotinoid insecticides

6.1. Introduction

The use of pesticides in agriculture has intensified in the past decades. Although the new generation of pesticides is deemed less bioaccumulative, the released quantities represent a potential threat to groundwaters, surface waters, and sediments (Aguilar et al. 2017; Chrétien et al. 2017; Hladik et al. 2018; Morrissey et al. 2015; Qu et al. 2017; Struger et al. 2017). Since surface and groundwaters are often used as sources of drinking water, pesticides may also be harmful to human populations (Klarich et al. 2017). The quality of the world's major freshwater hydrosystems is therefore under close scrutiny (Loos et al. 2009; Loos et al. 2017).

The St. Lawrence River (SLR) is one of the major hydrosystems in North America, draining a 1.3 million km^2 watershed which includes the Laurentian Great Lakes (Pham et al. 2000). The freshwater inputs of the St. Lawrence provide a source of drinking water production for more than half of the population of the province of Quebec (Canada). Increasing anthropogenic pressures contributed to deteriorating the water quality of the St. Lawrence during the 20^{th} century, but the situation has improved since the 1970s thanks to regulations and efficient sanitation (Giroux, Hébert and Berryman, 2016). For instance, decreasing trends of organochlorine pesticides in beluga whales from the St. Lawrence estuary were reported over a 1982–1994 period (Muir et al. 1996).

While legacy contaminants may gradually subside, there has been an increased mobilization of contaminants of emerging concern in intensive agriculture watersheds in southern Quebec and elsewhere. Neonicotinoid insecticides have been recently reported in environmental waters in Canada (Main et al. 2014; Giroux, Hébert and Berryman, 2016; Chrétien et al. 2017; Struger et al. 2017; Montiel-León et al. 2018). Thiamethoxam is classified as class C sales chemical (1-10 tons) in Quebec province (MDDELCC, 2016), although the report does not include the quantities from pre-treated seeds. Corn seeds and about half of soya seeds are treated with neonicotinoids since 2011 in Quebec (Giroux 2015). In Ontario, large areas of agricultural fields are also planted with seeds treated with thiamethoxam, clothianidin, and imidacloprid (Ontario, 2015). Thiamethoxam and clothianidin were also frequently applied in the European Union, and their use was initially restricted in 2013 due to their toxicity

to natural pollinators. A new regulation entered into force in 2018 allowing their use only in permanent greenhouses. In addition, five neonicotinoid compounds were on the first E.U. watch list (2015/495) and they are also included in the second watch list (2018/840) for E.U.-wide monitoring (acetamiprid, clothianidin, imidacloprid, thiacloprid, and thiamethoxam). Atrazine and glyphosate are the most used pesticide active ingredients in the U.S. (USEPA, 2017). Atrazine is also a high sales herbicide in Ontario and Quebec provinces, albeit in decreased usage in some applications for which glyphosate is now preferred (Farm and Food Care Ontario, 2015; MDDELCC, 2016). In Quebec, atrazine is especially applied in cultures of corn and soya; the treated areas in 2012 represented more than 320,000 acres (Giroux 2015). Atrazine has been classified as a possible carcinogen to humans (Health Canada, 2013), with potential endocrine disruption (USEPA, 2007). In its 2016 report on pesticides sold in Quebec, the Minister of Sustainable Development, the Environment and the Fight against Climate Change classified glyphosate potassium salt as class F substance with annual sales higher than 1,000 tons of active ingredient (MDDELCC, 2016). Glyphosate usage is diverse and includes weed control, desiccant to accelerate maturation, and non-agricultural uses where a large-spectrum weed control is important (e.g., farmyards, parks and railway tracks). Despite their potential environmental health effects, there is only limited information available about the distribution and dynamics of emerging pesticides at large spatial scale in major hydrosystems such as the SLR. Documenting the occurrence of emerging micropollutants and old ones under continued use may lead to a re-assessment of current water quality and health thresholds (Sauvé and Desrosiers, 2014).

In this study, we set out to determine the occurrence and levels of selected pesticides in a 200-km reach of the SLR and its major tributaries. Sampling was conducted in summer 2017 onboard the *Lampsilis* research vessel. One central hypothesis was that variations in pesticide concentrations will reflect changes in land use and hydrology at the scale of this large hydrosystem, where different agricultural activities may have an impact on ecosystems. How this risk is divided between the major water bodies is, therefore, an important research question to address. Land use vastly differs in St. Lawrence lowlands between south and north shores (i.e., agricultural *vs.* forest use). Additionally, the water masses that enter the fluvial St. Lawrence tend to show little to no mixing as far as Lake St. Pierre, with the brown waters from the Ottawa River near the north portion, and the blue-green waters from the Great Lakes in the center and south portions. To evaluate the spatial distribution of pesticides, samples were collected from a series of cross-sections within the St. Lawrence, and from bridges at the mouth

of tributaries. These sampling efforts resulted in the collection of 68 surface water samples that were quantitatively analyzed for glyphosate and atrazine herbicides, 8 systemic insecticides, and some of their degradation products. This work aimed to address two critical knowledge gaps regarding: i) the current quality status of Quebec surface waters with regard to glyphosate, atrazine, and neonicotinoids; and ii) their spatial distribution within the SLR and its tributaries. The study provides much-needed data on the occurrence and fate of pesticides of high current concern in a complex hydrographic network.

6.2. Experimental

6.2.1. Target compounds

Glyphosate, aminomethylphosphonic acid (AMPA), atrazine, desethylatrazine (DEA), acetamiprid, clothianidin, desnitro-imidacloprid, dinotefuran, fipronil, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam were the compounds targeted in the present study. Further details on native compounds, isotope-labeled internal standards, and other chemicals and materials are provided in the Supporting Information (SI).

6.2.2. Description of the sampling area

The hydrological features of the surveyed area are well described (Hudon, 2004; Pham et al. 2000). The St. Lawrence flows over ~1,200 km in a north-easterly direction from its source in Lake Ontario to the estuary mouth in the Atlantic Ocean. The fluvial St. Lawrence is home to many freshwater fish species, including perch, pike, and walleye, while the Gulf of St. Lawrence hosts resident and migratory fishes and cetaceans (Simond et al. 2017).

The fluvial section extends along a ~520 km waterway from its source near Kingston (ON, Canada) to the Orleans Island (immediately downstream Quebec City), which marks the current salinity limit. The mean annual discharge at this point is *circa* 12,000 $m^3 s^{-1}$ (Pham et al. 2000). The international section (Canada/U.S.A.) of the St. Lawrence extends from Kingston, ON, to about 110 km upstream from the city of Montreal, QC. This reach of the river is characterized by the major input from Lake Ontario (with an overall remarkably stable water flowrate with the regulation of the Cornwall dam), and minor contributions from south shore tributaries (Twiss et al. 2010). After its full entry into Canada, the St. Lawrence flows through

a region of large islands, where it also receives the major input of the Ottawa River. In its course toward its mouth, the fluvial St. Lawrence is joined by other notable tributaries from the north and south shores, most of which discharge into Lake St. Pierre, a 32-km long widening of the river (**Figure 6.1**). Considering the mean annual flowrate, about 65% of the St. Lawrence discharge at the Orleans Island (downstream Quebec City) originates from Lake Ontario, 16% from the Ottawa River, and the remaining 19% from 15 smaller tributaries (Pham et al. 2000).

The study area covers a 200-km reach of the St. Lawrence River, from Lake St. Francis (near Salaberry-de-Valleyfield) to a few kilometers after the confluence with the Batiscan and Sainte-Anne rivers, downstream from the city of Trois-Rivières (**Figure 6.1**). In this surveyed tract, the St. Lawrence River flows through a watershed populated by more than 4 million inhabitants, nearly half of the total population of Quebec province (Canada). Agricultural activities make up an important contribution of land use in lowlands of the southern shore, especially in the Yamaska, Nicolet, Richelieu, and Châteauguay river basins, in contrast to the northern shore with less agricultural activities and where most of the land use is attributed to forestry (Goyette et al. 2016).

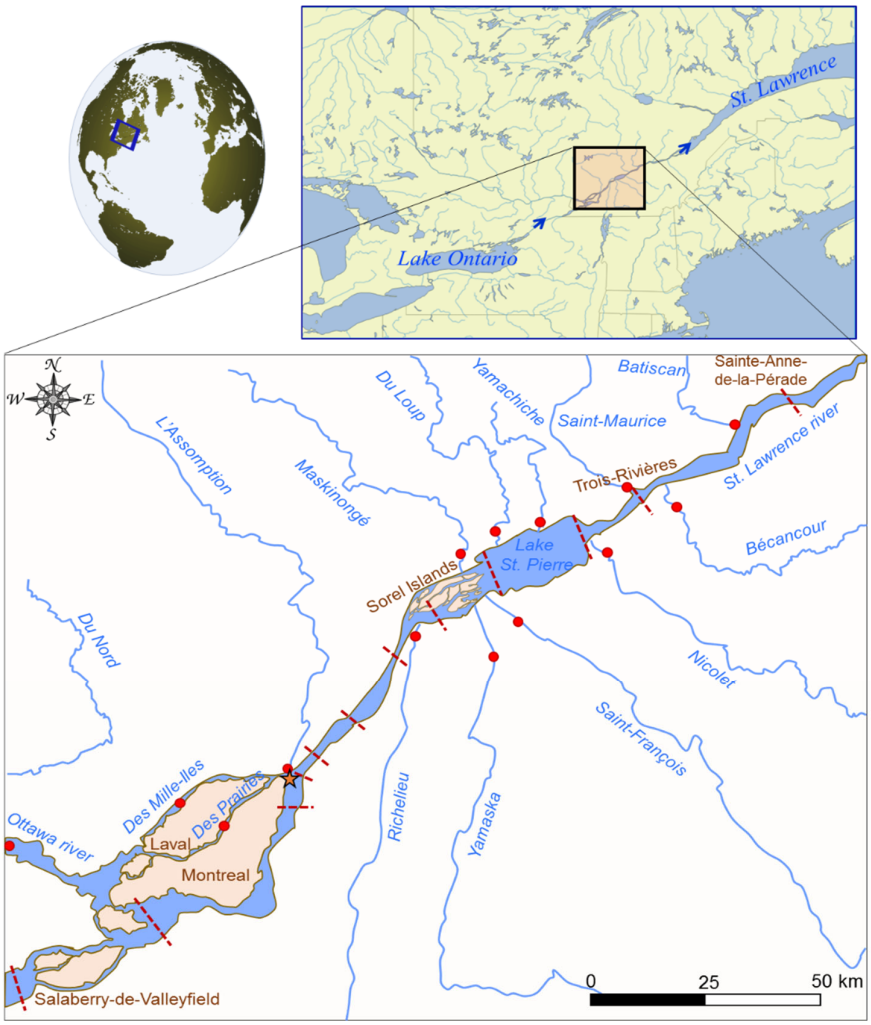

Figure 6.1. Overview of the study area, covering a reach of 200 km of the St. Lawrence River between Salaberry-de-Valleyfield and Sainte-Anne-de-la-Pérade, and major tributaries from the south and north shores including the Ottawa River. The series of orthogonal cross-sections realized with the *Lampsilis* research vessel are noted in red dashed lines (2-3 sampling sites for each transect), while samples collected from bridges near the mouth of tributaries are shown in red dots. Note that additional samples were collected across the Sorel Island channels (not shown on this map). The star symbol represents the effluent from the Montreal wastewater treatment plant. In the top panel, blue arrows indicate the path of the St-Lawrence river, which flows in a north-easterly direction from its source in Lake Ontario.

6.2.3. Sample collection

The sampling campaign was conducted in summer 2017 (9–16 July) aboard the *Lampsilis* research vessel. For a given kilometric point, biogeochemical features may vary transversally within the St. Lawrence, due to different water masses that flow in parallel with no or limited mixing. Multiple lines of evidence support this heterogeneity, including satellite imagery (Frenette et al. 2006) and measurements of parameters such as dissolved organic carbon or suspended particulate matter (Hudon and Carignan, 2008). Our sampling strategy involved a series of orthogonal cross-sections (2-3 sampling points each) performed at different areas along the St. Lawrence River. These include, from west to east, cross sections at the following locations: Lake St. Francis (upstream from Salaberry-de-Valleyfield), Lake St. Louis, Boucherville Islands, three locations immediately downstream from the Montreal Island, Repentigny, Contrecœur, Sorel-Tracy (upstream and downstream locations), Lake St. Pierre (immediately after the Sorel Islands for the upstream points, and immediately before the restriction of the river width, for the downstream points), Trois-Rivières, and Sainte-Anne-de-la-Pérade (**Figure 6.1**). An additional point was also collected at the Saint-Maurice River mouth. In parallel to the *Lampsilis* mission, surface water samples were collected from bridges near the mouth of major tributaries in July 2017. These included north shore tributaries to the SLR (L'Assomption, Maskinongé, Du Loup, Yamachiche, and Batiscan rivers) and south shore tributaries (Richelieu, Yamaska, Saint-François, Bécancour, and Nicolet rivers) (**Figure 6.1**). Additionally, surface water samples were collected in the Ottawa River (about 25 km upstream from its junction with Des Deux Montagnes Lake) and in the Du Nord River (an Ottawa River tributary), in the Des Prairies River (North of Montreal Island), and the Mille Iles River (North of Laval Island).

In total, 68 surface water samples were collected from the field sampling efforts (one sample per sampling site). Additional field blank samples were also collected in parallel to the samples (Section 2.5). At each site, the sampling of surface water was conducted as follows. A 15-20L subsurface water (1 m depth) was collected with a Niskin – Go Flow sampler, and aliquoted in different containers for analyses of diverse classes of targeted micropollutants. Amber glass bottles (1 L) previously cleaned at the laboratory and amended with omadine salt for preservation were filled to the brim, sealed, and stored at 4 °C until arrival at the laboratory where they were filtered on glass fiber filters (GFF; 0.3 μm). The samples were then stored at 4 °C until further processing (Section 2.4).

6.2.4. Sample preparation and analysis

Analytical methods were adapted from previously published studies, with some modifications to achieve sufficiently low limits of detection (Morissette et al. 2015; Montiel-León et al. 2018; Ibañez et al. 2006). Details on sample preparation and instrumental methods are given in the SI (**Tables S6.1-S6.3**). Briefly, atrazine and DEA were analyzed by on-line solid phase extraction (SPE) coupled to ultra-high-performance liquid chromatography electrospray ionization tandem mass spectrometry (UHPLC-MS/MS), using a TSQ Quantiva triple quadrupole mass spectrometer (Thermo Scientific, Waltham, MA, U.S.A.). Glyphosate and AMPA were subjected to FMOC derivatization procedure and processed by on-line SPE – UHPLC high-resolution mass spectrometry (HRMS), using a Q-Exactive Orbitrap mass spectrometer (Thermo Scientific, Waltham, MA, U.S.A.). Neonicotinoids and fipronil were analyzed by off-line solid phase extraction (250 mL loading volume) prior to small-volume injection (10 µL) into a UHPLC-HRMS system (Q-Exactive Orbitrap, Thermo Scientific, Waltham, MA, U.S.A.).

6.2.5. Quality assurance and quality control

Identification of the quantitatively targeted analytes relied on matching retention times with authentic standards. For UHPLC-MS/MS analyses, two transitions were followed for each compound. Both LC-MS/MS transitions should have chromatographic peaks with signal-to-noise ratios (S/N) ≥3 at the set LOD, and peaks with S/N ≥10 at the set LOQ. The other criterion for positive identification was the compliance of relative response ratios of the two LC-MS/MS transitions, which should not deviate more than ± 30% from those in the calibration curve (European Commission, 2017). For UHPLC-HRMS analyses, the exact mass tolerance of the extracted full-scan HRMS chromatograms was set at ± 5 ppm (Kaboré et al. 2018).

Injection blanks and laboratory procedural blanks (method blanks) were performed for each batch of analyses and did not show detectable levels of the targeted analytes except low levels of ATZ and AMPA (<2 ng L^{-1}). The blank contribution of ATZ and AMPA was therefore subtracted from the samples from the corresponding batch. Field blanks were performed in triplicate by filling 1-L amber glass containers with HPLC-grade water onboard the *Lampsilis* research vessel and storing and processing these blanks in parallel to the collected surface water samples. A sampler blank was also performed by passing HPLC-grade water through the Niskin - Go Flow system and retrieving the water in sampling bottles.

Analyte quantification relied on matrix-matched calibration curves with internal standards (Montiel-León et al. 2018). Method limits of detection (mLODs) were determined following the procedure described in SI. The linearity performance was considered acceptable as per international requirements with determination coefficients (R^2) >0.9950. The accuracy along the LC-MS sequence was controlled by inserting continued calibration verification (CCV) standards after every ~10 samples, immediately followed by an injection blank to control the absence of carryover. The accuracy of CCV standards was in the range of 84-123% for glyphosate, 91-130% for AMPA, 92-112% for triazines, and 81-113% for systemic insecticides, which falls within the accepted range of 70-130% (Smith et al. 2007). For glyphosate and AMPA, the whole-method precision was also evaluated by performing triplicate analyses on a subset (~10%) of the field samples (SI **Table S6.4**).

6.2.6. Statistical analyses

Statistical significance was set at a 0.05 p-value cutoff. Statistical analyses were conducted with the R statistical software. Non-detect data (<LOD) were taken into account in the computation of descriptive statistics (mean, median) and correlations, wherein non-detect data were substituted by 0. Correlations were examined for those compounds with high detection frequencies (i.e., those with sufficient datapoints). Spearman's rank order correlation was preferred to reduce the influence of high-end concentrations.

6.3. Results and discussion

6.3.1. Occurrence data and concentration levels

Overall, 99% of the surface water samples (n = 68) were found to be positive to at least one of the targeted pesticides. About two-thirds of the samples were positive to at least one neonicotinoid. Compound-specific descriptive statistics are summarized in **Table 6.1**. Out of the 14 quantitatively targeted compounds, 7 were found above the mLODs (**Table 6.1**). The most often detected pesticides were glyphosate (84% of the samples), atrazine (82%), thiamethoxam (59%), DEA (47%), and clothianidin (46%). Imidacloprid and AMPA were found less recurrently (10% and 16%, respectively).

Glyphosate concentrations spanned about three orders of magnitude (<2–3,000 ng L^{-1}), with a median at 27 ng L^{-1} (**Table 6.1**). Glyphosate was also quantified in Ontario, Canada,

with upper-range concentrations as high as 41,000 ng L^{-1} in some rivers (Struger et al. 2008) and 12,000 ng L^{-1} in urban creeks (Byer et al. 2008). Elsewhere, glyphosate concentrations in surface waters impacted by agricultural activities have been reported between ~100 ng L^{-1} and ~700,000 ng L^{-1} (Ruiz-Toledo et al. 2014; Sanchís et al. 2011; Mörtl et al. 2013, Peruzzo et al. 2008).

In the present study, atrazine concentrations were in the range of <4-666 ng L^{-1} (median = 11 ng L^{-1}); 56 out of 68 samples displayed concentrations above the mLOD (4 ng L^{-1}). Comparatively, atrazine was reported above the LOD (22 ng L^{-1}) in 24% of raw water samples in a large-scale survey of drinking water treatment plants in U.S. states, with concentrations between <22-323 ng L^{-1} (Glassmayer et al. 2017). Atrazine was reported between 26-241 ng L^{-1} in the Susquehanna River near its discharge point into the Chesapeake Bay, U.S.A. (Foster et al. 2000). It was systematically detected (100%) in Haihe River samples collected in 2009-2010 near Beijing (China), with a concentration range of 5-590 ng L^{-1} (Heeb et al. 2012). Even if atrazine was banned in 2003 in the E.U., it is still detected in several surface water systems. In a Pan-European survey of riverine surface waters (n = 122), Loos et al. (2009) reported detection frequencies of 68% for atrazine (LOD = 1 ng L^{-1}), with a range of <1-46 ng L^{-1} (mean = 3 ng L^{-1}). Palma et al. (2014) also observed high detection frequencies of atrazine (75–100%) in the Alqueva reservoir in Portugal (2011-2012) with overall low levels (<0.4-19 ng L^{-1}).

Among the targeted neonicotinoids, thiamethoxam and clothianidin showed the highest concentrations, respectively at <1-42 and <1-70 ng L^{-1} (**Table 6.1**). Thiamethoxam and clothianidin displayed average concentrations in surface waters from the present study (n = 68) of ~4 ng L^{-1} each. This is about 20 times lower than the median value from worldwide surface water monitoring data (Morrissey et al. 2015). Imidacloprid was the only other detected compound but at relatively lower concentrations (1.2-11 ng L^{-1}). This agrees with neonicotinoid profiles observed in 2015 for agricultural floodplain waters in Quebec (Montiel-León et al. 2018). In southwestern Ontario, residues of thiamethoxam and clothianidin were also reported in surface waters adjacent to maize-producing areas with high detection frequencies (98.7-100%) and maximum concentrations of 17 and 44 ng L^{-1}, respectively (Schaafsma et al. 2015). In the U.S.A., similar patterns were documented in streams in a high corn and soybean producing region (Hladik et al. 2014). In their study, detection frequencies were also higher for clothianidin and thiamethoxam than for imidacloprid (47-75% *vs.* 23%), as were maximum concentrations (185-257 *vs.* 43 ng L^{-1}) (Hladik et al. 2014).

Table 6.1. Descriptive statistics of the detected pesticides in surface water samples (n = 68) from the St. Lawrence River and tributaries, including detection frequency (% of samples ≥mLOD), concentration range (min-max), mean, and median (ng L^{-1}).

	mLOD ng L^{-1}	Detection frequency %	Range ng L^{-1}	Mean* ng L^{-1}	Median* ng L^{-1}
Glyphosate	2	84	<2-3,000	109	26.9
AMPA	10	16	<10-656	NC**	<10
Clothianidin	1	46	<1-70	3.6	<1
Imidacloprid	1.2	10	<1.2-11	NC	<1.2
Thiamethoxam	1	59	<1-42	3.8	1.6
Atrazine	4	82	<4-666	29.2	11.1
DEA	4	47	<4-192	18.7	<4
Σ_6Neonicotinoids***	-	63	<mLOD-115	7.8	3.5
$\Sigma_{ATZ+DEA}$	-	84	<mLOD-860	48	16.4

*The mean and median include those values below the detection limit (replaced by 0 for the purpose of calculation).

**NC: not calculated for those compounds with high censoring percentages.

***Sum of 6 priority neonicotinoids (acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam).

Spearman's rank correlation indicated significant positive relations between thiamethoxam and clothianidin (Spearman's ρ = 0.750). This is expected since clothianidin is also a degradation product of thiamethoxam (Jeschke et al. 2011). Atrazine and its metabolite DEA were also significantly correlated (ρ = 0.494). Interestingly, a significant correlation was found between glyphosate and neonicotinoids (ρ = 0.568-0.606), while neither ATZ nor DEA were correlated to the other targeted pesticides classes suggesting different sources or environmental dynamics. Data analysis revealed noteworthy spatial trends between pesticides, especially in the first surveyed tract of the St. Lawrence (Section 5.3.3). ATZ was more prevalent in the south portion of the river (Great Lakes water mass) while glyphosate and neonicotinoids were more recurrent in the water masses flowing north of Montreal Island (Ottawa River inputs) (Section 5.3.3). This does not mean that glyphosate and neonicotinoids are not used in the Great Lakes area, but rather that they are significantly trapped/degraded upon transport. This could explain why

they would only occur at low levels in the Lake St Francis – Lake St. Louis area (220 km downstream from the outlet of Lake Ontario), as opposed to Des Prairies and Mille Iles rivers with the much closer Ottawa River agricultural sources (Section 5.3.3). In surface waters, glyphosate dissipation may proceed from sorption onto sediment but also biodegradation from biofilms and sediment-dwelling microorganisms (Battaglin et al. 2014; Carles et al. 2019). Abiotic degradation is not expected to be a significant driver of glyphosate variations, while neonicotinoids can undergo hydrolysis and photolysis (Bonmatin et al. 2015; Lu et al. 2015; Todey et al. 2018).

6.3.2. Compliance to surface water quality criteria

Diverse quality criteria are available to determine the status of surface water chemical pollution (**Table 6.2**). The MELCC sets a criterion of 800 $\mu g \cdot L^{-1}$ for glyphosate and 1.8 $\mu g\ L^{-1}$ for atrazine in Quebec (CCME, 2012) (http://www.environnement.gouv.qc.ca/eau/criteres_eau/). Although glyphosate and atrazine were frequently detected, none of the samples were found to surpass the Quebec surface water quality guideline for the protection of aquatic life (chronic exposure). The highest concentration reported in the present study for glyphosate (3 $\mu g\ L^{-1}$) is also ~700× lower than the NOEC for aquatic plants, although formulations of glyphosate may have different toxicity due to the contribution of co-formulants (e.g., surfactants) (Mensah et al. 2015). The maximum atrazine concentration observed in the present study (0.67 $\mu g\ L^{-1}$) remained lower than the U.S. EPA chronic toxicity criterion for aquatic wildlife at 10 $\mu g\ L^{-1}$ (US EPA, 2006), and lower than the E.U. maximum admissible concentration (MAC-EQS) at 2 $\mu g\ L^{-1}$ (2008/105/EC). Chronic toxicity studies of atrazine indicated lowest observed adverse effect concentrations (LOAEC(21d)) in the order of 120 $\mu g\ L^{-1}$ for fish (*Salvelinus fontinalis*), 140-230 $\mu g\ L^{-1}$ for invertebrates (*Chironomus tentans*, *Gammarus fasciatus*), and no effect concentrations (NOEC(>10d)) in the order of 10 $\mu g\ L^{-1}$ for some freshwater plants (*Lemna gibba*) (US EPA, 2006). The highest concentration of atrazine reported in the present study is thus between 15-350× lower than the above-mentioned chronic endpoint values.

The Canadian Council of Ministers of the Environment proposed a preliminary water quality guideline of imidacloprid for the protection of aquatic life (long-term exposure) at 0.23 $\mu g\ L^{-1}$ (CCME, 2007). The criterion used by the Quebec Ministry of the Environment (MELCC) was revised in recent years to 0.0083 $\mu g\ L^{-1}$ (RIVM, 2014) for imidacloprid and the

sum of six priority neonicotinoids. In the present study, the sum of six priority neonicotinoids ranged from below the mLOD to a maximum of 115 ng L^{-1}. The maximum value is about an order of magnitude higher than the chronic exposure criterion for aquatic wildlife (8.3 ng L^{-1}) but remains below the acute exposure criterion (200 ng L^{-1}) (RIVM, 2014). Overall, 31% of the surface water samples from the present survey (n = 68) were found to surpass the chronic exposure criterion (8.3 ng L^{-1}) for the sum of priority neonicotinoids. Exceedances to the Σ_6Neonicotinoids chronic exposure criterion were more often observed in tributaries (67%) compared to the St. Lawrence River (22%) (Section 3.3).

Table 6.2. International guidelines for the protection of the aquatic life for the target analytes (neonicotinoids, triazines and glyphosate).

Compound	Guideline (µg L^{-1})	Organization	Reference
Imidacloprid	0.23	Canadian Council of Ministers of the Environment	CCME, 2007
ΣNeonicotinoids	0.0083	Quebec Ministry of the Environment	RIVM, 2014
Atrazine	1.8	Canadian Council of Ministers of the Environment	CCME, 2012
Atrazine	10	U.S. Environmental Protection Agency	USEPA, 2006
Atrazine	2	European Union Directive	2008/105/EC
Glyphosate	800	Canadian Council of Ministers of the Environment	CCME, 2012

6.3.3. Spatial distribution

The database gathered in the present survey was used to explore the spatial trends of pesticides within the St. Lawrence River and tributaries. After the junction of the Ottawa and St. Lawrence rivers, only limited transversal mixing between the different water masses is expected to occur until farther east. This is visible in satellite imagery showing the blue-green waters of the Great Lakes in the central and south portions of the fluvial St. Lawrence, while darker waters flow close to the north shore (Ottawa River). If pesticides would originate from different upstream sources (Great Lakes *vs.* Ottawa River), or if side tributaries discharge different loads of pesticides, this may be reflected in their spatial distribution within the St. Lawrence River. Sampling points located downstream from the Montreal Island may also be influenced by the Montreal wastewater treatment plant (WWTP) effluent.

Glyphosate and AMPA. Glyphosate concentrations in the St. Lawrence River ranged between <2 and 202 ng L^{-1} (**Figure 6.2**). Note that the contribution from tributaries other than

the Ottawa River is not shown in this map but can be consulted in **Table 6.3**. Glyphosate concentrations were either low (<10 ng L^{-1}) or non-detectable (5/8 sampling sites) in the SLR before its junction with Des Prairies and Mille Iles rivers. The main upstream source (Lake St. Francis) did not show any detectable levels of glyphosate. While glyphosate is used in Great Lakes watersheds, its strong sorption capacity to soils and sediments, where enhanced biodegradation may occur (Mensah et al. 2015), may explain the lack of detectable levels in the upper St. Lawrence and corresponding water mass south of Montreal Island. In contrast, higher concentrations were observed from the Ottawa River (**Figure 6.2a**), with a glyphosate concentration at ~100 ng L^{-1}. Relatively high glyphosate concentrations were also observed north of Montreal and Laval Islands, as well as in L'Assomption River, a north shore tributary impacted by agriculture (**Table 6.3**). The marked difference between the two water masses (first tract of the St. Lawrence *vs.* Ottawa/Des Prairies/Mille Iles rivers) contributed to distinct glyphosate contamination patterns until after the junction of the water masses. This is apparent in the glyphosate profile of cross-sections at Repentigny and Contrecœur (**Figure 6.2a**), as well as near the Sorel Islands area (**Figure 6.2b**). We observed higher values for the sampling points closer to the north shore (brown and agricultural waters of the Ottawa and L'Assomption rivers) and lower values for those in the middle and south portions of the river (blue-green waters from the Great Lakes).

Interestingly, different patterns were observed in Lake St. Pierre and beyond (**Figure 6.2c**). We noted decreased glyphosate levels near the north shore and increased levels near the south shore. Agriculturally-impacted south shore tributaries enter the St. Lawrence River at Sorel-Tracy and downstream, with high levels of glyphosate at their mouths (134-3,000 ng L^{-1}). In contrast, north shore tributaries discharging into Lake St. Pierre showed low or non-detectible levels of glyphosate (**Table 6.3**), in agreement with the low percentage of land use devoted to agriculture in these watersheds. The percent area of the basin devoted to maize and soja is typically 5-10% for north shore tributaries such as Maskinongé, Du Loup and Yamachiche rivers, compared to 18% and 34% for Nicolet and Yamaska rivers in the south shore (Giroux, Hébert and Berryman, 2016).

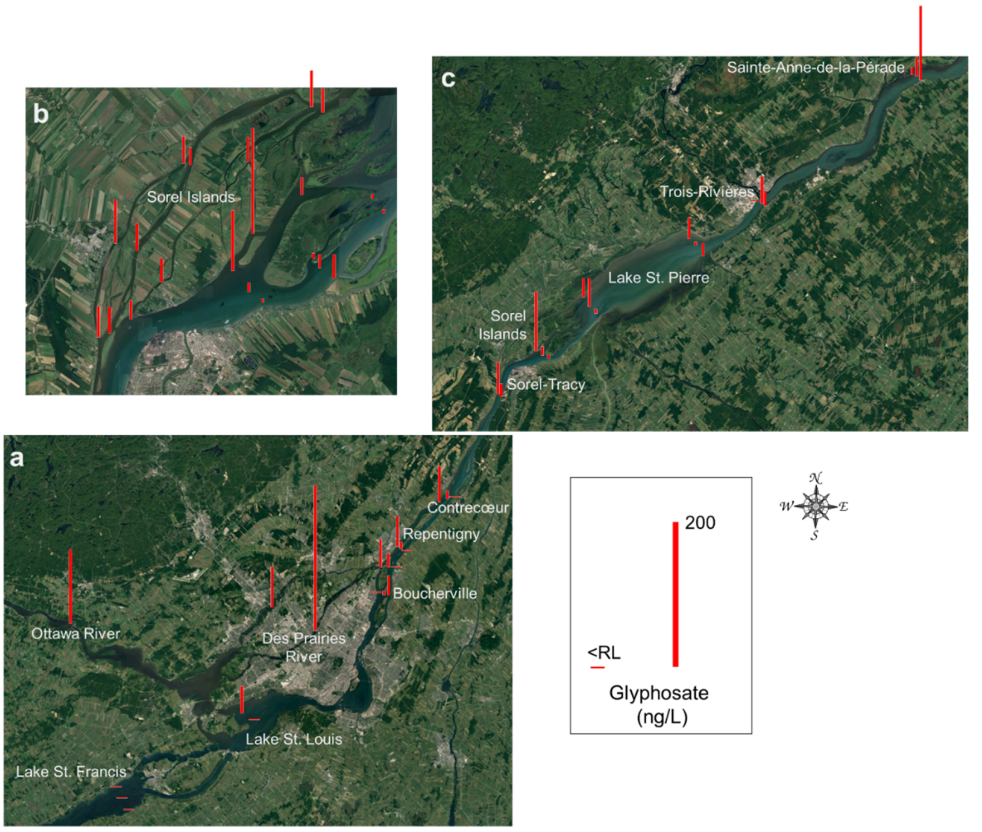

Figure 6.2. Spatial distribution of glyphosate concentrations (ng L^{-1}) in a 200-km reach of the St. Lawrence River. Panels **2a** and **2c** are arranged to facilitate the visualization of the SLR flow in a northeastern direction (see also **Figure 1**). **2a**: a first section including the mouth of the Ottawa River and the St. Lawrence from Lake St. Francis to Contrecœur; **2b**: zoom-in on Sorel Islands; **2c**: second section of the St. Lawrence from Sorel-Tracy to Sainte-Anne-de-la-Pérade.

AMPA was only detected at 6 monitoring locations within the St. Lawrence, at low concentrations (range: 12-88 ng L^{-1}). Similar to glyphosate, AMPA was less frequently detected in north shore tributaries compared to south shore tributaries where it was also reported at the highest concentrations (**Table 6.3**). In south shore tributaries, AMPA was detected when high concentrations of glyphosate occurred (mean ratio of 0.31 ± 0.07). Note that AMPA could originate not only from glyphosate but also from the degradation of phosphonic acids found in other types of household and industrial products such as detergents (Battaglin et al. 2014; Botta et al. 2009; Poiger et al. 2017; Struger, Van Stempvoort and Brown, 2015). This would seem a reasonable explanation for the AMPA detection in the Saint-Maurice River (where glyphosate was not detected), since this tributary is not impacted by high agricultural pressures but may be subjected to urban influence from the city of Trois-Rivières.

Table 6.3. Concentrations (ng L^{-1}) of the quantitatively targeted compounds in tributaries from the north and south shores of the St. Lawrence River. Analytes that were not found at any of the surveyed sites are not shown in this table (nd: analyte not detected).

		Surface water concentration (ng L^{-1})						
		Glyphosate	**AMPA**	**Clothianidin**	**Thiamethoxam**	**Imidacloprid**	**Atrazine**	**DEA**
North shore tributaries	L'Assomption River	105	nd	17	26	4.4	nd	nd
	Batiscan River	2.8	nd	nd	nd	nd	nd	nd
	Du Loup River	17	nd	9.2	3.4	nd	nd	nd
	Maskinongé River	24	nd	nd	1.6	nd	nd	nd
	Saint-Maurice River	nd	33	nd	nd	nd	nd	nd
	Yamachiche River	nd	nd	16	1.6	nd	nd	nd
South shore tributaries	Bécancour River	539	185	8.7	28	nd	213	83
	Saint-François River	134	40	10	5.1	1.8	15	18
	Nicolet River	3,000	656	24	14	nd	320	26
	Richelieu River	105	nd	3.4	nd	nd	17	nd
	Yamaska River	1,647	628	70	42	2.8	666	192
Other	Ottawa River	101	nd	1.6	2.1	nd	nd	nd
	Mille Iles River	56	88	6.2	14	nd	23	nd
	Des Prairies River	202	nd	3.7	nd	nd	7.7	nd
	Du Nord River	40	nd	nd	nd	11	nd	nd

Atrazine and DEA. Atrazine and desethylatrazine (DEA) showed quite distinct patterns than those of glyphosate, in relation with different contamination sources of the St. Lawrence and different environmental fate. Atrazine is characterized by a relatively high persistence and low sorption onto sediments, which may explain its long-range transport from the Laurentian Great Lakes. The $\Sigma_{ATZ+DEA}$ averaged 27 ng L^{-1} in the first track of the SLR between Lake St. Francis and Boucherville Islands (corresponding to the Great Lakes water mass), about 3 times higher than those water masses that flow north of Montreal Island (Ottawa River). After the junction of the rivers downstream from Montreal Island, the cross-sections showed consistent patterns.

The highest concentrations of atrazine and DEA within the St. Lawrence were observed near the effluent plume of the Montreal wastewater treatment plant ($\Sigma_{ATZ+DEA}$ = 43-106 ng L^{-1}), and in the middle and south parts of cross-sections from the eastern end of Lake St. Pierre to Sainte-Anne-de-la-Pérade ($\Sigma_{ATZ+DEA}$ = 57-119 ng L^{-1}; average = 92 ng L^{-1}). The Great Lakes have been identified as a major source of atrazine to the St. Lawrence River (Pham et al. 2000), which may explain the distinctive pattern between north and middle/south locations within transects of the St. Lawrence. In addition, neither atrazine nor DEA were reported in the surveyed north shore tributaries, compared to near-systematic detections in south shore tributaries (**Table 6.3**). This distinctive profile would also help interpret the results of a quality survey of tap water essentially covering the same area and time period. In a companion paper, we observed near-systematic detections and relatively high levels of atrazine and DEA in drinking water of municipalities located along the southern shore and using the St. Lawrence River as a source (Montiel-León et al., 2019). This signals the potential exposure of a large portion of the population of Quebec to low yet chronic levels of these herbicides in drinking water produced from the river.

Systemic insecticides. Detections of systemic insecticides were less frequent in the St. Lawrence River itself (55%) than in its surveyed tributaries (86%). We derived a median concentration of 1.6 ng L^{-1} for the sum of 6 priority neonicotinoids within the St. Lawrence (average Σ_6Neonicotinoids = 3.7 ng L^{-1}). Comparatively, the median concentration in tributaries was 15 ng L^{-1} (average Σ_6Neonicotinoids = 23 ng L^{-1}). Spatial patterns of neonicotinoids in the first surveyed tract of the river (**Figure 6.4a**) are similar to those of glyphosate, with few detection frequencies (25%) in the SLR from Lake St. Francis to Boucherville Islands, but higher levels for water masses flowing north of Montreal Island. This observation together with the contributions from L'Assomption River, explain the St.

Lawrence cross-sectional profiles observed downstream from the Montreal Island (**Figures 6.4a-4b).** Considering the tributaries to Lake St. Pierre, higher neonicotinoid levels were reported for those from the south shore (**Figure 6.4c** and **Table 6.3**). Barring the case of the Richelieu River, south shore tributaries generally surpassed the surface water quality criterion (8.3 ng L^{-1}), with the highest value for the Yamaska River (Σ_6Neonicotinoids =115 ng L^{-1}).

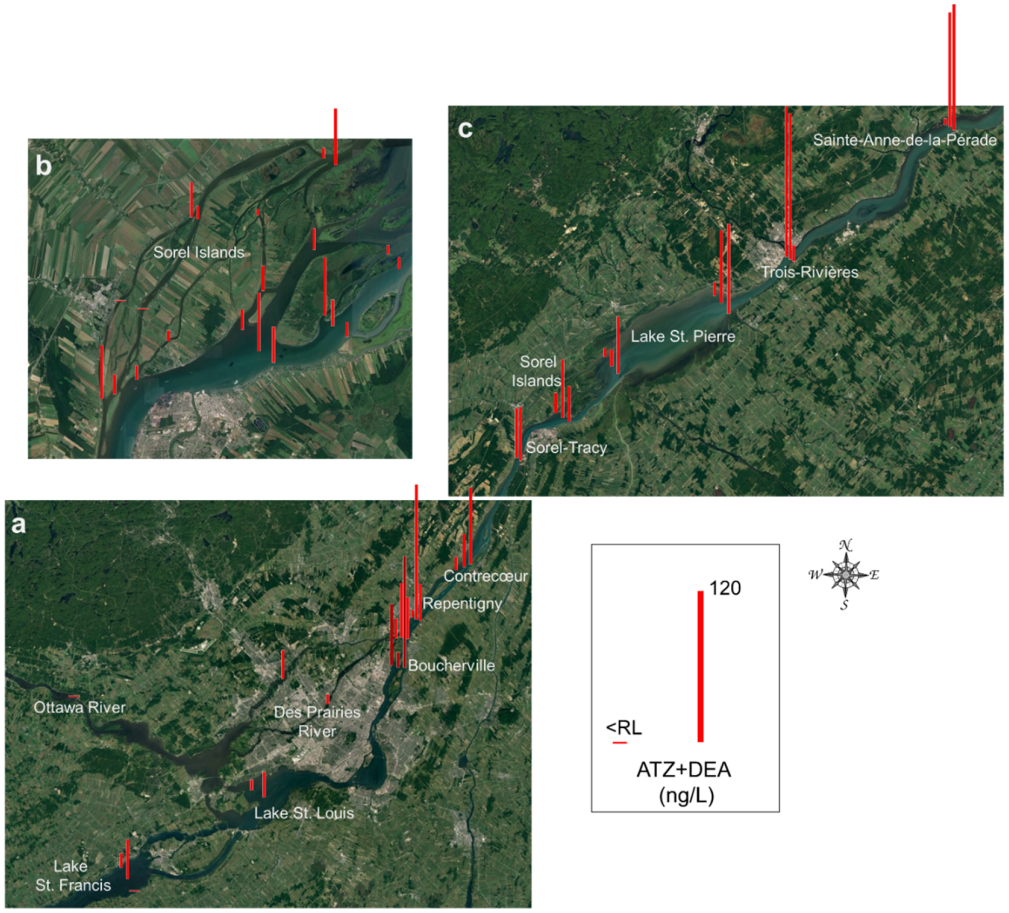

Figure 6.3. Spatial distribution of the sum of atrazine and desethylatrazine surface water concentrations (ATZ+DEA; ng L^{-1}) in a 200-km reach of the St. Lawrence River. Panels 3**a** and 3**c** are arranged to facilitate the visualization of the SLR flow in a northeastern direction. 3**a**: a first section including the mouth of the Ottawa River and the St. Lawrence from Lake St. Francis to Contrecœur; 3**b**: zoom-in on Sorel Islands; 3**c**: second section of the St. Lawrence from Sorel-Tracy to Sainte-Anne-de-la-Pérade. For clarity, the transect near the Boucherville Islands area is shown in a distinct shade of red.

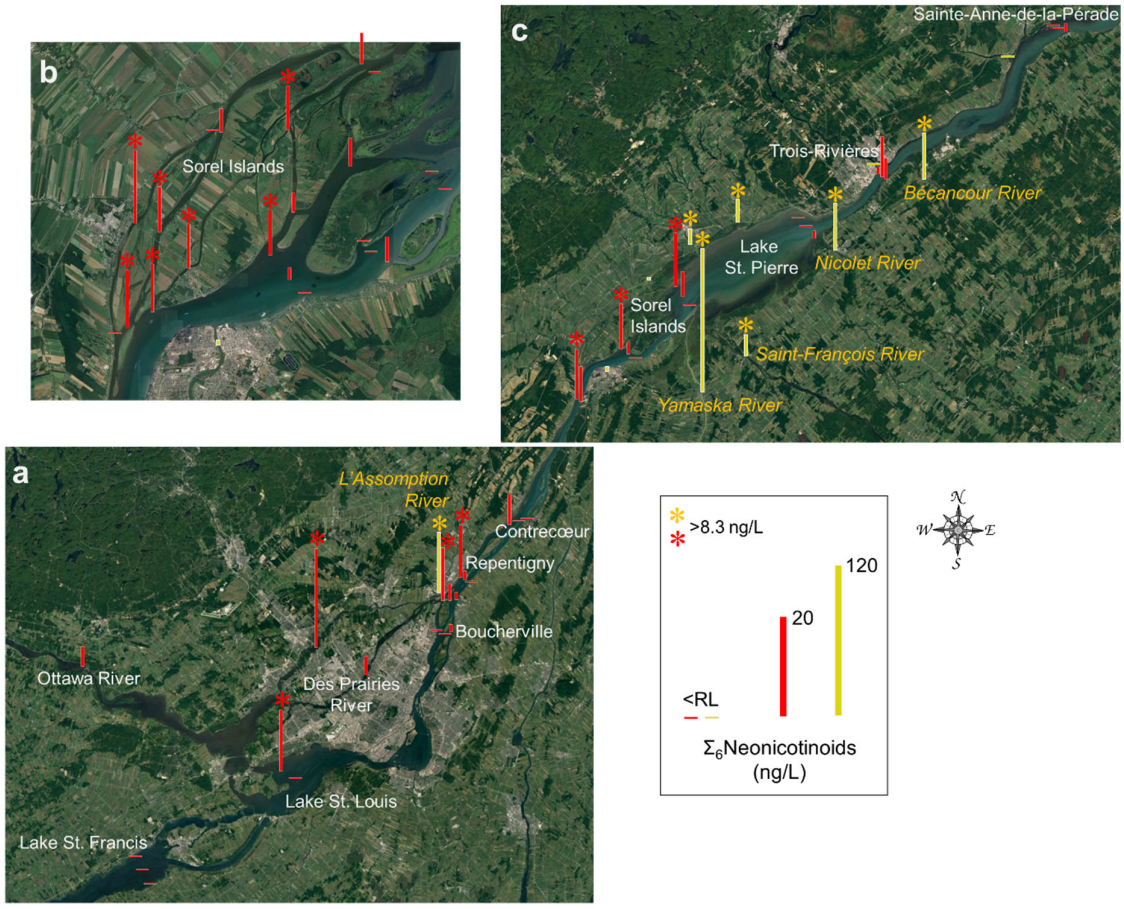

Figure 6.4. Spatial distribution of the sum of six priority neonicotinoids (Σ_6Neonicotinoids; ng L^{-1}) in the St. Lawrence River and tributaries. Yellow bars refer to side tributaries (north and south shores); note the different scales for yellow and red bars. Exceedances to the 8.3 ng L^{-1} surface water quality criterion (aquatic life, chronic exposure) are signaled with a (*) symbol. Panels **4a** and **4c** are arranged to facilitate the visualization of the SLR flow in a northeastern direction (see also **Figure 1**). **4a**: a first section including the mouth of the Ottawa River and the St. Lawrence from Lake St. Francis to Contrecœur; **4b**: zoom-in on Sorel Islands; **4c**: second section of the St. Lawrence from Sorel-Tracy to Sainte-Anne-de-la-Pérade.

6.3.4. Chemical load estimates

A preliminary assessment of the mass budget of pesticides in this reach of the St. Lawrence was conducted. Using a SLR flowrate of ~8,000 m^3 s^{-1} at Sorel for July months (www.planstlaurent.qc.ca), we estimate that the mass load of chemicals transiting in July 2017 through the first surveyed tract was 660 kg/month for glyphosate, 250 kg/month for atrazine and 80 kg/month for the sum of six priority neonicotinoids. This represents a relatively low chemical loading, especially for such a large hydrosystem (SI **Table S6.5**). The estimates for

atrazine in this reach of the SLR are about 130-250 kg/month when considering data from the present survey (July 2017) and those of Lemieux et al. (1995) covering a smaller tract of the SLR in Lake St. Pierre (1990-1991 data). Comparatively, the mass inventory of atrazine in summer months was estimated between 5,000-10,000 kg/month in the lower Mississippi River, and in the order of 2,400 kg/month in the Missouri River near its mouth (Pereira and Rostad, 1990) (see also SI **Table S6.5**).

Several tributaries enter the St. Lawrence in the Sorel - Lake St. Pierre area (**Figure 6.1**) and may contribute to discharge important loads of pesticides, especially following the application season. The flowrates (m^3 s^{-1}) used for this mass inventory calculation were as follows for north shore tributaries (Du Loup: 1; Maskinongé: 8; Yamachiche: 7) and south shore tributaries (Nicolet: 14; Richelieu: 290; Saint-François: 70; Yamaska: 28). (http://www.wsc.ec.gc.ca; https://eau.ec.gc.ca/mainmenu/historical_data_index_f.html). The combined discharge of these side tributaries to the SLR was estimated at 300 kg/month for glyphosate, 80 kg/month for atrazine, and 16 kg/month for neonicotinoids. Among these tributaries, those from the south shore contributed between ~97% (neonicotinoids) and >99.8% (glyphosate, atrazine) of the summed side tributary discharge during the surveyed period. The Nicolet, Richelieu, and Yamaska rivers contributed respectively to 33%, 24%, and 36% of the summed tributary discharge of glyphosate. The Yamaska River was an influential contributor to the summed tributary discharge of atrazine (64%) and Σ_6Neonicotinoids (53%) into the SLR - Lake St. Pierre area. The Saint-François River was responsible for minor contributions to the summed tributary discharge of glyphosate and atrazine, but represented 20% of that of neonicotinoids.

Side tributaries discharging into the Lake St. Pierre area accounted for a non-negligible contribution compared to the inflow from the SLR itself (**Figure 6.5**). For instance, the combined discharge of south shore tributaries contributed to a monthly input of ~300 kg glyphosate (July 2017), about 45% of the SLR input. South tributaries to Lake St. Pierre contributed to a monthly input of ~80 kg atrazine (July 2017), about 32% of the SLR input to the lake. Atrazine showed a conservative behavior, with the outflow of Lake St. Pierre nearly equating to the sum of the St. Lawrence inflow and inputs from side tributaries (**Figure 6.5b**), while for glyphosate an incomplete mass balance was observed, i.e., ~60% of glyphosate inputs exit Lake St. Pierre (**Figure 6.5a**). Significant attenuation of micropollutants may occur in the waters of the Sorel Islands archipelago and Lake St. Pierre through biodegradation processes. Additionally, while the central portion of the lake (blue-green waters of the Great Lakes) is

characterized by low suspended particulate matter (SPM) and higher current velocities, the north and south lake portions show higher SPM levels (Hudon and Carignan, 2008). This may also contribute to sequestration of the glyphosate inputs from Ottawa River (north lake portion) and south shore tributaries (south lake portion), the much lower flow velocities (Hudon and Carignan, 2008) allowing sedimentation and thus enhanced removal from the water column.

Consistent loads of atrazine were previously observed (August 1990) in the SLR at Les Grèves/Contrecœur (upstream Lake St. Pierre) and transects further downstream Lake St. Pierre, which seems to corroborate our findings (Lemieux and Lum, 1996). Our current data provide some insights into the spatial distribution of the various chemical contaminants. Note that while the present survey was conducted in the mid-summer, higher concentrations of pesticides may occur after pesticide applications in late spring/early summer (Byer et al. 2011). The spread of the St. Lawrence River watershed also complicates the interpretation of what happens in the field and how profiles evolve much further down the river. The long transport time may indeed contribute to the degradation and transformation of pesticides in the water column. The temporal variations due to seasonal changes, precipitation and agricultural activities must, therefore, be considered in future assessments of surface water quality, as illustrated earlier for drinking water produced from the river (Montiel-León et al., 2019).

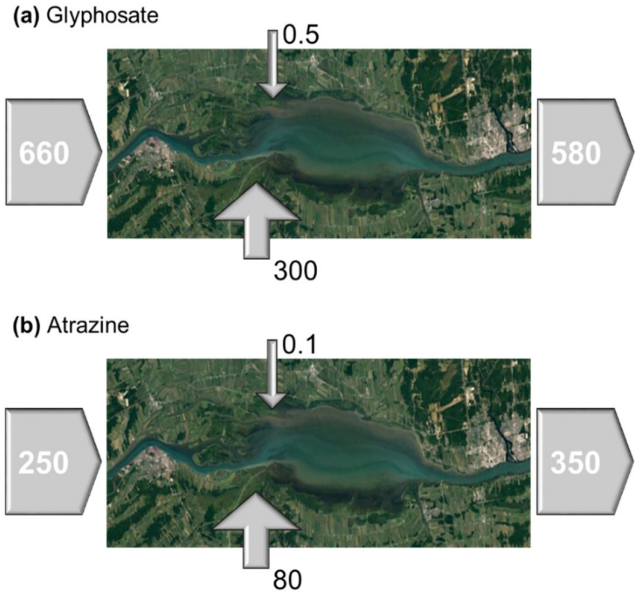

Figure 6.5. Schematic view of the chemical loads (kg/month) of glyphosate (**5a**) and atrazine (**5b**) transiting in and out of the Sorel – Lake St. Pierre area during the surveyed period (July 2017). Arrows indicate summed contributions from the surveyed north and south shore tributaries.

6.4. Conclusions

Glyphosate and atrazine were the most frequently detected compounds in surface water samples from a 200-km reach of the St. Lawrence River and its tributaries. However, their concentrations remained well below the Canadian water quality guidelines for the protection of aquatic life. Nearly one-third of the surface water samples had summed neonicotinoid concentrations above the criterion of 8.3 ng L^{-1}. Limited transversal mixing of the different water masses flowing within the SLR allowed to match specific contaminant types with major water masses. Atrazine was found predominately in the middle and south portions of transects (Great Lakes water mass), while glyphosate and neonicotinoids were rather found in the north portions (Ottawa River). Due to the different sources, the hydrological features of the system and different sensitivity to physical and biochemical degradations, pesticide concentrations were therefore quite variable within the St. Lawrence.

On a larger scale, the SLR is impacted by management decisions upstream and may affect the estuary and the marine system downstream. Upstream of the SLR, atrazine usage is believed to have declined in the last decades in Ontario but remains the second most widely used pesticide in the U.S. after glyphosate (USEPA, 2017). Due to slow environmental decay, some scenarios predict that ATZ concentrations may still increase in the Great Lakes (Rygwelski et al. 2012), remaining major contributors to the St. Lawrence ATZ loads. How much of the pollutant loads transported by the fluvial St. Lawrence would be exported into the St. Lawrence Gulf and the Atlantic Ocean has not been explored in the present study. Further monitoring efforts are underway to characterize contaminants of emerging concern within a longer reach of the St. Lawrence, from its source near Kingston (ON) to the Anticosti Island (QC).

Acknowledgments

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6.5 Supporting Information

Details on chemicals and materials

Native standards of acetamiprid (purity ≥99.9%), clothianidin (purity ≥99.9%), desnitro-imidacloprid hydrochloride (purity ≥99.8%), dinotefuran (purity ≥98.6%), imidacloprid (purity ≥99.9%), nitenpyram (purity ≥99.9%), thiacloprid (purity ≥99.9%), thiamethoxam (purity ≥99.6%), fipronil (purity ≥97.9%), atrazine (ATZ; purity ≥97%), desethylatrazine (DEA; purity ≥97%), glyphosate (purity≥98%), and aminomethylphosphonic acid (AMPA; purity≥99%) were all obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). Isotope-labeled internal standards imidacloprid-d_4 (purity ≥99.9%), acetamiprid-d_3 (purity ≥98%), clothianidin-d_3 (purity ≥99.9%) and thiamethoxam-d_3 (purity ≥99.9%) were also obtained from Sigma Aldrich (St. Louis, MO, U.S.A.), while fipronil-$^{13}C_4$ (purity ≥98%) was obtained from Santa Cruz Biotechnology (Dallas, TX, U.S.A.), atrazine-$^{13}C_3$ (purity ≥99.9%) from Cambridge Isotope Laboratories, Inc. (Andover, MA, U.S.A.) and glyphosate-$^{13}C_2$-^{15}N (purity ≥99%) from Toronto Research Chemicals (North York, ON, Canada).

Solvents were all HPLC-grade quality and were purchased from Fisher Scientific (Whitby, ON, Canada). Fluorenylmethyloxycarbonyl chloride (FMOC-Cl; purity ≥97%), ethylene diamine tetraacetic acid disodium salt dihydrate (purity ≥99%), sodium tetraborate decahydrate (purity ≥99.5%), and formic acid (purity ≥95%) were all acquired from Sigma-Aldrich (St. Louis, MO, U.S.A.). Glass fiber filters (GFF; 0.3 μm) and polyester membrane filters (0.2 μm) were obtained from the Sterlitech Corporation (Kent, WA, U.S.A.).

Determination of method limits of detection (mLODs)

For the analysis of atrazine and DEA by on-line SPE – UHPLC-MS/MS (TSQ Quantiva), the method limits of detection (mLODs) were determined as follows. A surface water matrix containing known concentrations of ATZ and DEA (at low level) was used for measurement of signal-to-noise ratio (S/N). Since two LC-MS/MS transitions were followed for each compound, the transition with the lowest S/N was used for the mLOD determination (S/N = 3) (Thompson et al. 2002). Since the matrix sample is submitted to on-line SPE – UHPLC-MS/MS, the mLODs integrate potential losses occurring during the on-line extraction process. For the analysis of glyphosate and DEA by derivatization – on-line SPE – UHPLC-HRMS (Q-Exactive Orbitrap) and other pesticides by off-line SPE – UHPLC-HRMS (Q-Exactive Orbitrap), the mLODs were determined as per the calibration curve method, i.e., derived from the error on the y-intercept and the slope of the regression of the calibration curve (Araujo, 2009).

Araujo, P. (2009). Key aspects of analytical method validation and linearity evaluation. *Journal of Chromatography B*, *877*(23), 2224-2234.

Thompson, M., Ellison, S.L.R., Wood, R. (**2002**) Harmonized guidelines for single-laboratory validation of methods of analysis. *Pure and Applied Chemistry*, 74(5), 835-855.

Analysis of glyphosate and AMPA

For glyphosate and AMPA analysis, a 4.75-mL aliquot of the filtered water sample was spiked with Glyphosate-$^{13}C_2$-^{15}N isotope-labelled internal standard (47.5 µL of a 10 ng/mL IS solution in HPLC-water) and subjected to the FMOC derivatization procedure described as follows.

The water sample was first acidified with 105 µL of HCl 6M (pH = 1.1-1.3). The sample was briefly vortexed (10 s) and left 1h at room temperature before adjusting the pH using KOH 6M (pH = 6-8). 0.3 mL of borate-Na (150 mM, pH = 9.5) was then added and the sample was briefly vortexed (10 s). Note that 0.2 mL of EDTA-2Na (0.1 M) was added in order to prevent further metal complexation of glyphosate. Following brief vortexing (10s) and a short wait time (5 min, room temperature), 0.6 mL of FMOC-Cl (12 mg/mL in ACN) was added to the sample. The resulting mixture was stirred at 150 rpm (24h, 40 °C, no light) to allow the derivatization reaction to take place.

After this step, the samples were acidified to pH ~2-3 with hydrochloric acid, briefly vortexed, and filtered onto GFF filters. The samples were then processed by on-line SPE – UHPLC-HRMS (**Table S6.1**). The injection volume was 2 mL. The sample was loaded onto a Thermo Hypersep Retain PEP on-line SPE column (20 mm × 2.1 mm) at 1,000 µL·min^{-1}. After this loading step, the on-line mobile phase was left to flow for an additional 1 min before the valve-switching process to allow washing of the residual salts and borax buffer (wash volume = 1 mL). The concentrated analytes were then back-flushed at 400 µL·min^{-1} following the gradient program, and separated onto a Thermo Hypersil Gold C18 column (50 mm × 2.1 mm, 1.9 µm particle size) thermostated at 30 °C. Detection was performed by a Q-Exactive Orbitrap mass spectrometer from Thermo Scientific (Waltham, MA, U.S.A.), operated in full scan MS mode (scan range: 100–450 m/z) and coupled to a polarity-switching ionization interface (allowing to acquire both positive and negative parent ions of the derivatized glyphosate, AMPA, and their corresponding internal standard). Details on chromatographic gradient conditions, source

parameters, Orbitrap parameters, and exact mass (m/z) of targeted compounds can be found in **Table S6.1**.

Table S6.1. LC-HRMS method parameters for the analysis of glyphosate and AMPA.

Instrument	Thermo Q-Exactive Orbitrap mass spectrometer Dionex Ultimate 3000 UHPLC chain
Ionization	Electrospray ionization source (fast polarity-switching mode)
Acquisition mode	Full Scan MS mode
Analytical column	Thermo Hypersil Gold C18 column (50 × 2.1 mm; 1.9 µm)
Column Temperature	30°C
Analytical Mobile Phases	A: 5 mM ammonium acetate in HPLC-water B: acetonitrile Flow rate (mL/min) 0.4
Gradient Profile (analytical column)	Time (min) % B 0.0 10 3.0 10 7.0 50 8.0 100 9.0 100 9.1 10 10 10
Injection Volume	2000 µL (on-line SPE)
On-line SPE column	Thermo Hypersep Retain PEP column (20 mm × 2.1 mm, 40–60 µm)
On-line SPE Mobile Phases	A: HPLC-water B: acetonitrile Flow rate (mL/min) 1
Gradient Profile (on-line SPE)	Time (min) % B 0.0 0 3.0 0 3.1 100 7.0 100 7.1 0 10 0
Source/gas	Sheath gas flow rate 55 Aux gas flow rate 10 Sweep gas flow rate 0 Spray voltage (|kV|) 3.5 Capillary temperature (°C) 320 Vaporizer temperature (°C) 350 S-lens RF level 70
Orbitrap parameters	Resolution 70,000 AGC target 3e6 Maximum Inject Time (ms) 100
Target compounds	Glyphosate ESI+ $[C_{18}H_{19}NO_7P]^+$ 392.08936 ESI- $[C_{18}H_{17}NO_7P]^-$ 390.07371 AMPA ESI+ $[C_{16}H_{17}NO_5P]^+$ 334.08389 ESI- $[C_{16}H_{15}NO_5P]^-$ 332.06824 Gly-$^{13}C_2{}^{15}N$ ESI+ $[C_{16}{}^{13}C_2H_{19}{}^{15}NO_7P]^+$ 395.09147

	ESI-	$[C_{16}{}^{13}C_2H_{17}{}^{15}NO_7P]^+$	393.07582

Analysis of atrazine and DEA

Atrazine and DEA were analyzed as follows. Briefly, a GFF-filtered water aliquot was amended with $^{13}C_3$-ATZ internal standard for a final concentration of 50 ng L^{-1} and processed by on-line solid phase extraction (SPE) coupled to ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). A HTC thermopal autosampler (CTC analytics AG, Zwingen, Switzerland) was used for in-loop sample injection (2 mL injection volume, using a 2-mL loop). The on-line enrichment column and analytical column were both from Thermo Fisher Scientific (San Jose, CA, U.S.A.). The first step consisted in loading 2 mL of sample at 1,500 µL·min^{-1} (on-line mobile phase: HPLC-water with 0.1% HCOOH) into a Thermo Hypersep Retain PEP column (20 mm × 2.1 mm, 40–60 µm particle size). After this step, target analytes were back-flushed at 350 µL·min^{-1} by a H_2O:acetonitrile gradient program (A_1:B_1) and separated onto a Thermo Hypersil Gold C18 column (50 mm × 2.1 mm, 1.9 µm particle size) thermostated at 30 °C. The TSQ Quantiva triple quadrupole mass spectrometer from Thermo Scientific (Waltham, MA, U.S.A.) was coupled to a heated electrospray ionisation (HESI) source. The analyzer was operated in selected reaction monitoring (SRM) mode. The first (Q1) and third (Q3) quadrupoles were operated at a resolution of 0.7 Da FWHM. The collision gas (CID) pressure in the second quadrupole (q2) was set at 1.5 mTorr. Further details on LC gradient programs, source parameters, and compound-dependent MS/MS acquisition parameters are provided in **Table S6.2**.

Table S6.2. LC-MS/MS method parameters for the analysis of atrazine and DEA.

Instrument	Thermo TSQ Quantiva triple quadrupole mass spectrometer Accela 1250 quaternary pump (analytical column) Accela 600 quaternary pump (on-line SPE)
Ionization	Electrospray ionization source (positive ionization mode)
Acquisition mode	SRM (MS/MS)
Analytical column	Thermo Hypersil Gold C18 column (100 × 2.1 mm; 1.9 µm)
Column Temperature	30°C
Analytical Mobile Phases	A: HPLC-water B: acetonitrile

Gradient Profile (Analytical column)

Time (min)	% B	Flow rate (µL/min)
0.0	20	350
1.5	20	350
5.4	95	350
7.4	95	350
7.5	20	350
10	20	350

Injection Volume	2000 µL (on-line SPE)
On-line SPE column	Thermo Hypersep Retain PEP (20 × 2.1 mm; 40–60 µm).
On-line SPE Mobile Phases	A: 0.1% formic acid in HPLC-water B: 0.1% formic acid in acetonitrile

Gradient Profile (On-line SPE)

Time (min)	% B	Flow rate (µL/min)
0.0	0	1500
1.5	0	1500
5.4	100	2000
7.4	100	1500
7.5	0	1500
10	0	1500

Source/gas	Sheath gas flow rate 60 A.U. Aux gas flow rate 20 A.U. Sweep gas flow rate 0 A.U. Ion transfer tube temperature (°C) 350 Vaporizer temperature (°C) 400 Ion Spray voltage (V) +3000

MS/MS parameters

	Parent Ion (m/z)	Product Ion (m/z)	Transition*	RF Lens (V)	Collision Energy (V)
ATZ	216.2	174.0	Q.T.	62	17
		104.0	C.T.	62	29
DEA	188.1	146.0	Q.T.	57	17
		103.9	C.T.	57	26
ATZ-$^{13}C_3$	219.2	176	I.S.	60	18

*Q.T.: quantification transition; C.T.: confirmation transition; IS : internal standard.

Neonicotinoids and fipronil were initially analyzed by processing a 2-mL filtered aliquot using on-line SPE (Montiel-León et al. 2018). In view of their low concentrations in the St. Lawrence River, these analytes were reanalyzed using an off-line SPE workflow (250 mL sample loading volume onto Phenomenex Strata X-AW cartridges (200 mg)) to improve the detection limits. The samples were processed as follows. Before sample loading, cartridges were sequentially washed with i) 2 x 4 mL of 0.2% NH_4OH in MeOH and ii) HPLC-water. A 250-mL aliquot of GFF-filtered surface water amended with isotope-labelled internal standards was loaded onto the SPE cartridges. After sample loading, the cartridges were rinsed with 5 mL of HPLC-water and left to dry for 1h under vacuum. Analytes were eluted with 2 x 4 mL of 0.2% NH_4OH in MeOH. After elution and concentration to 0.4 mL under a gentle stream of N_2 and moderate heating (40 °C), the methanolic extracts were subjected to small volume injection (10 µL) UHPLC-HRMS analysis (Q-Exactive Orbitrap mass spectrometer from Thermo Scientific, Waltham, MA, U.S.A.).

Details on chromatographic gradient conditions, source parameters, Orbitrap parameters, and exact mass (m/z) of targeted compounds can be found in **Table S6.3**.

Table S6.3. LC-HRMS method parameters for the analysis of neonicotinoids and fipronil.

Instrument	Thermo Q-Exactive Orbitrap mass spectrometer Dionex Ultimate 3000 UHPLC chain
Ionization	Electrospray ionization source (fast polarity-switching mode)
Acquisition mode	Full Scan MS mode
Analytical column	Thermo Hypersil Gold C18 column (100 × 2.1 mm; 1.9 µm)
Column Temperature	40°C
Mobile Phases	A: 0.1% formic acid in HPLC-water B: 0.1% formic acid in acetonitrile Flow rate (mL/min) 0.55
Gradient Profile	Time (min) % B 0.0 10 7 72.5 8.5 100 12.5 100 12.6 10 14.6 10
Injection Volume	10 µL
Source/gas	Sheath gas flow rate 45 Aux gas flow rate 10 Sweep gas flow rate 0 Spray voltage (|kV|) 3 Capillary temperature (°C) 320 Vaporizer temperature (°C) 350 S-lens RF level 55
Orbitrap parameters	Resolution 70,000 AGC target 3e6 Maximum Inject Time (ms) 50
Target compounds	Acetamiprid ESI+ $[C_{10}H_{12}ClN_4]^+$ 223.07505 Clothianidin ESI+ $[C_6H_9ClN_5O_2S]^+$ 250.01655 DN-Imidacloprid ESI+ $[C_9H_{12}ClN_4]^+$ 211.07505 Dinotefuran ESI+ $[C_7H_{15}N_4O_3]^+$ 203.11387 Fipronil ESI- $[C_{12}H_3Cl_2F_6N_4OS]^-$ 434.93088 Imidacloprid ESI+ $[C_9H_{11}ClN_5O_2]^+$ 256.06013 Nitenpyram ESI+ $[C_{11}H_{16}ClN_4O_2]^+$ 271.09618 Thiacloprid ESI+ $[C_{10}H_{10}ClN_4S]^+$ 253.03147 Thiamethoxam ESI+ $[C_8H_{11}ClN_5O_3S]^+$ 292.02711 Acetamiprid-d3 ESI+ $[C_{10}H_9D_3ClN_4]^+$ 226.09388 Clothianidin-d3 ESI+ $[C_6H_6D_3ClN_5O_2S]^+$ 253.03538 Fipronil-$^{13}C_4$ ESI- $[C_8{}^{13}C_4H_3Cl_2F_6N_4OS]^-$ 438.94430 Imidacloprid-d4 ESI+ $[C_9H_7D_4ClN_5O_2]^+$ 260.08524 Thiamethoxam-d3 ESI+ $[C_8H_8D_3ClN_5O_3S]^+$ 295.04594

Table S6.4. Whole-method precision as relative standard deviation (RSD, %) of the sample preparation process (derivatization and on-line SPE – UHPLC-HRMS) for glyphosate and AMPA analysis, evaluated on a subset of the field samples from the present survey.

	Glyphosate		AMPA	
	Concentration ($ng\ L^{-1}$)	RSD %	Concentration ($ng\ L^{-1}$)	RSD %
Sorel10-A	6.0		<LOD	
Sorel10-B	7.2	9.9	<LOD	
Sorel10-C	7.1		<LOD	-
LakeStFrancis1-A	ND		<LOD	
LakeStFrancis1-B	ND	-	<LOD	-
LakeStFrancis1-C	ND		<LOD	
StFrançoisRiver-A	144.2		33.0	
StFrançoisRiver-B	102.4	20.6	50.8	23.5
StFrançoisRiver-C	154.3		36.7	
SLR@Trois-Rivières2-A	20.3		<LOD	
SLR@Trois-Rivières2-B	18.2	14.3	<LOD	
SLR@Trois-Rivières2-C	15.2		<LOD	-
SLR@Sainte-Anne1-A	12.0		<LOD	
SLR@Sainte-Anne1-B	7.8	22.8	<LOD	
SLR@Sainte-Anne1-C	12.0		<LOD	-
DesPrairies-A	165.6		<LOD	
DesPrairies-B	235.8	17.4	<LOD	
DesPrairies-C	203.3		<LOD	-

Table S6.5. Comparison of atrazine load estimates in hydrosystems worldwide.

System	Atrazine load	Period	Reference
St. Lawrence river, QC, Canada	**250-350 kg/month**	**2017**	**This study**
Tributary rivers into Lake St. Pierre, QC, Canada	**80 kg/month**	**2017**	**This study**
St. Lawrence river at Contrecœur, QC, Canada	400 kg/month	August 1990	Lemieux & Lum, 1996
St. Lawrence river at Quebec City, QC, Canada	390 kg/month	August 1990	Lemieux & Lum, 1996
St. Lawrence river, QC, Canada	230 kg/month	1990	Lemieux et al. 1995
St. Lawrence river, QC, Canada	130 kg/month	1991	Lemieux et al. 1995
Inputs to Lake Superior (North American Great Lakes)	80 kg/month	1991-1994	Schottler & Eisenreich, 1997
Inputs to Lake Michigan (North American Great Lakes)	1,000 kg/month	1991-1994	Schottler & Eisenreich, 1997
Inputs to Lake Huron (North American Great Lakes)	700 kg/month	1991-1994	Schottler & Eisenreich, 1997
Inputs to Lake Erie (North American Great Lakes)	600-1,500 kg/month	1991-1994	Schottler & Eisenreich, 1997
Inputs to Lake Ontario (North American Great Lakes)	1,300-2,000 kg/month	1991-1994	Schottler & Eisenreich, 1997
Susquehanna river into Chesapeake Bay, U.S.A.	250 kg/month	1994-1995	Foster et al. 2000
Flux to upper Patuxent river estuary, U.S.A.	20 kg/month	1996	McConnell et al. 2004
Flux to lower Patuxent river estuary, U.S.A.	12 kg/month	1996	McConnell et al. 2004
Patuxent river estuary into Chesapeake Bay, U.S.A.	7 kg/month	1996	McConnell et al. 2004
Estuaries of the South Atlantic Bight, U.S.A.	50-470 kg/month	1994-1995	Alegria et al. 2000
Inner shelf of the South Atlantic Bight, U.S.A.	325 kg/month	1994	Alegria et al. 2000
Inner shelf of the South Atlantic Bight, U.S.A.	550 kg/month	1995	Alegria et al. 2000
Missouri river before discharge into Mississippi, U.S.A.	2,400 kg/month	1988	Pereira & Rostad, 1990
Mississippi river after its confluence with Missouri, U.S.A.	4,200 kg/month	1988	Pereira & Rostad, 1990
Mississippi river at Arkansas City, KS, U.S.A.	11,000 kg/month	1988	Pereira & Rostad, 1990
Mississippi river at Vicksburg, MISS, U.S.A.	9,200 kg/month	1988	Pereira & Rostad, 1990
Mississippi river at St Francisville, LA, U.S.A.	7,000 kg/month	1988	Pereira & Rostad, 1990
Mississippi river at Belle Chasse, LA, U.S.A.	4,900 kg/month	1988	Pereira & Rostad, 1990
Ebro river, Spain (U.E.)	60 kg/month	2002-2003	Gómez-Gutiérrez et al. 2006
Ebro river, Spain (U.E.)	72 kg/month	1995-1996	Gascón et al., 1998
Rhone river at Arles, France (U.E.)	480 kg/month	1994-1995	Tronczynski & Moisan, 1996
Riverine inputs to the Humber estuary (U.K.)	26 kg/month	1994-1997	Zhou et al. 1999
Humber estuary (U.K.) output to coastal zones	19 kg/month	1994-1997	Zhou et al. 1999
Haihe river downstream of Beijing, China	2.5 kg/month	2009-2010	Heeb et al. 2012
Yangtze River at Datong, China	830 kg/month	2009-2010	Qi et al. 2014

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Chapitre 7. Présence de pesticides dans les fruits et légumes issus de l'agriculture biologique et conventionnelle par extraction QuEChERS et chromatographie liquide couplée à la spectrométrie de masse en tandem

Article publié dans le journal *Food Control* 104 (2019) 74–82 :
"Occurrence of pesticides in fruits and vegetables from organic and conventional agriculture by QuEChERS extraction liquid chromatography tandem mass spectrometry". Auteurs: **Montiel-León, J. M., S. V. Duy, G. Munoz, M.-A. Verner, M. Y. Hendawi, H. Moya, M. Amyot and S. Sauvé.**

Description: Cet article présente une méthode d'extraction de 22 pesticides par QuEChERS dans quatre types differents de fruits et legumes vendus au Québec. La quantification montre les niveaux de contamination de produits issus de l'agriculture biologique et conventionnelle.

Contributions: J'ai participé à la conception du projet ainsi que la collecte d'échantillons, la réalisation de manipulations, le traitement de données et la rédaction de l'article.
Co-auteurs: Sung Vo Duy m'a aidé avec une partie des manipulations et à la rédaction. Gabriel Munoz m'a aidé avec la révision de l'article. Mohammed Hendawi et Hector Moya m'ont aidé avec les manipulations. Marc-André Verner m'a aidé avec une partie de la rédaction de l'article. Co-directeur: Marc Amyot m'a aidé à l'amélioration de la rédaction. Directeur : Sébastien Sauvé m'a aidé à la conception du projet et à la rédaction de l'article.

Abstract

Human exposure to pesticides commands the implementation of food safety control, but few studies have provided a comparative assessment of conventional and organic products. This study set out to examine 22 pesticides in four distinct commodities (lettuce, apples, grapes, and tomatoes) from conventional and organic agriculture available to consumers. A multiresidue procedure based on QuEChERS extraction and ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) was first validated for its robustness. Suitable determination coefficients (R^2>0.99) and recoveries generally between 70 and 110% were obtained. Intraday and interday variations were <20% and low matrix effects were noted with sample-to-sample variation. Method limits of detection (LOD) in the overall range of 0.05-2 μg kg^{-1} were obtained. The validated method was applied to 133 fruit and vegetable samples purchased in Canada, including conventional and organic culture samples. Overall, 47% of the 133 samples had levels above the LOD for at least one pesticide. Neonicotinoid insecticides were detected in all four product types. Imidacloprid (0.08–29 μg kg^{-1}), acetamiprid (0.11–108 μg kg^{-1}), and clothianidin (0.13–141 μg kg^{-1}) were the most recurrent. Atrazine was reported in approximately a third of the lettuce samples (0.25–7.5 μg kg^{-1}). For varieties with samples available from both organic and conventional agriculture, the proportion of insecticide levels >LOD was significantly higher (p<0.05) for samples from conventional agriculture (9.7%) than from organic agriculture (2.0%). Measured levels were compliant to Canadian Maximum Residue Limits (MRLs), but a thorough human health risk assessment has yet to be conducted for many of these pesticides.

Keywords

Pesticide residues; Fruits and vegetables; Organic and conventional culture; Neonicotinoids; Imidacloprid; QuEChERS extraction

7.1 Introduction

The extensive use of certain pesticides and their relative persistence go on par with the presence of residue levels in the environment, drinking water, and agricultural products available to consumers, with possible implications for human exposure. A legislative framework was established to control maximum residue limits (MRL) in foodstuff, representing the maximum concentration of a residue that is permitted for a food agricultural commodity (Holland, 1996). The compliance to MRLs is now a mandatory criterion of food security. The pesticides' MRLs may vary according to the country and particular commodity, as is apparent in online databases that summarize their regulatory status around the globe (Botitsi, Tsipi & Economou, 2017; Handford, Elliott & Campbell, 2015). The quantification of trace pesticides residues in food commodities has led to the application of diverse instrumental methods. For pesticides of emerging concern, liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) is currently the most widely used technique in pesticide residue analysis to reach world standards (Nunez, Gallart-Ayala, Martins & Lucci, 2012).

Pesticides of high current concern include neonicotinoids, triazines, carbamates, and organophosphates. They have usually been analyzed separately or in some combinations, but one central issue is to attain acceptable extraction efficiencies for a wide range of compounds during the multiresidue procedure (Nunez et al., 2012). Extraction procedures for solid samples such as solvent extraction (Iwafune, Ogino & Watanabe, 2014) and solid phase extraction (SPE) (Obana, Okihashi, Akutsu, Kitagawa & Hori, 2003) have been gradually replaced by the method developed by Anastassiades & Lehotay (2003), a multiresidue extraction procedure known as QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe). Thereafter, QuEChERS methods have been optimized, validated, and applied to analyze pesticides residues in diverse consumer products, including cereals (Zhang et al., 2013), flour (Bordin, Minetto, do Nascimento Filho, Beal & Moura, 2016), fruit and vegetables (Badoud, Ernest, Hammel & Huertas-Pérez, 2018; Chamkasem, Ollis, Harmon, Lee & Mercer, 2013; Chen, Tao, McLean & Lu, 2014; Mac Loughlin et al., 2018; Poulsen, Andersen, Petersen & Jensen, 2017; Sharma, Nagpal, Pakade & Katnoria, 2010), baby food (Kapoor et al., 2013), cooked food (Park et al., 2011), fish (Barbieri et al., 2019;

Wang, Shu, Li, Yang & Qiu, 2017), milk (Manav, Dinç-Zor & Alpdoğan, 2019), aromatic herbs (Nantia, Moreno-Gonzalez, Manfo, Gamiz-Gracia & Garcia-Campana, 2017), and honey (Jovanov et al., 2014; Proietto Galeano et al., 2013; Tanner & Czerwenka, 2011; Tette et al., 2016). Relatively few methods have been fully validated for their compliance with quality assurance and quality control criteria, including resilience to matrix effects, constituting a first knowledge gap to address in this study.

Another important research avenue relates to the assessment of commodities from different farming approaches. Previous studies have compared the impacts of conventional and organic farming on soil erosion (Reganold, Elliott & Unger, 1987), fertility (Mäder et al., 2002), and diversity of microbial and fungal communities in agroecosystems (Hartmann, Frey, Mayer, Mader & Widmer, 2015; Oehl et al., 2004). Some studies have also examined the nutritional quality of commodities from organic *vs.* conventional farming, including nutrient content and antioxidant properties, in red wines (Garaguso & Nardini, 2015), winter wheat (Mazzoncini, Antichi, Silvestri, Ciantelli & Sgherri, 2015), and fruit (Lombardi-Boccia, Lucarini, Lanzi, Aguzzi & Cappelloni, 2004). However, significant data gaps exist regarding the consumer exposure to pesticide residues in the two types of samples (Baranski et al., 2014), especially for chemicals of high current concern such as neonicotinoids.

The present work was initiated to document the occurrence and levels of various pesticides (insecticides, herbicides, and fungicides) in fruits and vegetables from conventional and organic agriculture. A QuEChERS extraction coupled to ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) was evaluated for this purpose. One particular objective was to obtain a sufficiently robust analytical method, with acceptable recovery, accuracy, and quality control performances. Different types of matrix effects were assessed, including dynamic and sample-to-sample matrix effects. We used this validated method to analyze samples (n=133) of tomatoes, lettuce, grapes, and apples available to consumers in Canada and compared the occurrence and levels between conventional farming and organic farming products.

7.2 Materials and methods

7.2.1 Chemicals

Desnitro-imidacloprid hydrochloride (purity ≥ 99.8%), dinotefuran (purity ≥ 98.6%), nitenpyram (purity ≥ 99.9%), thiacloprid (purity ≥ 99.9%), imidacloprid (purity ≥ 99.9%), acetamiprid (purity ≥ 99.9%), thiamethoxam (purity ≥ 99.6%), clothianidin (purity ≥ 99.9%), fipronil (purity ≥ 97.9%), tetrahydrophthalimide (purity ≥ 99.6%), desethylatrazine (purity ≥ 99.9%), atrazine (purity ≥ 99.9%), desisopropylatrazine (purity ≥ 99.9%), cyanazine (purity ≥ 99.9%), simazine (purity ≥ 99.9%), carbendazime (purity ≥ 99.9%), carbaryl (purity ≥ 99.9%), linuron (purity ≥ 99.9%), phosmet (purity ≥ 99.9%) and a mix of o,o,o triethylphosphorothioate, dimethoate, famphur, (purity ≥ 99.9%) were purchased from Sigma Aldrich (St. Louis, MO, U.S.A.). Isotope-labeled internal standards (ISs) imidacloprid-d_4 (purity ≥ 99.9%), acetamiprid-d_3 (purity ≥ 98%), clothianidin-d_3 (purity ≥ 99.9%) and thiamethoxam-d_3 (purity ≥ 99.9%) were also obtained from Sigma Aldrich (St. Louis, MO, U.S.A.), while fipronil-$^{13}C_2$-$^{15}N_2$ (purity ≥ 98%), linuron d_6 (purity ≥ 98%), carbaryl-d_6 (purity ≥ 98%) and phosmet-d_6 (purity ≥ 98%) were obtained from Santa Cruz Biotechnology (Dallas, TX, U.S.A.) and Atrazine-$^{13}C_3$ (purity ≥ 99.9%) from Cambridge Isotope Laboratories, Inc. (Andover, MA, U.S.A.).

Extraction salts including magnesium sulfate ($MgSO_4$), sodium chloride (NaCl), sodium citrate tribasic dihydrate, and sodium citrate dibasic sesquihydrate were from Sigma Aldrich (St. Louis, MO, U.S.A.). The PSA (primary and secondary amines) clean-up agent was from Sigma Aldrich (St. Louis, MO, U.S.A.). Solvents were all of HPLC grade quality and were purchased from Fisher Scientific (Whitby, ON, Canada). Formic acid (HCOOH; purity ≥ 95%) and ammonium acetate (CH_3COONH_4; purity ≥ 98%) were acquired from Sigma-Aldrich (St. Louis, MO, U.S.A.).

7.2.2 Sample collection

Sampling was carried out taking into account cultivar, organic or conventional farming, and origin for each of the four commodities, based on the 2014 report from the Ministry of

Agriculture, Fisheries and Food of Quebec, Canada (LEAA, 2014). A condensed description by commodity, variety, and farming method is provided in **Table 7.1**. Fresh lettuce (*Lactuca sativa*) samples (n = 39) were from Canada and the U.S.A. and included the following varieties: curly green leaf (conventional and organic), curly red leaf (conventional and organic), iceberg (conventional and organic), romaine (conventional and organic), Boston (conventional and organic), Boston live lettuce (conventional), chicory (conventional), and escarole (conventional). Apple (*Malus pumila*) samples (n = 37) were from Canada, the USA, New Zealand, Chile, Argentina, South Africa, and Japan and included the following varieties: Golden Delicious (conventional and organic), Cortland, Macintosh, Red Delicious (conventional and organic), Empire, Granny Smith (conventional and organic), Spartan, Pink Lady (conventional and organic), Gala (conventional and organic), Fuji (conventional and organic), Crispy Pink, Envy, Honey Crisp, Jazz, Eve, Smitten, Royal Gala, Divine and Green-acid. Grape (*vitis*) samples (n = 27) from the USA and Italy were from the following varieties: red and green seedless grapes (conventional and organic), green autumn crisp, sweet celebration, scarlotta, green perletts, red flames, and green muscat grape. Tomatoes (*Solanum lycopersicum)* samples (n = 30) from Canada and Mexico included the following varieties: red tomato greenhouse (conventional and organic), Roma, cherry, cocktail, Kumato (organic), pink, Zima, mini apero (conventional and organic), grape (conventional and organic), chopin, Yore, Angel Sweet, Campari, cherry pink, red cello, apero (organic), and concentrated tomatoes in puree can. All samples were purchased from supermarkets or organic markets located in Montreal (Quebec, Canada) by qualified university personnel. Samples were collected into individual plastic bags and transported to our laboratory facilities, where they were attributed to a unique sample identifier and labelled accordingly. Information on market location, date of purchase, variety, and country of origin was recorded for each sample. The samples were kept at 4°C until preparation and analysis, which were performed within 3 days of purchase.

Table 7.1. Commodity, variety and farming method (conventional or organic agriculture, signalled with an "X") of the samples collected in the present survey (n = 133).

Commodity	Variety	Conventional farming	Organic farming
Lettuce	Curly green leaf	X	X
	Curly red leaf	X	X
	Iceberg	X	X
	Romaine	X	X
	Boston	X	X
	Boston live lettuce	X	
	Chicory	X	
	Escarole	X	
Apple	Golden Delicious	X	X
	Cortlan	X	
	Macintosh	X	
	Red Delicious	X	X
	Empire	X	
	Granny Smith	X	X
	Spartan	X	
	Pink Lady	X	X
	Gala	X	X
	Fuji	X	X
	Crispy Pink	X	
	Envy	X	
	Honey Crisp	X	
	Jazz	X	
	Eve	X	
	Smitten	X	
	Royal Gala	X	
	Divine	X	
	Green-acid	X	
Grape	Green seedless grapes	X	X
	Red seedless grapes	X	X
	Green autumn crisp	X	
	Sweet celebration	X	
	Scarlotta	X	
	Green perletts	X	
	Red flames	X	

	Green muscat grape	X	
Tomatoes	Red tomato greenhouse	X	X
	Roma	X	
	Cherry	X	
	Cocktail	X	
	Kumato		X
	Pink	X	
	Zima	X	
	Mini apero	X	X
	Grape	X	X
	Chopin	X	
	Yore	X	
	Angel Sweet	X	
	Campari	X	
	Cherry pink	X	
	Red cello	X	
	Apero		X
	Canned tomatoes	X	

7.2.3 Sample preparation

Details on the analytical method development and optimization are provided in the SI (pages S-3 to S-6; see also SI **Figure S7.1-S7.4**). Whole samples (unpeeled) were ground with a domestic blender Nitrobullet no-NBR1202M from Homeland Housewares, LCC (Los Angeles, CA, U.S.A.). For the QuEChERS procedure, 5 g wet weight of each homogenized sample was placed into a 50 mL polypropylene centrifugation tube. Following the addition of 5 mL of acetonitrile (ACN), the samples were vortexed (1 min, 3200 rpm) with an LP Vortex mixer (Thermo Scientific, U.S.A.). Then 2 g of $MgSO_4$, 0.5 g NaCl, 0.5 g sodium citrate tribasic dihydrate, and 0.25 g sodium citrate dibasic sesquihydrate were added and the samples were vortexed (1 min, 3200 rpm), followed by further mechanical stirring (5 min, 40 rpm) using an orbital shaker by Scientific Equipment Products (MD, U.S.A.). Following centrifugation (15 min, 6000 rpm), a 4 mL aliquot of the supernatant was transferred into a 15 mL polypropylene tube containing 0.6 g $MgSO_4$ and 0.2 g PSA for

clean-up, and the samples were homogenized under vortex agitation (1 min, 3200 rpm). After centrifugation (15 min, 6000 rpm), 2 mL of the supernatant was transferred into a clean 15 mL polypropylene centrifugation tube and spiked with the isotope-labelled internal standard mixture for a final concentration of 50 ng mL^{-1} after reconstitution. For this purpose, the final extraction solvent was evaporated to dryness with a stream of N_2 at 40°C. The final residue was reconstituted in 200 µL of 0.1% formic acid in H_2O:MeOH (90:10 v/v). After brief vortexing and ultrasonication, the samples were centrifuged (10 min, 6000 rpm) and a 180 µL aliquot of the sample was transferred into a 250 µL injection vial for subsequent UHPLC-MS/MS analysis.

7.2.4 Instrumental analysis

An HTC thermopal autosampler (CTC analytics AG, Zwingen, Switzerland) was used for in-loop sample injection. The system was composed of a six-port valve from VICIs Valco Instruments Co., Inc. (Houston, TX, U.S.A.). A quaternary pump Accela 1250 (Thermo Finnigan, San Jose, CA, U.S.A.) was used for gradient elution. Chromatographic separation was performed with a Thermo Hypersil Gold C18 column (100 mm x 2.1 mm, 1.9 µm particle size) from Thermo Fisher Scientific (San Jose, CA, U.S.A.), thermostated at 50°C. The aqueous mobile phase (A) was 10mM CH_3COONH_4 HPLC-water with 0.1% HCOOH, and the organic phase (B) was MeOH with 0.1% HCOOH. The injection volume was 100 µL. Further details regarding the mobile phase gradient program are provided in the Supporting Information (SI **Table S7.1**).

The TSQ Quantiva triple quadrupole mass spectrometer from Thermo Scientific (Waltham, MA, U.S.A.) was coupled to a heated electrospray ionisation (HESI) source, operated in fast polarity-switching mode (all target analytes acquired within a single run). The optimized source parameters were as follows: sheath gas was set at 45 arbitrary units (A.U.), auxiliary gas at 15 A.U., sweep gas at 0 A.U., ion transfer tube temperature at 350°C, and vaporizer temperature at 400°C. The ion spray voltage was +3000V for neonicotinoids, herbicides and carbamates and -2900V for fipronil and linuron (Montiel-León, Duy, Munoz, Amyot & Sauvé, 2018). The analyzer was operated in selected reaction monitoring (SRM) mode. The first (Q1) and third (Q3) quadrupoles were operated at a resolution of 0.7 Da FWHM. The

collision-induced dissociation (CID) gas pressure in the second quadrupole (q2) was set at 1.5 mTorr. Compound-dependent parameters were optimized to select collision energies, quantification and confirmation transitions, and RF Lens values, as summarized in the SI (**Table S7.2**).

7.2.5 Quality assurance and quality control

The present QuEChERS method was validated in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision (intraday and interday), and whole-method recovery.

Procedural blanks were performed for the various extraction batches. Such blanks consisted of 5 mL of HPLC-water undergoing the whole preparation procedure and spiked with the isotope-labelled ISs before reconstitution as described in **Section 7.2.3**. None of the targeted analytes were detected in these blanks.

The positive identification of the targeted analytes was based on matching retention times with authentic standards, detectable signals (S/N >3) for both quantification (Q) and confirmation (C) transitions, and compliance of Q.T./C.T. ratios, that should not deviate more than 30% from the average of ratios observed in matrix-matched calibration curve (MMCC) levels (FAO-CAC/GL-56-2005; Mol et al., 2015; Murray et al., 2013; SANTE/11945/2015, 2015; F. Zhang, Li, Yu & Pan, 2012).

As part of the method linearity assessment, linearity range and determination coefficients (R^2) were evaluated. Calibration curves were constructed in the four different matrices by spiking native analytes at 8 levels (0.5, 1, 20, 100, 200, 300, 500, and 600 μg kg^{-1}) at the beginning of the extraction procedure, while isotope-labelled ISs were spiked before the reconstitution (concentration of 50 ng mL^{-1} in the final reconstituted extract). Each calibration level was prepared and analyzed in triplicate. Calibration curves were plotted using the native analyte to IS peak area ratio (y-axis) versus native analyte concentration (x-axis), fitted with an inverse-weighted (1/x) linear regression.

The limit of detection (LOD) was defined as the smallest analyte concentration in the matrix-matched sample that could be distinguished from the background with a signal-to-noise (S/N) ratio >3. In the present study, two MS/MS transitions were monitored for analyte

identification; the transition with the lowest S/N ratio was chosen for LOD calculation. The limit of quantification (LOQ) was defined in a similar way, but considering an S/N ratio >10 in accordance with the IUPAC definition (Thompson, Ellison, & Wood, 2002).

Accuracy was evaluated at two quality control levels (in quintuplicate) within the linear range of the calibration curve, but using levels not previously included in the calibration curve regression. Blank matrix samples were spiked at the selected concentrations and the accuracy (expressed as a percentage of the expected value) was derived from the concentration found per the MMCC quantification procedure. The low-end quality control level, labeled as QC_L, was set at 2.5 μg kg^{-1} for most neonicotinoids and herbicides, 5 μg kg^{-1} for dinotefuran, desisopropylatrazine, and organophosphates, and 25 μg kg^{-1} for phosmet and linuron. The high-end quality control level, labeled as QC_H, was set at 400 μg kg^{1} for all the targeted analytes. Intermediate precision was evaluated at both quality control levels. Intraday precision corresponded to the RSD of accuracy values of 5 preparations analyzed within a single work day. The process was repeated on a second (n = 5) and third (n = 5) work day, and the interday precision derived from the overall RSD (n = 15).

Whole-method recovery (%) was determined as per the general guidelines introduced by Matuszewski (2003). Matrix-specific samples with low or absent initial levels of the targeted pesticides were spiked either *before* or *after* the whole preparation procedure to derive the recovery, evaluated as per **Equation 1** (Munoz et al., 2016) as follows:

$$Recovery\ (\%)\ = \frac{SB-NS}{SA-NS} \times 100 \qquad \textbf{(Equation 1)}$$

Where *SB* is the native analyte to IS area ratio of the sample spiked with the native analyte mix before the start of the extraction procedure (left to equilibrate before carrying out the extraction), *SA* is the analyte to IS area ratio of the sample spiked with the analyte solution mix at the end of the extraction procedure but before the reconstitution step, and *NS* is the analyte to IS response ratio of the reference (non-spiked matrix blank sample). In all three cases, isotope-labelled ISs were added at the end of the extraction procedure but before the reconstitution step.

The quantification strategy retained was based on a matrix-matched calibration curve (MMCC) approach. Matrix samples were quantified based on the native analyte to IS area ratio in the particular sample, divided by the slope of the corresponding matrix-matched

calibration curve (constructed on a composite matrix blank sample for each commodity), and further applying a factor accounting for the weighed sample intake.
In order to demonstrate the method suitability in terms of residual matrix effects and stringent quality control for long samples series, two quality control tests were implemented. Continuing Quality Control (CQC) standards consisted of a matrix blank spiked with both target analytes (100 μg kg^{-1}) and internal standards. The CQC standards were injected every five samples along the LC-MS sequence immediately followed by a matrix blank to verify the absence of carryover.

For each matrix type, Standard Additions Quality Control (SAQC) samples consisted in spiking a subset of individual samples which were not part of the MMCC and controlling the accuracy of spiked concentrations when quantified against the MMCC. These individual samples were spiked at a concentration level of 80 μg kg^{-1} and analyzed in triplicate.

7.2.6 Statistical analyses

Data treatment was carried out with Xcalibur 3.0 software (Thermo Scientific, Waltham, MA, U.S.A.). Statistical analyses were performed with the R software (R Core Team, 2018). Statistical significance was set at $p \leq 0.05$. During the method optimization stage, ANOVA was used to test statistically significant differences in terms of analyte absolute response between 3 or more method treatments (e.g., extraction solvent, extraction salt variation, clean-up sorbents). When the test suggested that not all groups are equal (rejection of the null hypothesis), pairwise comparisons were run as a post-hoc test. Differences between organic and conventional samples in terms of pesticide detection frequency were evaluated using χ^2 (chi square) test.

7.3 Results and discussion

7.3.1 Method validation

The present method was validated as per SANCO 2013 guidelines (SANTE/11945/2015, 2015). A suitable linearity range was obtained in matrix-matched calibration curves (**Table 7.2**), covering about 3 orders of magnitude (generally between 0.15–600 or 1–600 μg kg^{-1}).

Suitable coefficients of determination (R^2) were also obtained for the scope of targeted pesticides in the four commodities, with R^2 in the range of 0.9960–1.0000 in lettuce matrix-mached calibration curves, 0.9901–0.9999 in apple, 0.9901–0.9983 in grape, and 0.9905–0.9992 in tomato (**Table 7.2**).

The LODs were compound-specific and somehow commodity-specific (**Table 7.2**). LODs were in the range of 0.05–0.7 μg kg^{-1} in lettuce, 0.08–1.2 μg kg^{-1} in apples, 0.5–1.8 μg kg^{-1} in grapes, and 0.4–2 μg kg^{-1} in tomatoes. The LOD performance is similar to or better than previously published works (Golge & Kabak, 2015). Compound-specific LOQs were in the range of 0.15–6 μg kg^{-1} (**Table 7.2**), which is suitable for food control since MRLs for the four commodities are typically in the range of 10–1000 μg kg^{-1} or higher in Canada and the European Union.

Accuracy and precision were also evaluated at two fortification levels in the four tested commodities (lettuce, apple, grapes, and tomato). The quantification approach performed satisfactorily, with accuracies in the range of 73–118% for the four matrices (SI **Tables S7.4 to S7.7**). Regardless of the spike level examined, intraday precision (n=5) remained between 0.1–20% for the targeted analytes. Interday precision (n=15) averaged 8.9%, 10.4%, 8.8%, and 8.7% for lettuce, apples, grapes, and tomatoes spiked samples, respectively (see also the SI for details).

The recovery of the method in the four tested commodities is presented in SI (**Table S7.3**). With a few exceptions, the recovery was generally within acceptable ranges as per U.S. EPA guidelines (70–130%) and European Union SANCO guidelines (70–120%). For instance, recovery in lettuce ranged from 82 to 106% (except for dinotefuran: 54%) at QC_L and from 70 to112 % at QC_H for the model compounds considered, with suitable precision (SI **Tables S7.4 to S7.7**).

Table 7.2. Coefficients of determination (R^2) of the matrix-matched calibration curves (corresponding linearity range: LOQ–600 μg kg^{-1}) and method limits of detection (LOD, μg kg^{-1}) and limits of quantification (LOQ, μg kg^{-1}) in the four tested matrices: lettuce, apple, grapes, and tomatoes.

	R^2				**LOD [μg kg^{-1}]**				**LOQ [μg kg^{-1}]**			
	Lettuce	**Apple**	**Grapes**	**Tomato**	**Lettuce**	**Apple**	**Grapes**	**Tomato**	**Lettuce**	**Apple**	**Grapes**	**Tomato**
Acetamiprid	1.0000	0.9999	0.9938	0.9931	0.05	0.10	1.1	1.2	0.15	0.32	3.3	3.5
Clothianidin	0.9999	0.9999	0.9924	0.9942	0.13	0.12	1.2	1.1	0.40	0.37	3.6	3.2
DN-Imi	0.9984	0.9993	0.9955	0.9914	0.51	0.31	0.94	1.3	1.5	0.92	2.8	3.9
Dinotefuran	0.9983	0.9992	0.9915	0.9937	0.48	0.32	1.3	1.1	1.4	0.97	3.9	3.3
Flonicamid	0.9993	0.9996	0.9980	0.9945	0.30	0.24	0.62	1.0	0.91	0.71	1.8	3.1
Imidacloprid	0.9999	1.0000	0.9903	0.9944	0.11	0.08	1.6	1.0	0.33	0.23	4.9	3.1
Nitenpyram	0.9960	0.9959	0.9937	0.9914	0.73	0.75	1.3	1.3	2.2	2.2	3.7	3.9
Thiacloprid	1.0000	0.9999	0.9901	0.9916	0.05	0.11	1.4	1.3	0.16	0.34	4.2	3.9
Thiamethoxam	1.0000	0.9999	0.9910	0.9914	0.05	0.10	1.3	1.3	0.16	0.29	4.0	3.9
Fipronil	0.9998	0.9999	0.9960	0.9911	0.18	0.14	0.99	1.3	0.55	0.41	3.0	4.0
Atrazine	0.9996	0.9995	0.9926	0.9949	0.25	0.27	1.7	1.0	0.71	0.81	5.1	3.0
DEA	0.9989	0.9999	0.9941	0.9905	0.39	0.12	1.3	1.4	1.2	0.36	3.9	4.1
DIA	0.9993	0.9995	0.9963	0.9910	0.30	0.27	1.3	2.0	0.90	0.81	3.9	6.1
Cyanazine	0.9996	0.9998	0.9918	0.9949	0.23	0.15	1.3	1.0	0.70	0.44	3.8	3.0
Simazine	0.9997	0.9999	0.9928	0.9984	0.20	0.16	1.2	0.56	0.59	0.47	3.6	1.7
Carbendazim	0.9981	0.9999	0.9926	0.9991	0.51	0.09	1.2	0.42	1.5	0.27	3.6	1.3
Carbaryl	0.9999	0.9999	0.9901	0.9989	0.12	0.15	1.4	0.46	0.36	0.44	4.2	1.4
Linuron	1.0000	0.9974	0.9953	0.9949	0.12	1.2	1.9	1.0	0.36	3.6	5.6	3.0
Phosmet	0.9989	0.9966	0.9983	0.9960	0.39	1.0	0.58	0.99	1.2	3.1	1.7	3.0
(EtO)3PS	0.9999	0.9901	0.9945	0.9935	0.14	1.2	1.0	1.1	0.43	3.5	3.1	3.4

Dimethoate	0.9998	0.9998	0.9948	0.9971	0.16	0.10	1.0	0.75	0.47	0.31	3.0	2.2
Famphur	0.9995	0.9984	0.9915	0.9992	0.25	0.47	1.3	0.38	0.75	1.4	3.9	1.1

7.3.2 Assessment of method robustness

The robustness of the method was also evaluated during its application to real samples. For each type of commodity, a triplicate extraction and analysis of non-spiked samples was performed for a subset (~ 20%) of the samples. This helped to confirm the positive (or negative) detection of the targeted pesticides by repeated analyses of real samples and provided a further evaluation of the whole-method precision.

In addition, two types of spiked quality control tests were included along with the several LC-MS sequences. To demonstrate the consistency of analytical performances along the entire LC-MS run, intermediate-level Continuing Quality Control (CQC) standards were inserted after every 5 samples (immediately followed by a matrix blank to control the absence of carryover). The precision performance of matrix-matched CQC samples proved satisfactory in the various UHPLC-MS/MS sequences, the RSD of the native analyte to IS area ratios remaining between 1–18% (SI **Table S7.8**). Regardless of the matrix type, the accuracy of CQC verification standards remained within 80–120% and chromatographic retention time stability was also suitable with RSDs between 0.1% and 0.5%.

Standard Additions Quality Control (SAQC) samples were also run for each type of commodity. This experiment was designed to verify that certain differences between samples from a common commodity (e.g., different varieties of apples, green grapes *vs.* red grapes, etc.) would not lead to significant deviations from the matrix-matched model sample used to construct the calibration curve. Note that the different varieties subjected to standard additions were selected randomly and the fruits were not peeled before grinding (following the same procedure as that implemented for the samples). For each commodity, five samples from different varieties were therefore submitted to standard additions (in triplicate) and the spiked concentrations were quantified against the MMCC to determine the accuracy. **Figure 7.1** provides an illustration of the accuracy performance obtained for the 22 target pesticides across 5 different varieties of apples. Limited deviations were observed for most analytes with matched isotope-labelled internal standards. For instance, the accuracy of imidacloprid or clothianidin remained within 95–105%, i.e., representing a *relative* matrix effect within ± 5%. Even in other instances, the accuracy was generally within the acceptable range of 70–120% across the different varieties (**Figure 7.1**). For

each type of commodity, the suitable accuracy performance across individual samples validated the use of matrix-matched calibration curves (MMCC), thereby avoiding detailed standard additions for quantification.

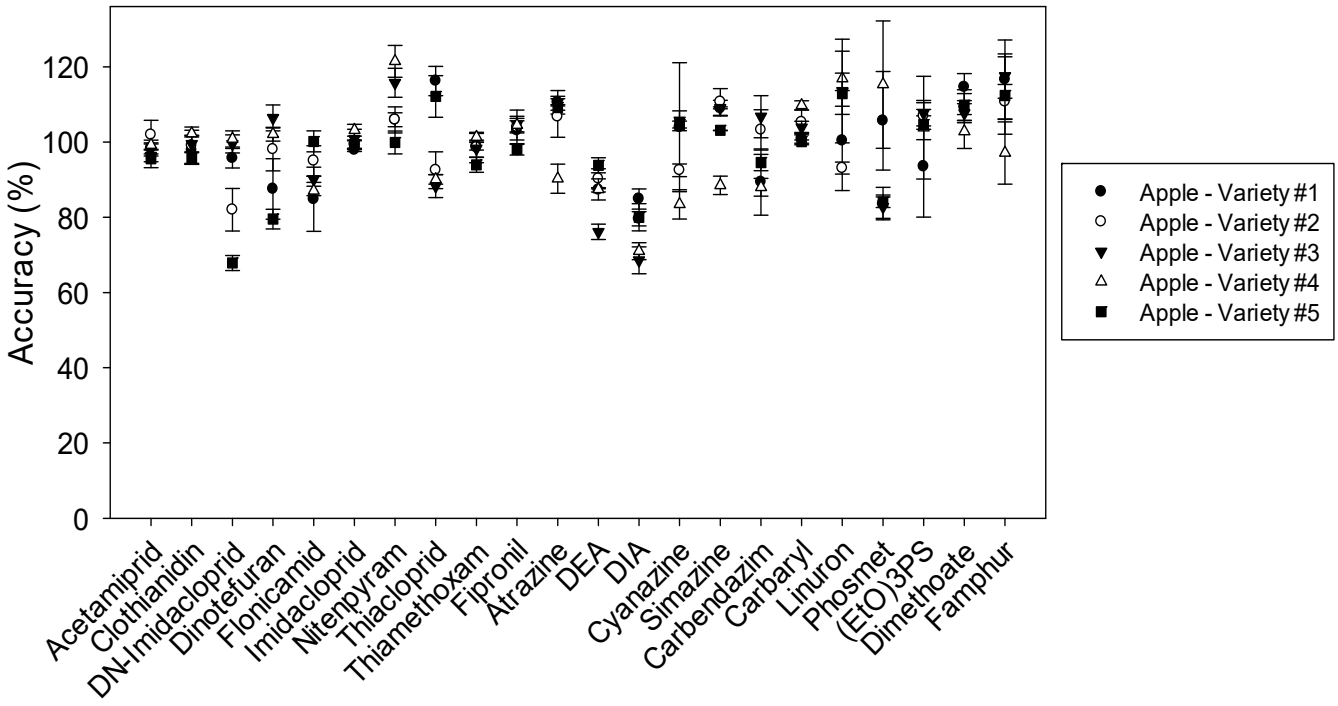

Figure 7.1. Accuracy performance of Standard Addition Quality Control (SAQC) samples illustrated for five individual samples from different varieties of apples (tested apple cultivars were, in order: Empire, organic Grany Smith, Envy, Cortland, and conventional Grany Smith). Error bars indicate standard deviations (n = 3).

7.3.3 Quality survey of fruits and vegetables

The applicability of the newly developed method was demonstrated through the analysis of 133 fruit and vegetable samples purchased in the province of Quebec (Canada), covering both conventional culture and organic culture.

7.3.3.1 Overview of occurrence and levels on the overall data set

When considering the overall dataset (N = 133), 47% of fruit and vegetable commodities had levels of at least one of the 22 pesticides above the LOD. Among these positive samples, about half (48%) had multiple pesticide residues. Neonicotinoids was the most

recurrently detected class, with 39% of the 133 tested samples presenting levels above the LOD for at least 1 out of the 8 targeted neonicotinoids (maximum 3 out of 8 neonicotinoids). Based on the overall dataset (N = 133), imidacloprid was the most often detected (16% of samples), followed by acetamiprid (13%), clothianidin (12%) and thiamethoxam (8%). Atrazine was detected in 10% of the samples, and carbendazim in 5%. Neonicotinoids was the only pesticide class that was detected across all four commodities (**Table 7.3**).

The sum of 22 target pesticides ranged from <LOD to a maximum of 435 μg kg^{-1}. Overall, concentrations of individual pesticides were compliant with Health Canada and the European Commission MRLs. The sum of Σ_8Neonicotinoids varied between <LOD and 193 μg kg^{-1}. Concentration ranges (in positive samples) were as follows for individual neonicotinoids: acetamiprid (0.11–108 μg kg^{-1}), clothianidin (0.13–141 μg kg^{-1}), dinotefuran (13–47 μg kg^{-1}), imidacloprid (0.08–29 μg kg^{-1}), thiacloprid (1.6–28 μg kg^{-1}), and thiamethoxam (0.10–54 μg kg^{-1}). Residues of neonicotinoid insecticides were also detected across various fruit and vegetable commodities in previous studies, at similar concentration levels. For instance, acetamiprid was reported in the range of 15–370 μg kg^{-1} in tomatoes (Golge and Kabak, 2015) and 0.2–10 μg kg^{-1} in tea (Liu et al., 2010). The average concentration of acetamiprid in positive samples from a comprehensive U.S. data set was 4.3 μg kg^{-1} for fruits and 1.1 μg kg^{-1} for vegetables (Craddock, Huang, Turner, Quiros-Alcala & Payne-Sturges, 2019). Imidacloprid was also reported at low concentrations in the data set reported by Craddock et al. (2019) with a mean concentration at 3.6 μg kg^{-1}, albeit sporadically high concentrations were reported for some samples (e.g., up to 2,300 μg kg^{-1} for imported grapes).

The herbicide atrazine was only found in one particular commodity from the present survey at low levels (0.25–7.5 μg kg^{-1} in positive lettuce samples). Atrazine residues were previously reported in diverse fruits and vegetables available to consumers in Argentina and China, with an overall concentration range of 35–310 μg kg^{-1} in positive samples (Mac Loughlin et al., 2018; Tian, Cheng, Ye, Liu & Jia, 2014). Carbendazim was only reported in one commodity from the present survey, at concentration levels of 0.5–28 μg kg^{-1} in apples. This falls in the same order of magnitude as previous reports of carbendazim

residues in oranges and aromatic herbs (Nantia et al., 2017; Zamora, Pozo, López & Hernández, 2004).

Table 7.3. Method application to food commodities (n = 133) available in Canada: summary of occurrence data (detection frequency, %) and concentration ranges (min–max of samples >LOD; µg kg^{-1}) of the targeted analytes in lettuce (n = 39), apple (n = 37), grapes (n = 27) and tomato (n = 30) samples.

	Detection frequency (%)				**Concentration range (min-max) (µg kg^{-1})**			
	Lettuce (n = 39)	Apple (n = 37)	Grapes (n = 27)	Tomato (n = 30)	Lettuce (n = 39)	Apple (n = 37)	Grapes (n = 27)	Tomato (n = 30)
Acetamiprid	0	41	4	3	<LOD	0.11–24	108	16
Clothianidin	23	3	19	3	0.14–2.4	0.13	2.1–141	3.2
DN-Imidacloprid	0	0	15	0	<LOD	<LOD	1.9–11	<LOD
Dinotefuran	0	0	4	7	<LOD	<LOD	47	13–20
Flonicamid	0	0	0	0	<LOD	<LOD	<LOD	<LOD
Imidacloprid	10	16	30	10	0.64–5.0	0.08–3.8	4.9–29	7.6–11
Nitenpyram	0	0	0	0	<LOD	<LOD	<LOD	<LOD
Thiacloprid	0	5	0	0	<LOD	1.6–28	<LOD	<LOD
Thiamethoxam	18	0	7	3	0.10–8.3	<LOD	2.7–54	1.4
Fipronil	0	0	0	0	<LOD	<LOD	<LOD	<LOD
Atrazine	33	0	0	0	0.25–7.5	<LOD	<LOD	<LOD
DEA	0	0	0	0	<LOD	<LOD	<LOD	<LOD
DIA	0	0	0	0	<LOD	<LOD	<LOD	<LOD
Cyanazine	0	0	0	0	<LOD	<LOD	<LOD	<LOD
Simazine	0	5	0	0	<LOD	0.22–0.43	<LOD	<LOD
Carbendazim	0	19	0	0	<LOD	0.49–28	<LOD	<LOD
Carbaryl	0	3	0	0	<LOD	1.1	<LOD	<LOD
Linuron	0	0	0	0	<LOD	<LOD	<LOD	<LOD
Phosmet	0	0	0	0	<LOD	<LOD	<LOD	<LOD

(EtO)3PS	10	0	0	0	0.23–219	<LOD	<LOD	<LOD
Dimethoate	8	0	0	0	6.3–215	<LOD	<LOD	<LOD
Famphur	0	0	0	0	<LOD	<LOD	<LOD	<LOD

7.3.3.2 Comparison between organic and conventional culture samples

Summed pesticides levels (Σ_{22}Pesticides) ranged between <LOD–435 µg kg^{-1} for conventional culture samples and <LOD–58 µg kg^{-1} for organic culture samples (see also the Supporting Excel file). Multiple pesticide residues were more often detected in conventionally produced samples (25% of samples with 2 or more pesticide residues) than in organically produced ones (8%) (**Figure 7.2**). Noteworthy differences were also observed when considering the detection frequencies in samples from conventional and organic culture. For varieties with samples available from both organic and conventional agriculture (n=16), the proportion of insecticide levels >LOD was significantly higher for samples from conventional agriculture (9.7%) than from organic agriculture (2.0%) (p = 1.4 E-05). This difference was most striking for neonicotinoids. For example, imidacloprid was detected in 8 conventional samples out of 16 (paired organic and conventional culture samples from the same variety; **Table 7.1**), whereas it was detected in only 1 organic sample out of 16. In these 16 varieties, at least one pesticide was detected in 15 samples of conventional culture versus 5 samples of organic culture.

To the authors' best knowledge, few studies have systematically examined differences between organic and conventional agriculture samples with regard to pesticide occurrence and levels. In their meta-analysis of published literature, Baranski et al. (2014) suggested that organically-grown crops could show different nutritional properties and lower incidence of pesticide residues than conventionally-grown ones. The authors also indicated that the mean frequency of occurrence of pesticides above the LOD in samples was statistically lower for organic samples compared to conventional samples in fruits (11% *vs.* 75%) as well as in vegetables (10% *vs.* 32%) (Baranski et al., 2014).

Lower occurrence and levels of pesticide residues in organically produced food compared to conventional ones were reported in some other studies (Baker, Benbrook, Groth III & Lutz Benbrook, 2002; Bourn & Prescott, 2002; Rembiałkowska, 2007; Vitali Cepo et al., 2018). Baker et al. (2002) analyzed pesticide residue data from U.S. data sets; conventionally grown fruits and vegetables had three times more pesticide residues compared to organically grown ones. In a comparative survey of organically and

conventionally produced wines, Vitali Cepo et al. (2018) also found lower levels of pesticide residues in those from organic production).

This consistent trend between organic and conventional products is also corroborated (indirectly) via human biomonitoring surveys. For instance, studies conducted in the U.S.A and Australia demonstrated that the level of organophosphorus pesticide metabolites in human urine was significantly lower for individuals with an organic diet, compared to individuals with a predominantly conventional food diet (Curl, Fenske & Elgethun, 2003; Hyland et al., 2019; Lu et al., 2006; Oates, Cohen, Braun, Schembri & Taskova, 2014).

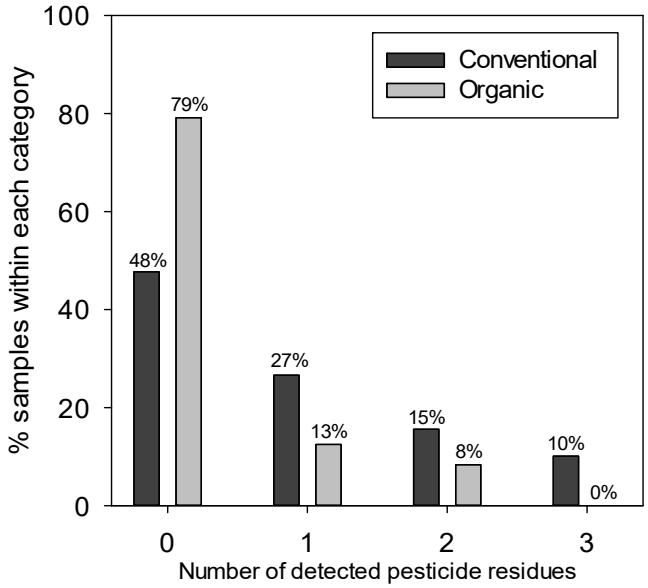

Figure 7.2. Percentage of samples (%) according to the number of detected pesticide residues for conventionally and organically produced fruits and vegetables.

7.3.3.3 Commodity-specific trends of pesticide residues

In lettuce (**Table 7.3**), 59% of the samples tested positive for at least one of the targeted pesticides (see also SI **Table S7.9**). Atrazine was reported at a relatively high detection frequency (33%), with a concentration range from <LOD to 7.5 μg kg^{-1}. Note that atrazine is not often reported in foodstuff (Gammon, Aldous, Carr, Sanborn & Pfeifer, 2005). The high water constitution of lettuce, the continued use of atrazine as an herbicide in Canada, and the use of herbicides in lettuce culture could, however, explain the occurrence and

concentrations in our study. The maximum concentration of atrazine in lettuce reported in the present study remained about one order of magnitude below the MRL of 50 μg kg^{-1} set by the European Commission (2004/248/EC). A less recurring pesticide found in lettuce samples was dimethoate, with a detection frequency of 8%, but a high concentration for one green curly lettuce sample from conventional farming (215 μg kg^{-1}). Note that this sample also contained a high level of triethylphosphorotioate (219 μg kg^{-1}). Limited reports are available regarding triethylphosphorotioate and dimethoate occurrence in foodstuff. In a previous study led by the *Laboratoire d'expertises et d'analyses alimentaires* (LEAA, 2014) in the province of Quebec, occasional detections of dimethoate were also reported in lettuce samples, with a maximum of 630 μg kg^{-1} (compliant with the maximum residue limit of 2000 μg kg^{-1}). Clothianidin, imidacloprid, and thiamethoxam were the three neonicotinoids recurrently found in lettuce samples, with detection frequencies of 23%, 10%, and 18%, respectively. Maximum concentrations of clothianidin, imidacloprid, and thiamethoxam in lettuce samples were 2.4, 5.0, and 8.3 μg kg^{-1}, respectively. Interestingly, two organic culture lettuce samples were found to contain detectable amounts of clothianidin (0.81 μg kg^{-1}) and/or atrazine (1.6 and 7.5 μg kg^{-1}, respectively). It should be highlighted, however, that these concentrations remained compliant to the European Commission MRLs.

Out of the 37 apple samples, 57% tested positive for at least one of the targeted pesticides (**Table 7.3**; see also SI **Table S7.10**). Acetamiprid was the most commonly found compound in apples, with a detection frequency of 41% and a maximum concentration of 24 μg kg^{-1} for a Cortland apple sample (conventional farming). This agrees with trends detected in a comprehensive U.S. data set, wherein acetamiprid was recurrently detected (32.5%) in apple commodities (Craddock et al., 2019). Residues of acetamiprid were previously reported in apples produced in the province of Quebec (LEAA, 2014) with maximum concentrations of 40 μg kg^{-1}, also in agreement with the range of values reported in the present study. The maximum acetamiprid concentration reported in the present study remained substantially below the MRL of the European Commission for apples (800 μg kg^{-1}) as well as that from Health Canada (1000 μg kg^{-1}). Other pesticides found in apple samples from the present survey included carbendazim (detection frequency of 19%),

carbaryl (3%), and simazine (5%), and other neonicotinoids such as clothianidin (detection frequency of 3%), imidacloprid (16%), and thiacloprid (5%).

In grapes (**Table 7.3**), 48% of samples tested positive for at least one of the targeted pesticides (see also SI **Table S7.11**). Imidacloprid was found in 30% of the samples (concentration range: <LOD to 29 μg kg^{-1}) and DN-Imidacloprid in 15% of the samples (concentration range: <LOD to 11 μg kg^{-1}). This agrees with a previous Québec report wherein imidacloprid tested positive in 34% of grapes samples, at concentration ranges between <LOD and 130 μg kg^{-1} (LEAA, 2014). Thiamethoxam was found in only two samples at concentration levels of 2.7 and 54 μg kg^{-1}. Clothianidin was detected in 19% of the samples, with a maximum concentration of 141 μg kg^{-1} in Autumnscrip green seedless grapes from the United States (conventional agriculture). Acetamiprid was found in only one out of the 27 samples, at a concentration of 108 μg kg^{-1} in Scarlotta red seedless grapes (conventional agriculture) from the United States (lower than the permitted MRLs at 500 μg kg^{-1} for the European Commission and 350 μg kg^{-1} for Health Canada). Dinotefuran was detected in one sample at 47 μg kg^{-1} (green seedless grapes from the United States, conventional agriculture).

Tomato samples from the present survey had 17% of positives to at least one of the targeted pesticides (**Table 7.3**; see also SI **Table S7.12**). Neonicotinoids were the only class found among the targeted pesticides. Acetamiprid was reported in one tomato sample only (detection frequency = 3%) at 16 μg kg^{-1}. Dinotefuran was found in two tomato samples (concentrations of 13 and 20 μg kg^{-1}) in the same order of magnitude as the European Commission and Health Canada MRL indicative default values at 10 μg kg^{-1}. Imidacloprid was detected in 10% of the samples (concentrations of 7.6, 10, and 11 μg kg^{-1} in positive samples); these values are compliant with the European Commission and Health Canada MRLs set at 500 μg kg^{-1} and 1000 μg kg^{-1}, respectively.

7.4 Conclusions

A method based on QuEChERS extraction and UHPLC-MS/MS was developed to provide a preliminary assessment of pesticide residues in conventionally and organically produced fruits and vegetables. The multiresidue method was validated for 7 different classes of pesticides in lettuce, apples, grapes, and tomatoes. The limits of detection were well below the corresponding MRLs, and acceptable whole-method recoveries were obtained in the four commodities. A rigorous quality control approach was implemented to demonstrate the consistency of quantification performance along the analytical sequence. The accuracy did not depend on sample-to-sample variations as indicated by limited matrix effects.

The method was applied to a pilot survey of 133 lettuce, apple, grapes and tomatoes samples which included between 13% and 22% of organic agriculture samples. Out of the 133 analyzed samples, 62 were tested positive (i.e., 47%) to at least one pesticide. Our results indicate the recurrent presence of neonicotinoid residues across all four commodities. Overall, 39% of the samples tested positive to at least one neonicotinoid insecticide. Imidacloprid was the most recurrently detected, followed by acetamiprid and clothianidin. Residues of the herbicide atrazine were reported in approximately a third of the lettuce samples; some of these samples also contained high levels of organophosphorus pesticides. For varieties available from both organic and conventional agriculture, the proportion of detectable pesticide levels was significantly higher for conventional agriculture than for organic agriculture, but concentration levels remained compliant to international and national MRLs.

Acknowledgments

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Compliance with ethical standards

The authors declare no conflict of interest.

7.5 Supporting Information

Details on method development and optimization

The development of the QuEChERS – UHPLC-MS/MS method was conducted based on literature precedent (Golge & Kabak, 2015; Tette et al., 2016). The optimization of influential method parameters, including extraction solvent nature, variations of salts, clean-up sorbents, mixing time, and injection volume, is as follows.

7.5.1 Extraction solvent nature

Variations of extraction solvents were tested to maximize the recovery of target analytes despite their different physicochemical properties, all the while ensuring appropriate precision. The solvent preselection was made in accordance with the literature. This involved testing acetonitrile (EN 12393-1:2013), amended or not with modifiers such as acetic acid (Golge & Kabak, 2015) and/or ethyl acetate at different percentages, which may help to reduce the yellow color after clean up (Tette et al., 2016).

The different extraction solvents (n = 3 replicates for each) included a mixture of acetonitrile and ethyl acetate (70:30 v/v) containing 1% acetic acid (Method #1), acetonitrile with 1% acetic acid (Method #2), and acetonitrile without acid (Method #3). It was critical to find a compromise between the precision (%RSD), number of target pesticides amenable to analysis, and the highest extraction efficiency. The different treatments exhibited no significant differences in terms of absolute area for 11 out of the 22 targeted compounds (**Figure S7.1**). When a statistically significant difference was noted, method #3 was found to be statistically superior to both methods #1 and #2 (10/22 compounds), or superior to one of the two methods. Method #3 also produced the lowest RSD, at 12% on average, compared to 24% and 16% for methods #1 and #2, respectively. Considering these results, acetonitrile without acid (Method #3) was selected as the extraction solvent (**Figure S7.1**).

7.5.2 Extraction salts, clean up sorbents, and vortex time

The extraction salts were evaluated following the European Union method reference (CEN, 2008), with $MgSO_4$ tested at 1 g, 2 g, 3 g, and 4 g in order to absorb the water content and promote the transfer of analytes into the organic phase. Additionally, different salts were also tested, including sodium acetate, sodium chloride, and sodium citrate tribasic dihydrate and dibasic sesquihydrate. The influence of using additional clean-up agents was also considered, including PSA, C18, and Florisil at 0.2 g (Tette et al., 2016). To standardize the high-speed agitation time in each extraction step, the influence of vortex time was investigated at 4 levels (0.5, 1, 2, and 4 minutes).

The amount of $MgSO_4$ added (tested at 1, 2, 3 and 4 grams) had a limited influence between treatments (**Figure S7.2**), suggesting some flexibility in the choice of this parameter, and an intermediate amount (2 grams of $MgSO_4$) was finally retained.

Variations in clean-up agents were subsequently investigated. NaCl can be used to reduce polar interferents and the buffered system sodium citrates to prevent losses of pH-sensitive analytes. PSA can be introduced with the aim to remove co-occurring matrix components such as fatty acids, polar organic acids, and sugars, florisil to reduce sugar in the matrix, and C18 for its ability to retain non-polar interferents such as long-chain fatty compounds and sterols. In the present study, the various clean-up tests were all conducted in a medium with PSA and additional $MgSO_4$ (for further reduction of the remaining water content), with either addition of C18 (Method A), both C18 and florisil (Method B), or without modification (Method C). When considering analyte response, method C was significantly superior to both methods A and B for 12 out of the 22 targeted compounds, while no significant differences were reported for 6/22 compounds (**Figure S7.3**). The use of C18 (Method A) caused a 5 × decrease in DN-Imidacloprid absolute area compared to Method C, while the decrease was even more pronounced (20 ×) with the combined use of C18 and florisil (Method B). Relative losses in the -30% to -60% range were also observed for Methods A and B in the case of the triazine compounds atrazine, cyanazine, and simazine Based on the above, methods A and B were discarded. Method C (without modification) showed either statistically superior or equivalent performances to methods A and B, and method C was therefore retained as the final clean-up method.

The vortex time was tested for the selected extraction method at four discrete steps (0.5, 1, 2 and 4 minutes), suggesting in most cases no statistically significant differences between treatments (**Figure S7.4**). Since the 0.5 min condition presented the disadvantage of a higher variability in for some compounds, we retained the 1 min vortex time condition for the final procedure.

7.5.3 Reconstitution solvent and injection volume

Following the optimization of extraction conditions, the influence of reconstitution solvent nature was considered, using either 100% H_2O or H_2O amended with variable percentages of organic solvent (MeOH at 10%, 20%, and 30%). The best solvent among the 4 previous conditions was furthermore tested with formic acid at 0.1% (Tette et al., 2016). The injection volume was also tested to improve method limits of detection comparatively with the literature (Golge & Kabak, 2015), but without compromising the chromatographic peak shapes and/or method robustness. For these tests, the injection volume was set at 25, 50, and 100 µL.

Deformations of the chromatographic peak shapes were previously reported in reversed phase liquid chromatography when injecting samples with a high percentage of organic solvent —i.e., with a different viscosity from that of the starting mobile phase (Keunchkarian, Reta, Romero, & Castells, 2006). The early-eluting analytes may be more liable to such phenomena, especially when operating at higher injection volumes. The influence of reconstitution solvent and injection volume was therefore considered.

A highly aqueous reconstitution medium can be desirable because it allows to increase the injection volume, without compromising analyte retention in reversed phase chromatography. Highest peak intensities and suitable chromatographic peak shapes were generally obtained with the H_2O:MeOH 90:10 (v/v) combination containing 0.1% formic acid. Note that further increasing the formic acid percentage did not present any significant advantage or led to decreased peak intensities, hence the choice of the 0.1% formic acid condition.

After reconstituting the sample in a modified aqueous mobile phase medium (as mentioned previously), we set out to evaluate the influence of the injection volume. A near-linear

increase in signal intensity was generally observed between 25 and 100 µL for the targeted analytes (unpublished data). Therefore, the final injection volume retained was 100 µL to maximize the proportion of final extract analyzed (*de facto* ensuring an improved method detection limit), but without sacrificing the chromatographic performances (**Figure S7.5**).

FIGURES

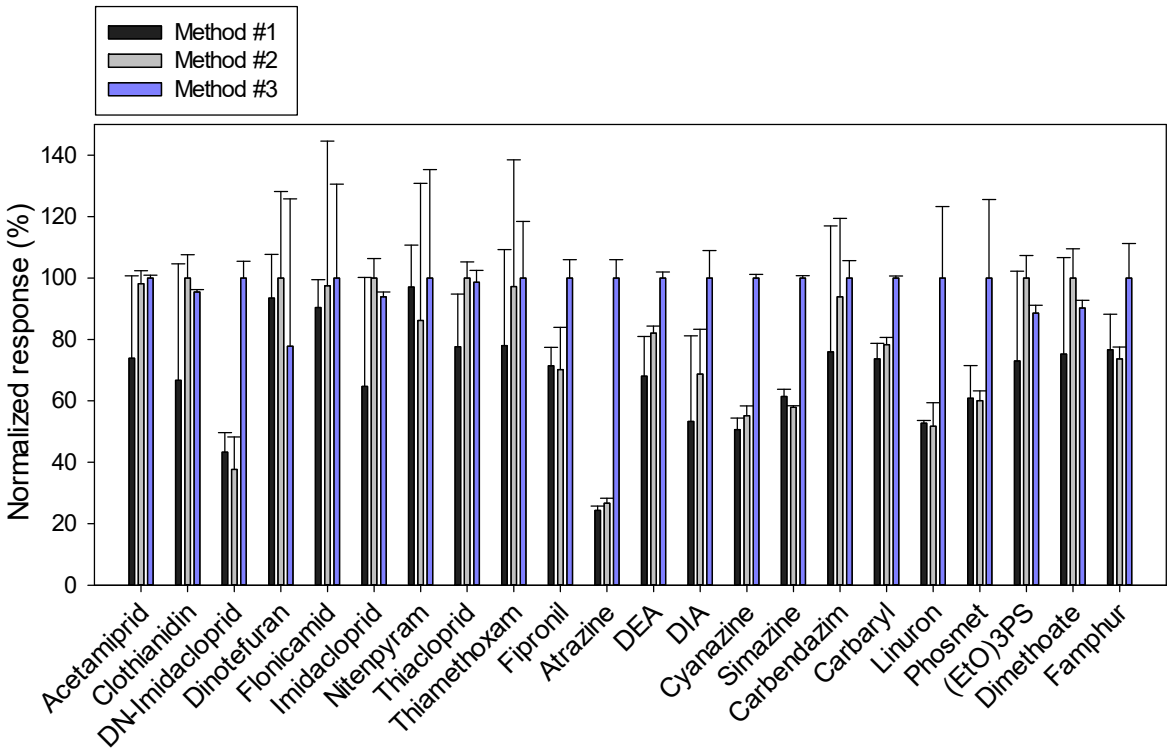

Figure S7.1. Influence of solvent and extraction salts variation (Method #1: Solvent: ACN:EtAc 70:30% v/v 1% Ac. Acid; Extraction salts: $MgSO_4$ + CH_3COONa; Method #2: Solvent: ACN with 1% Ac. Acid; Extraction salts: $MgSO_4$ + NaCl, Sodium Citrates; Method #3: ACN without acid; Extraction salts: $MgSO_4$ + NaCl, Sodium Citrates) on analyte absolute area. Error bars indicate standard deviations (n = 3 per condition).

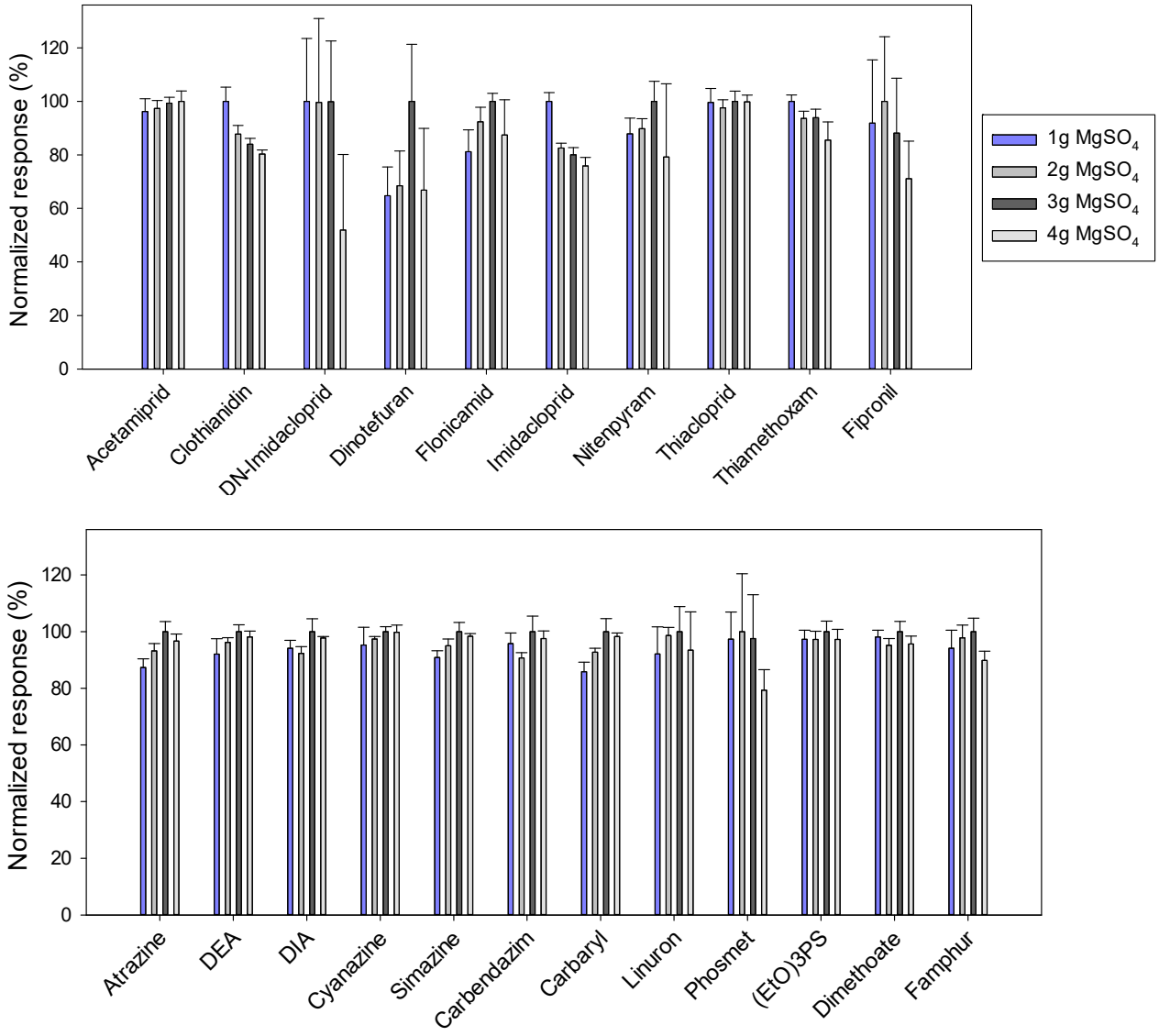

Figure S7.2. Influence of the $MgSO_4$ amount used in the extraction step (different tested amounts: 1, 2, 3, and 4 grams) on the analyte normalized response (for each compound, the area was set at 100% for the maximum observed across the 4 conditions). Error bars indicate standard deviations (n = 3 per condition).

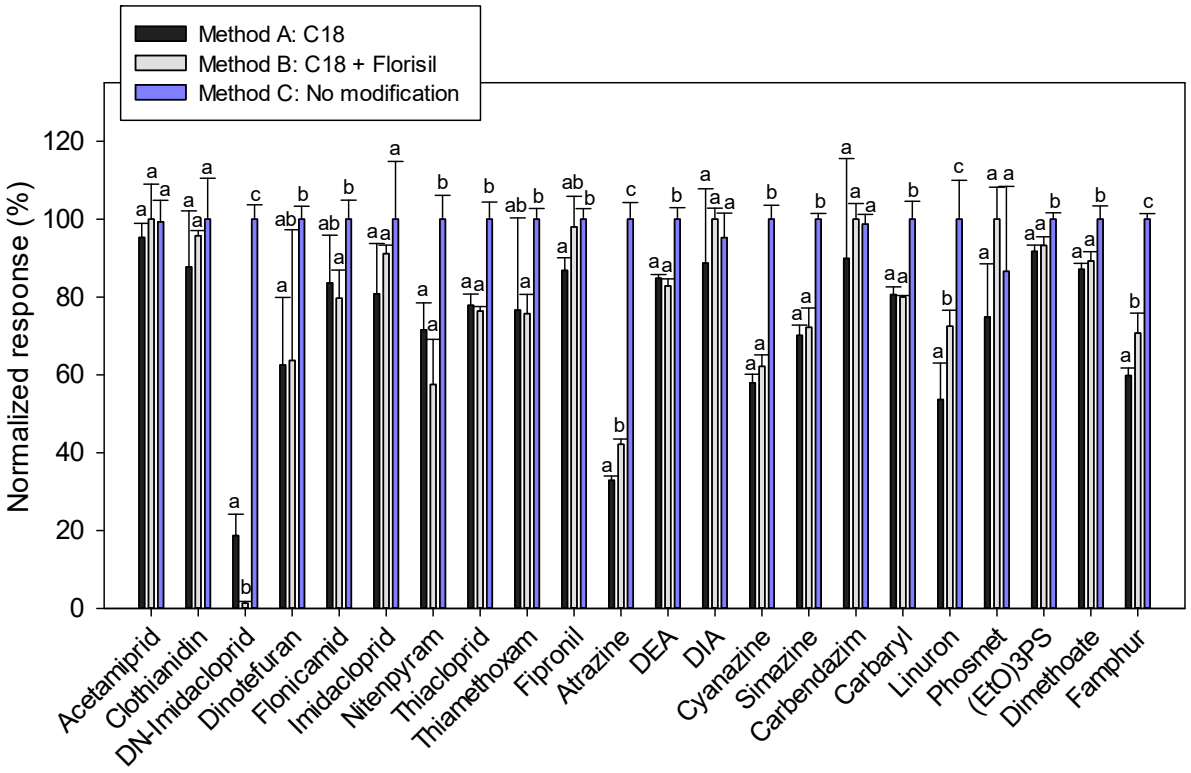

Figure S7.3. Influence of the sample clean-up procedure (Method A: $MgSO_4$ + PSA with the addition of C18; Method B: $MgSO_4$ + PSA with the addition of C18 + Florisil; Method C: $MgSO_4$ + PSA, without modification) on analyte normalized responses (for each compound, the area was set at 100% for the maximum observed across the 3 conditions), illustrated for spiked tomato. Error bars indicate normalized standard deviations (n = 3 per condition).

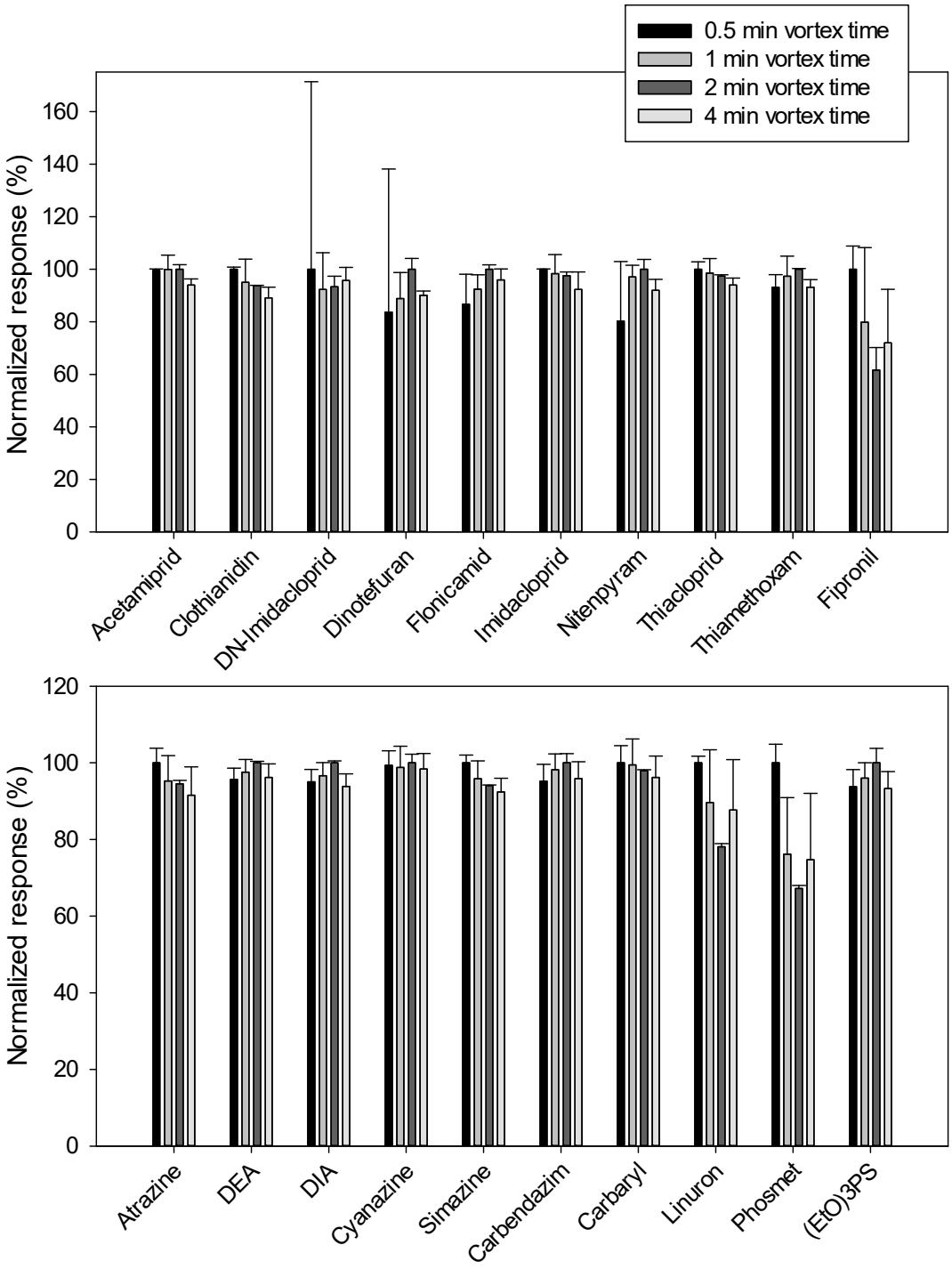

Figure S7.4. Influence of the high speed vortex time evaluated at 4 discrete steps (0.5, 1, 2, and 4 minutes) on the analyte normalized response (for each compound, the area was set at 100% for the maximum observed across the 4 conditions). Error bars indicate standard deviations (n = 3 per condition).

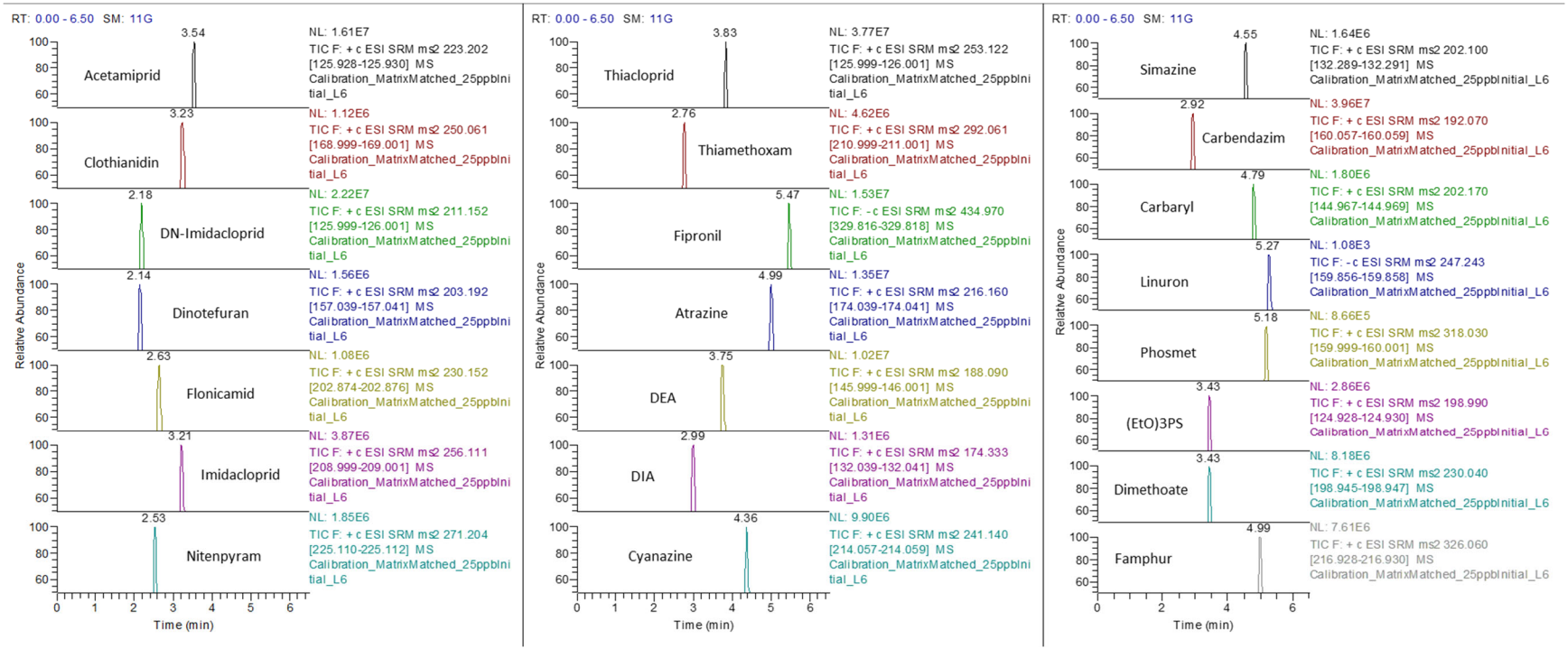

Figure S7.5. UHPLC-MS/MS chromatograms obtained with the final retained method (100 µL injection volume).

TABLES

Table S7.1. UHPLC gradient program.

Time *(min)*	A_1 (%)	B_1 (%)	Flow rate ($\mu L\ min^{-1}$)
0	95	5	500
3.5	45	55	500
4.5	5	95	500
6	5	95	500
6.1	95	5	500
8	95	5	500

A_1: HPLC-water with 10mM CH_3COONH_4 and 0.1% (v/v) HCOOH. B_1: MeOH with 0.1% (v/v) HCOOH.

Table S7.2. Mass spectrometry compound-dependent parameters of the targeted pesticides, and correspondence between native analytes and isotope-labelled internal standards. The MS/MS transition type is also specified: Q (quantification transition), C (confirmation transition), or IS (isotope-labelled internal standard).

Compound	Molecular formula	Precursor Ion (m/z)	Product Ion (m/z)	MS/MS Transition (type)	RF Lens (V)	Collision Energy (V)	IS
Acetamiprid	$C_{10}H_{11}ClN_4$	223.2	125.9	**Q**	51	23	Acetamiprid-d3
			99	C	51	39	
Clothianidin	$C_6N_5H_8SO_2Cl$	250.1	169	**Q**	36	13	Clothianidin-d3
			131.9	C	36	17	
DN-Imidacloprid	$C_9H_{11}ClN_4$	211.1	126	**Q**	75	25	Thiamethoxam-d3
			175	C	75	17	
Dinotefuran	$C_7H_{14}N_4O_3$	203.2	157	**Q**	30	10	Thiamethoxam-d3
			129.1	C	30	10	
Fipronil	$C_{12}H_4Cl_2F_6N_4OS$	434.9	329.8	**Q**	71	16	Fipronil-$^{13}C_2$-$^{15}N_2$
			249.9	C	71	28	
Imidacloprid	$C_9H_{10}ClN_5O_2$	256.1	209	**Q**	44	17	Imidacloprid-d4
			175	C	44	19	
Nitenpyram	$C_{11}H_{15}ClN_4O_2$	271.2	225.1	**Q**	48	19	Thiamethoxam-d3

			237	C	48	16	
Thiacloprid	$C_{10}H_9ClN_4S$	253.1	126	**Q**	56	22	Acetamiprid-d3
			185.9	C	56	15	
Thiamethoxam	$C_8H_{10}ClN_5O_3S$	292.1	211	**Q**	38	12	Thiamethoxam-d3
			180.9	C	38	22	
Atrazine	$C_8H_{14}ClN_5$	216.2	174.0	**Q**	62	17	Atrazine-$^{13}C_3$
			104.0	C	62	29	
DEA	$C_6H_{10}ClN_5$	188.1	146.0	**Q**	57	17	Atrazine-$^{13}C_3$
			103.9	C	57	26	
DIA	$C_5H_8ClN_5$	174.0	132.0	**Q**	61	17	Atrazine-$^{13}C_3$
			96.0	C	61	18	
Cyanazine	$C_9H_{13}ClN_6$	241.1	214.1	**Q**	65	17	Carbaryl-d6
			205.0	C	65	16	
Simazine	$C_7H_{12}ClN_5$	202.1	132.1	**Q**	64	19	Carbaryl-d6
			124.1	C	64	18	
Carbendazim	$C_9H_9N_3O_2$	192.1	160.1	**Q**	55	18	Carbaryl-d6
			132.1	C	55	30	
Carbaryl	$C_{12}H_{11}NO_2$	202.2	145.0	**Q**	48	10	Carbaryl-d6
			171.0	C	48	17	
Linuron	$C_9H_{10}Cl_2N_2O_2$	247.2	159.9	**Q**	49	12	Linuron-d6
			215.0	C	49	22	
Phosmet	$C_{11}H_{12}NO_4PS_2$	318.0	160.0	**Q**	38	15	Phosmet-d6
			276.9	C	38	10	

O,O,O-Triethyl phosphorothioate	$C_6H_{15}O_3PS$	199.0	124.9	**Q**	44	16	Phosmet-d6
			170.9	C	44	10	
Dimethoate	$C_5H_{12}NO_3PS_2$	230.0	198.9	**Q**	35	10	Phosmet-d6
			125.0	C	35	21	
Famphur	$C_{10}H_{16}NO_5PS_2$	326.1	216.9	**Q**	56	19	Phosmet-d6
			280.92	C	56	13	
Acetamiprid-d3	$C_{10}H_8D_3ClN_4$	226.2	126	IS	51	22	
Clothianidin-d3	$C_6N_5H_5D_3SO_2Cl$	253.1	172	IS	36	14	
Fipronil-$^{13}C_2$-$^{15}N_2$	$C_{10}{}^{13}C_2H_3Cl_2F_6N_2{}^{15}N_2OS$	439	333.9	IS	67	16	
Imidacloprid-d4	$C_9H_6D_4ClN_5O_2$	260.2	213	IS	44	18	
Thiamethoxam-d3	$C_5D_3H_{10}ClN_5O_3S$	295.1	214	IS	38	13	
Atrazine-$^{13}C_3$	$C_5{}^{13}C_3H_{14}ClN_5$	219.2	176	IS	60	18	
Carbaryl-d6	$C_{12}H_5D_6NO_2$	209.13	152	IS	30	10	
Linuron-d6	$C_9H_4D_6Cl_2N_2O_2$	253.13	160	IS	60	13	
Phosmet-d6	$C_{11}H_6D_6NO_4PS_2$	324.04	160	IS	33	13	

Table S7.3. Recovery (%) of the method (average ± SD), evaluated in 4 distinct matrices (lettuce, apple, grapes, and tomato) at two fortification levels (QC_L: 2.5-25 ng g^{-1}, compound-specific; QC_H: 400 ng g^{-1} for all compounds).

	Lettuce		Apple		Grapes		Tomato	
	QC_L n = 3	QC_H n = 3	QC_L n = 3	QC_H n = 3	QC_L n = 3	QC_H n = 3	QC_L n = 3	QC_H n = 3
Acetamiprid	101 ± 5	101 ± 7	99 ± 5	102 ± 6	97 ± 3	103 ± 1	101 ± 6	114 ± 1
Clothianidin	96 ± 5	99 ± 8	99 ± 5	100 ± 5	95 ± 4	97 ± 2	101 ± 7	96 ± 5
DN-Imidacloprid	95 ± 6	112 ± 15	50 ± 3	66 ± 6	89 ± 11	115 ± 13	48 ± 4	63 ± 4
Dinotefuran	54 ± 9	70 ± 14	99 ± 7	100 ± 10	74 ± 5	44 ± 4	97 ± 5	105 ± 10
Flonicamid	96 ± 9	102 ± 3	92 ± 3	106 ± 10	84 ± 9	104 ± 9	83 ± 6	111 ± 7
Imidacloprid	103 ± 6	105 ± 9	96 ± 5	101 ± 6	109 ± 10	97 ± 4	99 ± 8	101 ± 3
Nitenpyram	96 ± 8	93 ± 8	81 ± 2	94 ± 7	72 ± 3	95 ± 6	105 ± 5	114 ± 3
Thiacloprid	98 ± 6	95 ± 4	99 ± 6	97 ± 6	111 ± 2	111 ± 1	122 ± 12	114 ± 4
Thiamethoxam	100 ± 6	97 ± 5	97 ± 5	99 ± 7	97 ± 3	102 ± 2	110 ± 4	116 ± 4
Fipronil	100 ± 5	103 ± 7	100 ± 6	102 ± 7	92 ± 3	104 ± 2	116 ± 2	121 ± 4
Atrazine	99 ± 5	103 ± 4	72 ± 2	104 ± 9	101 ± 3	98 ± 1	99 ± 1	98 ± 2
DEA	89 ± 1	100 ± 6	93 ± 7	92 ± 7	100 ± 1	101 ± 2	116 ± 1	99 ± 2
DIA	82 ± 4	101 ± 5	90 ± 6	93 ± 5	96 ± 2	95 ± 2	71 ± 5	111 ± 1
Cyanazine	105 ± 6	102 ± 5	107 ± 10	103 ± 11	107 ± 16	94 ± 4	106 ± 1	98 ± 3
Simazine	99 ± 3	103 ± 6	99 ± 5	107 ± 6	91 ± 2	98 ± 4	118 ± 2	108 ± 2
Carbendazim	89 ± 5	98 ± 14	91 ± 4	90 ± 6	95 ± 13	93 ± 6	118 ± 12	95 ± 2

Carbaryl	100 ± 4	102 ± 6	100 ± 4	106 ± 8	99 ± 3	99 ± 3	112 ± 6	114 ± 1
Linuron	94 ± 5	91 ± 2	98 ± 4	92 ± 10	95 ± 4	97 ± 2	102 ± 6	119 ± 1
Phosmet	96 ± 3	101 ± 6	96 ± 2	76 ± 7	95 ± 1	68 ± 2	115 ± 3	92 ± 3
(EtO)3PS	102 ± 5	98 ± 24	105 ± 9	91 ± 6	86 ± 8	95 ± 2	104 ± 12	117 ± 4
Dimethoate	106 ± 7	102 ± 19	105 ± 10	91 ± 6	83 ± 7	92 ± 2	112 ± 16	111 ± 3
Famphur	95 ± 1	83 ± 6	98 ± 4	93 ± 3	95 ± 8	94 ± 1	110 ± 5	96 ± 3

Table S7.4. Precision (RSD, %) and accuracy (%) in **lettuce matrix**, at two fortification levels (QC_L: 2.5-25 µg kg^{-1}, depending on the particular compound; QC_H: 400 µg kg^{-1} for all compounds).

	Intraday Precision RSD %		**Interday Precision** RSD %		**Accuracy** %	
	QC_L n = 5	QC_H n = 5	QC_L n = 15	QC_H n = 15	QC_L n = 5	QC_H n = 5
Acetamiprid	13	3	8	4	93	108
Clothianidin	6	5	7	12	90	104
DN-Imidacloprid	20	9	18	15	115	104
Dinotefuran	5	7	19	16	99	104
Flonicamid	1	4	1	12	108	101
Imidacloprid	6	4	3	7	103	103
Nitenpyram	0	3	10	15	87	108
Thiacloprid	3	1	10	17	98	112
Thiamethoxam	4	3	1	1	91	106
Fipronil	3	4	3	1	102	105
Atrazine	5	4	4	5	77	113
DEA	6	3	4	1	77	102
DIA	9	4	5	10	77	104
Cyanazine	3	2	12	12	116	112
Simazine	1	0	12	9	89	106
Carbendazim	9	5	12	12	97	117
Carbaryl	5	2	11	9	115	109
Linuron	10	2	8	18	93	98
Phosmet	0	5	1	3	106	101
Triethylphosphorothioate	2	5	20	9	84	98
Dimethoate	4	2	10	4	93	109
Famphur	8	4	11	10	97	113

Table S7.5. Precision (RSD, %) and accuracy (%) in **apple matrix**, at two fortification levels (QC_L: 2.5-25 µg kg^{-1}, depending on the particular compound; QC_H: 400 µg kg^{-1} for all compounds).

	Intraday Precision RSD %		**Interday Precision** RSD %		**Accuracy** %	
	QC_L n = 5	QC_H n = 5	QC_L n = 15	QC_H n = 15	QC_L n = 5	QC_H n = 5
Acetamiprid	5	3	0	5	86	107
Clothianidin	3	4	4	4	90	108
DN-Imidacloprid	8	4	17	17	93	104
Dinotefuran	6	3	16	15	89	104
Flonicamid	6	3	18	13	101	111
Imidacloprid	4	2	14	8	109	105
Nitenpyram	3	3	20	17	106	104
Thiacloprid	2	2	14	8	103	92
Thiamethoxam	4	3	7	5	102	105
Fipronil	3	5	2	1	107	106
Atrazine	5	3	4	1	74	110
DEA	3	2	12	10	100	97
DIA	6	5	15	19	97	103
Cyanazine	6	2	15	14	106	111
Simazine	5	8	17	5	82	102
Carbendazim	10	4	17	9	82	94
Carbaryl	4	3	7	10	100	112
Linuron	9	9	1	4	96	101
Phosmet	2	5	2	14	75	108
Triethylphosphorothioate	18	4	12	13	102	101
Dimethoate	15	4	12	14	90	98
Famphur	8	4	11	18	82	98

Table S7.6. Precision (RSD, %) and accuracy (%) in **grapes matrix**, at two fortification levels (QC_L: 2.5-25 µg kg^{-1}, depending on the particular compound; QC_H: 400 µg kg^{-1} for all compounds).

	Intraday Precision RSD %		**Interday Precision** RSD %		**Accuracy** %	
	QC_L n = 5	QC_H n = 5	QC_L n = 15	QC_H n = 15	QC_L n = 5	QC_H n = 5
Acetamiprid	4	3	4	2	76	104
Clothianidin	2	1	8	6	110	104
DN-Imidacloprid	4	2	15	5	79	99
Dinotefuran	10	12	14	23	111	113
Flonicamid	10	3	9	1	91	98
Imidacloprid	4	1	14	16	80	102
Nitenpyram	9	1	1	6	104	107
Thiacloprid	7	3	16	10	80	92
Thiamethoxam	3	4	2	3	101	105
Fipronil	4	3	12	1	84	108
Atrazine	1	1	7	12	73	102
DEA	6	1	9	2	99	116
DIA	6	2	8	9	97	105
Cyanazine	5	3	13	9	80	106
Simazine	4	2	19	12	110	111
Carbendazim	5	0	20	10	114	111
Carbaryl	2	5	4	7	97	101
Linuron	11	5	13	4	103	100
Phosmet	1	7	1	13	117	99
Triethylphosphorothioate	4	7	6	10	118	108
Dimethoate	1	9	6	12	83	104
Famphur	3	8	3	9	103	103

Table S7.7. Precision (RSD, %) and accuracy (%) in **tomato matrix**, at two fortification levels (QC_L: 2.5-25 µg kg^{-1}, depending on the particular compound; QC_H: 400 µg kg^{-1} for all compounds).

	Intraday Precision RSD %		**Interday Precision** RSD %		**Accuracy** %	
	QC_L n = 5	QC_H n = 5	QC_L n = 15	QC_H n = 15	QC_L n = 5	QC_H n = 5
Acetamiprid	2	3	8	1	114	100
Clothianidin	2	3	10	1	99	100
DN-Imidacloprid	7	5	12	3	94	108
Dinotefuran	8	6	15	6	105	97
Flonicamid	4	3	20	9	81	109
Imidacloprid	2	6	11	3	104	101
Nitenpyram	1	5	10	1	91	102
Thiacloprid	3	2	2	8	84	111
Thiamethoxam	2	4	7	3	115	95
Fipronil	2	3	1	4	96	103
Atrazine	2	4	9	5	95	103
DEA	6	2	10	11	83	100
DIA	9	8	8	12	95	102
Cyanazine	1	3	13	14	90	106
Simazine	3	3	10	9	92	104
Carbendazim	1	3	11	4	96	92
Carbaryl	1	5	1	3	86	105
Linuron	6	5	2	2	103	96
Phosmet	6	5	10	19	109	99
Triethylphosphorothioate	16	2	23	22	111	101
Dimethoate	8	6	22	3	91	99
Famphur	18	5	18	9	103	97

Table S7.8. Illustration of the quality control strategy implemented in the present study. Variability (% RSD of analyte to IS area ratios) of the matrix-matched Continuing Quality Control (CQC) samples injected regularly along the UHPLC-MS/MS analytical sequences.

Variation of the analyte to IS response ratio (RSD, %)

	Lettuce	**Apple**	**Grapes**	**Tomato**
Acetamiprid	7	2	2	3
Clothianidin	7	3	3	2
DN-Imidacloprid	11	6	9	9
Dinotefuran	10	8	5	2
Flonicamid	13	5	2	16
Imidacloprid	7	2	12	3
Nitenpyram	9	11	9	5
Thiacloprid	8	5	2	3
Thiamethoxam	8	3	2	1
Fipronil	8	3	1	2
Atrazine	7	2	2	2
DEA	12	4	4	2
DIA	15	4	4	5
Cyanazine	12	7	3	4
Simazine	13	2	2	4
Carbendazim	6	11	6	6
Carbaryl	6	6	3	3
Linuron	12	13	9	5
Phosmet	7	13	5	4
(EtO)3PS	10	16	17	4
Dimethoate	10	16	18	4
Famphur	15	7	7	4

Table S7.9. Concentrations (µg kg $^{-1}$) of the 22 target analytes in lettuce samples (n = 39). Triplicate samples are signalled with a 't' in the sample label.

Variety	Label	Acetamiprid	Clothianidin	DN-Imidacloprid	Dinotefuran	Flonicamid
Lettuce Boston	L-001_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Lettuce Frisée verte	L-002	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Lettuce Frisée rouge	L-003	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Lettuce Romain	L-004	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Iceberg Lettuce	L-005	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Lettuce Boston	L-006	*<LOD*	**0.75**	*<LOD*	*<LOD*	*<LOD*
Lettuce Frisée rouge	L-007_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Lettuce Chicoree	L-008	*<LOD*	**0.72**	*<LOD*	*<LOD*	*<LOD*
Lettuce Escarole	L-009	*<LOD*	<LOD	*<LOD*	*<LOD*	*<LOD*
Lettuce Boston	L-010	*<LOD*	**2.37**	*<LOD*	*<LOD*	*<LOD*
Iceberg Lettuce	L-011	*<LOD*	**0.77**	*<LOD*	*<LOD*	*<LOD*
Spring Mix	L-012	*<LOD*	**0.24**	*<LOD*	*<LOD*	*<LOD*
Lettuce Romain	L-013	*<LOD*	**0.14**	*<LOD*	*<LOD*	*<LOD*
Letuce Red Leaf	L-014_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Lettuce Frisée verte	L-015	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Lettuce Frisée rouge	L-016	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Lettuce Frisée verte	L-017	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Lettuce Frisée rouge	L-018	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Lettuce Frisée verte	L-019	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Iceberg Lettuce	L-020	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Lettuce Frisée verte	L-021_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Lettuce Frisée verte	L-022	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Lettuce Frisée verte	L-023	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Lettuce Frisée rouge	L-024	*<LOD*	**0.24**	*<LOD*	*<LOD*	*<LOD*
Lettuce Frisée verte	L-025	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic Lettuce Romain	L-026	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Lettuce Frisée rouge	L-027	*<LOD*	**0.15**	*<LOD*	*<LOD*	*<LOD*
Lettuce Iceberg	L-028	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic Lettuce Frisée rouge	L-029	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Living Lettuce	L-030	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic Lettuce Frisée rouge	L-031	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic Lettuce Frisée verte	L-032	*<LOD*	**0.81**	*<LOD*	*<LOD*	*<LOD*
Lettuce Iceberg	L-033_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Living Lettuce Boston Premium	L-034	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic Iceberg Lettuce	L-035	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Lettuce Frisée verte	L-036	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Iceberg Lettuce	L-037	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Lettuce Romain	L-038	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Lettuce Frisée verte	L-039	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*

Table S7.9. (Continued).

Label	Imidacloprid	Nitenpyram	Thiacloprid	Thiamethoxam	Fipronil	Atrazine	DEA
L-001_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-002	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	**0.47**	*<LOD*
L-003	*<LOD*	*<LOD*	*<LOD*	**0.13**	*<LOD*	**0.56**	*<LOD*
L-004	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	**0.61**	*<LOD*
L-005	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-006	*<LOD*	*<LOD*	*<LOD*	**0.10**	*<LOD*	**1.18**	*<LOD*
L-007_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	**0.52 ± 0.01**	*<LOD*
L-008	*<LOD*	*<LOD*	*<LOD*	**2.18**	*<LOD*	**1.21**	*<LOD*
L-009	*<LOD*	*<LOD*	*<LOD*	**0.25**	*<LOD*	*<LOD*	*<LOD*
L-010	*<LOD*	*<LOD*	*<LOD*	**8.28**	*<LOD*	*<LOD*	*<LOD*
L-011	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-012	*<LOD*	*<LOD*	*<LOD*	**0.22**	*<LOD*	**0.90**	*<LOD*
L-013	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	**0.25**	*<LOD*
L-014_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	**0.49 ± 0.053**	*<LOD*
L-015	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-016	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-017	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-018	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-019	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-020	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-021_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-022	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-023	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-024	*<LOD*	*<LOD*	*<LOD*	**0.39**	*<LOD*	*<LOD*	*<LOD*
L-025	**4.99**	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-026	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-027	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	**1.90**	*<LOD*
L-028	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-029	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-030	**1.27**	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-031	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	**1.62**	*<LOD*
L-032	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	**7.54**	*<LOD*
L-033_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-034	**4.33**	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-035	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-036	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-037	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-038	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	**0.27**	*<LOD*
L-039	**0.64**	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*

Table S7.9. (Continued).

Label	DIA	Cyanazine	Simazine	Carbendazim	Carbaryl	Linuron
L-001_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-002	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-003	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-004	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-005	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-006	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-007_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-008	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-009	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-010	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-011	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-012	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-013	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-014_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-015	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-016	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-017	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-018	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-019	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-020	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-021_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-022	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-023	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-024	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-025	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-026	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-027	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-028	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-029	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-030	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-031	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-032	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-033_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-034	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-035	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-036	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-037	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-038	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-039	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*

Table S7.9. (Continued).

Label	Phosmet	(EtO)3PS	Dimethoate	Famphur
L-001_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-002	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-003	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-004	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-005	*<LOD*	**6.41**	**6.33**	*<LOD*
L-006	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-007_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-008	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-009	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-010	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-011	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-012	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-013	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-014_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-015	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-016	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-017	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-018	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-019	*<LOD*	**0.23**	*<LOD*	*<LOD*
L-020	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-021_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-022	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-023	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-024	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-025	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-026	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-027	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-028	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-029	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-030	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-031	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-032	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-033_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-034	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-035	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-036	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-037	*<LOD*	*<LOD*	*<LOD*	*<LOD*
L-038	*<LOD*	**8.00**	**8.10**	*<LOD*
L-039	*<LOD*	**218.73**	**215.37**	*<LOD*

Table S7.9. (Continued).

Variety	Label	Σ22 Pesticides
Lettuce Boston_Rep	L-001_t	*<LOD*
Lettuce Frisée verte	L-002	**0.47**
Lettuce Frisée rouge	L-003	**0.69**
Lettuce Romain	L-004	**0.61**
Iceberg Lettuce	L-005	**12.74**
Lettuce Boston	L-006	**2.03**
Lettuce Frisée rouge_Rep	L-007_t	**0.52 ± 0.01**
Lettuce Chicoree	L-008	**4.11**
Lettuce Escarole	L-009	**0.25**
Lettuce Boston	L-010	**10.65**
Iceberg Lettuce	L-011	**0.77**
Spring Mix	L-012	**1.35**
Lettuce Romain	L-013	**0.39**
Letuce Red Leaf_Rep	L-014_t	**0.49 ± 0.053**
Lettuce Frisée verte	L-015	*<LOD*
Lettuce Frisée rouge	L-016	*<LOD*
Lettuce Frisée verte	L-017	*<LOD*
Lettuce Frisée rouge	L-018	*<LOD*
Lettuce Frisée verte	L-019	**0.23**
Iceberg Lettuce	L-020	*<LOD*
Lettuce Frisée verte_Rep	L-021_t	*<LOD*
Lettuce Frisée verte	L-022	*<LOD*
Lettuce Frisée verte	L-023	*<LOD*
Lettuce Frisée rouge	L-024	**0.63**
Lettuce Frisée verte	L-025	**4.99**
Organic Lettuce Romain	L-026	*<LOD*
Lettuce Frisée rouge	L-027	**2.04**
Lettuce Iceberg	L-028	*<LOD*
Organic Lettuce Frisée rouge	L-029	*<LOD*
Living Lettuce	L-030	**1.27**
Organic Lettuce Frisée rouge	L-031	**1.62**
Organic Lettuce Frisée verte	L-032	**8.35**
Lettuce Iceberg _Rep	L-033_t	*<LOD*
Living Lettuce Boston Premium	L-034	**4.33**
Organic Iceberg Lettuce	L-035	*<LOD*
Lettuce Frisée verte	L-036	*<LOD*
Iceberg Lettuce	L-037	*<LOD*
Roman Lettuce	L-038	**16.37**
Lettuce Frisée verte	L-039	**434.73**

Table S7.10. Concentrations (µg kg $^{-1}$) of the 22 target analytes in apple samples (n = 37). Triplicate samples are signalled with a 't' in the sample label.

Variety	Label	Acetamiprid	Clothianidin	DN-Imidacloprid	Dinotefuran	Flonicamid
Golden Delicious	A-001	**3.72**	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Cortland	A-002	**23.79**	**0.13**	*<LOD*	*<LOD*	*<LOD*
Macintosh	A-003	**0.13**	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Red Delicious	A-004	**0.84**	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Empire	A-005	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Grany Smith	A-006	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Spartan	A-007_t	**2.85 ± 0.06**	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Pink Lady	A-008	**6.21**	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Gala	A-009	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic Grany Smith	A-010	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic Fuji	A-011	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic Crispps Pink	A-012	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic Gala	A-013	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic Red Delicious	A-014_t	**0.16 ± 0.0012**	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic Golden Delicious	A-015	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Envy	A-016	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Honey Crisp	A-017	**9.47**	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Jazz	A-018	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Eve	A-019	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Smitten	A-020	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Red Delicious	A-021_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Golden Delicious	A-022	**0.11**	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Cortland	A-023	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Pink Lady	A-024	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Fuji	A-025	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Grany Smith	A-026	**0.19**	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Macintosh	A-027	**17.59**	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Royal Gala	A-028_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Divine	A-029	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Empire	A-030	**1.21**	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Grany Smith	A-031	**0.21**	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Macintosh	A-032	**0.32**	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Surete (green-acid)	A-033	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Grany Smith	A-034	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Empire	A-035_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Grany Smith	A-036	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Fuji	A-037	**0.85**	*<LOD*	*<LOD*	*<LOD*	*<LOD*

Table S7.10. (Continued).

Label	Imidacloprid	Nitenpyram	Thiacloprid	Thiamethoxam	Fipronil	Atrazine	DEA
A-001	**0.14**	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-002	**3.79**	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-003	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-004	**0.08**	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-005	*<LOD*	*<LOD*	**1.56**	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-006	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-007_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-008	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-009	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-010	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-011	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-012	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-013	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-014_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-015	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-016	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-017	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-018	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-019	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-020	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-021_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-022	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-023	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-024	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-025	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-026	**0.16**	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-027	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-028_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-029	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-030	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-031	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-032	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-033	*<LOD*	*<LOD*	**28.38**	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-034	**0.13**	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-035_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-036	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*

A-037	**0.11**	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*

Table S7.10. (Continued).

Label	DIA	Cyanazine	Simazine	Carbendazim	Carbaryl	Linuron
A-001	*<LOD*	*<LOD*	*<LOD*	*<LOD*	**1.10**	*<LOD*
A-002	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-003	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-004	*<LOD*	*<LOD*	*<LOD*	**1.13**	*<LOD*	*<LOD*
A-005	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-006	*<LOD*	*<LOD*	*<LOD*	**3.08**	*<LOD*	*<LOD*
A-007_t	*<LOD*	*<LOD*	**0.22 ± 0.064**	*<LOD*	*<LOD*	*<LOD*
A-008	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-009	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-010	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-011	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-012	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-013	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-014_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-015	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-016	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-017	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-018	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-019	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-020	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-021_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-022	*<LOD*	*<LOD*	*<LOD*	**0.69**	*<LOD*	*<LOD*
A-023	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-024	*<LOD*	*<LOD*	*<LOD*	**0.58**	*<LOD*	*<LOD*
A-025	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-026	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-027	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-028_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-029	*<LOD*	*<LOD*	*<LOD*	**0.49**	*<LOD*	*<LOD*
A-030	*<LOD*	*<LOD*	**0.43**	*<LOD*	*<LOD*	*<LOD*
A-031	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-032	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-033	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-034	*<LOD*	*<LOD*	*<LOD*	**5.97**	*<LOD*	*<LOD*
A-035_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-036	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*

A-037	*<LOD*	*<LOD*	*<LOD*	**28.31**	*<LOD*	*<LOD*

Table S7.10. (Continued).

Label	Phosmet	(EtO)3PS	Dimethoate	Famphur
A-001	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-002	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-003	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-004	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-005	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-006	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-007_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-008	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-009	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-010	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-011	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-012	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-013	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-014_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-015	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-016	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-017	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-018	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-019	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-020	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-021_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-022	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-023	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-024	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-025	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-026	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-027	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-028_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-029	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-030	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-031	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-032	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-033	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-034	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-035_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*

A-036	*<LOD*	*<LOD*	*<LOD*	*<LOD*
A-037	*<LOD*	*<LOD*	*<LOD*	*<LOD*

Table S7.10. (Continued).

Variety	Label	Σ22 Pesticides
Golden Delicious	A-001	**4.96**
Cortland	A-002	**27.71**
Macintosh	A-003	**0.13**
Red Delicious	A-004	**2.04**
Empire	A-005	**1.56**
Grany Smith	A-006	**3.08**
Spartan	A-007_t	**3.07 ± 0.064**
Pink Lady	A-008	**6.21**
Gala	A-009	<LOD
Organic Grany Smith	A-010	<LOD
Organic Fuji	A-011	<LOD
Organic Crispps Pink	A-012	<LOD
Organic Gala	A-013	<LOD
Organic Red Delicious	A-014_t	**0.16 ± 0.0012**
Organic Golden Delicious	A-015	<LOD
Envy	A-016	<LOD
Honey Crisp	A-017	**9.47**
Jazz	A-018	<LOD
Eve	A-019	<LOD
Smitten	A-020	<LOD
Red Delicious	A-021_t	<LOD
Golden Delicious	A-022	**0.80**
Cortland	A-023	<LOD
Pink Lady	A-024	**0.58**
Fuji	A-025	<LOD
Grany Smith	A-026	**0.35**
Macintosh	A-027	**17.59**
Royal Gala	A-028_t	<LOD
Divine	A-029	**0.49**
Empire	A-030	**1.65**
Grany Smith	A-031	**0.21**
Macintosh	A-032	**0.32**
Surete (green-acid)	A-033	**28.38**
Grany Smith	A-034	**6.11**

Empire	A-035_t	<LOD
Grany Smith	A-036	<LOD
Fuji	A-037	**29.27**

Table S7.11. Concentrations (µg kg $^{-1}$) of the 22 target analytes in grapes samples (n = 27). Triplicate samples are signalled with a 't' in the sample label.

Variety	Label	Acetamiprid	Clothianidin	DN-Imidacloprid	Dinotefuran	Flonicamid
Red sedless table grapes	G-001	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Green sedless table grapes	G-002	*<LOD*	**73.13**	*<LOD*	**47.14**	*<LOD*
Organic green sedless table grapes	G-003	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic red sedless table grapes	G-004	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic green sedless table grapes	G-005_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Green sedless table grapes-Autumncrisp	G-006	*<LOD*	**140.77**	**1.91**	*<LOD*	*<LOD*
Green sedless table grapes-SweetCelebration	G-007	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic red sedless table grapes	G-008	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Red sedless table grapes-Scarlotta	G-009_t	**107.82 ± 4.25**	**85.02 ±1.51**	*<LOD*	*<LOD*	*<LOD*
Organic green sedless table grapes	G-010	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic red sedless table grapes	G-011	*<LOD*	**4.64**	*<LOD*	*<LOD*	*<LOD*
Green sedless table grapes-Perletts	G-012	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Red sedless table grapes-Flames	G-013	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Red table grapes with sed	G-014_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Green table grapes with sed-Muscats	G-015	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Green sedless table grapes	G-016	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Red sedless table grapes	G-017	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Green sedless table grapes	G-018	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic green sedless table grapes	G-019_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Green sedless table grapes	G-020	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Black sedless table grapes	G-021_t	*<LOD*	*<LOD*	**4.40 ± 0.08**	*<LOD*	*<LOD*
Red sedless table grapes	G-022	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Red/green sedless table grapes	G-023	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Green sedless table grapes	G-024	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Red sedless table grapes	G-025	*<LOD*	2.06	*<LOD*	*<LOD*	*<LOD*
Green sedless table grapes	G-026_t	*<LOD*	*<LOD*	**10.55 ± 0.39**	*<LOD*	*<LOD*
Organic red sedless table grapes	G-027	*<LOD*	*<LOD*	**3.57**	*<LOD*	*<LOD*

Table S7.11. (Continued).

Label	Imidacloprid	Nitenpyram	Thiacloprid	Thiamethoxam	Fipronil	Atrazine	DEA
G-001	**4.86**	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-002	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-003	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-004	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-005_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-006	**5.77**	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-007	**29.18**	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-008	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-009_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-010	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-011	*<LOD*	*<LOD*	*<LOD*	**53.85**	*<LOD*	*<LOD*	*<LOD*
G-012	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-013	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-014_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-015	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-016	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-017	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-018	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-019_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-020	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-021_t	**23.34 ± 1.01**	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-022	**12.94**	*<LOD*	*<LOD*	**2.72**	*<LOD*	*<LOD*	*<LOD*
G-023	**5.05**	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-024	**28.89**	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-025	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-026_t	**21.59 ± 0.49**	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-027	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*

Table S7.11. (Continued).

Label	DIA	Cyanazine	Simazine	Carbendazim	Carbaryl	Linuron
G-001	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-002	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-003	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-004	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-005_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-006	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*

G-007	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-008	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-009_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-010	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-011	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-012	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-013	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-014_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-015	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-016	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-017	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-018	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-019_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-020	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-021_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-022	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-023	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-024	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-025	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-026_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-027	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*

Table S7.11. (Continued).

Label	Phosmet	(EtO)3PS	Dimethoate	Famphur
G-001	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-002	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-003	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-004	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-005_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-006	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-007	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-008	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-009_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-010	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-011	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-012	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-013	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-014_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*

G-015	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-016	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-017	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-018	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-019_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-020	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-021_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-022	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-023	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-024	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-025	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-026_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
G-027	*<LOD*	*<LOD*	*<LOD*	*<LOD*

Table S7.11. (Continued).

Variety	Label	Σ22 Pesticides
Red sedless table grapes	G-001	**4.86**
Green sedless table grapes	G-002	**120.27**
Organic green sedless table grapes	G-003	*<LOD*
Organic red sedless table grapes	G-004	*<LOD*
Organic green sedless table grapes	G-005_t	*<LOD*
Green sedless table grapes-Autumncrisp	G-006	**148.45**
Green sedless table grapes-SweetCelebration	G-007	**29.18**
Organic red sedless table grapes	G-008	*<LOD*
Red sedless table grapes-Scarlotta	G-009_t	**192.84 ± 4.51**
Organic green sedless table grapes	G-010	*<LOD*
Organic red sedless table grapes	G-011	**58.49**
Green sedless table grapes-Perletts	G-012	*<LOD*
Red sedless table grapes-Flames	G-013	*<LOD*
Red table grapes with sed	G-014_t	*<LOD*
Green table grapes with sed-Muscats	G-015	*<LOD*
Green sedless table grapes	G-016	*<LOD*
Red sedless table grapes	G-017	*<LOD*
Green sedless table grapes	G-018	*<LOD*
Organic green sedless table grapes	G-019_t	*<LOD*
Green sedless table grapes	G-020	*<LOD*
Black sedless table grapes	G-021_t	**27.74 ± 1.01**
Red sedless table grapes	G-022	**15.66**
Red/green sedless table grapes	G-023	**5.05**
Green sedless table grapes	G-024	**28.89**
Red sedless table grapes	G-025	**2.06**
Green sedless table grapes	G-026_t	**32.14 ± 0.63**
Organic red sedless table grapes	G-027	**3.57**

Table S7.12. Concentrations (μg kg $^{-1}$) of the 22 target analytes in tomatoes samples (n = 30). Triplicate samples are signalled with a 't' in the sample label.

Variety	Label	Acetamiprid	Clothianidin	DN-Imidacloprid	Dinotefuran	Flonicamid
Cherry tomato	T-001	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Red Greenhouse tomato	T-002	*<LOD*	*<LOD*	*<LOD*	**12.76**	*<LOD*
Roma tomato	T-003	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Cocktail tomato	T-004	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Kumato	T-005_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Red Greenhouse tomato	T-006	*<LOD*	*<LOD*	*<LOD*	**19.87**	*<LOD*
Pink tomato	T-007	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Cherry mix tomato	T-008	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Zima tomato	T-009	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic mini Apero cherry tomato	T-010_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Grape tomato	T-011	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic grape tomato	T-012	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Grape tomato	T-013	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Red Greenhouse tomato	T-014	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Red chopin tomato	T-015_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Yore tomato	T-016	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Cherry tomato	T-017	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Pink tomato	T-018	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Angel sweet tomato	T-019	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Pink cherry tomato	T-020_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Campari tomato	T-021	**15.70**	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Red Cello tomato	T-022	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic red tomato	T-023	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Cocktail tomato	T-024	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic grape tomate	T-025_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Red grape tomato	T-026	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Organic Apero tomato	T-027	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Grape tomato	T-028	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
Tomato puree_1	T-029_t	*<LOD*	**3.21 ± 0.04**	*<LOD*	*<LOD*	*<LOD*
Tomato puree_2	T-030	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*

Table S7.12. (Continued).

Label	Imidacloprid	Nitenpyram	Thiacloprid	Thiamethoxam	Fipronil	Atrazine	DEA
T-001	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-002	**7.59**	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-003	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-004	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-005_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-006	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-007	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-008	**10.18**	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-009	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-010_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-011	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-012	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-013	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-014	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-015_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-016	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-017	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-018	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-019	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-020_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-021	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-022	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-023	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-024	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-025_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-026	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-027	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-028	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-029_t	**11.22 ± 0.03**	*<LOD*	*<LOD*	**1.37 ± 0.03**	*<LOD*	*<LOD*	*<LOD*
T-030	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*

Table S7.12. (Continued).

Label	DIA	Cyanazine	Simazine	Carbendazim	Carbaryl	Linuron
T-001	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-002	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-003	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-004	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-005_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-006	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-007	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-008	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-009	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-010_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-011	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-012	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-013	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-014	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-015_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-016	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-017	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-018	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-019	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-020_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-021	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-022	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-023	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-024	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-025_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-026	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-027	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-028	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-029_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-030	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*

Table S7.12. (Continued).

Label	Phosmet	(EtO)3PS	Dimethoate	Famphur
T-001	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-002	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-003	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-004	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-005_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-006	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-007	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-008	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-009	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-010_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-011	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-012	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-013	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-014	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-015_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-016	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-017	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-018	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-019	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-020_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-021	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-022	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-023	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-024	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-025_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-026	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-027	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-028	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-029_t	*<LOD*	*<LOD*	*<LOD*	*<LOD*
T-030	*<LOD*	*<LOD*	*<LOD*	*<LOD*

Table S7.12. (Continued).

Variety	Label	Σ22 Pesticides
Cherry tomato	T-001	*<LOD*
Red Greenhouse tomato	T-002	**20.35**
Roma tomato	T-003	*<LOD*
Cocktail tomato	T-004	*<LOD*
Kumato	T-005_t	*<LOD*
Red Greenhouse tomato	T-006	**19.87**
Pink tomato	T-007	*<LOD*
Cherry mix tomato	T-008	**10.18**
Organic zima tomato	T-009	*<LOD*
Organic mini Apero cherry tomato	T-010_t	*<LOD*
Grape tomato	T-011	*<LOD*
Organic grape tomato	T-012	*<LOD*
Grape tomato	T-013	*<LOD*
Red Greenhouse tomato	T-014	*<LOD*
Red chopin tomato	T-015_t	*<LOD*
Yore tomato	T-016	*<LOD*
Cherry tomato	T-017	*<LOD*
Pink tomato	T-018	*<LOD*
Angel sweet tomato	T-019	*<LOD*
Pink cherry tomato	T-020_t	*<LOD*
Campari tomato	T-021	**15.70**
Red Cello tomato	T-022	*<LOD*
Organic red tomato	T-023	*<LOD*
Cocktail tomato	T-024	*<LOD*
Organic grape tomate	T-025_t	*<LOD*
Red grape tomato	T-026	*<LOD*
Organic Apero tomato	T-027	*<LOD*
Grape tomato	T-028	*<LOD*
Tomato puree_1	T-029_t	**15.8 ± 0.03**
Tomato puree_2	T-030	*<LOD*

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Chapitre 8. Conclusions

8.1 Conclusions

Si l'utilisation de pesticides à des fins agronomiques n'est pas un phénomène nouveau, en revanche l'étude de leur devenir et de leurs effets n'a attiré l'attention de la communauté scientifique et civile que depuis relativement récemment. Selon le degré de contamination des divers compartiments, les résidus de pesticides peuvent affecter tant l'environnement que la santé humaine. L'application massive de pesticides, notamment insecticides et herbicides, entraîne une répartition de ces composés par mobilité dans l'eau d'inondation agricole et les sols. Les pesticides peuvent ensuite être transportés dans les eaux de surface et souterraines et finalement parvenir à l'eau potable. D'autres produits de consommation tels que les fruits et légumes peuvent conserver des teneurs résiduelles de pesticides.

Pour les matrices aqueuses éloignées des sources de contamination, une certaine dilution est prévisible, et les niveaux atteints peuvent être de l'ordre du ng L^{-1}. C'est ici que la nécessité de méthodes analytiques suffisamment sensibles, robustes et fiables apparaît, ce qui constitue un des principaux objectifs du présent travail de recherche.

Afin d'analyser les insecticides néonicotinoïdes dans l'eau de surface et l'eau potable, le principal défi était de diminuer le temps d'analyse ainsi que le volume d'échantillon en intégrant une extraction SPE en ligne. Dans le **Chapitre 4**, il est démontré que la méthode développée pour l'analyse de 8 néonicotinoïdes et du fipronil par SPE en ligne couplée à la spectrométrie de masse atteint des limites de détection de l'ordre de 0.1 à 5 ng L^{-1}, du même ordre voire meilleures que les performances publiées précédemment dans la littérature. Ce degré de performance a été rendu possible par une optimisation multivariable faisant intervenir la méthodologie des surfaces de réponses. Ce type d'optimisation a permis de prendre en considération les interactions possibles entre les variables critiques, telles que le volume de charge et la vitesse de charge. Ces variables peuvent affecter des paramètres clés de l'assurance de la qualité analytique comme le taux de récupération, la forme des pics chromatographiques, la sensibilité et la précision. L'approche par plan d'expérience faisant varier simultanément plusieurs variables,

présente un avantage important à mentionner par rapport aux optimisations variable par variable (un facteur à la fois) qui négligent les interactions possibles entre variables et couvrent une zone plus réduite du domaine expérimental. La possibilité de rencontrer les conditions optimales est donc a priori supérieure avec l'approche par plan d'expérience. Un autre avantage de la méthode analytique développée est sa relative simplicité. Suite à la collection des échantillons aqueux, la méthode consiste en une simple filtration des échantillons sur membranes de polyester (filtres PETE) avant l'analyse d'une aliquote de 2 mL par SPE en-ligne directement couplée à l'analyse LC-MS/MS (temps total d'analyse : 8 min), réduisant considérablement les manipulations de prétraitement d'échantillon. Certaines limitations ou restrictions de cette méthode sont cependant à noter. Bien que les limites de détection soient faibles et meilleures comparativement à d'autres méthodes publiées dans la littérature, la méthode semble moins pertinente pour les échantillons où de très faibles (<1 ng L^{-1}) concentrations seraient attendues. Lors de l'analyse des échantillons du fleuve Saint-Laurent tel que décrit dans le **Chapitre 6**, une méthode alternative reposant sur une extraction SPE hors ligne large volume (250 mL) a été appliquée pour couvrir les faibles niveaux de concentration anticipés. Par ailleurs, seulement 8 néonicotinoïdes et le fipronil étaient analysés dans la méthode SPE en-ligne – LC-MS/MS; il serait donc avantageux d'élargir la méthode multirésiduelle à d'autres composés pertinents. La diversité des composés de différentes caractéristiques physicochimiques demandera un compromis entre les paramètres de qualité désirés et le nombre d'analytes ciblés. C'est ainsi que l'atrazine ainsi que son produit de dégradation, la desethylatrazine (DEA), ont été par la suite intégrées à la méthode en-ligne décrite dans le **Chapitre 4**.

Concernant l'atrazine, il existait peu de données sur les variations spatio-temporelles de la contamination dans l'eau potable au Québec. Quelle est l'amplitude des variations saisonnières dans l'eau du robinet accessible à la population de la région de Montréal, et dans quelle mesure les concentrations d'atrazine varient selon que les municipalités puisent leur eau du fleuve, des tributaires, ou de l'eau souterraine ? Ce sont ces questions de recherche qui sont abordées dans le **Chapitre 5**. Un échantillonnage intensif d'eau potable dans la région de Montréal (échantillonnage durant 18 mois consécutifs, à raison de deux fois par semaine) a permis d'identifier les pics de

concentrations maximales de l'atrazine dans un cycle annuel complet (2015-2016). L'atrazine a été détectée dans la totalité des échantillons du suivi temporel à Montréal avec des concentrations entre 30-195 ng L^{-1}. Concernant les échantillons d'eau potable analysés, nous n'avons observé aucun dépassement des critères de qualité de l'eau en accord avec les normes de l'Amérique du Nord. Toutefois, dans certains cas, il existe un dépassement des normes Européennes. Il aurait été intéressant de réaliser un suivi de l'atrazine dans l'eau potable de Montréal pendant 24 mois à la place de 18 mois de suivi. Cela aurait permis de confirmer la saisonnalité des pics de concentrations. L'analyse systématique d'autres produits de dégradation de l'atrazine, tel que la deisopropyl-atrazine (DIA) ou l'hydroxy-atrazine (OH-atrazine), aurait également été pertinente afin d'examiner d'éventuelles différences de ratios entre l'atrazine et ses produits de dégradation. Ces composés ont été identifiés dans un échantillon d'eau potable prospectivement analysé par LC-HRMS (Orbitrap Q-Exactive) et pourraient être intégrés dans de futures activités de surveillance après acquisition de leurs étalons analytiques.

Dans un deuxième temps (2017-2018), les variations spatiales de l'atrazine ont été évaluées dans 52 municipalités du Québec méridional, ciblant quelques-unes des villes les plus peuplées de la province (Montréal, Québec, Laval, Sherbrooke, Saint-Jean-sur-Richelieu, Drummondville, etc.). Outre la source d'eau pour produire l'eau potable (eau de surface, souterraine, ou sources mixtes), le niveau de contamination peut dépendre du type de traitement suivi dans les usines de production d'eau potable, mais aussi de l'activité agricole du bassin versant. Dans notre étude de distribution spatiale de l'atrazine dans l'eau du robinet, nous avons noté que les villes qui présentaient les plus grandes concentrations étaient celles qui puisent l'eau du fleuve Saint-Laurent pour produire l'eau potable. Nous avons émis l'hypothèse que la source majeure dans ce cas pouvait être reliée aux applications d'atrazine dans les Grands Lacs en amont. À l'inverse, des villes situées sur la Rive Nord comme Terrebonne ou Laval qui puisent leur eau dans la rivière des Outaouais ou la rivière des Mille Iles présentaient des concentrations beaucoup plus faibles. Le lien avec la distribution de l'atrazine au sein des différentes masses d'eau qui s'écoulent de part et d'autre de l'île de Montréal a d'ailleurs été approfondi dans le **Chapitre 6**. D'autres sites avec des niveaux relativement élevés d'atrazine incluaient certaines municipalités qui

puisent leur eau dans les tributaires de la rive Sud (notamment la rivière Yamaska), en lien avec la forte proportion de terres agricoles dans ces bassins versants.

Suite aux tendances spatiales remarquées pour la contamination de l'eau potable de diverses villes, nous avons conçu le projet de documenter la présence des pesticides tel que les néonicotinoïdes, l'atrazine et le glyphosate le long d'un tronçon de 200 km du fleuve Saint-Laurent (**Chapitre 6**). Une partie de la mission a été conduite à bord du navire de recherche *Lampsilis* (en collaboration avec l'UQTR), permettant de recueillir des échantillons d'eau de surface en divers transects du fleuve. Les concentrations de pesticides varient en fonction de la masse d'eau échantillonnée au sein du fleuve Saint-Laurent : eaux brunes de la rivière des Outaouais qui s'écoulent le long de la rive nord *Vs.* eaux bleu-vert des Grands Lacs qui constituent la masse d'eau centrale. La distribution d'atrazine, tel qu'il a été conjecturé dans le **Chapitre 5**, est fortement liée à son transport conservatif (en raison de sa persistance) depuis sa source majeure en amont (Lac Ontario et autres Grands Lacs) vers l'Atlantique. L'atrazine va rester plus fortement présente dans la masse d'eau au centre des transects, avec un léger renforcement pour les points proches de la rive sud au niveau du Lac Saint-Pierre en raison des apports de tributaires tels que les rivières Nicolet, Saint-François et Yamaska. À noter que les néonicotinoïdes et le glyphosate ainsi que son produit de dégradation AMPA, présentent un profil différent à celui de l'atrazine. Les néonicotinoïdes et le glyphosate étaient plus fréquemment détectés dans la rivière des Outaouais ainsi que dans les points du Saint-Laurent situés le long de la rive nord en aval de l'île de Montréal (masses des eaux brunes), reflétant le mélange limité des masses d'eau. Ce profil est conservé pour les divers transects échantillonnés jusqu'au niveau du lac Saint-Pierre, où le profile s'inverse dû à la contribution majeure des tributaires de la rive Sud. Il serait intéressant de poursuivre les efforts d'échantillonnage dans les années suivantes et d'inclure d'autres pesticides ainsi que d'autres types de contaminants émergents qui pourraient avoir un impact sur la santé des écosystèmes du fleuve Saint-Laurent. La situation de la perchaude du Lac Saint-Pierre ou des bélugas du Parc Marin du Saguenay reste préoccupante, sans doute dû à une multiplicité de facteurs (stress lié aux activités humaines et au trafic maritime, changement climatique, fragilisation des organismes due aux contaminants, etc.).

Avec l'utilisation massive de pesticides et d'autres produits liés aux activités humaines, la probabilité de retrouver ces composés dans l'environnement est en augmentation, avec de possibles implications pour la santé publique. À quel degré et par quelles voies majoritaires l'être humain est-il exposé aux pesticides ? Cette question a été en partie abordée dans le **Chapitre 5** sur l'eau potable, mais également dans le **Chapitre 7** sur les fruits et légumes. L'adaptation d'une méthode d'extraction par phase solide dispersive aussi dénommée QuEChERS a permis d'évaluer la présence de 22 pesticides couvrant 7 classes différentes dans différents produits de consommation (laitue, pommes, tomate et raisin). Couplée à la LC-MS/MS, cette méthode a permis d'atteindre des niveaux de détection de l'ordre de 0.05 à 2 $\mu g\ kg^{-1}$, des seuils de détection 10 à 1000 fois inférieurs aux limites maximales admissibles de résidus (LMR) dans les produits alimentaires. Un criblage des différents pesticides dans 133 échantillons issus de la culture biologique et conventionnelle a été réalisé, afin de mettre en évidence les éventuelles différences entre ces deux types de méthodes de production. Les néonicotinoïdes ont été retrouvés dans l'ensemble des matrices alimentaires ciblées, en particulier l'imidaclopride, l'acétamipride et la clothianidine (0.08 à 141 $\mu g\ kg^{-1}$). Aucun dépassement de normes n'a été constaté selon les LMR canadiennes. Cette méthode est compétitive en termes de qualité des données générées, tant au niveau de la performance des LODs que de la méthode de quantification (élimination des effets de matrice par un étalonnage dans chaque matrice), mais elle reste relativement lourde à mettre en œuvre ce qui pourrait constituer un frein pour l'application à de plus grandes séries d'échantillons.

Ces travaux de doctorat ont permis d'appréhender un ensemble de techniques d'analyse de pesticides pour une diversité de matrices. Les différentes étapes des procédures analytiques ont été optimisées et validées afin d'assurer la meilleure qualité possible aux jeux de données établis. Les méthodes nouvellement développées ont permis de fournir une image générale du degré de contamination des écosystèmes aquatiques au Québec '*from source to tap*', depuis la source de contamination (plaines agricoles) jusqu'à l'eau du robinet que nous consommons. La contamination des eaux de surface notamment du fleuve Saint-Laurent, bien que généralement inférieure aux valeurs seuils des normes de qualité, reste préoccupante en ce qui concerne les effets chroniques à long terme. De futurs travaux pourraient s'intéresser au devenir environnemental des insecticides

néonicotinoïdes dans la zone estuarienne du fleuve Saint-Laurent et estimer les flux exportés dans le Golfe du Saint-Laurent. Les méthodes analytiques développées dans le cadre de cette thèse permettront de faciliter l'acquisition de données sur les pesticides présents au Canada dans l'environnement et la population, à des fins environnementales et de santé publique.

8.2 Perspectives

Les méthodes présentées dans cette thèse ont rendu possible des analyses aussi rapides, sensibles, fiables et robustes que possible, pour une diversité de matrices d'intérêt environnemental. Les limites de détection atteintes par la méthode de SPE en ligne, bien que compétitives, ne sont cependant pas suffisantes pour certains types d'échantillons aqueux comportant de très faibles concentrations (<0.1 ng/L). A cet égard, la préconcentration de larges volumes d'échantillons (250-1000 mL) par SPE hors ligne reste attractive pour certaines matrices (eau du robinet ou embouteillée, échantillons prélevés en milieu marin, etc.) en raison des facteurs de préconcentration élevés (typiquement >1000x). Une autre amélioration à noter pour de futurs travaux est l'intégration de davantage de classes de pesticides dans les méthodes analytiques. Les méthodes multirésiduelles publiées par Cotton et al. (2016) et Vazquez et al. (2015) intègrent entre 500 et 253 pesticides ciblés, pour l'analyse de l'eau et du pollen, respectivement. Ces méthodes peuvent aussi comporter certains inconvénients comme le nombre limité des étalons internes homologues aux composés natifs, avec pour conséquence une compensation sans doute insuffisante des effets matriciels. Par ailleurs, avec un grand nombre de pesticides à analyser, la précision et la sensibilité de la méthode peuvent aussi être affectées, notamment en raison des compromis à faire pour l'optimisation de la chromatographie, et du faible nombre de points par pic chromatographique dans la zone du gradient où de nombreuses transitions MS/MS se chevauchent.

Dans le **Chapitre 2**, les variations temporelles de l'atrazine dans l'eau potable ont été étudiées sur une fenêtre de deux ans (tendances saisonnières). Afin d'évaluer les tendances à plus long terme, nous avons également analysé des séries de données

temporelles issues de la littérature sur une période de temps étendue. Cette documentation est nécessaire non seulement pour mieux comprendre les possibles tendances sur plusieurs décennies (reflétant l'augmentation ou la diminution des usages), mais aussi pour prédire les moments de l'année où la population est exposée à des pics de contamination (reflétant notamment la contamination des eaux de surface). Un échantillonnage en 'continu' permettrait d'intégrer des pics de contamination qu'il est possible d'échapper lors d'un échantillonnage ponctuel.

Afin d'éviter de recourir à un échantillonnage ponctuel à haute résolution temporelle (mesures répétées dans le temps), une approche alternative pourrait être l'utilisation de techniques d'échantillonnage passif. Leur principe est basé sur l'accumulation de contaminants par diffusion passive dans des systèmes comportant une phase de réception (liquide, solide ou gel chélatant) laquelle a une affinité pour un certain type de contaminants. Le POCIS, « échantillonneur intégratif de composés chimiques organiques polaires » (en anglais Polar Organic Chemical Integrative Sampler) pourrait être un outil à intégrer à de futurs projets de surveillance de pesticides dans l'eau de surface et souterraine (Alvarez et al. 2007, Morin et al. 2012, Gong et al. 2018). Son utilisation reste toutefois complexe et nécessite la réalisation de calibrations (Morin et al. 2012) afin de convertir les concentrations dans la phase solide (en ng de composé par gramme d'adsorbant) en concentrations dans l'eau moyennées dans le temps (en ng/L, TWA – *time-weighted average concentrations*). La surveillance de pesticides par échantillonneurs passifs pourrait être étendue aux analyses d'air avec des PAS (*Passive Air Sampler*) tels que les PUF (*Polyurethane foam disks*), afin d'obtenir une image plus complète de la contamination (Estellano et al. 2015, Silva-Barni et al. 2018) et d'améliorer le calcul préliminaire des flux de pesticides entrants/sortants du Lac Saint-Pierre (**Chapitre 6**).

Concernant les pesticides récemment introduits dans l'environnement, tels que ceux utilisés en remplacement aux néonicotinoides (chlorantraniliprole et sulfoxaflor par exemple), les informations restent limitées concernant leur devenir et leurs effets dans l'environnement. Il serait intéressant de mener des études de persistance comme des analyses de biodégradation dans des microcosmes de sol aérobie, des études de toxicité aigüe et chronique par exposition aux organismes. Par exemple, Chevillot et al. (2017) ont

récemment montré que les néonicotinoïdes peuvent se bioaccumuler de façon sélective dans les tissus des vers de terre. Même si les néonicotinoïdes ne présentaient pas d'effets létaux aux concentrations testées, des effets sur l'ADN et la reproduction ont été notés. Des études de bioaccumulation sont également en cours pour une large gamme de pesticides, par exemple chez les insectes (El Khoury et al. 2019) et les mammifères insectivores (Poisson et al. 2019).

Les effets des pesticides sur les humains ne sont pas toujours bien connus, surtout en ce qui concerne les nouveaux pesticides récemment introduits sur le marché. Quelques études ont été réalisées pour mieux comprendre les voies métaboliques et la persistance des néonicotinoïdes chez l'être humain (Han et al. 2018), que ce soit à travers l'exposition chronique (Taira et al. 2014, Roca et al. 2014, Ueyama et al. 2014, Song et al. 2019, Zhang et al. 2018), par voie professionnelle (Kabata et al. 2016), par empoisonnement (Yamamuro et al. 2014; Mohamed et al. 2009), ou encore par exposition contrôlée (Harada et al. 2016). Les travaux précédemment cités fournissent de premières données utiles pour de futures évaluations gouvernementales et d'éventuelles restrictions de leur utilisation. Afin de mieux caractériser l'exposition de la population aux pesticides, il convient non seulement d'analyser les produits/matrices susceptibles d'atteindre le consommateur (aliments, eau potable, air, autres produits de consommation, etc.), mais également les fluides biologiques humains témoins de l'exposition (par exemple, lait maternel, sang ou urine). Pour y répondre, des tests préliminaires ont été réalisés dans le cadre de cette thèse afin de développer une méthode d'analyse des pesticides dans l'urine (**Annexe A**).

Le premier grand défi rencontré a été la complexité et variabilité de la matrice nécessitant une purification avant analyse LC-MS. Les méthodes actuellement utilisées sont la SPE et la LLE (Song et al. 2019). Après avoir testé quelques-unes des méthodes publiées, la SPE nous a semblé l'option plus avantageuse pour le groupe de pesticides ciblés (**Tableau A.1** et **Figure A.1**, **Annexe A**). Diverses cartouches ou combinaisons de cartouches SPE ont été testées (polymérique hydrophilique-lipophilique, échange de cations, échange d'anions fort et faible). Suite à la SPE hors ligne, la réalisation d'élutions par fractionnement (chaque fraction étant analysée séparément), peut cependant doubler ou tripler le temps d'analyse. Plus de 3000 composants ont été rapportés dans l'urine, dont

la plupart sont des anions faibles (Schlittenbauer et al. 2015). Ceci supporte le choix d'une cartouche de type échange d'anions faibles pour retenir les interférents plus fortement que les pesticides ciblés. Les paramètres préliminaires retenus sont l'acétonitrile pour le conditionnement des cartouches, une dilution de 1 : 10 mL de l'échantillon avant de réaliser le chargement SPE, et une élution avec acétonitrile sans agent modifiant. Ces paramètres permettront de retenir plus fortement les interférents dans la partie d'échange d'anions et d'éluer seulement ou plus facilement les analytes ciblés qui ont été retenus par la partie polymérique. Une estimation initiale de la LOD a été établie (**Tableau A.4**, **annexe A**), mais il faudrait poursuivre ces travaux en mettant l'accent sur l'amélioration des étapes d'extraction et de purification pour atteindre des LODs plus faibles et garantir la robustesse des analyses.

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Annexe A

Pesticides ciblés pour l'analyse dans l'urine : mode d'action et ces structures

Les benzothiadiazoles

La bentazone (**Figure A.1**) occasionne des lésions aux feuillages qui ont été en contact avec l'herbicide. À faibles doses elle peut provoquer les mêmes symptômes que les herbicides inhibiteurs de la photosynthèse et à une dose plus élevée perturber les membranes cellulaires des végétaux (OMAFRA, 2000).

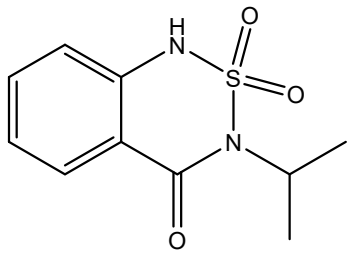

Figure A.1 Structure moléculaire de la bentazone.

Les herbicides inhibiteurs de la croissance de plantules (ou de cellules)

Les dinitroanalines et les chloro-acétamides

Cette catégorie d'herbicides est spécifique par son mode d'action. Les substances de type dinitroanalines telles que la pendiméthaline (**Figure A.2**) peuvent inhiber l'assemblage des microtubules de la protéine (tubuline) lors de la division cellulaire et aussi interrompre la mitose en inhibant les racines. En conséquence, les plantes restent rabougries avec des racines latérales courtes et épaisses. Les substances chloroacétamides (mobiles dans le xylème seulement) tels que la dimethènamide, l'alachlor et le métolachlor (y compris les métabolites metolachlor-ESA et metolachlor-OA) jouent plutôt un rôle sur la conjugaison de l'acétylcoenzyme A en inhibant les pousses. Par conséquent, les tigelles des plantes sont rabougries et les plantules malformées (OMAFRA, 2000).

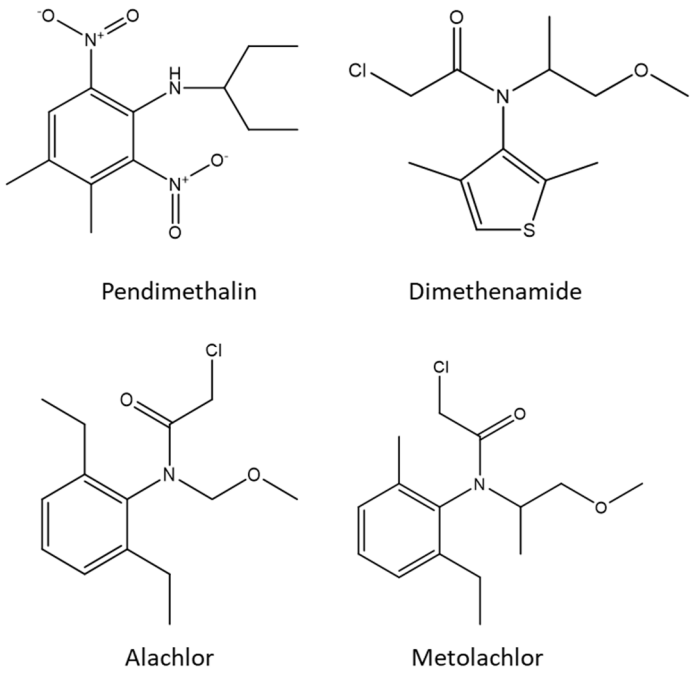

Figure A.2 Structures moléculaires des dinitroanalines et les chloro-acétamides sélectionnés.

Les herbicides perturbateurs de la membrane cellulaire

Les éthers de diphényle

Ces substances sont aussi couramment connues comme herbicides de contact. Parmi ce type d'herbicides, on retrouve les éthers de diphényle comme le fomesafen (**Figure A.3**). Le fomesafen est un inhibiteur de la protoporphyrinogène oxydase (PPO ou Protox), ce qui a comme effet le changement de la couleur de feuilles, dans un premier temps à jaune puis brun, provoquant l'arrêt du développement (Cobucci et al., 1998).

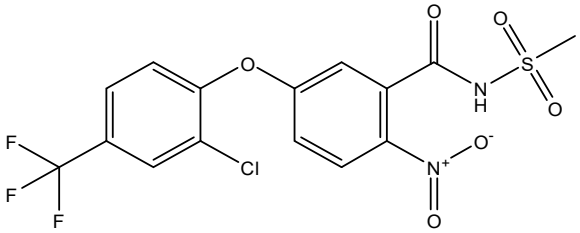

Figure A.3 Structure moléculaire du fomesafen.

Les acides phénoxys et les acides benzoïques

Les acides auxiniques, comme les acides phénoxys et les acides benzoïques, sont d'autres substances herbicides amplement utilisées dû à leur effet sur la croissance de plantes étant donné leur mobilité dans le phloème. Le MCPA et le mécoprop (**Figure A.4**) sont des herbicides de type acide phénoxys qui peuvent causer une torsion des tiges et une malformation des feuilles. Le dicamba, comme exemple d'acide benzoïque, peut causer une déformation de feuilles en « cuillère », mais peut également causer des déformations de tiges en « col de cygne » surtout dans le maïs et d'autres céréales comme le blé (Paszko et al., 2016).

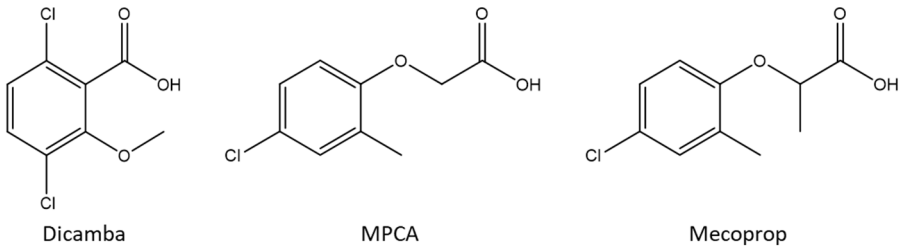

Figure A.4 Structure moléculaire de certains acides phénoxys et acides benzoïques avec effet herbicide.

Inhibiteurs de l'acétolactate synthétase (ALS)

Le flumetsulame, l'imazethapyr et le nicosulfuron

Ces pesticides (**Figure A.5**) sont absorbés par les racines et ils sont des inhibiteurs de l'ALS aussi appelée acétohydroxyacide synthétase (AHAS). Parmi les divers symptômes, le ralentissement de la croissance, la chlorose internervaire, la décoloration des nervures (violacées) et l'apparition graduelle de chlorose et nécrose foliaires (OMAFRA, 2000; Walsh et al., 2015; Dobbels et al., 1993).

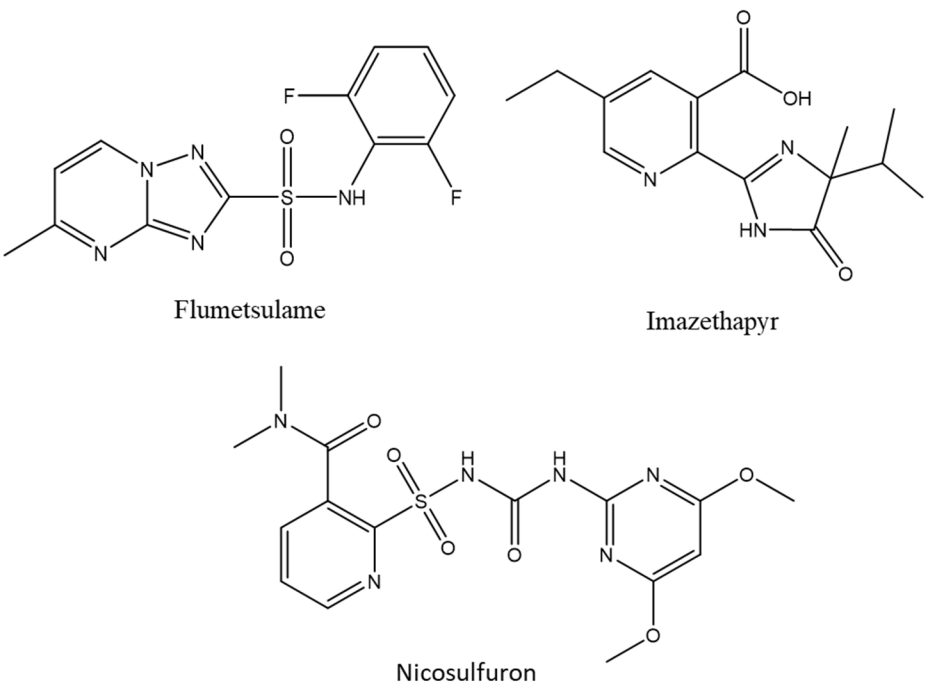

Figure A.5 Structure moléculaire des Inhibiteurs de l'acétolactate synthétase sélectionnés.

Le DEET

Le DEET (N,N-diethyl-m-toluamide) est un insecticide qui agit aussi comme un inhibiteur de l'acétylcholinestérase et qui est appliqué directement sur la peau ou les vêtements, **Figure A.6** (Swale et al., 2014).

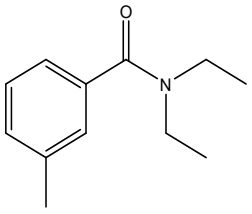

Figure A.6 Structure moléculaire du DEET (N,N-diethyl-m-toluamide).

Les fongicides

Le fluxapyroxad, le metconazole, le propiconazole, le tebuconazole et le pyrimethanil sont des fongicides de classes diverses (carboxamide, triazoles et anilinopyrimidines), **Figure A.7**. Ils inhibent la succinate déshydrogénase dans le complexe II de la chaîne respiratoire mitochondriale ou la biosynthèse des stérols (Vicentini et al., 2007; Snelders et al., 2012; Dong et al., 2012).

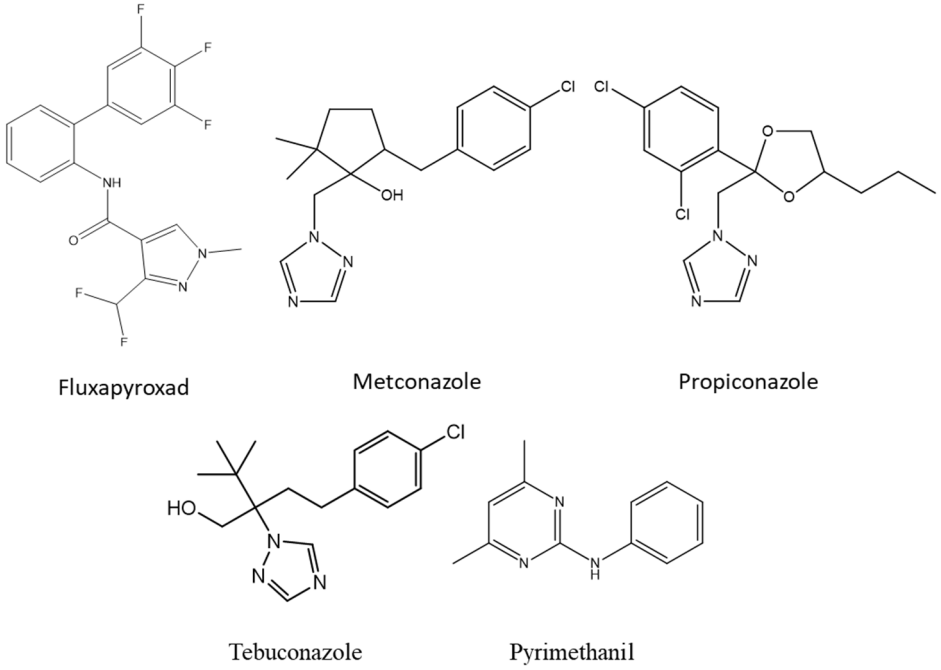

Figure A.7 Structure moléculaire des fongicides sélectionnés.

Optimisation et résultats préliminaires d'une méthode d'analyse de pesticides dans l'urine

Dans un premier temps, l'optimisation de la réponse instrumentale fut évaluée par un ciblage de différentes phases mobiles UHPLC. Une différence de sensibilité a été constatée entre la phase mobile acide typiquement utilisée jusqu'à présent (H_2O + 0.1 % Acide formique : ACN + 0.1 % Acide formique) et une phase mobile faisant intervenir le fluorure d'ammonium (H_2O + 0.1 mM NH_4F : MeOH + 0.1 mM NH_4F). L'introduction du NH_4F permet une augmentation des signaux instrumentaux en comparaison à la phase mobile acidifiée (voir **Tableau A.2**, **Figure A.8**, **annexe A**).

Différents adsorbants SPE furent testés, tel que HLB, Strata X polymérique et Strata X-AW pour trouver celui qui donnerait le meilleur compromis pour les 46 pesticides ciblés. La cartouche Strata X-AW donne les meilleurs rendements pour un plus grand nombre de pesticides dans l'urine, permettant l'analyse de 33 pesticides avec des rendements entre 73 % et 104 % avec un seul composé à 56 % (voir **Tableau A.3**, **Figure A.10**, **annexe A**).

Une fois choisies la phase mobile d'analyse ainsi que la cartouche d'extraction SPE, une optimisation des étapes de SPE a été conduite. Les étapes de lavage, charge et élution ont ainsi été évaluées (**Section 2.1.3**).

Parmi les tests réalisés pour évaluer l'effet de la charge matricielle. On peut noter le test de lavage de la cartouche après la charge de l'échantillon. Des solvants de lavage composés de H_2O 100 %, H_2O : MeOH (90 : 10 % ; v/v), H_2O : MeOH (80 : 20 % ; v/v) ont été évalués. L'augmentation du pourcentage de solvant organique dans le solvant de lavage entraine des pertes d'analytes, et un lavage exclusivement aqueux (H_2O à 100 %) a donc été retenu.

L'étape de conditionnement de la cartouche ainsi que l'étape d'élution sont les étapes les plus importantes de la SPE. Le conditionnement de la cartouche a été évalué en prenant en compte la nature de la plupart de pesticides et la nature de la cartouche SPE. Les conditions testées sont l'ACN neutre, basique et acide (pour l'élution), l'utilisation de buffer après la charge de l'échantillon, le volume de dilution de l'échantillon (1 : 1, 1 : 10

ou 1 : 30 mL d'urine: eau) avant chargement SPE, et aussi avec l'ajout de l'enzyme β-Glucuronidase comme prétraitement de l'échantillon.

ableau A.1. Aire absolue des analytes ciblés et dopés à 1 µg L^{-1} dans un échantillon d'urine, extraits par SPE vs. LLE (d'après la méthode de Song et al. 2019). Phase mobile utilisée (a) H_2O + 0.1 mM NH_4F, (b) MeOH + 0.1 mM NH_4F. Analyse instrumentale réalisée par UHPLC-Orbitrap HRMS.

	SPE		LLE	
Analyte	**Tr**	**Aire absolue**	**Tr**	**Aire absolue**
Acetamiprid	3.78	26637676	3.8	7857683
Alachlor	7.78	8353607	7.78	4086607
Atrazine	6.25	35026680	6.24	24391140
Atrazine-OH	4.16	49634466	4.26	44149367
Atrazine-DEA	4.08	21701568	nd	nd
Atrazine-DIA	2.94	10391273	2.96	8336926
Bentazone	3.06	113761156	nd	nd
Carbendazim	3.80	60908206	3.7	42008355
Carbaryl	5.74	7084241	5.87	24788020
Chlorantraniliprole	6.75	2084386	nd	nd
Clothianidin	3.28	47232262	3.27	44597746
Cyanazine	5.13	6422377	5.1	1939820
DEET	6.48	111082144	6.48	67032721
Dicamba	2.85	1787925	nd	nd
Dimethenamide	7.18	32049389	7.17	15129090
Dimethoate	3.59	7537303	3.57	2252513
Dinotefuran	1.75	4988785	1.75	8379836
Famphur	6.30	11004406	6.3	4456090
Fipronil	8.02	53508472	8.02	31360490
Flonicamid	2.48	744426	2.66	92572789
Flumetsulame	3.36	7856820	nd	nd
Fluxapyroxad	7.43	15436151	7.42	5016270
Fomesafen	6.82	21107491	6.62	15773334
Hexazinone	5.50	53921396	5.48	14404471
Imazethapyr	3.80	17171209	3.86	3301463
Imidacloprid	3.32	13916629	nd	nd
Linuron	6.95	4990260	6.96	425721
MCPA	4.99	8853765	4.92	3370304
Mecoprop	5.81	19010288	nd	nd
Metconazole	8.40	18216471	8.39	9899769
Metolachlor	7.86	38738615	7.86	25417015
Metolachlor-ESA	5.66	330156	5.83	15677358
Metolachlor-OA	5.60	127727982	nd	nd
Metribuzin	5.19	19472895	5.18	5989425
Nicosulfuron	4.68	1783846	4.77	385859

Nitenpyram	2.35	6931152	nd	nd
Pendimethalin	9.04	6073121	9.05	143692
Prometryn	7.64	89975165	7.63	43471045
Propiconazole	8.32	15516118	8.34	7449834
Pyrimethanyl	6.95	39256126	6.96	18405826
Simazine	5.33	44281267	5.35	106252091
Sulfoxaflor	3.96	5115968	3.93	1894337
Tebuconazole	8.21	20045924	8.2	8093275
Thiacloprid	4.25	35274116	4.24	10524966
Thiamethoxam	2.63	8461390	2.61	12747644
Thionazin	6.38	34590120	6.36	8608122

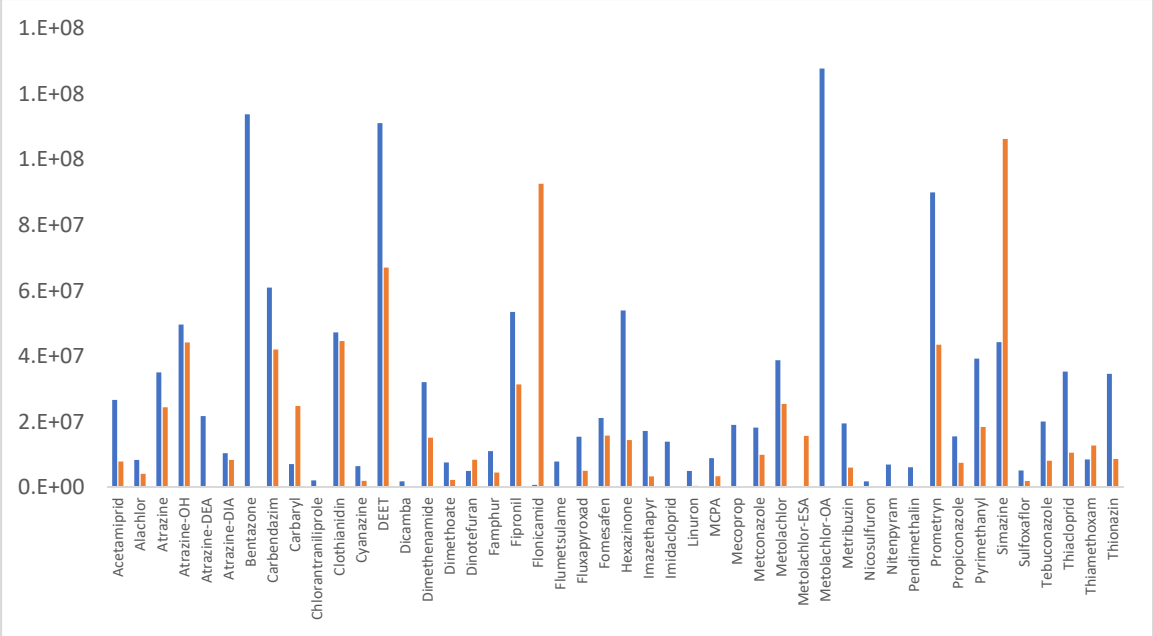

Figure A.8. Histogramme comparatif du teste des extractions SPE (bleu) et LLE (orange) d'après les résultats du **Tableau A.1**.

Tableau A.2. Comparaison des aires absolues des analytes ciblés, en variant la nature de la phase mobile UHPLC. (**Phase mobile A**) H2O + 0.1% Acide formique : ACN + 0.1 Acide formque, (**Phase mobile B**) H_2O + 0.1 mM NH_4F + 0.1% Acide formique: MeOH + 0.1 mM NH_4F + 0.1% Acide formique, et (**Phase mobile C**) H_2O + 0.1 mM NH_4F : MeOH + 0.1 mM NH_4F.

Analyte	Phase mobile A Aire absolue	Phase mobile B Aire absolue	Phase mobile C Aire absolue
Acetamiprid	9186336	36464974	76800746
Alachlor	2716815	12386155	12447628
Atrazine	86489488	98573345	116537906
Atrazine-OH	64740690	66160641	140225265
Atrazine-DEA	35975192	57588450	79736987
Atrazine-DIA	28242267	41437070	60633331
Bentazone	66872316	nd	104204091
Carbendazim	69831315	76604108	185098913
Carbaryl	2476560	19999890	21656877
Chlorantraniliprole	2930001	8098887	11896436
Clothianidin	2785042	9445475	26342696
Cyanazine	12504993	11530307	13529162
DEET	89504658	111227717	137429491
Dicamba	916465	nd	1292489
Dimethenamide	13347594	59971048	66291375
Dimethoate	4277169	15719005	23001121
Dinotefuran	9540171	43448283	72832664
Famphur	2871577	10922272	20635584
Fipronil	10104483	20333849	41658039
Flonicamid	7106625	3136641	3940816
Flumetsulame	5343456	214626	15324217
Fluxapyroxad	5411171	33722934	34207476
Fomesafen	7229263	4252162	12299049
Hexazinone	35982819	66593916	89376592
Imazethapyr	38628230	33012199	82869952
Imidacloprid	4794978	10781649	33573712
Linuron	5581272	22648957	22636455
MCPA	4439924	3026452	7624519
Mecoprop	7184418	5182777	12271868
Metconazole	27106450	21991822	20564323
Metolachlor	17739982	59709994	53494360
Metolachlor-ESA	1184469	2279321	2563883
Metolachlor-OA	5240789	8399918	7810267
Metribuzin	39063158	45513042	51823740
Nicosulfuron	1228076	2784675	4980259
Nitenpyram	22347459	36520112	55510824
Pendimethalin	3624483	8669529	5748043
Prometryn	80548404	91669927	130216564
Propiconazole	19194066	16470849	16319577
Pyrimethanyl	81214749	96589150	116926379
Simazine	61717236	73002578	94126114

Sulfoxaflor	2004169	9865340	17941092
Tebuconazole	27148192	45394083	26162449
Thiacloprid	12163830	50284038	123946170
Thiamethoxam	2647018	20075796	40686107
Thionazin	22835427	50505146	73530781

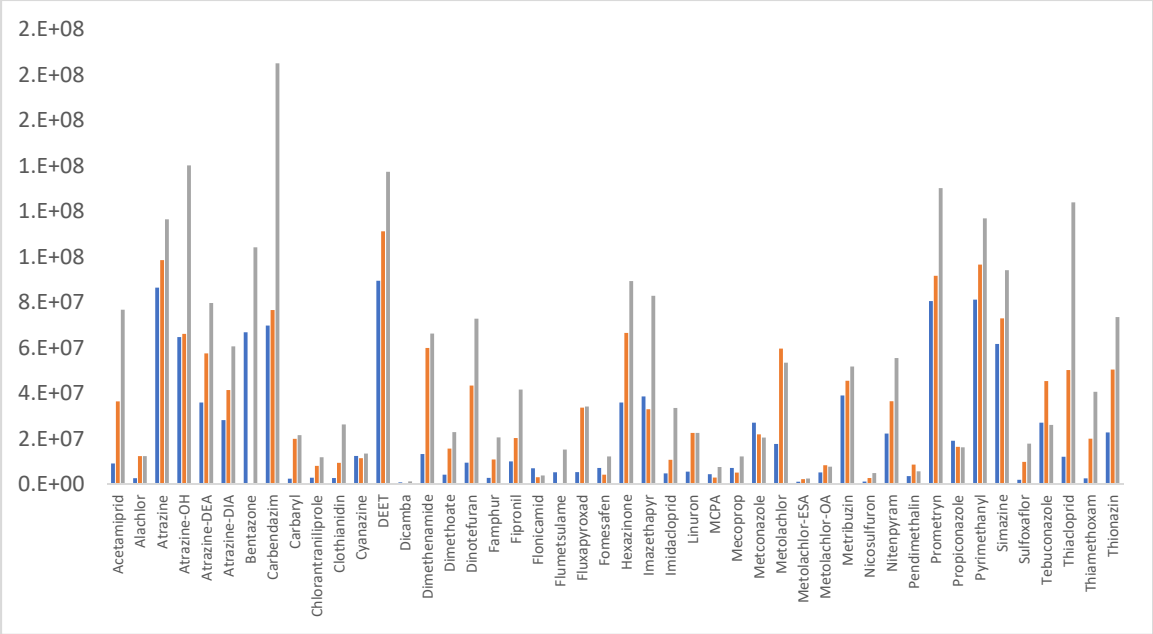

Figure A.9. Histogramme de la comparaison des phases mobiles dans l'analyse de pesticides ciblés dans l'urine par UHPLC-Orbitrap HRMS. (**Phase mobile A en bleu**) H2O + 0.1% Acide formique : ACN + 0.1 Acide formque, (**Phase mobile B en orange**) H_2O + 0.1 mM NH_4F + 0.1% Acide formique: MeOH + 0.1 mM NH_4F + 0.1% Acide formique, et (**Phase mobile C en gris**) H_2O + 0.1 mM NH_4F : MeOH + 0.1 mM NH_4F.

Tableau A.3. Aires absolues obtenues par SPE avec trois cartouches de diffèrents adsorbants, sur une base polymérique avec modifications comme échange d'anions faibles (**Strata X-AW**) et modification hydrophilique-lipophilique (**Strata X et Oasis HLB**). Les pesticides cibles étaient dopés dans une échantillon d'urine à 1 µg L^{-1}. Phase mobile utilisée : (a) H_2O + 0.1 mM NH_4F, (b) MeOH + 0.1 mM NH_4F. Analyse instrumentale par UHPLC-Orbitrap HRMS.

Analyte	Strata X-AW Aire absolue	Strata X Aire absolue	Oasis HLB Aire absolue
Acetamiprid	206867485	31300155	46979523
Alachlor	36018204	29557531	31055761
Atrazine	335826074	79300966	80659736
Atrazine-OH	179222	nd	nd
Atrazine-DEA	107077283	nd	nd
Atrazine-DIA	64212356	nd	425088302
Bentazone	3509100	nd	nd
Carbendazim	408825252	nd	nd
Carbaryl	228112193	nd	nd
Chlorantraniliprole	38979851	9537978	8448557
Clothianidin	103026637	23697466	28910900
Cyanazine	64246705	18055562	20499875
DEET	592762720	614415604	636283856
Dicamba	nd	nd	nd
Dimethenamide	191730802	113420523	125134349
Dimethoate	nd	15115147	13866472
Dinotefuran	48998366	9663228	13380639
Famphur	63526576	33744621	34981529
Fipronil	104700072	110330527	96043689
Flonicamid	16325797	2991413	5047519
Flumetsulame	nd	7967129	7445253
Fluxapyroxad	110063927	54883068	59660757
Fomesafen	nd	71018698	60994557
Hexazinone	410305462	31300155	46979523
Imazethapyr	nd	29557531	31055761
Imidacloprid	72574451	79300966	80659736
Linuron	77020358	294868700	203704283
MCPA	nd	nd	nd
Mecoprop	nd	753088576	919276604
Metconazole	78399987	12951288	14295997
Metolachlor	186197731	129124167	126863290
Metolachlor-ESA	nd	151087515	nd
Metolachlor-OA	nd	9537978	8448557
Metribuzin	154793870	23697466	28910900
Nicosulfuron	nd	50947008	65202163
Nitenpyram	87812945	nd	636283856
Pendimethalin	nd	nd	nd
Prometryn	408504977	113420523	125134349

Propiconazole	70467597	15115147	13866472
Pyrimethanyl	261327728	9663228	13380639
Simazine	228130498	33744621	34981529
Sulfoxaflor	28923359	110330527	96043689
Tebuconazole	113097955	2991413	5047519
Thiacloprid	311358568	7967129	7445253
Thiamethoxam	39067954	1605566	5112260
Thionazin	193313614	71018698	60994557

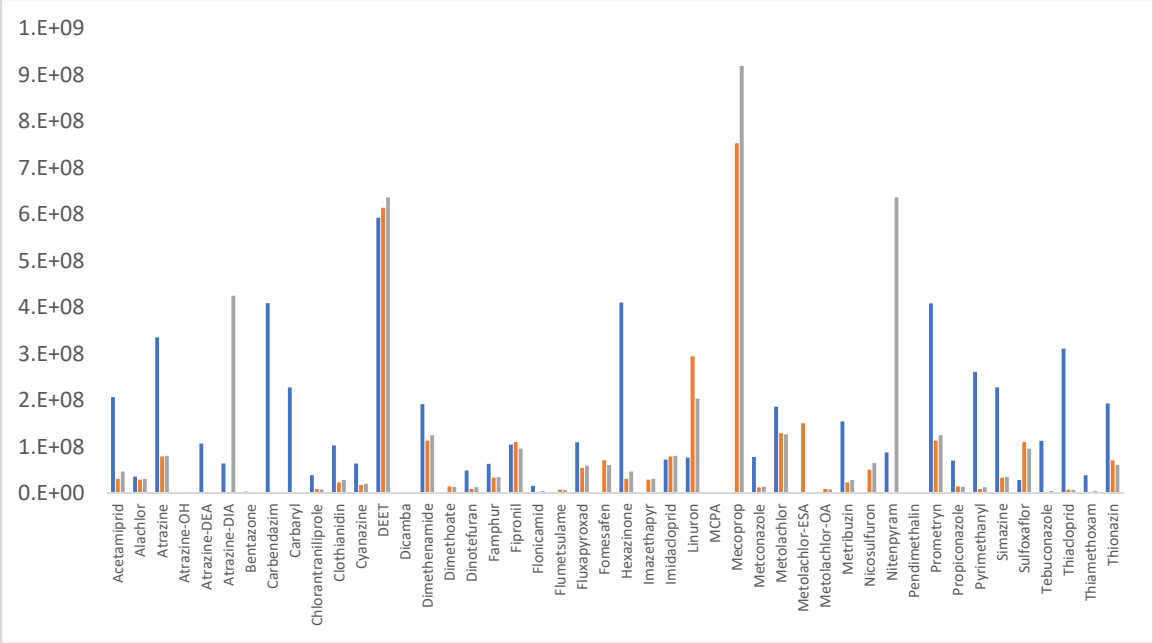

Figure A.10. Histogramme comparatif des adsorbants des cartouches SPE (Strata X-AW (**en bleu**), Strata X (**en orange**) et HLB (**en gris**)) pour l'analyse de pesticides ciblés dopés à 1 µg L^{-1} dans l'urine.

Tableau A.4. Limites de détection et de quantification obtenues pour les pesticides ciblés dans une matrice d'urine. Analyse instrumentale conduite par UHPLC-Orbitrap HRMS.

Analyte	**LOD [ng L^{-1}]**	**LOQ [ng L^{-1}]**
Acetamiprid	72	216
Alachlor	45	136
Atrazine	52	157
Atrazine-OH	129	387
Atrazine-DEA	141	424
Atrazine-DIA	121	364

Bentazone	nd	nd
Carbendazim	78	233
Carbaryl	nd	nd
Chlorantraniliprole	31	92
Clothianidin	165	496
Cyanazine	nd	nd
DEET	91	272
Dicamba	nd	nd
Dimethenamide	68	203
Dimethoate	13	40
Dinotefuran	64	191
Famphur	49	148
Fipronil	83	249
Flonicamid	86	259
Flumetsulame	nd	nd
Fluxapyroxad	28	85
Fomesafen	nd	nd
Hexazinone	108	324
Imazethapyr	nd	nd
Imidacloprid	71	214
Linuron	48	144
MCPA	nd	nd
Mecoprop	nd	nd
Metconazole	30	89
Metolachlor	42	125
Metolachlor-ESA	nd	nd
Metolachlor-OA	nd	nd
Metribuzin	18	55
Nicosulfuron	nd	nd
Nitenpyram	59	176
Pendimethalin	nd	nd
Prometryn	29	88
Propiconazole	49	147
Pyrimethanyl	65	196
Simazine	36	109
Sulfoxaflor	78	233
Tebuconazole	51	153
Thiacloprid	37	110
Thiamethoxam	155	465
Thionazin	85	254

Annexe B

Tableau B.1 Valeurs de MRL pour quatre différentes matrices (laitue, tomate, raisin et pomme) d'après Santé Canada, U.S. EPA, l'Union Européen et FAO/OMS.

	Santé Canada				U.S. EPA				European Union				FAO/WHO			
	Lettuce	**Apple**	**Grapes**	**Tomato**	**Lettuce**	**Apple**	**Grapes**	**Tomato**	**Lettuce**	**Apple**	**Grapes**	**Tomato**	**Lettuce**	**Apple**	**Grapes**	**Tomato**
Acetamipride	NF	1	0.35	0.2	3	1	0.35	0.4	3	0.8	0.5	0.5	NF	0.8	0.5	0.2
Clothianidine	NF	o.3	0.6	0.2	3	1	0.6	0.2	0.1	0.4	0.7	0.04	2	0.4	0.7	0.05
Dinotefuran	NF	NF	NF	NF	15	2	2.5	1	NF	NF	0.9	NF	6	NF	0.9	0.5
Fipronil	NF	NF	NF	NF	NF	NF	NF	NF	0.005	0.005	0.005	0.005*	NF	NF	NF	NF
Imidaclopride	NF	0.6	1.5	1	3.5	0.5	1	6	2	0.5	1	0.5	2	0.5	1	0.5
Nitenpyram	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Thiaclopride	NF	0.3	NF	NF	NF	0.6	NF	NF	1	0.3	0.01*	0.5	NF	0.7	NF	0.5
Thiamethoxame	NF	0.2	0.2	0.25	NF	NF	0.3	0.8	5	0.3	0.4	0.2	3	0.3	NF	0.7
Atrazine	NF	NF	NF	NF	0.25	NF	NF	NF	0.05	0.05	0.05	0.05	NF	NF	NF	NF
Cyanazine	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Simazine	NF	NF	NF	NF	NF	0.2	0.2	NF	0.01*	0.01*	0.2	0.01*	NF	NF	NF	NF
Carbendazim	NF	5	5	2.5	NF	NF	NF	NF	0.1*	0.2	0.3	0.3	5	3	3	0.5
Carbaryl	10	5	5	5	10	15	10	5	0.01*	0.01*	0.01*	0.01*	NF	NF	NF	5
Linuron	NF	NF	NF	NF	NF	NF	NF	NF	0.05*	0.05*	0.05*	0.05*	NF	NF	NF	NF

Phosmet	NF	10	10	NF	NF	10	10	NF	0.05*	0.5	0.05*	0.05*	NF	10	10	NF
(EtO)3PS	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Dimethoate	2	2	NF	0.5	2	NF	NF	2	0.01*	0.01*	0.01*	0.01*	0.3	NF	NF	NF
Famphur	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF