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DIETARY EXPOSURE OF THE BELGIAN POPULATION TO CONTAMINANTS – CONSIDERATION OF PROCESSING EFFECTS

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Philippe

Statement on the use of Artificial Intelligence tools

In the preparation of this thesis, several artificial intelligence (AI) tools were used in a limited, supervised manner. These include large language models (LLM) such as ChatGPT and Gemini, as well as language and translation tools (Grammarly and DeepL).

These tools were employed exclusively as support instruments, particularly for:

- improving the clarity, readability, and linguistic quality of the manuscript,
- suggesting reformulations and refining academic phrasing,
- assisting in learning and troubleshooting LaTeX, including formatting and document preparation issues.

In addition, ChatGPT was used to generate the illustration on the cover page of this manuscript. However, no AI tools were used to generate, analyze, or interpret experimental data, nor to produce scientific results or conclusions. Furthermore, AI tools were not used to generate bibliographic references, citations, or sources included in this manuscript. All outputs generated by these tools were critically reviewed and revised when necessary. Hence, no content was included without prior validation of the author.

Considering of the above, the author remains solely responsible for the content, the scientific integrity, and the conclusions presented in this manuscript. The AI tools used cannot be considered as authors or scientific contributors.

Abstract

Food safety remains a major public health concern worldwide, particularly in a context of globalised supply chains, diversified agricultural practices, and evolving dietary habits. Among chemical hazards, pesticides are widely used to improve agricultural yields, and their residues may remain in food and be ingested by consumers. In addition to pesticides, processing contaminants such as polycyclic aromatic hydrocarbons (PAHs) and acrylamide are of particular concern due to their genotoxic and carcinogenic potential. European regulatory frameworks ensure a high level of consumer protection. However, significant uncertainties persist regarding occurrence data for under-investigated foods, the impact of food processing and domestic preparation, and up-to-date dietary exposure assessments.

This doctoral thesis aimed to strengthen the dietary risk assessment of the Belgian population by integrating analytical innovation, updated occurrence data, and the quantification of processing effects, including consumer behaviour, in order to refine dietary exposure and risk assessment. The work focused on three relevant contaminant/matrix combinations: pesticides in tea and herbal infusions, PAHs in spices and dried herbs, and acrylamide in selected food products insufficiently or not documented in previous European assessments.

To achieve these objectives, sensitive, adaptable, and robust analytical methods were developed and optimised to address complex and heterogeneous food matrices. Multi-residue liquid chromatography and gas chromatography tandem mass spectrometry (LC-MSMS and GC-MS/MS) methods were implemented for tea and herbal tea leaves as well as their corresponding brews in order to determine pesticides transfer factors (TFs). For PAHs, a modular GC–MS/MS method inspired by the QuEChERS approach, combining a common extraction step with adaptable clean-up procedures, was designed to cope with the diversity and complexity of spice and herb matrices. This strategy enabled reliable quantification of the four regulated hydrocarbons (PAH4) across a wide range of products, including smoked spices and complex mixtures. The innovative character of this method led to its use as the basis for an interlaboratory ring trial within the national and european laboratories network, addressing the lack of harmonised methods capable of dealing with complex spice and herb matrices. For acrylamide, the QuEChERS method initially developed for pesticides was adapted through the implementation of matrix-specific clean-up procedures. This resulted in a sensitive LC–MS/MS method validated for heterogeneous and under-investigated food categories such as pastries, confectionaries, dried fruits, nuts and oil seeds, vegetable chips, black olives, and potato-based dough products.

These developed analytical methods were subsequently applied to generate missing occurrence data necessary for accurate exposure and risk assessment. The occurrence results revealed several important findings. In tea, 38 % of the 55 analysed samples

contained at least one pesticide exceeding the EU maximum residue level (MRL) in dry material or contained prohibited pesticides, confirming the relevance of continued monitoring. Furthemore, up to 15 pesticides has been identified in one tea sample. However, a key innovation of this work lies in the systematic quantification of pesticide transfer from dry leaves to infusion under brewing conditions representative of Western consumption habits. Experimentally determined TFs ranged from 0 % to 100 %, revealing clear relationships with physico-chemical properties: highly lipophilic pyrethroids exhibited limited transfer (<10 %), whereas polar neonicotinoids frequently exceeded 50 %.

New occurrence data also revealed relatively high acrylamide concentrations in several less-studied food categories, such as sterilised black olives, potato-dough-based products, and particularly vegetable-based crisps. In spices and dried herbs, PAH4 were detected in several samples, especially in products subjected to smoking, confirming their contribution to dietary exposure.

A distinctive and innovative aspect of this thesis is the integration of consumer behaviour into exposure assessment. For tea, experimentally derived and modelled TFs reflecting realistic brewing practices were incorporated. Results showed that brewing parameters such as temperature and infusion time had limited influence on transfer factors under typical preparation conditions, whereas intrinsic characteristics of the tea matrix and the presence of flavouring ingredients played a more significant role. A comprehensive TF database was established, and four predictive models based on physico-chemical parameters were developed, enabling transfer estimation for non-tested compounds and providing a tool for future risk assessment and regulatory use. Regarding acrylamide, participants were recruited to prepare food products according to their usual cooking habits. Questionnaires combined with analytical measurements revealed that acrylamide levels were generally well below current benchmark levels. Interestingly, participants tended to stop cooking when a golden colour was reached, corresponding to their preferred sensory characteristics, which aligns with the colour recommended by EU regulation to limit acrylamide formation. These studies highlight the importance of integrating consumer behaviour into research in order to obtain more realistic occurrence data and exposure assessments.

Once occurrence data and processing factors were acquired, risk assessment was performed using approaches adapted to contaminant toxicological profiles. Hazard indices and hazard quotients were calculated for acute and chronic exposure to pesticides, while the margin of exposure (MOE) approach was applied for genotoxic and carcinogenic substances such as PAHs and acrylamide. No significant acute or chronic risk associated with pesticide intake via tea infusion was identified, despite the high proportion of MRL exceedances in dry leaves, demonstrating that MRL exceedance is not necessarily synonymous with consumer health risk. For PAHs in spices and herbs, integration of new occurrence data into national exposure

calculations resulted in only a limited increase in total PAH intake, with MOE remaining largely above levels considered of a concern for the consumer. For acrylamide, new food categories analysed moderately modified overall intake estimates, and updated exposure assessment confirmed that MOE for the Belgian population remain a public health concern, in line with EFSA conclusions of 2015, thereby reinforcing the need for continued mitigation measures.

Overall, this thesis contributes to reducing knowledge gaps in dietary exposure assessment by combining analytical innovation, updated occurrence data, modelling of processing effects, and integration of consumer behaviour. Importantly, the TF database and predictive models developed during this work are already being used by the Belgian Food Safety Agency to support risk evaluation in cases of MRL exceedances in tea. Furthermore, the acrylamide occurrence data generated were transmitted to EFSA to support the revision of the EU regulation and the establishment of benchmark levels for new food categories, thereby strengthening consumer protection and improving risk characterisation at EU level.

Résumé

La sécurité des aliments reste une préoccupation majeure en matière de santé publique dans le monde entier, en particulier dans un contexte de mondialisation des chaînes d'approvisionnement, de diversification des pratiques agricoles et d'évolution des habitudes alimentaires. Au sein des risques chimiques, les pesticides sont largement utilisés pour améliorer les rendements agricoles, et leurs résidus peuvent rester dans les aliments et être ingérés par les consommateurs. Outre les pesticides, les contaminants issus de la transformation, tels que les hydrocarbures aromatiques polycycliques (HAP) et l'acrylamide, sont particulièrement préoccupants en raison de leur potentiel génotoxique et cancérigène. Les cadres réglementaires européens garantissent un niveau élevé de protection des consommateurs. Cependant, d'importantes incertitudes persistent concernant la présence de ces substances dans certains aliments peu étudiés, l'impact quantitatif de la transformation des aliments et de leur préparation à domicile et le manque d'évaluations actualisées de l'exposition alimentaire.

Cette thèse de doctorat vise à renforcer l'évaluation des risques alimentaires pour la population belge en intégrant des innovations analytiques, la généralisation de données actualisées sur la présence de contaminants et des effets de la transformation, y compris le comportement des consommateurs, afin d'affiner l'évaluation de l'exposition alimentaire et des risques. Les travaux se sont concentrés sur trois combinaisons contaminant/matrice pertinentes : les pesticides dans le thé et les infusions à base de plantes, les HAP dans les épices et les herbes séchées, et l'acrylamide dans certains produits alimentaires insuffisamment ou non documentés dans les évaluations européennes précédentes.

Pour atteindre ces objectifs, des méthodes d'analyse sensibles, adaptables et robustes ont été mises au point et optimisées afin de traiter des matrices alimentaires complexes et hétérogènes. Des méthodes multi résidus utilisant la chromatographie liquide et gazeuse couplée à la spectrométrie de masse en tandem (LC-MS/MS et GC-MS/MS) ont été mises en œuvre pour les feuilles de thé et de tisane, ainsi que pour leurs infusions correspondantes afin de déterminer les facteurs de transfert (TFs) des pesticides. Pour les HAPs, une méthode GC-MS/MS modulaire inspirée de l'approche QuEChERS, combinant une étape d'extraction commune avec des procédures de purification adaptables, a été conçue pour faire face à la diversité et à la complexité des matrices d'épices et d'herbes. Cette stratégie a permis une quantification fiable des quatre HAPs normés (HAP4) dans une large gamme de produits, y compris les épices fumées et les mélanges complexes. Le caractère innovant de cette méthode a conduit à son utilisation comme base pour un essai inter laboratoire au sein du réseau des laboratoires nationaux et européens de référence, afin de pallier le manque de méthodes harmonisées capables de traiter les matrices complexes d'épices et d'herbes. Pour l'acrylamide, la méthode QuEChERS initialement développée pour

les pesticides a été adaptée grâce à la mise en œuvre de procédures de purification spécifiques à la matrice. Ceci a abouti à une méthode LC-MS/MS sensible, validée pour des catégories d'aliments hétérogènes et peu étudiées telles que les pâtisseries, les confiseries, les fruits secs, les noix et les graines oléagineuses, les chips de légumes, les olives noires et les produits à base de pâte de pomme de terre.

Ces méthodes analytiques ont ensuite été utilisées pour générer les données manquantes nécessaires à une évaluation précise de l'exposition et des risques. Les résultats ont révélé plusieurs conclusions importantes. Dans le thé, 38 % des 55 échantillons analysés contenaient au moins un pesticide dépassant la limite maximale de résidus (LMR) fixée par l'UE ou contenaient des pesticides interdits, ce qui confirme la pertinence d'une surveillance continue. En outre, jusqu'à 15 pesticides ont été identifiés dans un échantillon de thé. Cependant, une innovation majeure de ces travaux réside dans la quantification systématique du transfert des pesticides des feuilles de thé vers l'infusion dans des conditions de préparation représentatives des habitudes de consommation occidentales. Les facteurs de transfert déterminés expérimentalement variaient de 0% à 100 %, révélant des relations claires avec les propriétés physico-chimiques: les pyréthroïdes hautement lipophiles présentaient un transfert limité (<10 %), tandis que les néonicotinoïdes polaires dépassaient fréquemment 50 %. De nouvelles données ont également révélé des concentrations relativement élevées d'acrylamide dans plusieurs catégories d'aliments moins étudiées, telles que les olives noires stérilisées, les produits à base de pâte de pomme de terre et, en particulier, les chips à base de légumes. Dans les épices et les herbes séchées, des HAP4 ont été détectés dans de nombreux échantillons, en particulier dans les produits soumis à un fumage, confirmant leur contribution à l'exposition alimentaire.

Un aspect distinctif et innovant de cette thèse est l'intégration du comportement des consommateurs dans l'évaluation de l'exposition. Pour le thé, des TFs déterminés expérimentalement et modélisés reflétant des pratiques d'infusion réalistes ont été intégrés. Les résultats ont montré que les paramètres d'infusion tels que la température et le temps d'infusion avaient une influence limitée sur les TFs dans des conditions de préparation typiques. En revanche, les caractéristiques intrinsèques de la matrice du thé et la présence d'ingrédients aromatisants jouaient un rôle plus important. Une base de données complète sur les facteurs de transfert a été créée et quatre modèles prédictifs basés sur des paramètres physico-chimiques ont été développés, permettant d'estimer le transfert pour des composés non testés et fournissant un outil pour l'évaluation future des risques et l'utilisation réglementaire. En ce qui concerne l'acrylamide, des candidats ont été recrutés pour préparer des produits alimentaires selon leurs habitudes culinaires. Les questionnaires combinés à des mesures analytiques ont révélé que les niveaux d'acrylamide étaient généralement bien inférieurs aux niveaux de référence actuels. Il est intéressant de noter que les candidats avaient tendance à arrêter la cuisson des aliments lorsque

la couleur dorée était atteinte. Cette couleur correspondait à leurs caractéristiques sensorielles préférées et à celle recommandée par la réglementation européenne pour limiter la formation d'acrylamide. Ces études soulignent l'importance d'intégrer le comportement des consommateurs dans la recherche afin d'obtenir des données plus réalistes sur les occurrences et les mesures d'expositions.

Une fois les données sur la présence et les facteurs de transformation obtenues, une évaluation des risques a été réalisée à l'aide d'approches adaptées aux profils toxicologiques des contaminants. Des indices de risque et des quotients de risque ont été calculés pour l'exposition aiguë et chronique aux pesticides, tandis que l'approche de la marge d'exposition (MOE) a été appliquée pour les substances génotoxiques et cancérigènes telles que les HAPs et l'acrylamide. Aucun risque aigu ou chronique significatif lié à l'ingestion de pesticides via l'infusion de thé n'a été identifié, malgré la forte proportion de dépassements des LMR dans les feuilles de thé, ce qui démontre que le dépassement des LMR n'est pas nécessairement synonyme de risque pour la santé des consommateurs. Pour les HAPs présents dans les épices et les herbes, l'intégration de ces nouvelles données d'occurrence dans les estimations d'exposition nationales n'a entraîné qu'une augmentation limitée de l'ingestion totale de HAPs. Les MOEs restant largement supérieures au seuil considéré comme préoccupants pour les consommateurs. En ce qui concerne l'acrylamide, les nouvelles catégories d'aliments analysées n'ont modifié que modérément les mesures d'exposition. L'évaluation actualisée du risque a confirmé que les MOEs pour la population belge restent préoccupantes pour la santé publique, conformément aux conclusions de l'EFSA de 2015, renforçant ainsi la nécessité de poursuivre les mesures d'atténuation.

Cette thèse contribue à réduire les lacunes relatives à l'évaluation de l'exposition alimentaire en combinant innovation analytique, données actualisées sur la présence de substances, modélisation des effets de la transformation et intégration du comportement des consommateurs. Il est important de noter que la base de données de TFs et les modèles prédictifs développés au cours de ces travaux sont déjà utilisés par l'Agence belge pour la sécurité alimentaire (AFSCA)afin d'étayer l'évaluation des risques en cas de dépassement des LMR dans le thé. En outre, les données générées sur la présence d'acrylamide ont été transmises à l'EFSA afin d'étayer la révision de la réglementation européenne et l'établissement de niveaux de référence pour les nouvelles catégories d'aliments. Ces données contribuent ainsi à renforcer la protection des consommateurs et à améliorer la caractérisation des risques au niveau européen.

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List of acronyms

Acronym	Definition
ACN	: Acetonitrile
Acrylamide D3	: Deuterated acrylamide
ADI	: Acceptable daily intake
aHI	: Acute hazard index
ALARA	: As low as reasonably achievable
AO	: Animal origin
ARfD	: Acute reference dose
ASE	: Accelerated solvent exposure
ATSDR	: Agency for Toxic Substances and Disease Registry
BaA	: Benzo[a]anthracene
BaP	: Benzo[a]pyrene
BbF	: Benzo[b]fluoranthene
BfR	: German Federal Institute for Risk Assessment
BjF	: Benzo[j]fluoranthene
BMDL	: Benchmark dose lower confidence level
$BMDL_{10}$	: Benchmark dose lower confidence level 10 %
BML	: Benchmark level
Bw	: Body weight
CAG	: Cumulative assessment group
CHR	: Chrysene

Acronym	Definition
CRM	: Certified reference material
DCM	: Dichloromethane
d-SPE	: Dispersive solid phase extraction
EC	: European Commission
ECD	: Electron capture detector
ECHA	: European Chemicals Agency
EDI	: Estimated daily intake
EDTA	: Ethylenediaminetetraacetic acid
EFSA	: European Food Safety Agency
EMA	: European Medicines Agency
EMR	: Enhanced matrix removal
ESI	: Electrospray ionisation
EU	: European Union
EURL	: European reference laboratory
FAIM	: Food additives intake model
FAO	: Food and Agriculture Organization of the United Nations
FASFC	: Federal Agency for the Safety of the Food Chain
FCS2014	: Food consumption survey of 2014
FDA	: Food and Drug Administration
FDE	: FoodDrinkEurope
FLD	: Fluorescence detector
GC	: Gas chromatography
GCB	: Graphite carbon black
GPC	: Gel permeation chromatography
H	: Henry's constant
HPLC	: High performance liquid chromatography
HQ	: Hazard quotient

Acronym	Definition
HR	: Highest residue level
HRMS	: High resolution mass spectrometry
IARC	: International Agency for Research on Cancer
IB	: Intermediate bound
ICC	: Ion charge control
ICP	: Inductively coupled plasma
IESTI	: International estimated short-term intake
IGG	: Intragovernmental Group
IL-IS	: Isotopically labelled internal standard
IPCS	: International Programme on Chemical Safety
IS	: Internal standard
ISO	: International Organization for Standardization
JECFA	: The Joint FAO/WHO Expert Committee on Food Additives
JMPR	: Joint Meeting on Pesticide Residues
LB	: Lower bound
LC	: Liquid chromatography
LOD	: Limit of detection
LOQ	: Limit of quantification
LP	: Large portion
MB	: Middle bound
MC	: Maximum concentration
ME	: Matrix effect
MIP	: Molecularly imprinted polymer
ML	: Maximum limit
MN	: Mann–Whitney test
MOE	: Margin of exposure
MRL	: Maximum residue limit

Acronym	Definition
MRM	: Multiple reaction monitoring
MS/MS	: Tandem mass spectrometry
MU	: Measurement uncertainty
NOAEL	: No adverse effect level
NRL	: National reference laboratory
P	: Polarity
PAH	: Polycyclic aromatic hydrocarbon
PAH EU 15+1	: Sixteen priority polycyclic aromatic hydrocarbons
PAH4	: Four regulated PAHs (BaA, CHR, BbF and BaP)
PC	: Processing contaminant
PLE	: Pressurized liquid extraction
PO	: Plant origin
Pow	: n-octanol/water partition coefficient
PPP	: Plant protection products
PRIMo	: Pesticide residue intake model
PSA	: Primary secondary amines
PSTI	: Predictable short-term intake
PT	: Proficiency test
PTFE	: Polytetrafluoroethylene
PTV	: Programmable temperature vaporization
PV	: Vapour pressure
PVDF	: Polyvinylidene difluoride
QuECheRS	: Quick, Easy, Cheap, Effective, Rugged and Safe
QuPPe	: Quick Polar Pesticides
RASFF	: Rapid alert system for food and feed
RL	: Reporting level
RSD	: Robust standard deviation

Acronym	Definition
RSDr	: Relative standard deviation under repeatability conditions
RSDrw	: Relative standard deviation under reproducibility conditions
RSLC	: Rapid separation liquid chromatography
RT	: Retention time
S	: Solubility
S/N	: Signal-to-noise ratio
SAFE	: Safe Food Advocacy for Europe
SCF	: Scientific Committee on Food
SD	: Standard deviation
SI	: Silica
SPE	: Solid phase extraction
SPS	: Smart parameter setting
SRM	: Single residue method
TDI	: Tolerable daily intake
TDS	: Total diet study
TF	: Transfer factor
TOF	: Time of flight
TWI	: Tolerable weekly intake
U	: Expanded uncertainty
u	: Uncertainty
U.S.	: United States
UHPLC	: Ultra high-performance liquid chromatography
UK	: United Kingdom
ULiège	: Université de Liège
UB	: Upper bound
UPLC-LC	: Ultra high-performance liquid chromatography
UV	: Ultraviolet

Acronym	Definition
VP	: Vapour pressure
WHO	: World Health Organization

Chapter 1

Context and general introduction

1 CONTEXT OF THE THESIS

Food safety is a major public health concern worldwide. In a context marked by supply chain globalisation, diversification of agricultural and industrial practices, and continuous dietary habit evolution, consumers may be exposed to a wide range of chemical contaminants present in foodstuffs. These substances, originating from various sources, may be intentionally used or unintentionally formed throughout the food chain, from primary production to domestic preparation. Their presence raises increasing concern due to their potential effects on human health, particularly when compounds exhibit genotoxic, carcinogenic, or endocrine-disrupting properties.

In response to these challenges, a highly structured regulatory and scientific framework has been established within the European Union. Over recent decades, the European Commission (EC), in close collaboration with the European Food Safety Authority (EFSA), has developed a set of regulations, coordinated monitoring programmes, and risk assessment tools aimed to ensure a high level of consumer protection. These mechanisms address a broad range of chemical hazards, whether intentionally introduced or unintentionally formed along the food chain, and are based on the assessment of dietary exposure and the characterisation of risks to human health.

In addition to surveillance and risk assessment activities, the management of non-compliances and food safety incidents at the European level relies on the Rapid Alert System for Food and Feed (RASFF). Established in 1979, the RASFF serves as an information exchange platform between Member States, the European Commission, and EFSA, enabling the rapid notification of situations that may pose a risk to human health. Its primary objective is to ensure a swift and coordinated response, including the withdrawal or recall of affected products, as well as the dissemination of detailed information regarding the nature of the hazard, the food matrix involved, the origin of the product, and the measures implemented.

The RASFF is a transversal tool applicable to all hazards monitored within the food safety framework. It covers approximately thirty hazard categories, including pesticide residues, process contaminants such as acrylamide and polycyclic aromatic hydrocarbons (PAHs), as well as heavy metals, mycotoxins, unauthorised additives, allergens, and microbiological hazards. Beyond its operational role in crisis management, the RASFF also represents a valuable source of information for identifying emerging trends, evaluating the effectiveness of regulatory measures, and guiding control and research priorities.

Nevertheless, despite the extensive efforts undertaken by academic research, national authorities, and EFSA, knowledge gaps remain in certain areas, particularly with regard to occurrence data and the understanding of factors influencing food contamination. These limitations may reduce the accuracy of dietary exposure assessments and, consequently, hinder an optimal characterisation of risks to consumer health.

It is within this context that the objective of the present thesis was to contribute to reducing some of the existing knowledge gaps related to food contamination, dietary exposure, and risk assessment. Given the diversity of chemical and biological hazards that may occur throughout the food chain, it was necessary to define a coherent and manageable scope for the present doctoral work. Therefore, the selection of target contaminants was guided, on the one hand,

by the expertise of the Belgian National Reference Laboratory for pesticides and process contaminants in food hosting this thesis and, on the other hand, by the priorities identified by the Belgian and European competent authorities.

The choice of pesticides, polycyclic aromatic hydrocarbons (PAHs), and acrylamide was motivated by both their current relevance to food safety and the complementary nature of these contaminant groups. Together, they represent three major contamination pathways occurring in the food chain. Pesticides are intentionally applied during agricultural production and may remain as residues in raw or processed food commodities. PAHs are environmental and process contaminants originating from combustion processes and food manufacturing practices such as drying and smoking. Acrylamide is a process contaminant formed during high-temperature cooking through Maillard reactions. Hence, these contaminants illustrate the food contamination from primary production to industrial processing and final consumer practices.

Finally, the present thesis forms part of a broader research framework aimed at improving knowledge on food contaminants relevant to risk assessment and food safety management. It complements previous doctoral and research projects conducted within the laboratory and elsewhere on other major food contaminants, including mineral oil hydrocarbons (MOH) and furans, and alkylated furans. Together, these studies contribute to reducing the knowledge gaps that may limit the accuracy of dietary exposure and risk assessments and, thereby supporting decision-making and consumer protection policies.

2 GENERAL INTRODUCTION

2.1. Pesticides Residues in Food

2.1.1. Description and uses

Pesticides are a broad group of chemical substances intentionally applied to prevent, control, or eliminate organisms that are harmful to agricultural production, food preservation, and public health. Their applications include, but are not limited to, fungicidal, insecticidal, herbicidal, acaricidal, nematocidal, rodenticidal, molluscicidal, repellent, and plant growth–regulating purposes. In addition, some pesticides are used for disease vector control or as repellents to limit human exposure to pests (BCPC 2009; FAO 2024; Pesticides | EFSA 2026).

Before proceeding further, it is important to emphasise that the term “pesticide” is a generic designation whose meaning may vary depending on the context. In the present chapter, “pesticide” refers either to the active substance or to the plant protection product applied to control target organisms, whereas “pesticide residues” refer to the active substances, their isomers, and their relevant metabolites detected in matrices following pesticide application.

On a global scale, the World Health Organization (WHO) estimated that more than 1,000 active substances are used to protect crops and food commodities against pests and diseases. The use of pesticides is constantly growing over the last decades reaching 3.7 Mtons in 2022 fig. 1.1) (FAO 2024). The major pesticide users are the Americas (notably Brazil, the USA, and Argentina) and Asia (particularly Indonesia, China, and Vietnam), reflecting the intensive

agricultural systems of the world's leading food exporters.

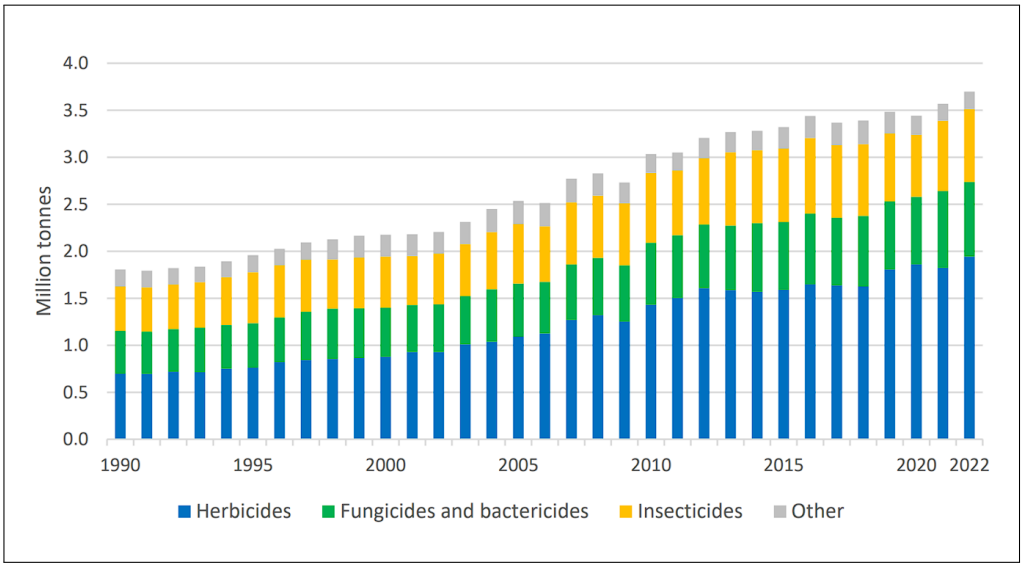

Figure 1.1: Global pesticide use by category (from FAO 2024)

This extensive use is accompanied by a high diversity of chemical compounds. Pesticides encompass a wide range of chemical classes that differ in their intended use, physicochemical properties, and biochemical mechanisms of action. Given this diversity, classifying pesticides into major chemical families provides a useful framework for understanding their properties and behaviour. Selected examples of representative pesticide classes, together with their primary uses and mechanisms of action, are summarized in table 1.1 (BCPC 2009; FAO 2024; Pesticides | EFSA 2026).

Table 1.1: Examples of pesticide chemical classes, uses, and mechanisms of action

Chemical class	Use	Examples	Mechanism of action
Carbamates	Insecticides	Carbaryl; Methomyl	Acetylcholinesterase inhibitors
Dithiocarbamates	Fungicides	Mancozeb; Thiram	Multi-site inhibitors
Neonicotinoids	Insecticides	Acetamiprid; Imidacloprid; Thiamethoxam	Nicotinic acetylcholine receptor agonists
Organochlorine	Insecticides	DDT; Lindane	Na channel modulators Cl channel antagonists
Organophosphates	Insecticides	Chlorpyrifos; Diazinon	Acetylcholinesterase inhibitors

Table 1.1 (continued)

Chemical class	Use	Examples	Mechanism of action
Phenoxy acids	Herbicides	2,4-D; Mecoprop	Growth regulators (synthetic auxins)
Pyrethroids	Insecticides	Cypermethrin; Deltamethrin; Pyrethrin	Voltage-gated sodium channel modulators
Strobilurin	Fungicides	Azoxystrobin; Pyraclostrobin	Mitochondrial respiration inhibitors
Triazine	Herbicides	Atrazine; Propazine	Photosystem II inhibitors
Triazoles	Fungicides	Tebuconazone; Difenoconazole	Sterol biosynthesis inhibitors

Because pesticides are biologically active substances that may remain as residues in food, their use is strictly regulated in order to protect consumer health and ensure food safety. In the European Union, a comprehensive regulatory framework governs both the approval of active substances and the establishment of maximum residue levels in food commodities. The main principle of this regulatory framework is presented in the following section.

2.1.2. Regulatory framework for pesticides in the European Union

Due to their extensive and diverse applications, determining the exact number of pesticides currently available worldwide is challenging. According to the European Union Reference Laboratory (EURL) DataPool website, 1,966 pesticide residues are currently listed. However, these residues may correspond to parent compounds, isomers, or metabolites (EURL DataPool). In contrast, the European Union MRLs Pesticides Database currently reports 679 residue definitions, which group active substances together with their relevant metabolites and isomers (EU Pesticides Database - MRLs). These figures highlight not only the diversity of pesticide chemistry but also the complexity associated with residue monitoring and risk assessment.

These numbers are subject to continuous revision due to regulatory reassessments and scientific advances. Despite the diversity of chemical structures, biological targets, and modes of action, pesticides share a common objective: increasing agricultural yields and reducing post-harvest losses. However, not all registered or historically used pesticides are currently authorised. Several substances or entire chemical classes, such as organochlorines, have been banned or severely restricted due to their environmental persistence, bioaccumulation potential, carcinogenicity, endocrine-disrupting properties, or other unacceptable toxicological risks (European Commission 2009).

Given this complexity and the potential risks associated with pesticide residues, a strict regulatory framework is essential to ensure food safety and consumer protection.

In the European Union, Regulation (EC) No 1107/2009 governs the approval of active substances, setting criteria related to their safety, including the absence of unacceptable risks such as environmental persistence, bioaccumulation, carcinogenicity, mutagenicity,

reproductive toxicity, or endocrine-disrupting effects. Approved substances are subject to time-limited authorisations and periodic re-evaluation (European Commission 2009).

Complementary to this framework, Regulation (EC) No 396/2005 establishes maximum residue limits (MRLs) for pesticide residues in food and feed of plant and animal origin. These limits are based on good agricultural practices, while ensuring that consumer exposure remains below toxicological reference values. MRLs are defined for each pesticide residue–food commodity combination (European Commission 2009).

Within this regulatory framework, 421 active substances, including herbicides, insecticides, and fungicides, were approved in the European Union as of February 2026 (EU Pesticides Database - Active substances).

2.1.3. Toxicological basis of pesticide risk assessment

Pesticides are designed to exert biological activity against target organisms. Because of their intrinsic bioactivity, these substances may also interact with physiological pathways shared by non-target species, including humans. Although pesticide regulation aims to minimise adverse effects, the presence of residues in food commodities raises concerns regarding potential chronic or acute health effects following dietary exposure.

Acute toxic effects are primarily associated with occupational exposure, particularly among agricultural workers and professional pesticide applicators, whereas chronic effects are also more relevant to the general population through long-term dietary intake. Moreover, exposure patterns differ substantially between occupational and consumer contexts, with occupational exposure involving additional routes such as dermal contact and inhalation, while consumer exposure mainly occurs via ingestion (EFSA 2022a).

Pesticides encompass diverse modes of action depending on their chemical class and intended use, resulting in a wide spectrum of toxicological profiles. For instance, organochlorine pesticides, now largely banned such as DDT, raised major public health concerns due to their high environmental persistence, bioaccumulation potential, neurotoxicity, and associations with carcinogenic and endocrine-disrupting effects (Jayaraj et al. 2016). Organophosphate and carbamate insecticides act primarily through inhibition of acetylcholinesterase, leading to overstimulation of the nervous system, characterised by symptoms such as hypersalivation, bradycardia, diaphoresis, and muscle spasms, and are associated with well-documented acute neurotoxicity in humans (Pope 1999; BCPC 2009).

Pyrethroid insecticides were developed as less persistent alternatives and target voltage-gated sodium channels in nerve cells, which are present in both insects and mammals. Although generally considered less acutely toxic to humans, exposure at higher doses may still induce neurotoxic effects, including tremors, headaches, nausea, salivation, and muscle spasms (Ramchandra et al. 2019). Long-term exposure has also been associated with oxidative stress, immunotoxicity, and endocrine-disrupting effects potentially contributing to carcinogenic outcomes (Yang et al. 2018).

More recently introduced neonicotinoids function as agonists of nicotinic acetylcholine receptors, selectively targeting insects but potentially affecting mammalian neurological pathways at sufficient exposure levels. Increasing evidence suggests possible impacts on neurodevelopment, particularly during early life stages (Xu et al. 2022). Additionally, experimental studies have reported associations with hepatotoxicity and reproductive toxicity linked to oxidative stress mechanisms (Wang et al. 2018). Triazole fungicides

interfere with sterol biosynthesis in fungi. However, some compounds have demonstrated endocrine-mediated and developmental effects in mammalian toxicological studies. These examples illustrate that pesticide efficacy is intrinsically linked to biological activity that may also produce unintended effects in humans (Taxvig et al. 2007; van der Ven et al. 2020).

In regulatory toxicology, a clear distinction is made between hazard and risk. Hazard refers to the intrinsic capacity of a substance to cause adverse effects, whereas risk depends on both the hazard and the level of exposure. Toxicological studies conducted in experimental models are used to characterise dose–response relationships and to identify reference points such as the No Observed Adverse Effect Level (NOAEL). These values are subsequently used to determine health-based guidance values through the application of uncertainty factors accounting for inter- and intra-species variability (Solecki et al. 2005).

Within the European regulatory framework, the main health-based guidance values established for pesticides include the Acceptable Daily Intake (ADI), defined as the amount of a substance that can be ingested daily over a lifetime without appreciable health risk, and the Acute Reference Dose (ARfD), which estimates the quantity that can be consumed within a short period, typically within one meal or one day, without adverse effects (BCPC 2009; EFSA 2024).

2.1.4. Risk assessment of pesticides in food in the European Union

EFSA uses the Pesticide Residue Intake Model (PRIMo, version 3.1) to assess both acute and chronic exposure scenarios within the framework of Regulations (EC) No 396/2005 and No 1107/2009.

PRIMo is a standardized Excel-based tool designed to automate the calculation of dietary intake of pesticide residues by combining concentration data with food consumption patterns. It serves as the harmonized European benchmark for verifying that the expected residue levels do not exceed safety thresholds for any population group.

PRIMo integrates food consumption data from different European populations and age groups, including infants, toddlers, children, adolescents, adults, and elderly individuals. Acute exposure is estimated using the International Estimated Short-Term Intake (IESTI), while chronic exposure is assessed via the International Estimated Daily Intake (IEDI). Risk characterisation is performed by comparing the IESTI with ARfD and the IEDI with the ADI. This concept will be further explained in chapter 3. If these toxicological thresholds are exceeded, a potential risk to consumer health is identified. The model is used for the establishment of MRL. While MRLs are primarily based on Good Agricultural Practice (GAP) to reflect the minimum amount of residue necessary to achieve pest control, they are systematically validated against toxicological safety limits to ensure consumer protection. This model is also used in enforcement contexts, particularly in response to RASFF notifications to evaluate the impact on consumer safety (EFSA 2026).

However, this classical risk assessment framework presents limitations when addressing endocrine-disrupting substances. Endocrine disruptors may exhibit non-monotonic dose–response relationships, meaning that adverse effects can occur at very low exposure levels that are not predicted by traditional threshold-based approaches. As a result, conventional toxicological reference values may not adequately describe the risks associated with endocrine disruption. Another limitation lies in the fact that this framework does not account for the cocktail effect of pesticides. Multiple pesticides may induce similar

deleterious effects through shared toxicological mechanisms, leading to cumulative effects (EFSA 2013; Santonja 2022).

2.1.5. Fate and transfer of pesticide residues during raw food materials processing

Beyond their occurrence in raw agricultural commodities, pesticide residues may undergo substantial changes during post-harvest processing and preparation prior to consumption. These processes can induce partial degradation of parent compounds through mechanisms such as hydrolysis, oxidation, photolysis, or thermal degradation, and may also lead to the formation of metabolites with distinct physicochemical properties and toxicological relevance. In plant-derived products, technological steps including drying, fermentation, or enzymatic oxidation can further alter residue profiles by affecting pesticide stability and distribution within the matrix. Food processing operations performed either by consumers or by food business operators, such as washing, peeling, trimming, milling, or cooking can result in significant reductions of pesticide residues. For instance, Bonnechère et al. (2012a) reported reductions of up to 90 % during the washing of carrots, while washing of spinach led to residue decreases ranging from 10 to 50 %, and microwave cooking resulted in reductions of up to 39 % (Bonnechère et al. 2012b). In the case of melons, peeling was shown to be particularly effective, with residue reductions exceeding 90 % (Bonnechère et al. 2012c). These variations demonstrate that pesticide residue levels in raw matrices rarely reflect actual consumer exposure. These observations highlight the importance of accounting for food transformation when assessing dietary exposure and evaluating potential health risks. They also illustrate the high complexity of this issue, given the wide range of possible combinations involving food matrices, processing conditions, and the large number of pesticides currently used worldwide. Among these transformation processes, the preparation of beverages through infusion represents a critical but complex pathway for exposure.

Consequently, this thesis partly focuses on the transfer factors (TFs) of pesticide residues from tea leaves and herbal teas to the brewed infusion. This focus is justified by the substantial lack of available TF data for many pesticide residues detected on the Belgian market (AFSCA-FAVV 2020), which considerably hampers risk assessment in cases of MRL exceedance (FAO 2014; BfR 2019). Although several studies have investigated pesticide residues transfer during tea infusion, they often rely on tea types that are not representative of the Belgian market or on infusion protocols that do not reflect Belgian consumption habits (Chen et al. 2015; Xiao et al. 2017; Wang et al. 2019). In the Belgian cultural context, tea is frequently blended with fruits, herbs, or flavouring oils, which may further influence pesticide residues mobility (Pincemaille et al. 2014).

Pesticide residue transfer during infusion is a compound-specific phenomenon influenced by multiple factors, including intrinsic physicochemical properties such as water solubility, polarity, pKa, and octanol–water partition coefficient (Hou et al. 2013; Wang et al. 2014; Wang et al. 2019). From an analytical perspective, this complexity requires robust methods capable of covering a broad range of chemistries while maintaining sufficiently low limits of quantification (LOQ) to reliably determine TFs in diluted infusion samples. Addressing these knowledge gaps is therefore essential to better characterize pesticide exposure and associated risks for the Belgian population. Addressing these knowledge gaps is therefore essential to better characterize pesticide exposure and associated risks for the Belgian population.

2.1.6. Analytical methods for pesticides determination in food

Given the large number of pesticides currently in use, providing a comprehensive review of all existing analytical methods is particularly challenging, due to the wide diversity of available approaches. Nevertheless, these methods can generally be classified into two main categories: multi-residue methods, which enable the simultaneous determination of several tens to several hundreds of compounds, and single-residue methods, which focus on a single pesticide or a specific class of pesticides, typically because they present particular analytical challenges. Once extracted, samples are typically analysed by liquid chromatography and gas chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS and GC-MS/MS). Regarding multi-residue methods, three main approaches can be distinguished:

The Dutch mini-luke

The Dutch mini-Luke method is a historically important extraction technique that has been widely adopted in analytical laboratories for the multi-residue determination of pesticides in food, particularly matrices with high water content such as fruits and vegetables. The methodology is based on a liquid–liquid partitioning approach designed to efficiently separate a broad spectrum of pesticide residues from the sample matrix. The procedure begins with sample homogenization, followed by an initial extraction using a polar solvent, most commonly acetone. This step allows the efficient solubilization of both polar and non-polar pesticides. Subsequently, the analytes are transferred from the aqueous–acetone phase into a less polar organic phase through liquid–liquid partitioning, typically using a mixture of solvents such as petroleum ether and dichloromethane. This step contributes to matrix clean-up while maintaining a broad extraction scope. The resulting organic extract is then concentrated and filtered prior to reconstitution in an appropriate solvent, such as methanol for LC-MS/MS analysis or toluene for GC-MS/MS analysis, enabling the simultaneous determination of several hundred analytes at trace concentration levels (de Kok et al. 1987; De Kok and Hiemstra 1992).

Despite its broad analytical scope and historical relevance, the Dutch mini-Luke method presents several significant drawbacks. In particular, it requires the use of large volumes of organic solvents, many of which are highly toxic and environmentally hazardous. In 2015 Lozano et al. (2016) optimized the method by reducing by a factor 2 the volume of dichloromethane and petroleum ether used. However, the use of dichloromethane is increasingly restricted or prohibited in many European Union countries due to its classification as a hazardous solvent, limiting the current applicability of this method in routine pesticide analysis (European Commission 2006).

SweEt method

The Swedish ethyl acetate extraction method (SweEt method), based on direct extraction with ethyl acetate, represented an important intermediate step between traditional liquid–liquid partitioning approaches and modern QuEChERS-based methods. It was developed with the aim of simplifying sample preparation while reducing the use of toxic chlorinated solvents. Extraction with ethyl acetate enables the efficient recovery of a broad spectrum of target analytes, particularly semi-polar and non-polar compounds, thereby providing a wide analytical scope. The procedure typically involves direct extraction of the

homogenized sample with an appropriate volume of ethyl acetate, often assisted by vigorous shaking or blending. A key early step is the addition of anhydrous sodium sulfate to remove co-extracted water, thereby improving extraction efficiency and preventing interference in subsequent processing steps. After extraction, the organic phase is concentrated under a gentle nitrogen stream and subsequently reconstituted in a suitable solvent, such as toluene or isooctane for GC-MS/MS analysis, or methanol for LC-MS/MS analysis (Andersson and Pålsheden 1991; Pihlström et al. 2007).

However, the applicability of the SweEt method to LC-MS/MS remains limited, mainly due to the moderate polarity of ethyl acetate, which is insufficient for the efficient extraction of highly polar pesticides typically analyzed by liquid chromatography.

QuEChERS method

More recently, the introduction of the QuEChERS method, developed by Lehotay et al. in 2003, addressed many of the limitations associated with both the SweEt and Dutch mini-Luke methods by combining a simplified extraction procedure with improved matrix clean-up, reduced solvent consumption, and enhanced compatibility with both GC- and LC-based multi-residue pesticide analysis (Anastassiades et al. 2003). This method has gained increasing popularity due to the advantages it offers that are highlighted in its own acronym, QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe). As a result, it is now widely adopted in analytical laboratories due to its straightforward implementation. Briefly, the procedure consists of an extraction with water and acetonitrile followed by the addition of salts, which induces phase separation and thus allows extraction and mild purification to occur in a single step. A subsequent clean-up step based on dispersive solid-phase extraction (d-SPE) may be performed depending on the matrix/pesticide combination, using sorbents such as PSA, C18, or GCB, or by applying freezing-out, to remove pigments, sugars, lipids, and other co-extractives (Anastassiades et al. 2003).

Several variants of the original QuEChERS protocol have been developed for specific pesticide classes, such as the use of acidified or buffered extraction conditions to either maximize the stability of target analytes or to prevent their degradation during sample preparation (Majors et al. 2026), allowing in specific case to add challenging pesticides to multi-residue methods). Such adaptations extend the scope of conventional multi-residue methods and make it possible to include particularly challenging compounds within a single analytical workflow. Due to these improvements, several hundred pesticides with a broad polarity range can now be determined using a single generic approach. Another major advantage is the compatibility of QuEChERS-based extracts with both LC-MS/MS and GC-MS/MS (Anastassiades et al. 2003; Majors et al. 2026).

Single residue methods

Another advantage of the QuEChERS method is that, with slight modifications of the protocol, it can be used as a single-residue method for specific problematic compounds. For instance, a recent concern has been raised regarding acidic pesticides, as active substances can be present in the matrix not only in their free form but also conjugated with a wide range of naturally occurring plant compounds, such as sugars, polysaccharides, amino acids, proteins, and epoxy groups (Mehltretter et al. 1974; Feung et al. 1975; Riederer and Schönherr 1986). To accurately determine the total amount of these active substances, the analytical method

must therefore account for all conjugated forms. However, since many conjugates are not fully identified, it remains challenging to quantify these compounds comprehensively. A solution provided by Steinborn et al. (2017) consists of introducing an alkaline hydrolysis step (using a NaOH solution) at the beginning of the QuEChERS extraction, converting conjugated forms of acidic pesticides into their free forms. The extract is then neutralised with an H_2SO_4 solution, after which the classical procedure is followed prior to LC-MS/MS analysis.

Due to the versatility of the QuEChERS extraction, it is relevant to evaluate its application to tea. As this method was initially developed for matrices with high water content, it would be particularly interesting to assess its applicability to tea infusions for the determination of pesticides TFs. Furthermore, its ability to cover a wide range of molecular polarities and to be adapted to specific classes of compounds, such as acidic pesticides, makes it a promising candidate for the analysis of pesticides also detected in tea (AFSCA-FAVV 2020).

To ensure the reliability of the analytical results, the method must be validated according to several performance criteria. The four European Union Reference Laboratories (EURLs) have elaborated a guidance document describing analytical quality control and method validation procedures for pesticide residue analysis in food and feed (European Commission 2026). This document notably defines the validation parameters as well as the corresponding acceptance criteria (table 1.2).

Table 1.2: Method validation criteria according to SANTE/11312/2021 v2 guidelines for pesticide residue analysis in food

Parameter	What / how	Acceptance criterion
Calibration / quantification	Calibration function check using five concentration levels	Deviation of back-calculated concentration from true concentration $\leq \pm 20\%$
Matrix effect	Difference of response between standard prepared in matrix extract and standard in solvent	$\leq \pm 20\%$
LOQ	Lowest spike level meeting identification criteria and method performance requirements (recovery and precision)	$\leq$ MRL
Specificity	Response observed in reagent blank and blank control samples	$\leq 30\%$ of reporting level (RL)
Recovery	Average recovery calculated for each spike level tested	70–120 %
Precision (RSD_r)	Repeatability expressed as RSD_r for each spike level tested	$\leq 20\%$

Table 1.2 (continued)

Parameter	What / how	Acceptance criterion
Precision (RSD_{wR})	Within-laboratory reproducibility derived from ongoing method validation or verification	$\leq 20\%$
Robustness	Evaluation based on average recovery and RSD_{wR} obtained during ongoing validation/verification	See criteria above
Ion ratio	Compliance with identification requirements for MS techniques	According to SANTE identification criteria (Table 3)
Retention time	Agreement of analyte retention time with calibration standards	± 0.1 min

High resolution mass spectrometry

More recently, LC and GC coupled to high-resolution mass spectrometry (HRMS), such as Orbitrap or time-of-flight (ToF) mass spectrometers, are progressively implemented in analytical laboratories. In contrast to low-resolution mass spectrometry, HRMS provides accurate mass measurements that significantly improve compound identification capabilities. Besides providing accurate mass measurements of precursor ions, HRMS instruments can acquire MS/MS data through different acquisition strategies, including targeted MS/MS, data-dependent acquisition (DDA), and data-independent acquisition (DIA). In DDA workflows, precursor ions are automatically selected for fragmentation according to predefined criteria, whereas DIA approaches fragment all ions within predefined consecutive m/z windows. The resulting product ion spectra provide complementary structural information that can be exploited during data processing for compound confirmation, suspect screening, and the identification of previously unmonitored contaminants. Combined with full-scan accurate-mass acquisition, these approaches enable the simultaneous detection of a very large number of compounds, typically using extensive spectral or exact-mass databases that may contain several hundreds to several thousands of substances, including pesticides, metabolites, and other contaminants (Rajski et al. 2021; Huérfano Barco et al. 2022).

Another major advantage of HRMS lies in its capability for retrospective data analysis. Once data have been acquired, new compounds can be searched for posteriori by updating or expanding databases, without the need to reanalyse the samples (Huérfano Barco et al. 2022). This feature is particularly valuable in the context of emerging contaminants, evolving regulatory requirements.

Despite these advantages, the routine implementation of HRMS for pesticide analysis remains limited. This is primarily due to the high level of expertise required for method development, data processing, and result interpretation, as well as the substantial computational resources needed and well-trained personnel. HRMS generates large data files, leading to increased demands in terms of data storage capacity and processing time. Moreover, sensitivity and quantitative performance for certain compounds may still be inferior

to those achieved with optimized LC-MS/MS or GC-MS/MS methods, especially in complex matrices. As a result, HRMS is currently more often applied as a complementary or screening tool rather than as a replacement for established targeted MS/MS methods in routine pesticide analysis (Rajski et al. 2019, (Anastassiades, personal communication, January 29, 2026).

2.2. Acrylamide as a Process Contaminant

2.2.1. Description and formation

Acrylamide is a low-molecular-weight organic compound with high water solubility fig. 1.2. It is widely used in the chemical industry, primarily in its polymerised form, notably as a flocculant for the clarification of drinking water and the treatment of industrial effluents (Joint FAO/WHO 2011; ATSDR 2012). Polyacrylamide is also extensively used in the paper, cosmetic, and textile industries, as well as for the preparation of electrophoresis gels (IARC 1994; NICNAS 2002; Joint FAO/WHO 2011).

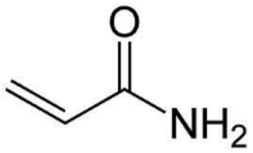

Figure 1.2: Acrylamide structure

The concern regarding dietary exposure to acrylamide has emerged in 2002, when its presence was first reported in foods prepared at temperatures above 120 °C under low-moisture conditions (Biedermann et al. 2002; Tareke et al. 2002; EFSA 2015). Although the formation mechanisms of acrylamide are not yet fully understood, it is primarily formed through the Maillard reaction between certain amino acids, notably asparagine, and reducing sugars such as fructose or glucose (Mottram et al. 2002; Stadler et al. 2002) as the most prominent route. Acrylamide formation from asparagine requires the presence of a reactive carbonyl substance such as reducing sugars (glucose, fructose and maltose) to form an intermediate product - a Schiff base. This product will further undergo a decarboxylation which are the starting point of multiple reaction cascades leading to acrylamide (Rifai and Saleh 2020) as it can be seen in fig. 1.3 (route A).

In addition to reducing sugars, other carbonyl-containing compounds may also react with asparagine to form corresponding Schiff bases, which can subsequently undergo similar reaction cascades leading to acrylamide formation (fig. 1.3, Route B).

These different formation pathways illustrate the complexity of acrylamide formation in food. In addition, the rate and scale of acrylamide formation depends on many key factors such as water activity. For instance, (Biedermann et al. 2002) demonstrated that cooking wet potatoes at 120 °C under pressure resulted in acrylamide levels below 20 µg kg^{-1}, whereas heating dry potato powder under similar temperature conditions produced approximately 10,000 µg kg^{-1}. This observation highlights the critical role of water evaporation prior to

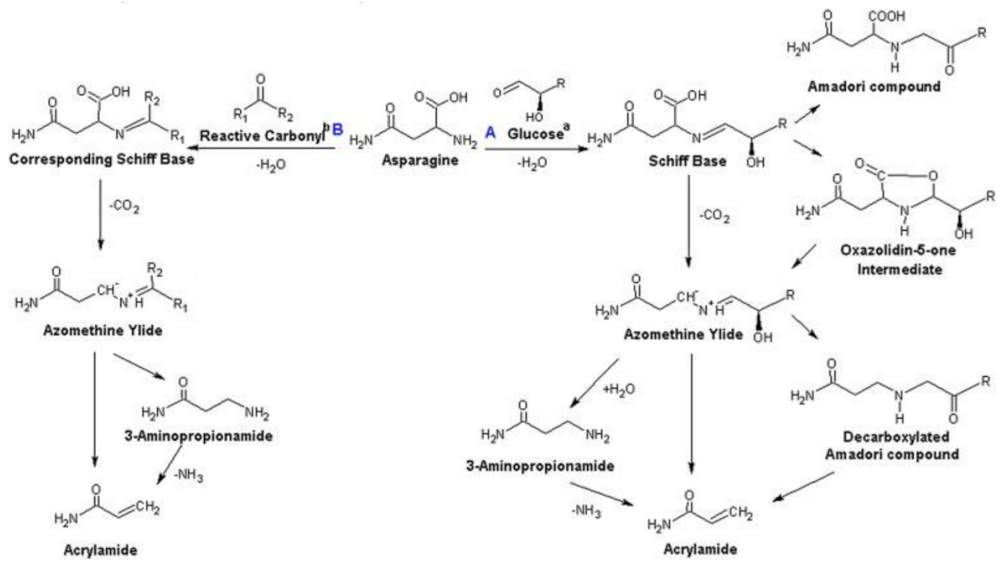

Figure 1.3: Mechanisms for the formation of acrylamide based on asparagine. Adapted from Jin et al. 2013

acrylamide formation.

In some cases, acrylamide formation does not involve the Maillard reaction, as the compound has also been detected in raw or minimally processed foods, such as olives, suggesting the existence of alternative formation pathways or sources (Casado et al. 2010; European Commission 2019; Breitling-Utzmann et al. 2023).

2.2.2. Toxicological properties and first risk assessment of acrylamide in food

In 2015, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) – an expert group on contaminants in food - published a scientific opinion on the risk to human health related to the presence of acrylamide in food (EFSA 2015). Based on the data available at that time, the Panel concluded that acrylamide exhibits clear genotoxic potential. In vitro studies showed that acrylamide is a weak mutagen but an effective clastogen (provoques DNA strand breakages) in mammalian cells, whereas its metabolite glycidamide (GA) is a strong mutagen and clastogen, inducing mutations through a DNA adduct–mediated mechanism. In vivo, acrylamide was shown to be clearly genotoxic in both somatic and germ cells (Koszucka et al. 2020). The genotoxicity of acrylamide is primarily mediated through its metabolic conversion to GA by cytochrome P450 2E1 (CYP2E1) (Sumner et al. 1999; Mei et al. 2008; Koszucka et al. 2020), although additional mechanisms involving the generation of reactive oxygen species (ROS) and oxidative DNA damage have also been described (Kim et al. 2015; Koszucka et al. 2020).

Given its genotoxic properties, the EFSA experts used the margin of exposure (MOE) approach, as recommended by EFSA for substances that are both genotoxic and carcinogenic (EFSA 2005). The reference point selected was a benchmark dose lower confidence limit for a 10 % response ($BMDL_{10}$) of 0.17 mg kg body weight day^{-1}. This $BMDL_{10}$ corresponds to

the estimation of the lowest dose which is 95 % certain to cause no more than a 10 % cancer incidence in rodents (EFSA 2005). This value was derived from the incidence of Harderian gland (adenomas and adenocarcinomas in male B6C3F$_1$ mice exposed to acrylamide for two years (EFSA 2015). Although the Harderian gland (pigmented lacrimal gland) (Miedel et al. 2015) is absent in humans, it represents a sensitive target tissue in rodents for genotoxic carcinogens compounds. The Panel therefore considered this endpoint to be a conservative basis for the assessment of neoplastic risk in humans, acknowledging that tumour target tissues may differ between species (EFSA 2015).

Using the BMDL$_{10}$ of 0.17 mg kg^{-1} bw day^{-1}, comparisons with human dietary exposure estimates revealed MOE values ranging from approximately 50 to 425, depending on the exposure scenario and population group. These values are substantially below the MOE of 10,000 (table 1.3), which the EFSA Scientific Committee considers to be of low concern for substances that are both genotoxic and carcinogenic (EFSA 2005). Consequently, the CONTAM Panel concluded that current dietary exposure to acrylamide shows a concern with respect to neoplastic effects (EFSA 2015).

Table 1.3: MOE values for neoplastic effects of acrylamide across surveys and age groups (from EFSA 2015)

Age group	Mean		P95	
	Minimum LB	Maximum UB	Minimum LB	Maximum UB
Infants ($n^{(a)} = 6/5$)	340	106	121	68
Toddlers ($n = 10/7$)	189	89	121	50
Other children ($n = 17/17$)	189	106	121	53
Adolescents ($n = 16/16$)	425	189	189	85
Adults ($n = 16/16$)	425	283	213	131
Elderly ($n = 13/13$)	425	340	243	170
Very elderly ($n = 11/9$)	425	340	283	170

n: number of surveys; LB: lower bound; UB: upper bound.
(a): Number of surveys used to derive the minimum/median/maximum mean exposure levels / number of surveys used to derive the minimum/median/maximum 95th percentile exposure levels.

Regarding the neurotoxicity of acrylamide, the EFSA CONTAM Panel also characterised the risk using the MOE approach but based on a BMDL$_{10}$ of 0.43 mg kg^{-1} bw day^{-1} and a threshold MOE of 100. For this endpoint, the MOE values obtained across all population groups and exposure scenarios ranged from 172 to 1,075, indicating a low level of concern for neurotoxic effects (table 1.4) (EFSA 2015).

Table 1.4: MOE values for neurotoxicity of acrylamide across surveys and age groups (from EFSA 2015)

Age group	Mean		P95	
	Minimum LB	Maximum UB	Minimum LB	Maximum UB
Infants ($n^{(a)} = 6/5$)	860	269	307	172
Toddlers ($n = 10/7$)	478	226	307	126
Other children ($n = 17/17$)	478	269	307	134
Adolescents ($n = 16/16$)	1 075	478	478	215
Adults ($n = 16/6$)	1 075	717	538	331
Elderly ($n = 13/13$)	1 075	860	614	430
Very elderly ($n = 11/9$)	1 075	860	717	430

n: number of surveys; LB: lower bound; UB: upper bound.
(a): Number of surveys used to derive the minimum/median/maximum mean exposure levels / number of surveys used to derive the minimum/median/maximum 95th percentile exposure levels.

Claeys et al. (2016) combined Belgian acrylamide monitoring data with food consumption data from the Belgian Food Consumption Survey conducted in 2004 by Sciensano (formerly the Scientific Institute of Public Health) (De Vriese et al. 2005). Using these datasets, they calculated MOE values for mean and 95th percentile (P95) dietary intakes. When based on the endpoint for neoplastic effects, MOE values ranged from 515 to 236 for mean intake and from 155 to 71 for P95 intake, respectively, thereby confirming the public health concern with respect to the neoplastic effects of acrylamide (Claeys et al. 2016).

Later evaluations, including the EFSA reassessment published in 2022, extended the available toxicological database and confirmed the conclusions of the 2015 Opinion (EFSA 2022b). Additional studies provided further evidence of chromosomal damage induced by acrylamide both *in vitro and in vivo*, with generally higher sensitivity observed in mice compared with rats. These recent studies showed that acrylamide was capable to induce DNA strand breaks in several human cell lines, including monocytes (Xiao et al. 2016), lymphocytes (Zamani et al. 2018; Hansen et al. 2018) and liver-derived cells (Mandon et al. 2019). Overall, these new data supported the previous assessment of 2015 and the MOE approach remains therefore valid for the risk characterisation of acrylamide (EFSA 2022b).

Despite this additional data on carcinogenicity in humans, no more recent risk assessment has been carried out in Europe. The most recent papers, such as (Costa et al. 2022), still base their risk assessments on occurrence data from the 2015 EFSA Opinion. Furthermore, as will be discussed in the following sections, the establishment of EU regulations on acrylamide, the implementation of mitigation measures, and the identification of additional dietary sources of acrylamide justify the need for an updated risk assessment.

2.2.3. Development and Updates of benchmark Levels for acrylamide in Foodstuffs and mitigation measures

Following the 2005 opinion of EFSA, which identified acrylamide as a potential concern for consumer health (EFSA 2005), the European Commission adopted Recommendation 2007/331/EC. This recommendation requested Member States to monitor the occurrence of acrylamide in selected food categories between 2007 and 2009, including potato-based products (French fries and crisps), soft bread, cereals and cereal snacks, biscuits and baby biscuits, coffee, and cereal-based baby foods.

In parallel, FoodDrinkEurope (FDE), the European trade association representing the food and drink industry, developed an Acrylamide Toolbox compiling mitigation measures for reducing acrylamide formation in foods. These measures cover multiple stages of the production chain, including raw material selection, storage conditions, and process optimisation, in accordance with the ALARA principle (As Low As Reasonably Achievable). For example, for potato-based products, the toolbox recommends selecting potato varieties with low levels of acrylamide precursors, such as asparagine and reducing sugars, avoiding cold storage below 6 °C to limit senescent sweetening, and optimising cooking conditions. In particular, it advises frying potatoes at temperatures not exceeding 175 °C or baking them in an oven at 180–200 °C until a golden colour is obtained, rather than a dark brown colour. Similar mitigation measures are provided for all food categories covered by Regulation (EU) 2017/2158. The Acrylamide Toolbox has been regularly updated to reflect advances in scientific knowledge and monitoring data, with the most recent consolidated edition published in 2019 (FDE 2019).

As uncertainties remained regarding the effectiveness of mitigation strategies proposed in the FDE “toolbox”, and because occurrence data were still insufficient for several food categories, the European Commission adopted Recommendation 2010/307/EU to extend the monitoring period and improve the assessment of mitigation measures. This was followed by Recommendation 2013/647/EU, which requested continued monitoring and introduced indicative values for acrylamide in various foodstuffs. These values were intended as reference levels to support process control and identify cases requiring further investigation, rather than as maximum limits or safety thresholds, and exceeding them did not in itself trigger enforcement actions.

Based on monitoring data collected under Recommendations 2010/307/EU and 2013/647/EU, together with an updated EFSA risk assessment, the European Commission adopted Regulation (EU) 2017/2158, establishing benchmark levels for acrylamide in several food categories (table 1.5). Unlike pesticides or polycyclic aromatic hydrocarbons, acrylamide is regulated through benchmark levels rather than maximum limits. These benchmark levels serve as performance indicators to verify the effectiveness of mitigation measures and are set according to the ALARA principle. They are not safety limits, but reference points intended to support continuous improvement. The mitigation measures to be implemented by food business operators are described in Annex II of the regulation (EU) 2017/2158.

Table 1.5: Benchmark levels for acrylamide in selected foods according to Regulation (EU) 2017/2158

Food	Benchmark level (μg kg^{-1})
French fries (ready-to-eat)	500
Potato crisps from fresh potatoes and from potato dough	750
Potato-based crackers	
Other potato products from potato dough	
Soft bread	
(a) Wheat based bread	50
(b) Soft bread other than wheat based bread	100
Breakfast cereals (excl. porridge)	
— bran products and whole grain cereals, gun puffed grain	300
— wheat and rye based products[(1)]	300
— maize, oat, spelt, barley and rice based products[(1)]	150
Biscuits and wafers	350
Crackers with the exception of potato based crackers	400
Crispbread	350
Ginger bread	800
Products similar to the other products in this category	300
Roast coffee	400
Instant (soluble) coffee	850
Coffee substitutes	
(a) coffee substitutes exclusively from cereals	500
(b) coffee substitutes from a mixture of cereals and chicory	[(2)]
(c) coffee substitutes exclusively from chicory	4 000
Baby foods, processed cereal based foods for infants and young children excluding biscuits and rusks[(3)]	40
Biscuits and rusks for infants and young children[(3)]	150

(1) Non-whole grain and/or non-bran based cereals. The cereal present in the largest quantity determines the category.
(2) The benchmark level to be applied to coffee substitutes from a mixture of cereals and chicory takes into account the relative proportion of these ingredients in the final product.
(3) As defined in Regulation (EU) No 609/2013.

The underlying principle of benchmark levels is to periodically assess (typically every three years) the effectiveness of mitigation measures based on updated occurrence data, with the potential to lower benchmark levels over time or, eventually, to convert them into maximum limits. In certain food categories, mitigation strategies have proven effective. For instance, long-term monitoring data of potato crisps indicates a substantial reduction in acrylamide levels in potato crisps over time from 763 µg kg^{-1} in 2002 to 421 µg kg^{-1} in 2016, accompanied by a decrease in the frequency of extreme values 4.8 % in 2002 vs 0.6 % in 2016. In contrast, for other food categories, no clear downward trend has been observed (Powers et al. 2017).

To date, benchmark levels have not been revised since the application of Regulation (EU) 2017/2158 in 2018 and no other food has been added either. Several factors contribute to this situation. First, significant data gaps persist for certain food categories not covered by the regulation. Second, acrylamide formation is subject to strong seasonal, climatic, and geographical variability, particularly linked to raw material composition, leading to high variability in acrylamide occurrence levels (fig. 1.4). Third, regulatory discussions remain influenced by diverging stakeholder positions. Consumer advocacy organisations, such as Safe Food Advocacy for Europe (SAFE), have called for the introduction of maximum limits, especially for foods intended for infants and young children (Sarion et al. 2021). However, food business operators highlight the technical and economic challenges of consistently complying with benchmark levels, even when mitigation measures are fully applied, due to natural variability in raw materials (Powers et al. 2017; FoodDrinkEurope 2021; F. Verstraete, personal communication, September 2025).

Hence, industry representatives, including FoodDrinkEurope, support the continued use of benchmark levels rather than maximum limits and advocate for maintaining the ALARA principle, which allows greater flexibility while encouraging continuous risk reduction (FoodDrinkEurope 2021; F. Verstraete, personal communication, September 2025).

In order to address remaining data gaps, the European Commission adopted Recommendation (EU) 2019/1888 in December 2019. This recommendation acknowledges that insufficient data are available for certain foods covered by Regulation (EU) 2017/2158, despite existing monitoring obligations, as well as for additional foods outside its scope that may contribute significantly to dietary acrylamide exposure. A non-exhaustive list of food categories was therefore established for further monitoring, including additional potato products (e.g. rösti, croquettes, potato meal), bakery products not previously covered, rice and maize crackers, cereal snacks, honey-roasted muesli, roasted nuts and oilseeds, dried fruits, cocoa beans and cocoa-derived products, olives in brine, coffee substitutes not based on chicory or cereals, and confectionery products such as fudge, caramel, and nougat (European Commission 2019).

Finally, Regulation (EU) 2017/2158 also establishes analytical performance criteria, including requirements for recovery, limits of detection (LOD), LOQ, and precision, for methods used to quantify acrylamide in foodstuffs table 1.6. These criteria apply both to foods covered by the

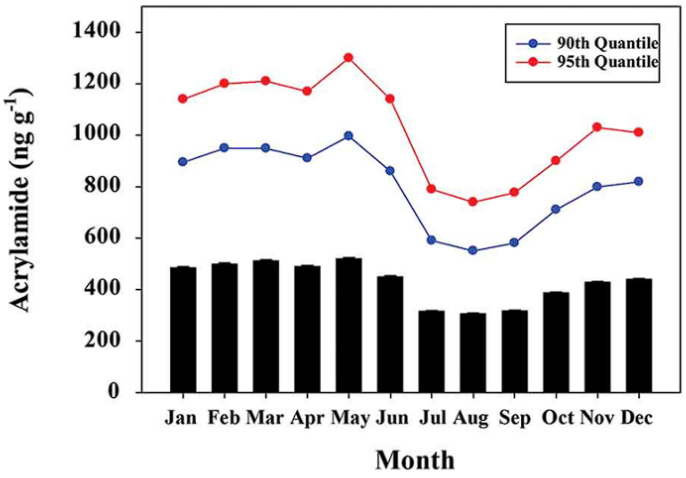

Figure 1.4: Seasonal variation of acrylamide content in potato crisps (from Powers et al. 2017)

regulation and to those monitored under Recommendation (EU) 2019/1888.

Table 1.6: Performance criteria for acrylamide analysis according to the Regulation (EU) 2017/2158

Parameter	Criterion
Applicability	Foods specified in this Regulation
Specificity	Free from matrix or spectral interferences
Field blanks	Less than Limit of Detection (LOD)
Repeatability (RSD_r)	0.66 times RSD_R as derived from the (modified) Horwitz equation
Reproducibility (RSD_R)	As derived from the (modified) Horwitz equation
Recovery	75–110 %
Limit of Detection (LOD)	Three tenths of LOQ
Limit of Quantification (LOQ)	For benchmark level < 125 μg kg^{-1}: $\leq$ two fifths of the benchmark level (however not required to be lower than 20 μg kg^{-1})
	For benchmark level ≥ 125 μg kg^{-1}: ≤ 50 μg kg^{-1}

It is within this regulatory context that the present thesis contributes to addressing Recommendation (EU) 2019/1888 by generating missing occurrence data. Furthermore, since the entry into force of Regulation (EU) 2017/2158 and the implementation of mitigation measures, no recent risk assessment has been conducted to determine where the MOE currently stands in comparison with the 2015 assessment. Achieving this objective required the development of an analytical method and an appropriate validation strategy capable of reliably analysing a wide variety of matrices while meeting the performance criteria established by Regulation (EU) 2017/2158.

2.2.4. Analytical methods for acrylamide determination in foods

Extraction and clean-up

As acrylamide is a highly polar compound, most extraction procedures rely on water as the primary solvent, which has historically been used in many analytical methods. Sample homogenization is commonly performed using devices such as an Ultra-Turrax or a Waring blender. In some protocols, organic solvents (e.g., n-hexane or cyclohexane) are added during extraction to remove non-polar interferences (Wenzl et al. 2007; Pan et al. 2020a). After extraction, samples are typically centrifuged to separate particulate matter. This classical aqueous extraction approach has been employed since the early development of acrylamide analysis and allows excellent extraction efficiencies, often close to 100 %. However, the resulting extracts are frequently heavily loaded with matrix components. For samples requiring additional clean-up, particularly those with high starch content, ultracentrifugation may be applied to frozen sub-samples to separate starch from the aqueous phase. Aqueous extraction of starch-rich matrices such as biscuits or bread may also lead to the formation of stable colloidal emulsions that are difficult to remove and may cause clogging of clean-up devices such as SPE cartridges or filters. Although ultracentrifugation (>15,000 rpm) can mitigate these issues, such equipment is not always available in laboratories. Another limitation of water-based extraction is the difficulty of concentrating the extract when a lower LOQ is required. For clean-up, hydrophilic–lipophilic balance (HLB) SPE sorbents are frequently used (Ferrer-Aguirre et al. 2016).

More recently, QuEChERS-based extraction has been applied to acrylamide analysis. The addition of NaCl to an acetonitrile–water mixture promotes the transfer of acrylamide into the acetonitrile phase via the salting-out effect. Although recoveries obtained with this approach are generally lower than those achieved with aqueous extraction (typically 40–90 %) (De Paola et al. 2017; Surma et al. 2017), these losses can be effectively compensated for by the use of isotopically labelled internal standards, such as deuterated acrylamide (acrylamide-d_3). In addition, this extraction procedure is faster and produces cleaner extracts, as most matrix components remain in the aqueous phase. Unlike water, acetonitrile does not promote the formation of colloidal suspensions of proteins or starch, thereby reducing matrix load (Anastassiades et al. 2003; Mastovska and Lehotay 2006). Due to the lower matrix load associated with acetonitrile extraction, clean-up steps are generally simplified and may consist of a rapid dispersive SPE (d-SPE), typically using PSA and/or C18 sorbents. For more complex matrices, such as cocoa-based beverages or coffee, or in the presence of specific interferences, strong cation-exchange SPE sorbents may be required (Bortolomeazzi et al. 2012). An additional advantage of acetonitrile is its volatility, which allows extract concentration and consequently facilitates a reduction of the method LOQ.

In this thesis, the QuEChERS method, combined with a range of d-SPE sorbents, will be

explored to develop a modular analytical approach capable of addressing the wide variety of food matrices targeted by Recommendation (EU) 2019/1888 and Regulation (EU) 2017/2158.

Separation and detection

As for pesticides and PAHs, the two main analytical techniques used for the separation and detection of acrylamide in food matrices are LC–MS/MS and GC–MS/MS (Pan et al. 2020b).

GC–MS/MS is less widely applied, as it requires a derivatization step based on bromination, which is complex and may lead to thermally unstable products. In addition, this procedure relies on toxic reagents such as potassium bromide, hydrogen bromide, and bromine. Bromination converts acrylamide into a more volatile derivative, facilitating GC–MS/MS analysis; however, this approach is time-consuming, as it typically requires an overnight derivatization step (Wenzl et al. 2003). To overcome the drawbacks associated with brominated derivatives, Yamazaki et al. (2012) developed a GC–MS-based method using xanthydrol derivatization. This approach avoids brominated compounds and results in a more robust analytical method applicable to multiple food matrices, with reported limits of quantification ranging from 4 to 20 µg kg^{-1}. Nevertheless, the chromatographic run time remains relatively long (20–30 min) (Wenzl et al. 2003; Yamazaki et al. 2012; Negoiță et al. 2020).

In contrast, LC–MS/MS methods do not require derivatization and are currently the most widely used techniques for acrylamide analysis in foods. Their popularity is mainly justified by very short run times (typically 1.5–5 min), enabling high analytical throughput. However, several analytical challenges arise from the intrinsic physicochemical properties of acrylamide, which is highly polar and has a low molecular weight (72 Da). As a consequence, conventional reversed-phase columns generally provide insufficient retention. Porous graphitic carbon columns, such as Hypercarb, are therefore commonly employed. Although this stationary phase is highly hydrophobic, acrylamide retention is still achieved through π–π interactions, allowing adequate retention times and preventing elution at the void volume (Rosén et al. 2007; Thermo Scientific Hypercarb Columns 2009). Alternatively, modified reversed-phase columns compatible with highly aqueous mobile phases have been developed, such as the Luna Polar Pesticides column from phenomenex (Phenomenex 2025) or the Atlantis dC18 column from Waters (Hancock and Krol 2004). More recently, dedicated UPLC columns specifically designed for acrylamide analysis have been introduced, including the Allure Acrylamide column from Restek (Supelco 2019), which offers improved column lifetime compared with carbon-based stationary phases.

Acrylamide is typically ionized using an electrospray ionization (ESI) source coupled to a triple quadrupole mass spectrometer. Sensitivity limitations may be encountered due to its low molecular mass and poor ionization efficiency. Moreover, detection occurs in a low-mass region characterized by a high background of small molecules, which can increase baseline noise and, consequently, the limit of quantification. Despite these challenges, UPLC–MS/MS remains the reference technique for acrylamide determination in food, offering short analysis times and satisfactory limits of quantification, generally between 10 and 20 µg kg^{-1}, in compliance with Commission Regulation (EU) 2017/2158. In the low mass range where acrylamide is detected, chromatographic interferences may occur, and longer retention times generally improve peak separation. For example, coffee and cocoa-based products are known to exhibit interfering signals, particularly due to the presence of the amino acid valine (Granby 2019). Gökmen and Senyuva (2006) also identified valine as a major interference in LC–MS

analysis of acrylamide, especially for fragment ions at m/z 72 and 118. They reported that valine "increased the baseline signal, preventing accurate and precise quantification, and resulted in poorer sensitivity in selected ion monitoring mode." Such interferences can be mitigated by applying an instrumental delay or by introducing a sample clean-up step, such as solid-phase extraction, prior to injection.

2.3. Polycyclic Aromatic Hydrocarbons in Food

2.3.1. Description and formation

PAHs constitute a large and diverse class of organic contaminants composed exclusively of carbon and hydrogen atoms arranged in two or more fused aromatic rings (fig. 1.5). Their conjugated ring structures confer high chemical stability and hydrophobicity, which contribute to their persistence in the environment. Several hundred individual PAH compounds have been identified in environmental matrices, varying in molecular weight, volatility, and physicochemical properties (Zhang et al. 2021).

PAHs are predominantly formed through processes involving incomplete combustion or pyrolysis of organic matter. Such processes occur both naturally and as a result of anthropogenic activities, leading to their widespread distribution in the atmosphere, hydrosphere, and geosphere. Natural sources of PAHs include forest fires, volcanic activity, and the natural carbonisation of organic material. Anthropogenic sources, however, represent the dominant contribution to global PAH emissions (Zhang et al. 2021). Stationary sources such as residential burning of wood, coal, oil, gas, and charcoal; power generation in industrial plants; waste incineration; and metallurgical industries (e.g., aluminium, iron, and steel production), are estimated to account for approximately 80 % of total annual emissions. Additional industrial processes such as petroleum refining, asphalt production, coal tar distillation, and coke manufacturing also release significant quantities of PAHs. Mobile sources, particularly exhaust emissions from gasoline- and diesel-powered vehicles, constitute another major contributor, especially in urban environments (ATSDR 1995).

2.3.2. Dietary exposure to PAHs

Given their widespread occurrence in air, soil, and water, polycyclic aromatic hydrocarbons (PAHs) can enter the human food chain through multiple pathways. Environmental contamination arising from atmospheric deposition, runoff from urban areas, and industrial discharges can lead to the accumulation of PAHs in crops, livestock, and aquatic organisms. For individuals who do not smoke and are not subject to occupational exposure, such as asphalt workers, aluminium smelter operators, or coke-oven employees, dietary intake is considered the major route of exposure (Scientific Committee on Food 2002a).

Food contamination by PAHs may occur at several stages, from primary production to domestic preparation. Environmental sources play a key role, particularly via atmospheric fallout, which can deposit particle-bound high-molecular-weight PAHs on the surface of fruits, vegetables, and grains. The waxy cuticle of fruits and vegetables facilitates the adsorption of low-molecular-weight PAHs, while particulate-bound forms adhere to outer surfaces (Speer et al. 1990). Aquatic products such as mussels and fish may also accumulate PAHs directly from polluted waters (Guillén et al. 1997; Phillips 1999).

In addition to environmental inputs, technological and culinary processes can generate or enhance PAH contamination. Industrial operations such as direct-fire drying of cereals

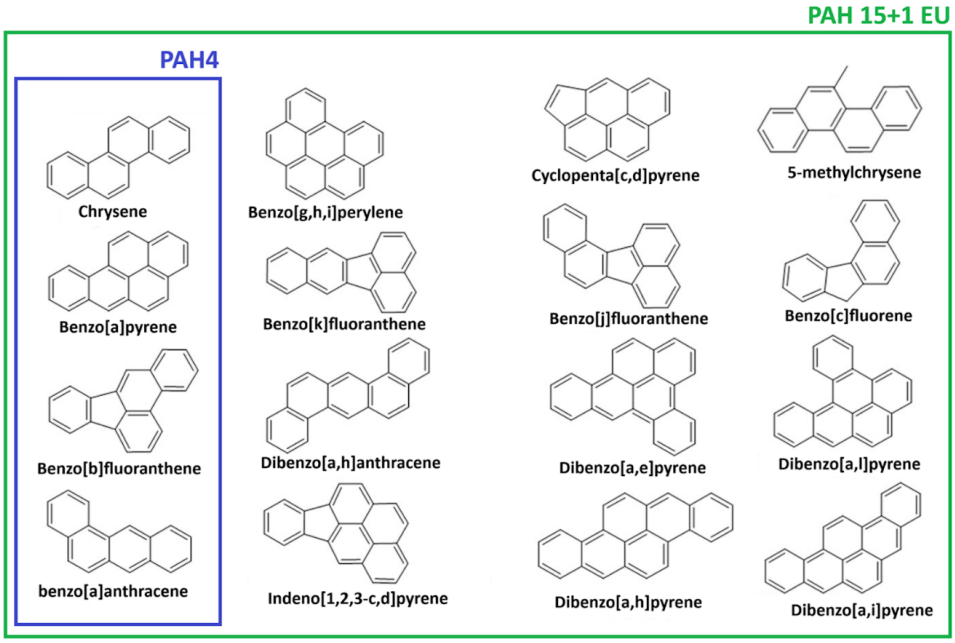

Figure 1.5: Chemical structures of the PAH 15+1 EU and the PAH4 indicator group

and oilseeds, or traditional smoking of fish and meat, allow combustion products to come into direct contact with food, resulting in elevated PAH levels (Karl and Leinemann 1996). Vegetable oils, including seed oils and olive-pomace oils, may be contaminated during production steps involving combustion (Speer et al. 1990; European Commission 2001a). Certain domestic cooking methods such as grilling, roasting, and pan-frying also contribute to PAH formation through pyrolysis of fats and incomplete combustion of fuel. High PAH concentrations have been reported in charcoal-grilled or barbecued meats, especially when fatty cuts are cooked over prolonged periods at high temperatures. Similarly, traditionally smoked foods, such as fish, often exhibit elevated PAH contents, and their contribution to dietary intake can be substantial in populations where such products are consumed frequently (Guillén et al. 1997; Phillips 1999).

PAHs have also been detected in products not typically associated with direct combustion processes, such as coffee. In this case, contamination may originate from environmental deposition on green beans during drying, or from endogenous formation during high-temperature roasting (Houessou et al. 2005; Houessou et al. 2008; Zhang et al. 2021).

2.3.3. Toxicological properties and risk assessment of PAHs

Based on the 1987 International Agency for Research on Cancer (IARC) monograph and subsequent evaluations by the International Programme on Chemical Safety (WHO/IPCS 1998) and the European Commission (2001b), the Scientific Committee on Food (SCF) identified 15 PAH compounds having clear evidence of mutagenicity or carcinogenicity in experimental animals (Scientific Committee on Food 2002b) (fig. 1.5). These were later complemented by the EFSA to form a group of 15+1 priority PAHs for monitoring in food also named PAHs 16. Consequently, these compounds are regarded as potentially carcinogenic to humans. While most epidemiological data and biomarker studies relate to occupational or environmental exposures, concerns have also been raised regarding dietary exposure, which constitutes the primary source for the general non-smoking population (Mei et al. 2008).

The carcinogenicity of PAHs has been demonstrated following dermal, subcutaneous, inhalation, and oral administration in laboratory animals. Tumor development generally occurs at the site of exposure, such as gastric tumors after oral dosing or skin tumors after dermal application, although systemic carcinogenicity has also been reported. In particular, oral administration of benzo[a]pyrene, the most extensively studied PAH, induced tumors in the gastrointestinal tract, liver, lungs, and mammary glands of rodents (Culp et al. 1998; Kroese et al. 2002). Fewer oral carcinogenicity data are available for other PAHs, which complicates the risk assessment of PAH mixtures (Scientific Committee on Food 2002b).

The genotoxicity of PAHs is primarily due to the metabolic activation of PAHs into reactive intermediates that form covalent DNA adducts, predominantly at the N2 position of guanine (Alexandrov et al. 2010). Toxicokinetic studies in rodents have shown that PAHs and their metabolites distribute widely across tissues, with higher concentrations in lipid-rich compartments (WHO/IPCS 1998). In oral exposure studies, significant accumulation of benzo[a]pyrene and other PAHs has been observed in both muscle and adipose tissue (Culp et al. 1998; Kang et al. 2007). Despite their carcinogenic potential, PAHs generally exhibit low acute toxicity, with reported LD50 values exceeding 1600 mg kg^{-1} body weight for benzo[a]pyrene (WHO/IPCS 1998). Hence the focus is made on the genotoxicity of PAHs.

Like acrylamide, PAHs are both mutagenic and carcinogenic. For substances with genotoxic and carcinogenic properties, EFSA recommends the use of the benchmark dose lower confidence limit for a 10 % response ($BMDL_{10}$) and the calculation of the MOE, rather than relying solely on no observed adverse effect levels (NOAELs) (EFSA 2005).

Based on occurrence data collected under EU Recommendation 2005/108/EC, EFSA evaluated dietary exposure to PAHs and, in its 2008 scientific opinion, calculated MOE values for different PAH groupings. For median consumers, MOE values were generally above 15,000, while for high consumers (97.5th percentile), MOEs ranged approximately from 9,500 to 10,800.

EFSA evaluated different groups of PAHs to address the issue that BaP was frequently absent in samples where other carcinogenic PAHs were present, thereby questioning its suitability as an indicator. Hence EFSA examined other groupings of PAHs based on occurrence data and toxicological relevance. Hence group of BaP alone, including PAH2 (BaP + chrysene), PAH4 (BaP, chrysene, benz[a]anthracene, and benzo[b]fluoranthene), and PAH8 (the PAH4 compounds plus benzo[k]fluoranthene, benzo[ghi]perylene, dibenz[a,h]anthracene, and indeno[1,2,3-cd]pyrene) fig. 1.5. MOE calculations indicated that BaP, PAH2, PAH4, and PAH8 yielded similar levels of concern. Thus, the choice of indicator was primarily driven by occurrence data rather than differences in toxicological potency. On this basis, EFSA concluded that BaP alone or PAH2 were not sufficiently representative, while PAH8 provided little improvement compared to PAH4. Consequently, PAH4 was identified as the most appropriate marker group for the occurrence and toxicity of genotoxic and carcinogenic PAHs in food (EFSA 2008).

Since this evaluation in 2008, no comprehensive reassessment of indicator PAH groupings has been identified in the literature. Some studies have performed risk characterisation in specific food categories; for example, Rozentāle et al. (2015) reported MOE values above 20,000 for smoked meat products, although this assessment did not account for total dietary exposure from other PAH sources. Other studies have applied the toxic equivalent factor (TEF) approach (Samiee et al. 2020). However, EFSA does not consider this approach appropriate for dietary risk assessment, as the TEF values are not derived from oral carcinogenicity studies (EFSA 2008).

Although the EFSA evaluation is now relatively old, it provided the scientific basis for the establishment and subsequent evolution of EU regulatory frameworks on PAHs in food.

2.3.4. Development and updates of EU maximum levels for PAHs in foodstuffs

In 2002, the Scientific Committee on Food (SCF) recommended BaP as a marker for the presence of PAHs in food and called for further investigation of priority PAHs. To support data collection, Recommendation 2005/108/EC requested Member States to monitor 16 targeted PAHs in foods and to study processing factors influencing their formation.

Regulation (EC) No 1881/2006 subsequently established maximum levels (MLs) for BaP in several food categories including oils and fats, smoked meats, smoked and non-smoked fish, crustaceans, molluscs, and cereal-based foods intended for infants and young children (EFSA 2008) (table 1.7). However, data collected across Member States and evaluated by EFSA in 2007–2008 showed that BaP alone was not a reliable marker, as other genotoxic and carcinogenic PAHs were frequently detected in its absence. EFSA concluded therefore, a monitoring system based on four indicator PAHs (PAH4): benzo[a]anthracene (BaA), chrysene

(CHR), benzo[b]fluoranthene (BbF), and BaP (EFSA 2008).

In response, Regulation (EU) No 835/2011 amended Regulation (EC) No 1881/2006 by introducing MLs for PAH4, extending the scope to additional foods, and lowering certain limits in line with technological improvements, while maintaining a separate ML for BaP to ensure comparability with historical data (table 1.7). Transitional derogations were nevertheless granted to certain Member States whose traditional smoking methods could not meet the revised MLs without compromising organoleptic characteristics.

Table 1.7: Evolution of maximum levels (MLs) for BaP and PAH4 in different food products according to EU regulations 1881/2006, 835/2011 and 2015/1933

Food product	ML BaP (μg kg^{-1}) (2006)	ML BaP (μg kg^{-1}) (2011)	ML PAH4 (μg kg^{-1}) (2011)	ML BaP (μg kg^{-1}) (2015)	ML PAH4 (μg kg^{-1}) (2015)
Oils and fats	2.0	2.0	10.0		
Coconut oil	-	2.0	20.0		
Smoked meat and smoked meat products	5.0	5.0 until 31.8.2014 2.0 as from 1.9.2014	30.0 as from 1.9.2012 until 31.8.2014 12.0 as from 1.9.2014	2.0	12.0
Smoked fish and smoked fish products	5.0	5.0 until 31.8.2014 2.0 as from 1.9.2014	30.0 as from 1.9.2012 until 31.8.2014 12.0 as from 1.9.2014	2.0	12.0
Muscle meat of fish	2.0	-	-	-	-
Bivalve molluscs	10.0	6.0	35.0	6.0	35.0
Processed cereal-based foods and baby foods	1.0	1.0	1.0	1.0	1.0
Food Supplements	-	-	-	10.0	50.0
Banana chips	-	-	-	2.0	20.0
Dried spices	-	-	-	10.0	50.0
Dried herbs	-	-	-	10.0	50.0

In parallel, analytical performance criteria for the determination of BaP, later extended to PAH4, were established in Regulation (EC) No 333/2007 and Regulation (EU) No 836/2011 (table 1.8). Further developments in EU legislation led to the adoption of Regulation (EU) 2015/1933, which established new maximum levels for PAHs, particularly for spices and dried herbs (table 1.7) for which some authors report high concentration in PAH4 (Bogdanović et al. 2019; Berki et al. 2020). However, at the time this regulation entered into force, only a limited number of analytical methods were fit for purpose for these particularly complex matrices. Conventional methods often resulted in highly dirty extracts, or significant matrix

interferences (Bratinova et al. 2016; Urban and Lesueur 2017).

Table 1.8: Performance criteria for PAHs analysis in food according regulation (EU) 836/2011

Parameter	Value / Comment
Applicability	Foods specified in Regulation (EC) No 1881/2006
LOD	Less than 0.3 μg kg^{-1}
LOQ	Less than 0.9 μg kg^{-1}
Precision	HORRAT$_r$ or HORRAT$_R$ values lower than 2
Recovery	50–120 %
Specificity	Free from matrix or spectral interferences, verification of positive detection

This analytical challenge was further reinforced by the wide diversity of spices and dried herbs available on the market. Consequently, the development of a robust and reliable analytical method capable of handling this diversity was a prerequisite for accurately assessing PAH occurrence in spices and dried herbs available on the Belgian market and for integrating these data into updated risk assessments.

2.3.5. Analytical methods for PAHs determination in food

Extraction and clean-up

Historically, accelerated solvent extraction (ASE) and extraction with saponification were commonly applied for the determination of PAHs in food. ASE offers the advantages of automation, and the possibility of using different solvent mixtures (Yusà et al. 2005), but it is not always effective in breaking the strong associations between PAHs and lipids. As an alternative, saponification with methanolic KOH has been widely employed (Bansal et al. 2017). In food matrices rich in fats and proteins, saponification, followed by liquid–liquid extraction and purification, is required to efficiently release and isolate PAHs (Ziegenhals et al. 2009; Raters and Matissek 2014). For example, in meat products, methanolic KOH hydrolyses proteins and lipids, releasing PAHs bound to these macromolecules. Subsequent liquid–liquid extraction with organic solvents such as hexane or cyclohexane isolates the analytes of interest while simultaneously removing polar compounds and other impurities (Zelinkova and Wenzl 2015). Although this method provides good accuracy and limits of quantification (LOQs) compliant with Regulation (EU) No 836/2011, it is time-consuming, as the saponification step alone can take up to three hours (Sampaio et al. 2021).

Purification steps after such an extraction protocol was traditionally a solid-phase adsorption using silica, florisil, or alumina to remove matrix components, particularly lipids. Gel permeation chromatography (GPC) has also been widely reported for food sample clean-up, but this approach is labor-intensive and requires large solvent volumes (50–400 mL per sample), which limits its practicality (Zelinkova and Wenzl 2015; Bansal et al. 2017).

More recently, QuEChERS-based methods have been adapted for PAH analysis, offering significant reductions in extraction time, solvent consumption, and overall costs. For example, Urban and Lesueur (2017) successfully applied QuEChERS to determine PAHs in curry powder.

However, extracts can remain highly matrix-dependent: acetonitrile co-extracts large amounts of matrix constituents, and the subsequent dispersive solid-phase extraction (d-SPE) step is not always sufficient to remove co-extractives, particularly in fatty samples such as meat or fish. Furthermore, ACN is not a suitable solvent for GC injection and it tends to highly expand in the GC liner. To overcome these limitations, new sorbents have been tested, including enhanced matrix removal-lipid (EMR-Lipid), which efficiently removes fats while ensuring good PAH recoveries (Zhao 2023; Zhao 2023), and zirconia-based sorbents (Z-Sep), which provide deeper clean-up for lipids and pigments (Supelco 2019). The implementation of these new materials has enabled the development of methods combining reduced sample preparation time and solvent consumption with LOQs as low as 0.05–1 µg kg^{-1}, fully compliant with EU regulations (Urban and Lesueur 2017; Sampaio et al. 2021).

Molecularly imprinted polymer (MIP) SPE cartridges have also recently been introduced for PAHs. These cartridges exhibit selective recognition for specific analytes or structurally related groups. For PAHs, dedicated MIP cartridges have been designed to efficiently purify them from oils and fats, ensuring good recoveries with minimal solvent use and short processing times (Supelco 2014; Affinisep). This recent development in purification products will be valuable to develop new modular method for complex matrices or food category characterized by a wide variety such as the spices and herbs category that have been selected for this work thesis. it will allow to modernise method by saving solvent consumption and time. a little bit like the QuEChERS extraction for the pesticides. Such recent advances in purification technologies offer promising opportunities for the development of modular and flexible analytical methods suitable for complex food matrices or food categories characterized by high diversity, such as spices and herbs that has been selected for this thesis. In this context, it may contribute to the modernization of PAH analytical methods by significantly reducing solvent consumption and sample preparation time, in a manner comparable to the impact of QuEChERS extraction on pesticide analysis.

Separation and detection

The most widely applied techniques for separation and detection of PAHs in food are high-performance liquid chromatography with fluorescence and UV detection (HPLC-FLD/UV) and gas chromatography coupled to tandem mass spectrometry (GC-MS/MS). Thanks to the aromatic structures of PAHs, HPLC-FLD achieves high sensitivity, with LOQs reported in the range of 0.05–0.29 ng g^{-1} (Bansal et al. 2017). This technique also provides good selectivity and separation of PAH isomers. Nevertheless, fluorescence detection remains susceptible to matrix interferences in complex food matrices such as spices and herbs, and it is not structurally specific, unlike mass spectrometry-based techniques (EURL-PC report 2015). Moreover, FLD/UV detectors do not allow the use of isotopically labelled internal standards, such as deuterated PAHs, since they cannot discriminate between native and labelled compounds.

GC-MS/MS methods typically show slightly higher LOQs than HPLC-FLD, due to the poor ionization efficiency of PAHs with electron impact (EI) sources (Bansal et al. 2017). Nevertheless, GC-MS/MS achieves LOQs that meet the criteria set by Regulation (EU) No 836/2011 and provides shorter run times (15–35 min per sample; Agilent application note). Importantly, the MS/MS system allows the use of isotopically labelled internal standards for each of the PAH4, thereby improving accuracy by correcting for matrix effects and analytical variability (Sampaio et al. 2021). A limitation of GC-MS/MS lies in the insufficient resolution of certain isomers (e.g., Bbf, benzo[k]fluoranthene, and benzo[j]fluoranthene) when using traditional columns such as DB-5MS, VF-5MS, or HP-5MS. Recently, manufacturers have addressed this issue by developing dedicated GC columns, such as Agilent Select PAH (15 and 30 m) and Restek Rxi-PAH (20 and 30 m), which provide improved separation of these critical isomers (Oostdijk 2010; Restek 2025).

Chapter 2

Objectives and structure of the thesis

1 Objectives

The overall objective of this thesis is to improve the Belgian risk assessment related to food contaminants. To this end, sensitive and validated analytical methods were developed for three specific contaminant–matrix pairs, namely pesticides in tea leaves, PAHs in spices and dried herbs and acrylamide in selected food products. The data generated were subsequently used to assess dietary exposure and associated health risks for the Belgian population.

To achieve this main objective, several research questions have been addressed:

Pesticides in tea leaves:

- What are the levels of pesticides in tea and herbal teas available on the Belgian market?

Numerous publications exist on tea in the literature; however, these studies are often from China or India and concentrate on traditional teas like black and green tea or examine a limited number of pesticides. In contrast, in retail shops in countries such as Belgium, a wide range of teas, including green, black, blue, herbal, and flavoured teas, as well as teas mixed with fruits, are available. In past years, FAFSC detected more than 50 different pesticides in tea samples. Addressing this diversity, an extensive sampling encompassing this variety of teas has been made. Then, 2 multi-residue methods were developed considering the most detected pesticides, to determine their occurrence in tea samples collected.

- To what extent are pesticides transferred from the tea leaves to the infusion water that is consumed? Which parameters influence this transfer? Is it possible to develop a predictive tool to estimate the amount of pesticides that would be transferred based only on certain physico-chemical parameters?

Tea leaves are rarely consumed but are mostly infused before drinking the brew. It is essential to consider this process to determine the pesticide uptake through tea consumption. Hence, transfer factors (TFs) have been determined for the most detected pesticides, but also with variating infusion parameters to evaluate their influence on pesticide transfer. Furthermore, thousands of pesticides are used worldwide, and new ones are created yearly. Even if all of them are not used for tea agriculture, the potential detection of previously unstudied pesticides in tea exists, and research for TF determination necessitates substantial time and financial resources. In response to this challenge, 4 predictive models for the pesticide transfer from tea leaves to brew have been created based on the physico-chemical properties of pesticides.

- What is the actual exposure of Belgian consumers to pesticide residues through the consumption of tea and herbal teas? What are the risks?

When evaluating the risk for consumers through pesticide uptake, it is essential to consider both acute and chronic exposure. To achieve this, occurrence data from our monitoring have been crossed with TFs data to determine the pesticide uptake. Then, these values were compared to toxicological thresholds to evaluate if a risk was present for adults and children.

Acrylamide in certain food products:

- What is the occurrence of acrylamide in certain foods present on the Belgian market?

Acrylamide has been widely studied in certain food commodities undergoing a heating

process and known to produce substantial acrylamide (i.e. fries, chips, bread and coffee). However, various other foods, such as pastries, cocoa powder, vegetable chips, black olives, and potato-based dough products, among others, can also potentially contain acrylamide. To address this issue, a representative sampling of the foods commonly consumed by the Belgian population has been created. Then, sensitive analytical methods have been developed to analyse this wide variety of food products and complete the missing data.

- What is the exposure of the Belgian population in the light of the latest data available? What is the risk to the health of the Belgian population?

Previously risk assessment of acrylamide in food made by EFSA in 2015 and identify a health concern for the population regarding carcinogenic and mutagenic effects of acrylamide. However, this assessment did not incorporate occurrence data from limited food products. To address this gap, the Food Consumption Survey 2014 (FCS 2014) has been crossed with our occurrence data and recent data from the Federal Agency for the Safety of the Food Chain (FASFC). This new exposure has been used to determine an updated margin of exposure (MOE) and re-evaluate the risk for the different Belgian population groups.

- Does the Belgian way of cooking pose an additional health risk?

The production of acrylamide is not exclusive to the food industry; consumers cooking at home can also generate this contaminant through oven cooking, frying, and toasting. To assess this, participants were recruited, provided with various food items, and instructed to prepare them using their usual cooking methods. Subsequently, participants completed a survey to provide insights into their cooking practices and determine if their behaviour poses a risk.

Polycyclic aromatic hydrocarbons in spices and herbs (PAHs):

- What is the occurrence of the four regulated PAHs (PAH4) in spices and herbs present on the Belgian market? What are their concentrations?

High amount of PAH4 have been reported in spices and herbs. However, the number of studies dealing with PAHs in this broad food category remained limited. One of the contributing factors to this limitation could be the analytical challenge posed by this food/contaminants combination. Consequently, a modular method for PAH4 determination in GC-MS/MS capable of dealing with the whole variety of spices and herbs has been developed.

- To what extent does the presence of PAH4 in spices and herbs increase consumers' exposure through their diet? What are the risks?

If a high amount of PAH4 can be found in spices and herbs, it is essential to evaluate how it increases the overall Belgian consumer exposure and if it poses a risk to his safety. To address this issue, occurrence data of PAH4 in spices and herbs have been crossed with food consumption data to determine the exposure related to this food category. Then, these data have been summed to the previous overall exposure made by EFSA before re-calculating the margin of exposure.

2 Structure of the thesis

This thesis will be structured around these 3 specific issues and the questions it will attempt to answer: pesticides in tea and infusion waters, acrylamide in certain food categories and PAHs in spices and dried herbs.

This thesis is structured into seven chapters. **Chapter 1** provides the general introduction and scientific background. It reviews the current state of knowledge related to the different topics covered in this thesis, identifies existing knowledge gaps, and defines the scope and direction of the research. **Chapter 2** presents the objectives of the doctoral work. **Chapter 3** focuses on the issue of pesticide contamination in tea leaves and herbal teas. It investigates the occurrence of pesticides in products available on the Belgian market, the determination of transfer factors from raw materials to infusion water, and the development of new tools enabling the assessment of dietary exposure and associated risks for the Belgian population. **Chapter 4** is dedicated to the reassessment of the dietary risk associated with acrylamide for the Belgian population. It details the sampling strategy, analytical procedures, and the development of consumption scenarios, which together allowed an updated risk assessment based on newly generated occurrence data. **Chapter 5** addresses the contamination of spices and dried herbs by polycyclic aromatic hydrocarbons and the corresponding risk assessment for Belgian consumers. This chapter describes the development of a modular analytical approach, its application to a representative sampling of spices and herbs from the Belgian market, and the resulting re-evaluation of consumer exposure and health risks. **Chapter 6** provides a general discussion of the exposure of the Belgian population to the various food contaminants investigated in this thesis. It evaluates the extent to which the research objectives have been met. Finally, **Chapter 7** concludes the thesis by summarising the studies conducted, highlighting the main findings and limitations, and discussing perspectives for future research.

Chapter 3

Pesticides in tea, determination of their transfer factors and risk assessment for the consumer

Outline

Pesticide residues in tea have long raised concern due to frequent exceedances of maximum residue limits. Since pesticides are widely used in tea cultivation, consumers may be exposed through tea consumption. To estimate this exposure and related health risks, the transfer of pesticides from tea leaves to infusion must be determined. However, few data exist, particularly for teas prepared according to Western brewing habits.

This study first established an inventory of pesticides in 55 tea and herbal tea samples from the Belgian market. Transfer factors were determined to estimate actual dietary intake, and the resulting data were used to assess potential health risks. The work also involved collaboration with other Sciensano units for the analysis of trace elements and pharmaceuticals. A second study further investigated more deeply the pesticide transfer, identifying key parameters and developing a predictive model for different tea types. All generated data were compiled to support refined risk assessment of pesticides in tea.

This chapter is an adapted version of the two following scientific articles:

- **Ph. Szternfeld**, D. Montalvo, J. Broos, K. Cheyns, L. Joly & C. Vanhee, 2023. Chemical contaminants present in tea samples available in Belgian retail shops, occurrence of pesticides, trace elements and pharmaceuticals and the risk associated upon acute and chronic exposure. Food Additives & Contaminants: Part B, 16(1):58-68 DOI: 10.1080/19393210.2022.2145617.
- **Ph. Szternfeld**, C. Demoury, W. Brian, J-Y. Michelet, V. Van Leeuw, E. Van Hoeck & L. Joly, 2023. Modelling the pesticide transfer during tea and herbal tea infusions by the identification of critical infusion parameters. Food Chemistry, 15:429:136893, DOI: 10.1016/j.foodchem.2023.136893.

Contribution

Article 1 (occurrence and risk assessment): Philippe Szternfeld conceived and supervised the project. Philippe Szternfeld and Julie Broos developed and validated the analytical methods and performed the pesticide analyses. Daniela Montalvo and Céline Vanhee performed the analyses of trace elements and pharmaceuticals, respectively. Philippe Szternfeld conducted the exposure and risk assesment on pesticides, Daniela Montalvo on trace elements, and Céline Vanhee on pharmaceuticals. Philippe Szternfeld, Daniela Montalvo, and Céline Vanhee drafted the manuscript. Philippe Szternfeld, Daniela Montalvo, Céline Vanhee, and Laure Joly contributed to manuscript revision and data visualization. All authors approved the final version.

Article 2 (transfer and modelling): Philippe Szternfeld, Laure Joly, and Els Van Hoeck acquired funding. Philippe Szternfeld supervised the project. Philippe Szternfeld, Jean-Yves Michelet, Wendy Brian, and Virginie Van Leeuw developed and validated the analytical methods and performed the pesticide analyses. Philippe Szternfeld and Claire Demoury performed the statistical analyses. Claire Demoury developed the pesticide transfer model. Philippe Szternfeld and Claire Demoury drafted the manuscript. Philippe Szternfeld, Claire Demoury, Laure Joly, and Els Van Hoeck contributed to manuscript revision and data visualization. All authors approved the final version.

I. Chemical contaminants present in tea samples available in Belgian retail shops, occurrence of pesticides, trace elements and pharmaceuticals and the risk associated upon acute and chronic exposure

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Abstract

The last decade, the consumption of tea and herbal tea has gained more and more popularity across the globe, but the potential presence of chemical contaminants (e.g. pesticides, trace elements, synthetic drugs) may raise health concerns. This study analysed selected teas available in Belgian retail stores and performed a risk assessment for these samples. No chemical adulteration could be detected in dry tea material. More than 38 % of the dry leaves samples contained at least one pesticide exceeding the maximal residue level (MRL) set by the EU. However, further risk assessment, based on the values of pesticide residues and the toxic trace elements encountered in the brew, demonstrate that the consumption of these teas will not give rise to health concerns. Nonetheless, attention should be given to the leaching potential of nickel from teas and the presence of arsenic in brews from algae containing teas.

Keywords: Tea and herbal teas - Pesticide residue analysis - Trace elements analysis - Risk assessment studies

1 INTRODUCTION

The last decade the consumption of tea and herbal tea has gained more and more popularity across the globe. Moreover, market research reports estimate that the consumption of tea and herbal teas have increased almost two fold during the last decade, in a mainly coffee-drinking culture nation such as Belgium (Tea - Belgium | Statista Market Forecast 2021). This increasing trend is mainly attributed to younger people that are consuming less coffee in favour of more fashionable tea-based drinks. Traditional tea, produced from the leaves of Camellia sinensis, can be categorized based on the degree of fermentation of the leaves during its processing. Green teas are non-fermented, oolong teas are semi-fermented and black teas are fully fermented (Karak and Bhagat 2010; Schwalfenberg et al. 2013). Additionally, there are herbal teas containing fresh or dried flowers, fruit, leaves, seeds or roots from various herbs or mixtures of herbs which may or may not include C. sinensis leaves

The global increase in the consumption of tea and herbal teas can be attributed to their pleasant flavor and their potential health benefits. Tea contains polyphenols, tannic acid and antioxidants which have been associated with a reduced risk of cancer, diabetes, cardiovascular and neurological diseases and antiaging effects (Khan and Mukhtar 2019). Although the number of studies that explored the clinical efficacy of its use and safety is small, no reporting occurred of any detrimental effects from the consumption of medicinal grade plant species as herbal teas (Poswal et al. 2019). Nevertheless, safety aspects need to be evaluated since studies have reported the presence of various contaminants including pesticides, toxic trace elements (i.e. lead, arsenic and cadmium) and chemical adulteration by spiking product with pharmaceutical drugs (Abd El-Aty et al. 2014).

A plethora of different synthetic chemical pesticides are currently being used to combat the great variety of plant pathogens, which could otherwise affect the quality and the quantity of the desired herbal product. The most widely spread pesticides include organophosphates, carbamates, synthetic pyrethroids, neonicotinoids and benzimidazoles. One of the major disadvantages of the use of pesticides resides in the fact that pesticide residues may remain on the plant at amounts higher than the maximum residue level (MRL). Such pesticide residues can then be transferred to the infusion, posing health hazards. The amount of pesticide that can transfer from the herbal material into the infusion depends on the physico-chemical properties of the pesticide in question and the brewing time. Indeed, the transfer rate of organophosphorus pesticides were reported to range from 0.7 % (chlorpyrifos) to 90.1 % (methamidophos) (Cao et al. 2018). Moreover, the transfer rate of chlorpyrifos also differed amongst different studies ranging from a transfer rate of 0.7 % to 3.14 %, 9.12 % or 11.7 % (Jaggi et al. 2001; Manikandan et al. 2009; Chen et al. 2015; Heshmati et al. 2021). These discrepancies can be explained by differences in the type of brewing procedure and the type of tea. Nevertheless, general tendencies can be seen, for example pyrethroid pesticides exhibited a low transfer tendency, while pesticides belonging to the neonicotinoid group generally have a high transfer potential (Wang et al. 2019). The above-mentioned studies mainly focused on traditional green tea or black tea, as these are the most consumed type of tea in their respective countries. In Belgium, herbal teas are becoming popular and it is therefore also essential to determine the amount and type of pesticides that are present in the dried material and the amount of pesticide that transfers in the infusion. Furthermore, as the herbal material for herbal teas generally do not require further processing like fermentation,

these teas might potentially contain higher levels of pesticide residues (Xiao et al. 2017).

In addition to the occurrence of polyphenols, tannic acid and antioxidants, the regular consumption of tea can be considered a source of dietary essential elements (i.e. calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P) and zinc (Zn) (Schwalfenberg et al. 2013). However, several studies have also shown the presence of potentially toxic trace metals like lead (Pb), cadmium (Cd) and metalloids such as arsenic (As) in herbal teas and their infusions (Han et al. 2006; Shekoohiyan et al. 2012; Schwalfenberg et al. 2013; Zhang et al. 2018; Milani et al. 2018). In a recent study, de Oliveira et al. (2018) measured As, Cd, Cr and Pb in 47 traditional teas and herbal teas commercialized in the U.S. The authors found that the mean concentrations of As (0.26 mg kg^{-1}), Cd (0.19 mg kg^{-1}) and Pb (2.32 mg kg^{-1}) in tea leaves were higher in herbal teas compared to traditional teas but in all cases the levels were below the WHO limits for medicinal plants, except for one sample. When the concentrations of As, Cd, Cr and Pb were measured in the infusions, all were below the WHO guidance values for drinking water (de Oliveira et al. 2018). The low concentrations of certain trace elements (i.e. As, Cd, Cr, Pb) can be related to their poor extractability as they are bound to the organic matrix and thus remain in the leaves (Szymczycha-Madeja et al. 2012). Another study conducted in Spain by (Martín-Domingo et al. 2017) showed that nearly 5 % of the analysed herbal teas (n=220) contained Cd levels that surpassed the recommended WHO limit for medicinal plants. High Cd levels were mostly detected in thyme and chamomile teas. Though the concentrations of trace metals in the infusions were not shown, the authors concluded that based on the low percentage solubilization of Cd in the herbal infusion ($\sim$11 %) the intake of Cd from such infusions do not pose a health risk for consumers. The large compilation of studies by Karak and Bhagat (2010) also reported that trace element concentrations in tea infusions are generally low and within safe limits in most of the cases. Nevertheless, in order to assure safe products for human consumption, it is important to regularly monitor the amount of metals and metalloids in tea and herbal teas.

Although many of these teas and herbal teas contain a large amount of beneficial substances, several reports have documented the chemical adulteration of these herbal products with either synthetic pharmaceutical drugs approved by the U.S. Food and Drug Administration (U.S. FDA and/or the European Medicines Agency (EMA) or non-approved drugs (Al Lawati et al. 2017; Hackl 2019; Jairoun et al. 2021). The majority of these adulterated herbal teas encountered in Belgian market originate from samples purchased online, however, the incidence of such products in retail shops can never be 100 % excluded (Hackl 2019).

The overall aim of this study was to generate a global cumulative overview of the risks associated with the consumption of teas and herbal teas commercialized in Belgium to better asses if they pose a risk to human health. Thus, the objectives of the study were to (1) determine the content of pesticides, toxic trace elements and synthetic pharmacological residues in various tea and herbal tea samples available on the Belgian market, (2) to determine the transfer rate of these contaminants in the infusions and (3) to estimate the short-term and long-term risk for the consumer health.

2 MATERIALS AND METHODS

2.1. Sampling

In order to be the most representative of the Belgian consumption habits, 55 tea samples of the most widespread brands were bought in local stores. Specific shop such as organic shops and dedicated tea shops were also included. Different tea types were selected i.e. black, green, white, flavoured, oolong, as well as commonly used herbal teas, i.e. chamomile, mint, rooibos, senna in order to reflect the variety of products available in Belgium. To ensure the representativeness of samples taken at retail stores level, one package or unit were taken. For samples in bulk, a minimum of 100 g of tea leaves were purchased.

2.2. Tea and herbal tea preparation and infusion

After arrival in laboratory, half of each tea and herbal tea leaves samples were ground to a fine powder and homogenized by a mixer (IKA M20, IKA®-Werke GmbH & Co. KG, Staufen, Germany) prior to contaminant analysis in dry material. The remaining part were kept to generate the brew. Tea infusions were prepared following ISO 3103 (2019): 2 g of dry tea leaves were placed in blank tea bags and 100 mL of boiled distilled water was added. The tea bags were left to stand for 6 min and thereafter removed without stirring. Solvents, reagents, standard solutions, supplementary information, quality assurance procedures, pesticide chromatograms at the LOQ, and detailed descriptions of the extraction procedures used for contaminants in both dry materials and infusions are provided in Appendix A1.

2.3. Risk assessment

2.3.1. Pesticides

To evaluate the acute risk for the consumer health an acute hazard risk index (aHI) was calculated. The aHI expressed as a percentage is calculated as the ratio between the IESTI (International Estimated Short Term Intake) values (expressed in mg kg^{-1} body $weight^{-1}$ day^{-1} and the acute reference dose (ARfD), for both adults and children, according to following equations:

$$aHI = \left(\frac{IESTI}{ARfD}\right) \times 100 \tag{3.1}$$

$$IESTI = \frac{LP \times HR \times TR}{bw} \tag{3.2}$$

where LP (Large portion) is the 97.5th percentile of the tea leaves portion size taken by the consumer (0.008 and 0.021 kg day^{-1} for children and adult respectively). HR is the highest residue level found in tea leaves for a specific pesticide (mg kg^{-1}), TR is the transfer rate of pesticides from tea leaves into tea infusion during brewing and bw is the mean body weight for the targeted population (children or adult) in kg. According to FAO (FAO JMPR experts 2015), a risk exists when the aHI is greater than 100 %. All these values have been calculated with the use of the pesticide residue intake model (Primo) made available by the European Food

Safety Authority (EFSA 2019). No ADI and ARfD values were available for anthraquinone and biphenyl. However, based on toxicological studies, the European chemical agency (ECHA) has put the no adverse effect level (NOAEL) for oral administration of anthraquinone at 1.36 mg kg^{-1} body weight per day and of biphenyl at 38 mg kg^{-1} body weight per day (Anthraquinone - Registration Dossier - ECHA 2022). A common way to approximate the ADI is to use a 100 fold factor on the NAOEL (Chemicals Regulation Directorate 2013). Hence an ADI of 0.0136 and 0.38 mg kg^{-1} bw per day have been used. To evaluate the chronic risk for the consumer health, the estimated daily intake (EDI) is calculated according to the following equation

$$\mathrm{EDI} = \frac{\mathrm{IR} \times \mathrm{MC} \times \mathrm{TR}}{\mathrm{bw}} \tag{3.3}$$

Where IR is the mean tea ingestion rate (kg day^{-1}). As no clear average consumption values were available for both children and adults in the primo model, we decided to utilize a daily intake value of 0.010 kg day^{-1} as this represents the median value of the different ingestion rates reported in the literature. Indeed, a review of the literature demonstrates that amount of tea consumed can vary widely among different population groups and habits. Ingestion rates of tea of 6 g day^{-1} (Polechońska et al. 2015; Martín-Domingo et al. 2017), 10 g day^{-1} (Yuan et al. 2007; Sofuoglu and Kavcar 2008; Na Nagara et al. 2021) and 13 g day^{-1} (Zhang et al. 2018) have been reported in previous work. Moreover, as no reliable information on the chronic intake for children was available, hence, the chronic risk was done for adults only. MC is the mean concentration of residue level found in tea leaves for a specific pesticide (mg kg^{-1}), TR is the transfer rate and bw is the mean adult body weight of 70 kg. Then, the health hazard quotient (HQ) expressed as the ratio between the EDI and the ADI has been calculated according to Eq. (4).

$$\mathrm{HQ} = \left(\frac{\mathrm{EDI}}{\mathrm{ADI}}\right) \times 100 \tag{3.4}$$

Also here a chronic risk exists when the percentage of HQ is greater than 100 %.

2.3.2. Trace elements

For trace elements the potential chronic health risk of exposure from drinking tea infusion was assessed from the EDI calculated for each target element using eq. (3.3). MC is the mean concentration of trace elements in tea leaves (mg kg^{-1}). TR is the transfer rate of trace elements from the dry tea leaves to the tea infusion which was calculated from the selected samples that were brewed (n=17)

For risk evaluation, the calculated EDI for each trace element was compared against the appropriate health-based guidance values (i.e. tolerable daily intake, tolerable weekly intake, tolerable upper intake level) or the benchmark dose lower confidence limit (BMDL). For As, the benchmark dose lower confidence limit ($BMDL_{10}$) of 0.3 µg kg^{-1} bw day^{-1} was used as recommended by EFSA (Arcella et al. 2021). For Cd, the tolerable weekly intake (TWI) of 2.5 µg kg^{-1} bw was used (EFSA 2012a). For Ni the tolerable daily intake (TDI) of 13 µg kg^{-1} bw was used (EFSA CONTAM Panel 2010). The guidance value used for Pb was the $BMDL_{10}$ of 0.63 µg kg^{-1} bw day^{-1} for nephrotoxic effects on adults (EFSA CONTAM Panel 2010). For Cu and Zn which are also essential elements, the tolerable upper intake level determined by EFSA was considered, 25 mg day^{-1} for Zn and 5 mg day^{-1} for Cu (EFSA 2006).

Risk values were calculated using two different approaches: For elements where the health-based guidance value is indicated as TDI, TWI or tolerable upper level, i.e. Cd, Cu, Ni and Zn, the EDI was divided by their respective guidance value and thus presented as a percentage of it. For As and Pb which are both genotoxic and carcinogenic contaminants and for which no threshold values are established, the margin of exposure (MOE) approach was used following recommendation by EFSA 2005 (EFSA 2005). In this case the BMDL was divided by their respective EDI. The MOE approach evaluated the potential risks associated with the presence of contaminants in the tea infusion. Acute toxicity was determined only for As. A minimal risk level of 5 µg kg bw^{-1} day^{-1} for acute-duration oral exposure to inorganic As derived by the Agency for Toxic Substances and Disease Registry (ATSDR) was used (U.S. Department of health and human services 2007). In this case the risk value was calculated as the ratio between EDI and the acute minimal risk level and presented as a percentage.

3 RESULTS AND DISCUSSION

3.1. Amount and type of pesticide residues in dry material

A total of 55 different tea and herbal tea samples were analyzed for the presence of a total of 250 different pesticides. The number and type of pesticide residues varied amongst the samples studied. A total of 7.3 % of the samples did not contain any detectable amount of the analyzed pesticide residues, while 65.5 % contained 1 to 5 residues, 14.5 % contained 6 – 10 residues and 12.7 % contained more than 10 residues with a maximum of 14 residues. From the 55 analyzed samples, 21 samples, corresponding to 38.2 % of the analyzed dry material, contained at least one pesticide in quantities that exceeded its corresponding maximum residue level (European Commission 2005) and are consequently not compliant to the EU legislation (expended measurement uncertainty of 50 % taken into account) (table 3.1). The detailed occurrence data of the pesticide residues present in the dry material of each sample can be found in the **??**.

These finding are concurrent with recent findings by Heshmati et al. (2021), where they found that approximately 40 % of the black tea samples, analyzed for the presence of 33 pesticides, exceeded the corresponding MRLs and were therefore not compliant. The most abundant pesticide residues that exceeded the MRL belonged to either the pyrethoid and PAH family as can be seen in table A6. The insecticide cyhalothrin-L was responsible for 9 of 21 non-compliant samples while the polycyclic aromatic hydrocarbon anthraquinone was responsible for 6 out of these 21 samples. These families were also present in the list of families of the most frequently encountered pesticides (n > 5 detected in the sample set) as demonstrated in table 3.2. This list is very similar to the findings of (Chen et al. 2015) and Fan et al. (2022) who analyzed the tea leaves produced in China. This similarity make sense since most of tea sold in Belgium originate from this area. In addition to the PAHs (e.g. anthraquinone and biphenyl) and the pyrethroîds (e.g. bifenthrin, cyhalothrin-L, cypermethrin), also the neonicotinoïds (e.g. acetamiprid, imidacloprid, thiamethoxam, thiachloprid) were abundantly present. These later two classes of insecticides are known to be commonly used for pest control on tea plantations as a result of their broad-spectrum activity (Chen et al. 2014; Abd El-Aty et al. 2014; Xiao et al. 2017; Ikenaka et al. 2018; Zhang et al.

2020; Thompson et al. 2020; Nimako et al. 2021).

Additionally, also the PAH anthraquinone has been detected 16 times, making it the second most detected pesticide, although its use has been banned by the EU. These findings are consistent with the findings of (Díaz-Galiano et al. 2021) where they found that 48 % of their tea samples, from different European countries, contain detectable amounts of this compound. However, prior studies have shown that origin of a contamination with this residue is not necessarily due to illegal use of phytosanitary products, but could also be due to an environmental pollution from natural and man-made processes of combustion (e.g., mineral oils, wood, and charcoal, and their products of combustion) or could be the result of a contamination occurring through drying or roasting processes (EFSA 2012b; Wang et al. 2018; Anggraini et al. 2020). A similar argument could also be made for biphenyl (EFSA 2012b).

Table 3.1: Overview of the occurrence of pesticides present in quantities exceeding the MRL

Sample	Tea description		Pesticides	
	Tea type	Organic	exceeding MRL/EU banned substance detected	Total # detected
T-1-02	Green	N	none	7
T-1-09	Green	N	Anthraquinone* Cyhalothrin-L Zoxamide	11
T-1-11	Green	N	none	2
T-1-12	Green	N	Carbendazim* Chlorothalonil	9
T-1-22	Green	N	Anthraquinone* Carbendazim* Cyhalothrin-L	12
T-1-24	Green	N	none	5
T-1-25	Green	N	Anthraquinone* Cyhalothrin-L	11
T-1-26	Green	N	Anthraquinone* Cyhalothrin-L	3
T-1-27	Green	N	Anthraquinone* OPP	10
T-1-28	Green	N	Pyraclofos*	3
T-1-29	Green	N	Cyhalothrin-L Mepronil* Propargite*	8
T-1-30	Green	N	Cyhalothrin-L	7

Table 3.1 (continued)

Sample	Tea description		Pesticides	
	Tea type	Organic	exceeding MRL/EU banned substance detected	Total # detected
T-1-33	Green	N	Carbendazim*	4
T-1-35	Green	N	none	2
T-1-38	Green	N	Boscalid Carbendazim* Cyhalothrin-L	13
T-1-39	Green	N	Anthraquinone* Carbendazim* Cyhalothrin-L Tecnazene*	13
T-2-99	Green	N	none	2
T-1-19	Green	Y	Anthraquinone* Chlorothalonil	3
T-1-20	Green	Y	Anthraquinone* Tebufenpyrad	4
T-1-34	Green	Y	none	1
T-2-27	Green	Y	Carbendazim* Disulfoton*	6
T-1-03	White	N	none	1
T-1-13	White	N	Cyhalothrin-L Disulfoton* Tolfenpyrad	14
T-1-32	White	N	Diphenylamine*	2
T-1-01	Black	N	Quinalphos*	2
T-1-05	Black	N	Anthraquinone* Biphenyl*	2
T-1-06	Black	N	Mepronil*	5
T-1-07	Black	N	none	1
T-1-10	Black	N	Mepronil*	2
T-1-14	Black	N	Anthraquinone* Biphenyl*	4
T-1-23	Black	N	Carbendazim* Propetamphos*	9

Table 3.1 (continued)

Sample	Tea description		Pesticides	
	Tea type	Organic	exceeding MRL/EU banned substance detected	Total # detected
T-2-160	Black	N	Carbendazim* Mepronil* Propargite*	5
T-2-162	Black	N	none	1
T-1-04	Oolong	N	Anthraquinone*	1
T-1-15	Oolong	N	none	1
T-1-31	Oolong	N	Anthraquinone* Dichlorobenzophenone-4,4 Tolfenpyrad	6
T-1-16	Smoked	N	Anthraquinone* Biphenyl* Carbendazim*	12
T-1-17	Smoked	N	Anthraquinone* Biphenyl*	3
T-1-18	Herbal	N	none	1
T-1-36	Herbal	N	none	1
T-1-37	Herbal	N	Propoxur*	4
T-2-28	Herbal	N	Biphenyl* Carbendazim*	3
T-2-93	Herbal	N	Anthraquinone* Mepronil*	3
T-2-98	Herbal	N	Biphenyl*	4
T-2-164	Herbal	N	Biphenyl*	3
T-1-21	Herbal	Y	Tebufenpyrad	1
T-2-25	Herbal	Y	none	1
T-2-26	Herbal	Y	none	n.d.
T-2-30	Herbal	Y	none	n.d.
T-2-58	Herbal	Y	Biphenyl* Mepronil*	6

Table 3.1 (continued)

Sample	Tea description		Pesticides	
	Tea type	Organic	exceeding MRL/EU banned substance detected	Total # detected
T-2-59	Herbal	Y	Biphenyl* Imidacloprid	3
T-2-61	Herbal	Y	Diphenylamine*	n.d.
T-2-64	Herbal	Y	none	1
T-2-167	Herbal	Y	none	n.d.
T-2-168	Herbal	Y	Chlorothalonil	4

* EU banned substances

3.2. Amount of pesticide residues present in the tea brew

A typical first step, common to all contaminants, is to determine the quantity of the contaminants that are transferred into the brew and thus ingest by the consumer, also known as the transfer rate (TR). For this purpose, tea contaminated with sufficiently high pesticides content have been infused to determine their TR. The obtained values are listed in table 3.2 and varied from 1 % (e.g. bifenthrin and cypermethrin) to 78 % (imidacloprid). The different TRs are related to the amount present in the dry material, their solubility rate in water and their partition coefficient (log P) (Abd El-Aty et al. 2014). While the high value of thiamethoxam (62 %), imidacloprid (78 %) and acetamiprid (54 %) are in line with the transfer rates obtained by Hou et al. (Hou et al. 2013), the low TR values (≤10 %) for bifenthrin, buprofezin, chlorothalonil and cypermethrin are very likely due to their high partition coefficients (log P > 4). For this reason and due to the lack of relevant pesticides content in dry tea, no reliable TR could have been determined experimentally for biphenyl, chlorfenapyr, chlorpyriphos-ethyl and cyhalothrin-L. As their log P are > 4, a maximal theoretical TR of 10 % has been used for these compounds in order to perform a risk assessment. Mepronil has not been found in any tea infusion. As its log (P) is < 4 (3.66). The theoretical TR of 10 % has not been used and no risk assessment has been performed for this compound. However, considering it can be assumed that the concentration of mepronil does not represent a risk for consumer's health.

Table 3.2: Occurrence, concentration range, average concentration and number of MRL exceedance in dry herbal material and the concentration range and average concentration of the different pesticides present in the infusion. The average percentage transfer rate of the different pesticide residues after 6 min brewing time are listed in the TR (%) column. The obtained values take into account a measurement uncertainty of 50 %, for the most frequently detected pesticides (n > 5 out of 55 samples)

Pesticide	Pesticide group	Dry herbal material (n=55)			MRL (μg kg^{-1})	# MRL exceeded	TR (%)	Tea infusion	
		Occurrence	Min – Max (μg kg^{-1})	Average content (μg kg^{-1})				Min – Max (μg L^{-1})	Average content (μg L^{-1})
Bifenthrin	Pyrethroid	19	5.6 – 600	137	30 000	0	1	N.D. – 1.3	0.7
Anthraquinone	PAH	16	6.2 – 241	48	20	6	6	3.0 – 4.7	3.9
Cyhalothrin-L	Pyrethroid	15	10 – 445	68	10	9	10*	-	-
Ortho-phenylphenol	OPP	13	9.0 – 663	68	100	1	12	32	32
Chlorothalonil	OCP	12	11 – 222	55	50	3	8	1.3 – 1.6	1.5
Imidacloprid	Neonicotinoïd	12	7.3 – 235	40	50	2	78	2.2 – 6.8	4.6
Acetamiprid	Neonicotinoïd	12	6.2 – 59	18	50	0	54	0.63 – 7.5	3.1
Carbendazim	benzimidazol carbamate	11	8.6 – 22	14	100	0	28	0.20 – 1.5	1
Thiamethoxam	Neonicotinoïd	11	8.2 – 161	43	20 000	0	62	1.1 – 16	7.4
Chlorfenapyr	halogenated pyrroles	10	11 – 826	117	50 000	0	10*	-	-
Biphenyl	PAH	9	12 – 218	53	50	2	10*	-	-

Table 3.2 (continued)

Pesticide	Pesticide group	Dry herbal material (n=55)			MRL (μg kg^{-1})	# MRL exceeded	TR (%)	Tea infusion	
		Occurrence	Min – Max (μg kg^{-1})	Average content (μg kg^{-1})				Min – Max (μg L^{-1})	Average content (μg L^{-1})
Buprofezin	thiadiazin	9	7.2 – 72	25	50	0	10	1.4	1.4
Chlorpyriphos -ethyl	OPP	8	6.5 – 49	20	2 000	0	10*	-	-
Thiacloprid	Neonicotinoïd	7	10 – 73	37	10 000	0	15	0.30	0.30
Mepronil		6	14 – 228	51	50	1	-	N.D.	N.D.
Cypermethrin	Pyrethroid	5	16 – 436	155	500	0	1	0.66 – 0.86	0.76

N.D. Not detected

* Theorical maximum transfer rate fixed at 10 %

** TF estimated by an in-house model.

3.3. Total element concentration in dry tea leaves

The measured trace element concentrations for the different samples are given in table A7, while table 3.3 summarizes the mean, minimum and maximum concentrations of the analyzed elements. In general, Mn and Fe were the most abundant elements in tea leaves and the measured concentrations compared well with values reported for tea samples elsewhere (Polechońska et al. 2015; Podwika et al. 2018; Milani et al. 2018). The highest Mn concentration was observed in a type of green tea, "greeting pine", whereas the lowest concentration was obtained in two types of herbal teas that do not contain C. sinensis. Tea is a plant that grows well in acidic soils (pH 5-5.6) (Mehra and Baker 2007), and in such conditions metals can be freely available for plant uptake. Therefore, the large concentrations of Mn and Fe in tea leaves can be explained by their high bioavailability and the capacity of the plant to accumulate such metals.

The concentration of Zn, Cu and Ni vary greatly between the different types of tea analyzed. Nevertheless, the mean total concentrations were comparable to those reported in previous work. For example, Milani et al. (2016) analyzed 30 tea samples that were purchased in Brazil and found Zn concentrations ranged between 21 and 44 mg kg^{-1}, Cu between 12 and 19 mg kg^{-1}, and Ni between 3.53 and 5.30 mg kg^{-1}.

The trace elements As, Cd, and Pb are listed in the WHO top 10 of chemicals that pose a public health concern. They are considered environmental pollutants and although they are not essential elements for plants they can be taken up by plant roots and accumulate in plant tissue. Although no global limits are set for herbal tea material, the matrix is quite similar to herbal drugs, and therefore the limits set by the WHO for, Cd and Pb are often utilized for risk assessment on dry herbal tea material or dry material for herbal infusion. These limits for, Cd and Pb correspond in mg kg^{-1} to 0.3 and 10, respectively (World Health Organization 2007). In this study only one tea sample exceeded the limits of Pb by almost 4-fold and one tea sample was just above the limits for Cd with 0.34 mg kg^{-1} table A7.

Table 3.3: Element concentrations in dry tea material and tea infusion, transfer rate and WHO limits

Element	Dry tea material (n=53)		Tea infusion (n=17)		Average transfer rate(%)	WHO drinking water limits (μg L^{-1})
	Average (mg kg^{-1})	Range (mg kg^{-1})	Average (μg L^{-1})	Range (μg L^{-1})		
As	0.31	0.003 – 6.42	5.81	0.052 – 49.6	29.6	10
Cd	0.07	0.01 – 0.34	0.14	0.049 – 0.33	9.8	3
Co	0.33	0.08 – 1.7	2.23	0.22 – 4.76	32.2	-
Cu	11.9	2.07 – 26.3	53.2	6.67 – 110	23.8	2000
Fe	345	40 – 3153	51.2	15.2 – 153	0.97	-
Mn	703	37 – 1852	2874	117 – 4929	-	-
Ni	4.54	0.57 – 13.8	57.4	5.97 – 181	-	-
Pb	1.49	0.05 – 38.9	1.41	0.23 – 6.20	-	-
Zn	26.3	8.89 – 47.7	129	24.3 – 369	-	-

3.4. Total element concentration in tea infusions

Trace element analysis was conducted on 17 infusions that were prepared following the standard procedure. It is well known that the concentration of elements in the infusion may depend on various factors, i.e. type of tea, initial total elemental content and speciation, infusion time, tea to water ratio, water temperature and the extraction efficiency of each element (Karak and Bhagat 2010; Welna et al. 2012). As it is shown in table 3.3 the transfer rate of each element varied widely. Iron, Pb and Cd had the lowest percent release in the infusions (<10 %), whereas Ni, As and Co had the highest release (between 30 to 57 %). Based on the extraction percentage, Welna et al. (2012) classified elements in three groups: highly extractable (>55 %, i.e. Co and Ni) moderately extractable (20 – 55 %, i.e. Cu, Mn and Zn) and poorly extractable (<20 %, i.e. Fe). Elements that are highly and moderately extractable can be of concern if the total concentration in the leaves is high because the expected levels in the infusions can be as well high.

The elemental concentration of toxic trace elements As, Cd, Ni and Pb are summarized in table 3.3. Arsenic was detected in all tea infusions but concentrations were in general low (<3 µg L^{-1}), except for two samples (with high total As levels in dry tea leaves) for which the measured concentrations were exceptionally high (38 µg L^{-1} and 50 µg L^{-1}). If the WHO guidelines for drinking water is considered to estimate potential risk, then those two samples were 3.8 and and 5 times higher than the 10 µg L^{-1} limit for drinking water (World Health Organisation 2017). The two tea samples high in As contain bladderwrack (Fucus vesiculosus), a type of seaweed used in herbal teas that claim slimming properties. Since, the toxicity of As depends on the As species exposed to, speciation analysis is necessary for a correct risk assessment. Studies that determined As concentration and speciation in seaweed

species found that inorganic As is generally a minor component and that arsenosugars are the dominant water-extractable species in seaweeds (Taylor and Jackson 2016). Therefore, a minor experiment was performed to analyze the species distribution of As extracted from the two algae-based infusions using high-performance liquid chromatography (HPLC) coupled with ICP-MS (data not shown). Results showed that inorganic As represented less than 1 % of the total As in the infusion (0.29 µg L^{-1}). The speciation results are relevant for risk assessment because inorganic As is the most toxic species to human and current regulatory guidelines are based on this species. The toxicity of the arsenosugars is not well documented yet, which complexes the risk interpretation.

Concentrations of other toxic elements Cd, Ni and Pb in the infusions were below the WHO drinking water guidelines (World Health Organisation 2017), except for Ni in five samples (table 3.3 and **??**). Cadmium and Pb are less of a concern since these elements practically do not extract in tea. However, Ni may be an element of interest since it is highly extractable and its contribution in the infusion may be large if the initial concentration in the leaves is high too.

3.5. Pharmacological residues in dry material

None of the samples bought in retail shops contained a detectable quantity of synthetic active pharmaceutical compounds, indicating that chemical adulteration is unlikely for those products brands present in popular retail shops. However, both samples T-1-36 and T-2-164 were positive for the presence of sennosides, known anthraquinone glycosides, but did not contain detectable amounts of anthraquinone (table 3.1). These sennosides are very likely originating from the senna plant Senna alexandrina, conform with the herbal ingredients listed on the label of the samples. The occurrence of this plant in herbal teas is by Belgian law not forbidden and does not automatically classify this tea as a medicinal herbal preparation. However, in 2018 the presence of sennosides in food supplements became under scrutiny by EFSA. The agency gave the advice to not use products containing senna for more than two consecutive weeks and the recommended daily intake should not exceed 18 mg (expressed as sennoside B) nor should it be administered to (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) et al. 2018). Moreover, also European medicines agency set a typical recommended dose for herbal teas containing 15 to 30 mg of sennosides once or in case of 15 mg, twice a day, and this for a very short term use only as put forward in the assessment report of the European medicines agency (Committee on herbal Medicinal Products 2018).

3.6. Risk assessment

3.6.1. Pesticides

As summarized in table 3.4, all calculated IESTI values and concurrent acute hazard risk (aHI) index, did not exceed 100 %, for both adults and children, thus indicating that there was no acute risk associated with the consumption of these teas. This can be explained by the fact that for those pesticides, where relative high concentrations were found in the samples, with low ADI and/or ARfD, so being the most toxic, low transmission rates were observed.

All EDI values calculated (table 3.4) and concurrent hazard quotient (HQ) did not exceed 100 %, indicating that there is no risk associated with the daily consumption of tea leaves. These results are in accordance with Fan et al. (2022) and Chen et al. (2015) and Wang et al. (2019)

were they determined HQ much lower than 1 %.

Taken together, solely based on the risk assessment performed for pesticide residues, it can be stated that the consumption of these teas does not represent any acute nor chronic health risk for the consumer, although 40 % of the samples were not compliant to EU regulation. However, the MRL encompass not only the acute risks to human health upon oral consumption but are also set taking into account the good agricultural practices.

Table 3.4: The acute and chronic risks associated with the intake of the encountered pesticide residues

Pesticides/ Elemental	ARfD (mg/kg bw per day)	ADI (mg/kg bw per day)	IESTI (mg/kg bw/day)		aHI		EDI (mg/kg bw/day)	HQ
			Children	Adults	Children	Adults	Adults	Adults
Bifenthrin	0.030	0.015	5.52E-06	1.81E-06	0.02	0.01	1.96E-07	0.01
Anthraquinone	n.a.	0.136*	1.33E-05	4.48E-06	0.1	0.03	4.11E-07	0.01
Cyhalothrin-L	0.0025	0.0012	4.09E-05	1.38E-05	1.6	0.55	9.71E-07	0.08
Ortho-phenylphenol	n.a.	0.4	7.32E-05	2.47E-05	-	-	1.17E-06	0.01
Acetamiprid	0.025	0.025	2.93E-05	9.88E-06	0.12	0.04	1.31E-06	0.01
Imidacloprid	0.08	0.06	1.69E-04	5.68E-05	0.21	0.07	4.35E-06	0.01
Chlorothalonil	0.6	0.015	1.63E-05	5.51E-06	0.01	0.01	1.10E-06	0.01
Carbendazim	0.02	0.02	5.67E-06	1.91E-06	0.03	0.01	5.20E-07	0.01
Thiamethoxam	0.5	0.026	9.18E-05	3.09E-05	0.02	0.01	3.81E-06	0.01
Chlorfenapyr	0.015	0.015	7.60E-05	2.56E-05	0.51	0.17	1.67E-07	0.01
Buprofezin	0.5	0.01	6.62E-06	2.23E-06	0.01	0.01	3.43E-07	0.01
Biphenyl	n.a.	0.38*	3.21E-05	2.01E-05	0.01	0.01	1.21E-06	0.01
Chlorpyriphos-ethyl	0.005	0.001	4.51E-06	1.52E-06	0.09	0.03	2.86E-07	0.03
Thiacloprid	0.02	0.01	1.01E-05	3.39E-06	0.05	0.02	7.93E-07	0.01
Mepronil	n.a.	0.05	-	-	-	-	-	-
Cypermethrin	0.2	0.05	4.01E-06	1.35E-06	0.01	0.01	2.21E-07	0.01

n.a.: not applicable.

* Value estimated by applying a factor 100 on the NOAEL.

3.6.2. Trace elements

The EDIs of trace elements from tea consumption are presented in table 3.5. For As it was assumed that the concentration of As in the brew was inorganic As, except for the two samples where measured As concentration in the infusion was high. For these two particular samples, the calculated EDI was multiplied by 0.01 since the results from the speciation analysis showed that just 1 % of total As in the infusion was inorganic As. Since we cannot rule out that the other tea infusions with much lower concentrations contain only inorganic As, we propose this approach as the worst case estimation.

As indicated in Materials and Methods different approaches were followed for risk characterization of the different contaminants. This distinction was done considering the type of health based guidance value. For elements where a chronic maximum intake rate has been established and expressed either as a TDI, TWI or upper level, the risk was evaluated as the percentage contribution of the guidance value. As shown in table 3.5, the average calculated exposure for Cd, Cu, Ni and Zn was below 3 % and thus it is unlikely that the consumption of tea can pose a risk of adverse health effects for those elements. The highest calculated risk exposure value was for Ni in one tea sample, which contributed with about 9 % of the TDI. This value is somewhat close to the range values reported by (Milani et al. 2016) for C. sinensis tea (0.3-6.8 % of TDI).

For Arsenic and Pb the MOE methodology was used to interpret possible risks associated to its exposure. The average and range MOE values for As and Pb are presented in table 3.5. There are not clear guidelines on how these values should be interpreted nor a desired MOE have been given for As. However, a MOE = 100 as a threshold of concern was proposed by Cheyns et al. (2021) as an indicative of low health concern for food supplements. For Pb, the EFSA CONTAM Panel (2010) concluded that a MOE > 10 would be sufficient to ensure no appreciable health risk. Even for a MOE > 1 the risk would be very low. The MOEs for Pb were in general very high except for one tea for which the MOE was only 2. However, based on the conclusion by EFSA this low MOE as it is larger than 1 is of low health concern.

For acute exposure to inorganic As the average calculated exposure was 0.1 % with a range between 0.003 % to 0.56 % of the minimal risk level for acute toxicity. These results indicate that no acute toxic effects are to be expected from drinking herbal teas.

3.6.3. Drugs and medicines

As no synthetic drug was encountered in the analyzed samples, it can be stated that at least for the presence of synthetic drugs, there was no risk associated for the consumption of these teas. However, two samples were positive for the presence of sennosides originating from the senna plant. Although, the use of over the counter senna containing herbal teas, for only a limited period of time by healthy adults, has been associated with few side effects (Senna 2012), an acute overdosis of sennosides or a patients/consumers sensitivity to sennosides could result in symptoms such as griping pain and severe diarrhea. Long term use or abuse can lead to “cathartic” colon with diarrhea, cramps, weight loss and darkened pigmentation of the colonic mucosa. Moreover, even toxic hepatitis has been reported (Vanderperren et al. 2005; Ish et al. 2019; Haoudar et al. 2021). Additionally, both EMA and EFSA state that the use of these preparations should be limited in time and should not be administered to children under the age of 12 and pregnant or lactating women. Unfortunately, for these 2 specific samples, the external packaging and the tea bags did not disclose any warning on

the contraindication for the consumption by children or pregnant or lactating women nor was there information on the restriction in time associated with the use of such a potent herbal food commodity.

Table 3.5: Overview of the occurrence of pesticides present in quantities exceeding the MRL

Element	EDI ($\mu g\ kg^{-1}$ bw day^{-1})		Calculated risk values			
			Chronic toxicity		Acute toxicity	
	Average	Range	Average	Range	Average	Range
As	0.005	0.0001 – 0.028	225	11 – 2267	0.1	0.003 – 0.56
Cd	0.001	0.0001 – 0.005	0.28	0.02 – 1.34		
Cu	0.40	0.07 – 0.89	0.57	0.10 – 1.25		
Ni	0.37	0.05 – 1.12	2.82	0.35 – 8.61		
Pb	0.01	0.0003 – 0.27	264	2 – 1953		
Zn	0.89	0.3 – 1.61	0.25	0.08 – 0.45		

A limitation of the present study is that exposure estimates were based on the total concentrations measured in tea infusions. Consequently, the assessment reflects external exposure and does not account for element-specific bioaccessibility or bioavailability. Bioaccessibility refers to the fraction of a trace element released from the food matrix and remaining available for absorption during gastrointestinal digestion, whereas bioavailability refers to the fraction that effectively crosses the intestinal barrier and reaches the systemic circulation.

Several approaches have been developed to assess the bioaccessibility of trace elements in foods and beverages. For example, Alnaimat et al. (2021) evaluated the bioaccessibility of trace elements in different tea infusions using an in vitro gastrointestinal digestion model simulating physiological temperature, gastric and intestinal pH conditions, digestive enzymes, and mechanical agitation. The bioaccessibility values reported for selected trace elements are presented in table 3.7. Previous studies have shown that bioaccessibility may vary substantially depending on tea type, manufacturing process, formulation, and the presence of additional ingredients such as flavourings or sugars (Erdemir et al., 2018; Schmit et al., 2019).

In contrast, relatively few studies have investigated the bioavailability of trace elements from tea infusions. Milani et al. (2020), using a Caco-2 intestinal cell model, reported bioavailability values of approximately 3–4 % for manganese in tea infusions. These findings suggest that the absorbed fraction of certain trace elements may be substantially lower than the amount measured in the beverage. Therefore, the present assessment should be regarded as a conservative exposure assessment. Although the incorporation of bioaccessibility and bioavailability data would allow a more refined estimation of internal exposure, the absence

Table 3.6: Bioaccessibility of trace elements reported in the literature

Trace element	Bioaccessibility (%)	Reference
Cd	∼80–100	Schmite et al. (2019)
Co	∼20–30	Alnaimat et al. (2021)
Cu	∼20–30	Alnaimat et al. (2021)
Fe	∼36	Apaydin et al. (2026)
Mn	∼5–12	Alnaimat et al. (2021)
Ni	∼20–60	Alnaimat et al. (2021)
Pb	∼80	Schmite et al. (2019)
Zn	∼40–80	Alnaimat et al. (2021)

Bioaccessibility values are reported as ranges or average values found in the cited studies.

of such data is unlikely to change the overall conclusion that no significant health concern was identified for the analysed tea samples. Future studies could combine in vitro digestion models with Caco-2 intestinal cell assays to better estimate the bioaccessible and potentially absorbable fraction of trace elements present in tea infusions.

4 CONCLUSION

This study assessed the health risks associated with the consumption of herbal teas marketed in Belgium. Our findings demonstrate that intake of teas sold in retails shops in Belgium is rather safe. However, care has to be taken as this study does not take into account the arise concern about the presence of pesticide cocktails or pesticides with endocrine disruptors effect. Moreover, as a lot of tea samples exceeded EU MRLs for pesticides residues. The MRL values encompass not only the acute risks to human health upon oral consumption but are also set taking into account chronic exposures at the consumers and production end and environmental effects to the ecosystem.

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Disclosure statement
The authors report there are no competing interests to declare

II. Modelling the pesticide transfer during tea and herbal tea infusions by the identification of critical infusion parameters

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Abstract

Pesticide residues in tea and herbal tea often exceed EU maximum residue limits. Consideration of the transfer of pesticides from the leaves (called transfer factors) to the brew is essential to assess the associated risk. This study identified infusion parameters influencing the transfer behaviour of 61 pesticides and elaborated a predictive model for pesticides with unknown transfer factors in black, green, herbal and flavoured teas. Tea type and the presence of flavours were the criteria that most influenced the pesticide transfer. Interestingly, infusion parameters that are individual and area dependent such as infusion time, temperature and water hardness, did not play a significant role. Beta regression models developed to characterise pesticide behaviour during infusion showed good predictions for most pesticides and revealed that log (P) was the main physico-chemical parameter to estimate the pesticide transfer. The transfer factors database and validated models are valuable tools for improving risk assessment.

Keywords: Pesticides - tea - infusion - transfer factor - modelling

1 INTRODUCTION

Recently, the consumption of tea and herbal tea has become more popular across the globe. More specifically, an almost twofold increase in (herbal and non-herbal) tea consumption during the last decade was estimated in a mainly coffee-drinking culture nation such as Belgium (da Silva Pinto 2013; Tea - Belgium | Statista Market Forecast 2021). Two reasons may explain this increasing trend. Firstly, tea is mainly attributed to younger people consuming less coffee in favour of more fashionable tea-based drinks. Secondly, tea is known to possess some potential health benefits. For example, tea contains polyphenols, tannic acid and antioxidants, which have been associated with a reduced risk of cancer, diabetes, cardiovascular and neurological diseases and antiaging effects (da Silva Pinto 2013; Khan and Mukhtar 2019).

Besides this beneficial effect, it is also important to consider some safety aspects related to the presence of contaminants in tea. Pesticides are one of the major concerns regarding consumer health. Indeed, the Rapid Alert System for Food and Feed (RASFF) reported 20 cases of non-compliant tea samples (European Commission 2005) for the period 2021-2022, either because of a pesticide exceeding the EU maximum residue limits (MRLs) or because of the detection of an illegal pesticide (RASFF 2022). Moreover, Szternfeld et al. (2022) have demonstrated that approximately 38 % of (herbal) tea samples bought on the Belgian market exceeded the MRLs for at least one pesticide. The same conclusion was drawn by Heshmati et al. (2021), who found that 40 % of samples exceeded the EU MRLs.

Since the MRLs are fixed on the leaves, the actual exposure through consumption of the brew will be different since the transfer factors (TF) need to be considered. Indeed, tea and herbal tea are rarely consumed as such, but they are infused with boiling water before the brew is consumed. However, pesticides are not always fully transferred into the brew, and the transfer rate strongly depends on the physico-chemical properties of the pesticides (Jaggi et al. 2001; Chen et al. 2015; Wang et al. 2019). For instance, pyrethroids, one of the most detected pesticide classes in tea, are only slightly transferred into the brew (TF < 10 %) (Chen et al. 2015; Xiao et al. 2017; Wang et al. 2019). On the opposite, neonicotinoids, another pesticide class very often used to treat tea fields, are largely transferred during the infusion (50 < TF < 100 %) due to their higher n-octanol-water partition coefficient, also called log (P) (Hou et al. 2013; Wang et al. 2014; Wang et al. 2019) This wide range of TFs shows the importance of a database containing all of these TFs.

A European database exists for TFs of pesticides in various types of food. However, only data for spiromesifen and hexythiazox are mentioned in tea (Scholz et al. 2018). Another database, created by the German Federal Institute for Risk Assessment (BFR), reported only TFs for glyphosate and fenazaquin in tea. A guidance document from the Intergovernmental Group on tea from the Food and Agricultural Organization (FAO-IGG) on the risk evaluation of pesticides in tea also contained TFs for 5 and 6 pesticides in black and green tea, respectively (Food and Agriculture Organization of the United nation 2014). Additionally, in recent decades, numerous studies investigated TFs, but focused on a limited number of pesticides. Wang et al. (2019) reported TFs for some classes of pesticides in green tea, such as pyrethroids (0 – 8 %), carbamates (6 – 115 %) and neonicotinoids (80 – 102 %), while Manikandan et al. (2009) reported TFs for 3 organophosphorus pesticides (2 – 9 %), 5 organochlorine pesticides (1 – 2 %) and 3 pyrethroids (< 1 %) in black tea. Nevertheless, much data is still lacking on pesticides

often detected in tea on the European market. Furthermore, the tea preparation applied in the different studies is not standardised and might differ between Asian and Occidental countries. For instance, in China, tea is often rinsed with boiled water that is discarded before starting the infusion that will be consumed (Gao et al. 2019). As a result, pesticides may be washed away, and hence TFs can be underestimated, causing bias in the risk assessment. Other studies often based their infusion protocol on ISO 3103 (ISO 3103 2019). Although this would allow comparing the different studies, it does not reflect the consumers habits, nor the difference in infusion temperature for the preparation of different types of tea.

Another aspect to consider is the tea type. Numerous studies focused on green or black tea (Manikandan et al. 2009; Wang et al. 2014; Scholz et al. 2018; Fan et al. 2022). However, in Europe, tea is often mixed with herbs or various flavours (e.g. orange, lemon, mint, apple, cinnamon) that may change the physical and chemical properties of water and affect the contaminants' behaviour during the brewing process, as shown for polycyclic aromatic hydrocarbons by Pincemaille et al. (2014).

Therefore, TFs for a broader range of pesticides based on the most detected at the EU border need to be determined, and existing TFs need to be confirmed or updated according to occidental infusion protocols. Additionally, the role of infusion parameters on TFs (tea type, flavourings) has to be assessed. Lastly, finding with high amount of multiple pesticides is a gamble. A less laborious approach, such as fortified teas, should be investigated.

This study aimed to fill these gaps by generating a database of TFs for the most detected pesticides in different types of tea (black tea, green tea, flavoured tea, herbal tea) abiding by occidental practices. This database will be an important tool for the competent authorities to improve the risk evaluation for the consumer in case of a non-compliant sample. Thus, the objectives of the study were to: (i) evaluate if fortified teas can be used to determine TFs when adequate incurred samples are lacking (ii) experimentally determine pesticides TFs the most found in EU and representative for occidental practices; (iii) identify the infusion parameters influencing these TFs; (iv) create a model to determine theoretically TFs for pesticides not yet studied.

2 MATERIALS AND METHODS

2.1. Sampling and sample preparation

Seventy non-organic teas (13 black teas, 9 green teas, 22 flavoured teas, 26 herbal teas) have been purchased in local stores to maximise the chance of identifying samples containing substantial concentrations of pesticides. A sample consists of one box of tea bags (10-20 units) for packed tea or 100 g for bulk tea. Those containing relevant traces of pesticides were selected for determining the TFs. In addition, eight organic teas (1 black tea, 1 green tea, 3 flavoured tea, 3 herbal tea) have also been purchased in a dedicated organic store. The absence of pesticides in these samples has been confirmed in the laboratory. These organic teas were used for procedural calibration and TF determination via fortification.

After arrival in the laboratory, half of the dry leaves material was ground to a fine powder

and homogenised by a mixer (IKA M20, IKA® Werke GmbH & Co. KG, Staufen, Germany) prior to analysis. The remaining (non-ground) sample was homogenised and kept to generate the brew.

2.2. Tea infusion

A standardised infusion protocol must be applied to study the pesticide transfer from dried leaves to the brew. However, as indicated on their labels, teas and herbal teas require different infusion times and temperatures. To best reflect consumption practices, three categories were distinguished: i.e. green tea (including flavoured green tea), black tea, and herbal tea. The brewing protocols were based on the EFSA report on the TFs database (Scholz et al. 2018) and the instructions available on the websites of the major brands. Basically, 1.5 g of each tea type was weighed in a tea bag and infused with 200 mL of tap water. Infusion temperatures were tea-type dependent (100 °C for black and herbal tea and 80 °C for green tea/flavoured green tea). Infusion time was also adapted (5 min for herbal tea, 3 min for black tea and 2 min for green/flavoured green tea). These protocols have been used as a starting point. Next, several parameters have been modified to reflect differences in infusion practices made by consumers (e.g. infusion time, water temperature).

To investigate the TF using the fortified approach, 1.5 g of organic dried leaves (free from pesticides) was weighed in a paper tea bag and spiked with 300 µL of a pesticide mixture containing two µg mL^{-1} of each pesticide (corresponding to 400 µg kg^{-1}). Then, the sample was dried for 45 min to ensure total evaporation of the solvent before applying the corresponding infusion protocol (table 3.1).

2.3. Determination of pesticides in dry leaves material and brew

Solvents, reagents, standard solutions, extraction and quantification of the pesticides from tea leaves and the brew are described in Appendix A3. Basically, the extraction of pesticides was adapted from the QuEChERS method from Lehotay et al. (2007). Analysis of the brew was also adapted from Lehotay et al. (2007) and (Szternfeld et al. 2022).

2.4. Determination of the transfer factors

The TFs have been determined according to eq. (3.5)

$$\text{TF}\ (\%) = \frac{\text{concentration in brew} \times \text{brew volume}}{\text{concentration in tea leaves} \times \text{tea quantity}} \times 100 \tag{3.5}$$

The concentration of the pesticides in tea leaves is the pesticide concentration in the commercial tea leaves or the fortified tea leaves, depending on the analyte and/or tea type.

2.5. Relationship between transfer factors and pesticide physico-chemical parameters

Plots and Spearman correlation tests have been performed with R 4.2.0 (R Core Team, 2022) statistical software on the whole dataset to describe the relationship between the pesticides' physico-chemical parameters (e.g. n-octanol-water partition coefficient (log (P)), solubility (S), Henry (H) and vapour pressure (VP)). Measurements inferior to the limit of quantification (LOQ) were replaced with zero. A p-value < 0.05 was considered statistically significant.

2.6. Identification of influencing infusion parameters

The infusion parameters that may influence the TFs, were infusion time, tea type, tea flavour, herbal tea type, infusion temperature and water hardness. The influence of the infusion time has been evaluated by varying the infusion time between 2, 3, 5 and 10 min. This parameter has been assessed for black tea and green tea. This dataset has also been used to evaluate the influence of the classic tea type (black vs green). The focus has been made on these two tea types as they are the most consumed (da Silva Pinto 2013). The influence of the flavour has been assessed by comparing TFs between the most common flavours in Belgium (orange, lemon, mint) with non-flavoured green tea. Regarding the impact of herbal teas, the most consumed herbal tea types (chamomile, linden, mint) have been compared. Next, the impact of the infusion temperature on the TFs was evaluated by comparing TFs acquired with black tea infused at 80 and 100 °C. The same experiment was conducted with green tea. The influence of water hardness was evaluated by comparing infusions made with two extreme water hardness types. Tap water (38 °f) from Bruxelles City has been used to simulate very hard water. Very soft water has been simulated using Milli-Q water. Only LC-MS/MS analyses were performed to determine the influence of infusion temperature and water hardness. This method covered the whole range of log (P), S, H and PV of the pesticides. All experiments were performed in triplicate.

Differences between groups (defined by the parameters mentioned above) were assessed by Mann Whitney (MW) tests, comparing the two groups for each pesticide (Mann and Whitney 1947), with a significance level of 1 % (α-value = 0.01). Applying this strict criterion is motivated by the large dispersion of the data observed in the laboratory and scientific literature when using tandem mass spectrometry. A p-value < 0.01 indicates significant differences between groups suggesting that the tested parameter can influence the transfer factor.

2.7. Modelling of pesticides transfer from leaves to the brew

Four models have been developed, one for each tea type (i.e., black tea, green tea, flavoured tea, herbal tea), to predict the pesticide TFs, using the physico-chemical properties of the pesticides. Log (P), log (H), log (VP), and log (S) were used to predict the TFs from in-house experiments using beta regression models (used for rates or proportions) (Ferrari and Cribari-Neto 2004) eq. (3.6)

$$\text{logit}\left(\frac{\text{TF}}{100}\right) = \beta_0 + \beta_1 \log(P) + \beta_2 \log(H) + \beta_3 \log(VP) + \beta_4 \log(S) \qquad (3.6)$$

Where: TF corresponds to the average TFs observed for a specific type of tea (all infusion time t=2, 3, 5 and 10 min in the case of black tea and green tea), log (P) is the n-octanol-water partition coefficient, VP is the vapour pressure (mPa), S is the solubility (mg L^{-1}), and H is Henry (Pa).

A stepwise regression with backward elimination has been used to select the variables significantly influencing the TFs. Only the pesticides with log (P) ≤ 4.5 and log (S) > -2 were used in the models (see Results). Among the dataset, ≈ 80 % of the pesticides were included in the prediction model, while six pesticides (≈ 20 %) were randomly selected for model validation.

These six pesticides were selected throughout the full range of TFs (i.e. 2 in the range of 0 – 20 %, 2 in the range of 40 – 60 % and 2 in the range of 80 – 100 %). Cross-validation was performed to assess the model's generalisation performance, i.e. all possible combinations of validation sets of 6 pesticides were selected, and the model was applied to the remaining pesticides (i.e. training set). The averaged mean squared error of all combinations' validation sets was used as a performance indicator.

Statistical analyses were carried out with R 4.2.0 (R Core Team, 2022) using the betareg package.

3 RESULTS AND DISCUSSION

3.1. Applicability of fortified teas

TFs obtained from fortified tea leaves and incurred samples (available in local stores or sprayed explicitly in experimental tea fields) were compared to ensure they behave similarly (table 3.7). Particular attention was paid to select studies using similar infusion protocols to minimise bias and ensure coverage of the whole log (P) values. Fortified and incurred TFs are strongly consistent throughout studies (including the present study) for polar and non-polar pesticides considering the results' dispersion (table 3.7). This conclusion is also valid for systemic pesticides (i.e. thiamethoxam, acetamiprid and thiacloprid). Hence, incurred approaches such as expensive experimental field studies or random potentially market-contaminated teas could be superseded by fortifying tea, thus highly simplifying the study design. Only the TF of triazophos (0 – 2 %) in the study from Yadav et al. (2017) is significantly lower than the TFs in the study of Chen et al. (2015); Wang et al. (2019) and the present study (27, 22-23, 27 %, respectively).

Table 3.7: Reported transfer factors of selected pesticides from tea leaves to infusion

Pesticide	Log (P)	Pesticide application type	Infusion time (min)	Pesticide concentration (µg kg^{-1})	Transfer factor $\pm$ standard deviation (%)	Reference
Thiamethoxam	-0.13	Incurred #	10	56 – 239	93	J. Wang et al. 2014
		Incurred *	5	n.m.	70	X. Wang et al. 2019
		Incurred #	5	47 – 675	72 $\pm$ 10	H. Chen et al. 2015
		Incurred #	5	39 – 515	71 $\pm$ 14	H. Chen et al. 2015
		Fortified	2 – 10	400	97 $\pm$ 16	Present study
Acetamiprid	0.8	Incurred *	2	25	47 $\pm$ 0.6	M. Gupta et al. 2009
		Incurred *	5	25	51 $\pm$ 0.5	M. Gupta et al. 2009
		Incurred *	10	25	53 $\pm$ 0.4	M. Gupta et al. 2009
		Incurred *	5	n.m.	59	X. Wang et al. 2019
		Incurred #	5	43 – 1520	54 $\pm$ 12	H. Chen et al. 2015
		Incurred #	5	20 – 2780	51 $\pm$ 10	H. Chen et al. 2015
		Fortified	2 – 10	400	57 $\pm$ 10	Present study
Thiacloprid	0.74	Incurred #	10	18 – 114	50	J. Wang et al. 2014
		Incurred *	5	n.m.	47	X. Wang et al. 2019
		Fortified	2 – 10	400	43 $\pm$ 8	Present study
Carbendazim	1.38	Incurred #	5	49 – 1470	63 $\pm$ 10	H. Chen et al. 2015
		Incurred #	5	39 – 1211	65 $\pm$ 14	H. Chen et al. 2015

Table 3.7 (continued)

Pesticide	Log (P)	Pesticide application type	Infusion time (min)	Pesticide concentration (µg kg^{-1})	Transfer factor $\pm$ standard deviation (%)	Reference
		Not specified	5	n.m.	83	L. Zhou et al. 2018
		Not specified	5	n.m.	89	L. Zhou et al. 2018
		Fortified	2 – 10	400	66 $\pm$ 14	Present study
Triazophos	3.34	Fortified	2	n.m.	1^{γ}	S. Yadav et al. 2017
		Fortified	5	n.m.	4^{γ}	S. Yadav et al. 2017
		Fortified	10	n.m.	2^{γ}	S. Yadav et al. 2017
		Incurred *	5	n.m.	27	X. Wang et al. 2019
		Incurred #	5	19 – 906	23 $\pm$ 4.6	H. Chen et al. 2015
		Incurred #	5	15 – 838	22 $\pm$ 5.2	H. Chen et al. 2015
		Fortified	2 – 10	400	27 $\pm$ 9	Present study
Anthraquinone	3.52	Incurred *	5	460	8.5	X. Wang et al. 2018
		Incurred *	5	2150	14	X. Wang et al. 2018
		Fortified	2 – 10	400	18 $\pm$ 6	Present study
Bifenthrin	7.3	Fortified	2	n.m.	1^{γ}	S. Yadav et al. 2017
		Fortified	5	n.m.	2^{γ}	S. Yadav et al. 2017
		Fortified	10	n.m.	2^{γ}	S. Yadav et al. 2017

Table 3.7 (continued)

Pesticide	Log (P)	Pesticide application type	Infusion time (min)	Pesticide concentration (μg kg^{-1})	Transfer factor ± standard deviation (%)	Reference
		Incurred #	5	334 – 3515	0	H. Chen et al. 2015
		Incurred #	5	437 – 7495	0.5 ± 0.5	H. Chen et al. 2015
		Incurred *	5	525	0	S.K. Cho et al. 2014
		Incurred *	5	525	0	S.K. Cho et al. 2014
		Incurred *	5	525	0	S.K. Cho et al. 2014
		Fortified	2 – 10	400	2 ± 2	Present study

[#] from local stores

[*] sprayed in experimental field

[γ] estimated from graphic

n.m. not mentioned

3.2. Relationship between transfer factors and pesticide physico-chemical parameters

fig. 3.1 depicts the TF for each tea type depending on the physico-chemical parameters (log (P), log (S), log Henry and log VP). For each tea type, an inverse linear relationship was suggested between TF and log (P) when log (P) was less than 4.5. Then, above this value, an asymptotic behaviour towards 0 (fig. 3.1-a). This strong relationship between the TF and the log (P) is similar to observations from Wang et al. (2019) and Chen et al. (2015). However, hexythiazox (log (P)=2.5, TF < 5 %) tends to deviate from this general trend. This deviation may be related to its low log (S) (-0.301), which is generally associated with pesticides with log (P) > 4.5 (table A9). However, hexythiazox TF is in line with the general trend, considering its log (S) (fig. 3.1-b). Spearman correlation test confirmed the strong negative correlation between log (P) and TFs (fig. 3.1-a) for all tea types with a rho ranging from -0.78 (herbal tea) to -0.88 (black tea) ($p < 0.0001$ in all cases).

The strong positive correlation between log (S) and TFs (fig. 3.1-b) were also confirmed by Spearman correlation tests for all tea type with rho between 0.67 to 0.83 ($p < 0.0001$ in all cases). A moderate correlation was found between log H and TFs (fig. 3.1-c) and all types of tea with rho ranging from -0.57 to -0.74 ($p <0.0001$ in all cases). Although some authors report the influence of volatility on TFs during a long brewing time (Jaggi et al. 2001; Gupta and Shanker 2009), only a weak relationship was observed between the log (VP) and TFs (fig. 3.1-d) with rho values ranging from -0.12 to -0.25. This weak relationship between volatility and TFs may be explained by the difference between the ”reference” temperature at which the VP is determined (typically 20 – 25 °C) (The Pesticide Manual 2009), and the temperature at which the TFs determination was performed (80 – 100 °C). Indeed, the VP strongly depends on the temperature depending on the compound considered, i.e. orthophenyl phenol has a VP of 0.07 Pa at 25 °C and 133 Pa at 100 °C (National Toxicology Program 1991). All these physico-chemical parameters will be further considered for the development of the model.

The standard deviation (SD) of replicate results may be high for some pesticides, with a maximum of 22 % for imidacloprid in black tea (table A10, fig. 3.1). This substantial variation is also observed in other studies using triple quadrupole detectors (Wang et al. 2014; Chen et al. 2015; Witczak et al. 2018; Wang et al. 2019) but less in studies using an ECD detector (Jaggi et al. 2001; Manikandan et al. 2009; Xiao et al. 2017), which is known to be more precise but limited to halogenated or nitrogenous compounds.

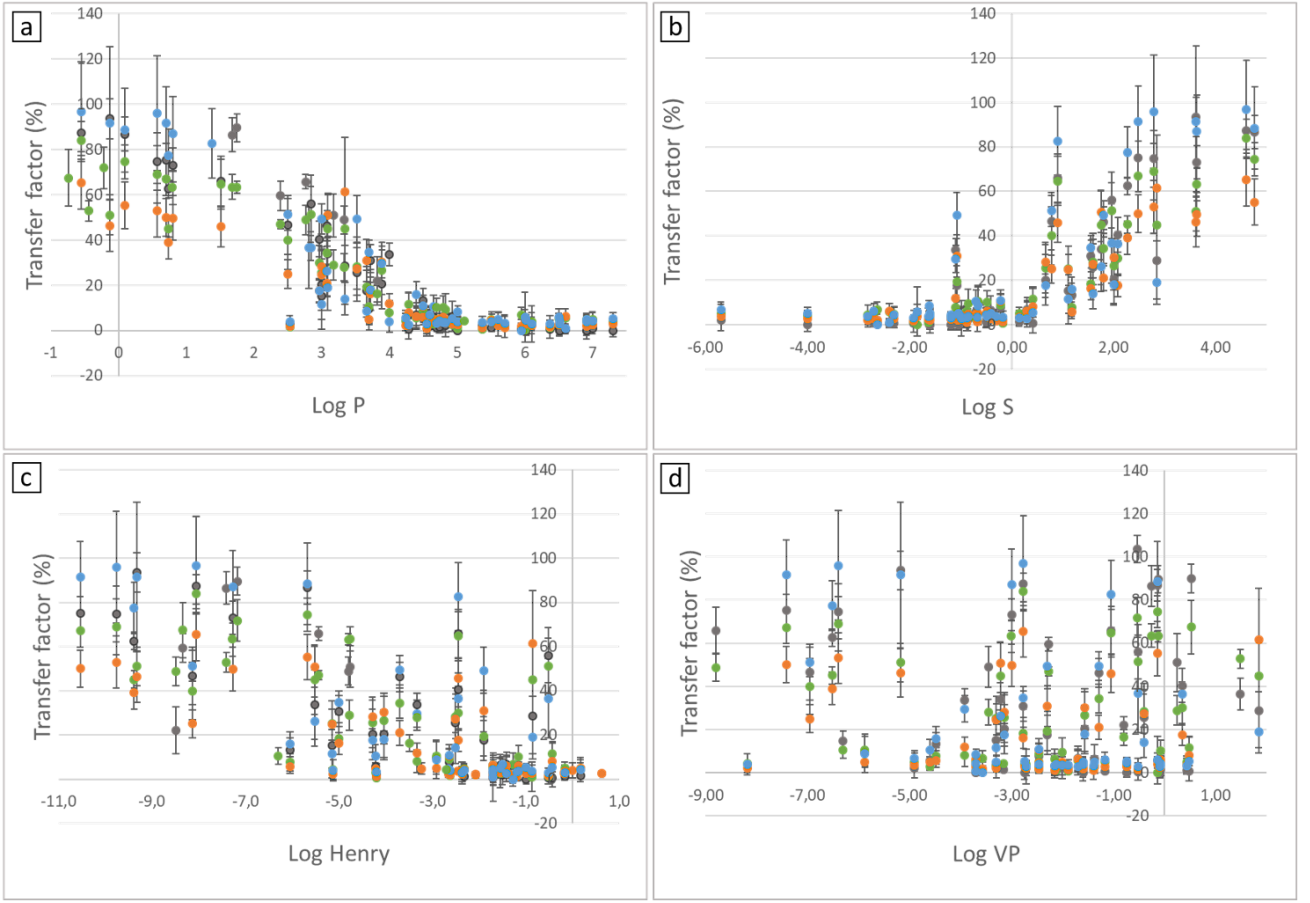

Figure 3.1: Relationship between transfer factor (TF) (%) and some physico-chemical parameters: log (P) (a), log (S) (b), log (Henry) (c), log (Vapour pressure) (d) for black tea (grey dot), green tea (green dot), flavoured tea (orange dot) and herbal tea (blue dot)

3.3. Identification of influencing infusion parameters

3.3.1. Infusion time

The most critical parameter to consider is the infusion time, as this may strongly differ among consumers. Considering the average TFs for each infusion time, an increase may be observed for some pesticides with the infusion time (2 – 10 min) (i.e. acetamiprid from 67 to 87 %, dinotefuran from 83 to 102 % both in black tea) (tables A10 and A11), especially for black tea. However, MW statistical tests did not show significant differences ($\alpha = 0.01$) between groups (2 min vs 3 min,2 min vs 5 min, 2 min vs 10 min (tables A10, A11 and 3.8) for black tea and green tea, suggesting that the infusion time does not affect the TFs. These results align with those of Xue et al. (2014) for the 5 – 10 min range. However, they are in contradiction with some other studies. Gupta and Shanker (2009) observed a very slight increase in TFs with the infusion time (2 – 10 min) for imidacloprid (38.6 ± 0.5 – 43.9 ± 0.4 %) and acetamiprid (47.1 ± 0.6 – 53.3 ± 0.4 %) in black tea. (Jaggi et al. (2001) also observed slightly increasing trends for some pesticides in correlation with increasing infusion time. This confirmed increase may be due to the detector (Electron capture detection, ECD), which is more precise than modern triple quadrupole mass spectrometry. However, the ECD detector drastically limits the pesticides that can be studied. Yadav et al. (2017) showed that TFs increase during a brewing time of 2 - 5 min, but observations are less clear for a brewing time of 5 – 10 min. Furthermore, the dispersion of results or statistical tests is not mentioned, making a reliable conclusion difficult, considering the MW test results and that other studies confirmed only a slight increase in the TF. Hence, we did not consider the infusion time to estimate the risk assessment for a population which make it easier as the information is not known. A possible alternative is to use maximum TF observed during experiments to simulate a worse-case scenario.

Table 3.8: Summary of the number of pesticides affected by tested infusion parameters

Parameters	Tested values	Nb of pesticides with a significant statistical difference* (n = 61)
Time	2 min vs 3 min	0
	2 min vs 5 min	0
	2 min vs 10 min	0
Water temperature	80 °C vs 100 °C	0#
Water hardness	0 °f vs 38 °f	0#
Classical tea type	Black tea vs green tea	29
Flavours	Green tea vs green flavoured orange tea	21
	Green tea vs green flavoured lemon tea	8
	Green tea vs green flavoured mint tea	3
Herbal tea type	Chamomile vs linden	0
	Chamomile vs mint	0
	Linden vs mint	0
Classical tea vs herbal tea	Herbal tea vs black tea	15
	Herbal tea vs green tea	23

*significant difference for $p < 0.01$ (confidence interval 99 %)

#n = 29

3.3.2. Classic teas (unflavoured)

The tea type (green vs black) significantly influences the TF of 29 out of 61 pesticides MW statistical tests, (tables A12 and 3.8). More polar pesticides (log (P) <2.5) tend to be transferred less during the infusion of green tea than black tea, whereas this trend is reversed for highly non-polar pesticides (log (P) >2.5) (fig. 3.2-a). Higher TFs in green tea than in black tea for non-polar organochlorine pesticides (log (P) > 3.5) were similarly reported by Witczak et al. (2018). To the best of our knowledge, this is the first time it has been shown that the tea type influences pesticide TF. Although the importance of the tea type on TFs may influence the risk assessment, most studies focused on black tea (Manikandan et al. 2009; Heshmati et al. 2021)

or green tea (Jaggi et al. 2001; Cho et al. 2014; Fan et al. 2022) or determined TFs without discriminating and comparing tea types (Szternfeld et al. 2022).

3.3.3. Tea flavour

Knowing the influence of the flavour is essential as in occidental countries, many flavoured teas with a mix of several flavours (between 2-5) are available on the market. A comparison of pesticide TFs from unflavoured and flavoured green teas showed higher TFs for flavoured teas than for unflavoured teas in general, especially for the most detected pesticides (polar and less non-polar pesticides such as neonicotinoids) (tables A13 and 3.8). Indeed, MW tests showed significant differences between unflavoured and orange teas for 21/61 pesticides but only for 8 and 3 pesticides for lemon and mint flavour teas, respectively. The absolute TF difference between green and flavoured teas decreased linearly with the pesticide log (P) (Pearson coefficient = 0.82 for orange flavour and 0.96 for lemon flavour) (fig. 3.2-b). These results contradicted Pincemaille et al. (2014), who hypothesised that flavoured oils might promote the transfer of non-polar compounds such as PAHs into the brew (Pincemaille et al. 2014). However, the present study clearly shows that the flavour type may significantly influence the pesticide TF.

3.3.4. Herbal teas

MW statistical tests did not show any statistical differences in TFs depending on the herbal type infused (chamomile, linden and mint) (tables A14 and 3.8). Only one study on the TFs from herbal tea of the same nature has been found. Ozbey and Uygun (2007) estimated the chlorpyrifos TF at 12 % in peppermint, which is the same magnitude order as this study for mint: 5 ± 5 % (table A14). This study focused on the most widespread herbal tea in Belgium, but it cannot be ruled out that these observations would differ for other herbs.

3.3.5. Teas and herbal teas

There were significant differences (MW tests) between herbal tea TFs and black tea TFs (for 15 pesticides) as well as for green tea TFs (for 23 pesticides) (fig. 3.2-c, tables A15 and 3.8). In most cases, TFs for herbal teas were higher than TFs for black and green teas, especially for polar pesticides such as neonicotinoids (thiamethoxam, thiacloprid, imidacloprid) (fig. 3.2-c). Hence, the tea type seems to be an important parameter for pesticide TFs.

3.3.6. Infusion temperature

Black tea and green tea infused with water at 80 °C and 100 °C did not show significant differences in TFs (tables A16, A17 and 3.8), which is in agreement with the results of Xue et al. (2014). However, Cho et al. (2014) demonstrated a positive correlation between the TFs and the infusion temperature (80 – 100 °C) for some pesticides, ranging from small (e.g. triflumizole from 3.0 to 3.3 %) to higher (e.g. fenitrothion from 10.8 to 14.7 %) and an exceptionally high correlation for azoxystrobin (from 61.9 to 97.8 %) had been observed.

3.3.7. Water hardness

No statistical difference was found between the TFs obtained from Brussels tap water (very hard - 38°f) and Milli-Q water (very soft) (tables A18 and 3.8 and **??**). It was impossible to compare with other studies, as it seems to be the first time this parameter has been investigated.To the best of our knowledge, this is the first time that the impact of so many

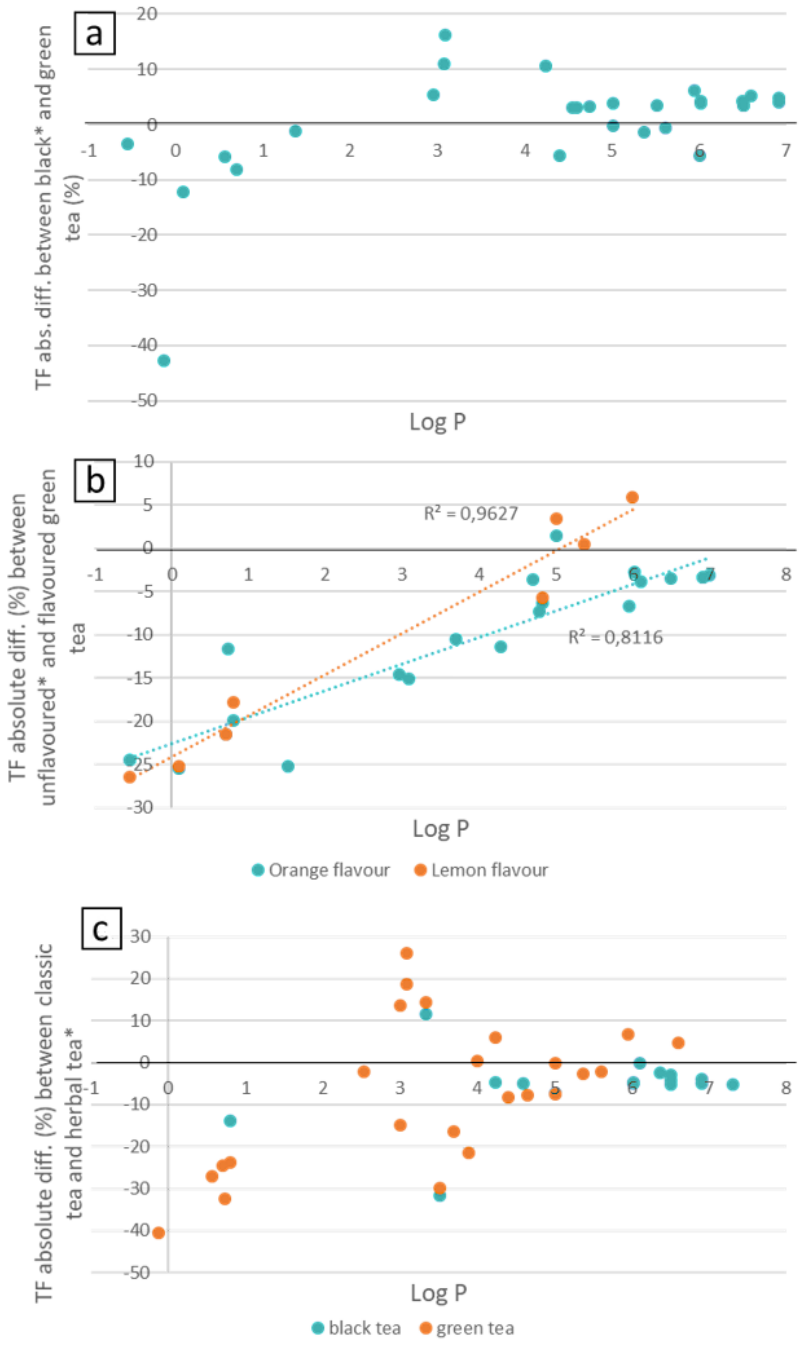

Figure 3.2: TF absolute difference (%) in function of the log (P) for pesticides showing a significant statistical difference in TF between a) black* and green tea, b) flavoured and unflavoured* green tea and c) between herbal tea* and classic tea. * as reference

infusion parameters has been investigated simultaneously.

Interestingly, individual-dependent infusion parameters (infusion time, water temperature) or area-dependent (water hardness) do not play a significant role in the pesticide transfer and should not be considered for a risk evaluation. Parameters mostly influencing pesticide behaviour and required for risk evaluation are the tea type and the presence of flavours, which are present on the label. The only issue remaining is dealing with tea/herbal tea composed of several flavours (2-5), especially for very polar pesticides (fig. 3.1-b). All the TFs from these experiments have been compiled in a database, and the average per tea type (black tea, green tea, flavoured tea and herbal tea) has been included for a more accurate risk assessment.

3.4. Modelling

Four models (one for each tea type) have been built with TFs from experimental data. All models were restricted to the range log (P) < 4.5 and log (S) > -2.0, as TFs are no longer sensitive to these physico-chemical parameters outside this range (fig. 3.1-a, fig. 3.1-b). In their study, Wang et al. (2019) insisted on restraining their modelling area to pesticides with log (S) > 3 as above this value, TFs already reached 100 %. This phenomenon was not observed in the present study and may be due to differences in infusion protocol, and the pesticides investigated in both studies were not always the same.

In the four final models, log (P) was a significant variable (table 3.9). Log (VP) was also significant for the green and flavoured green tea models. Contrary to Wang et al. (2019), log (S) was not significant in any of the four models, which can be explained by the link that exists between log (S) and log (P) (Hughes et al. 2008): these variables give nearly the same information.

Table 3.9: Beta coefficients determined for black tea, green tea, flavoured green tea and herbal tea models for eq. (3.6)

Coefficient	Black tea		Green tea		Flavoured tea		Herbal tea	
	Estimated	std error	Estimated	std error	Estimated	std error	Estimated	std error
β0 (intercept)	2.00	0.34	1.63	0.24	0.95	0.36	2.54	0.37
β1 (log (P))	-0.86	0.12	-0.76	0.07	-0.56	0.10	-1.11	0.12
β2 (log (H))	NS	-	NS	-	NS	-	NS	-
β3 (log (VP))	NS	-	0.07	0.03	0.11	0.06	NS	-
β 4 (log (S))	NS	-	NS	-	NS	-	NS	-

NS: coefficient not significant and equal to 0 in the formula eq. (3.6)
std error: standard error

Good adequacy can be observed between observed and predicted TFs for the four models (fig. 3.3). Better adequacy was obtained for green and herbal teas (pseudo R-squared = 0.84 and 0.86, respectively) than for black and flavoured green teas (pseudo R-squared = 0.66 for both models). Despite these good relations, some deviations are observed. For instance, in black tea, epoxiconazole and flutolanil predicted TFs were 1.6 and 1.7 times lower than the observed TFs. The maximum deviation was observed for anthraquinone in herbal tea with a predicted TF 2.4 times lower than the observed TF. This phenomenon may originate from the large TF variation for pesticides with a comparable log (P) and/or log (VP).

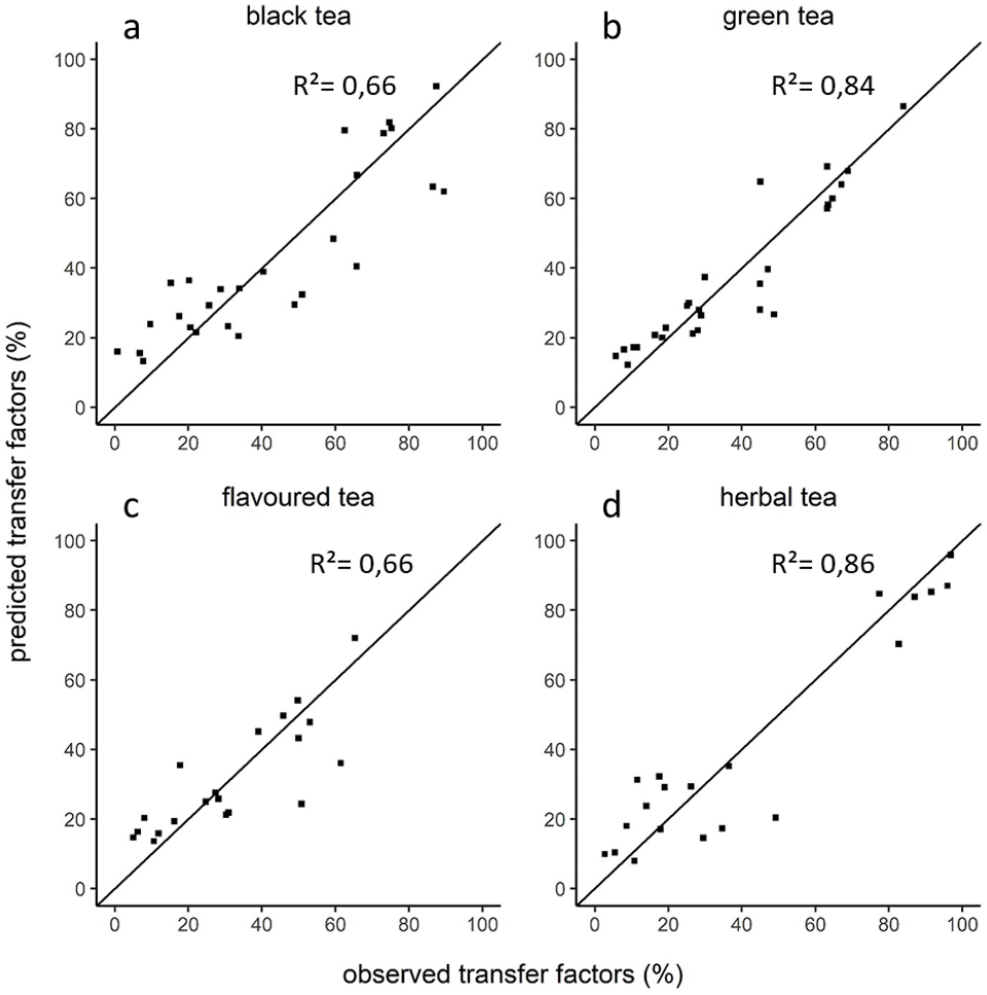

Figure 3.3: Predicted transfer factors (%) versus observed transfer factors (%) for black tea (a), green tea (b), flavoured green tea (c), and herbal tea (d)

Difenoconazole, hexythiazox, azoxystrobin, linuron, methomyl and thiamethoxam were selected for the four models' validation. For most pesticides, good prediction can be observed (fig. A6), except for hexythiazox, for which the models overestimated its TF. This overestimation was predictable as hexythiazox behaviour deviated from the general trend. Hexythiazox has a TF well below (TF < 5 %) to other pesticides (TF ≈ 20 – 60 %) with a similar log (P) (log (P) = 2.5) (fig. 3.1-a).

Next, cross-validations were performed using 4200 combinations of training and validation sets for black and green tea models and 3465 combinations of flavoured green and herbal tea models. The averaged mean squared errors of all the combinations (except for the model presented in this contribution) were respectively 317 (SD: 146), 148 (SD: 101), 196 (SD: 108) and 268 (SD: 100) for black tea, green tea, flavoured green tea and herbal tea, meaning that the green tea model showed the best generalisation performance and the black tea model the lowest performance (**??**). Besides, the averaged mean squared errors were lower than the mean squared errors of the validation sets of the black tea, green tea, flavoured tea and herbal tea models (324, 389, 341, 334, respectively) (**??**), which can be explained by the presence of the hexythiazox in the validation set, whose rate is difficult to predict.

Hence, modelling results suggest that even if a strong correlation exists between TF, tea type and log (P), they cannot explain pesticide behaviour completely. The model from Wang et al. (2019) came to the same conclusion: a good prediction on average, but with a deviation up 3.6 higher than the observed values for some isolated pesticides. Two reasons may explain this phenomenon. The first is that physico-chemical parameters are temperature dependant and typically determined at 20 – 25 °C, contrary to infusion experiments conducted between 80 – 100 °C. For instance, ortho-phenyl-phenol has a log (VP) of 1.85 at 25 °C and 5.12 at 100 °C (National Toxicology Program 1991). Another reason that may introduce uncertainty to the modelling is that physico-chemical parameters may strongly differ according to the sources or the determination technique (experimental or modelling), i.e. log (P) for hexythiazox is 2.5 (experimental) according to The Pesticide Manual (2009) and 5.57 (modelling) according to EPA website (US EPA 2014). Furthermore, Hughes et al. (2008) stated that the lack of measured values at the extremes (for log (P) and (S)) is a significant source of error. Physico-chemical parameters have been taken as much as possible from one source for consistency.

The modelling attempt reinforced the conclusion that a TF database (case-by-case approach) is needed to provide confident and accurate TFs. However, the model can be a valuable first approximation tool for unknown TF calculation, considering that significant variations may occur and the model's limitations must be considered.

4 CONCLUSION AND RECOMMENDATIONS

A database containing TFs and predictive models for unknown TFs has been established to support the risk evaluation for tea brewed according to occidental practices.

The database compiled more than 260 consolidated average TFs for various tea types and pesticide combinations and can be used for countries with similar infusion practices. This study showed the advantage of using fortified teas as a valid alternative to study TFs of pesticide residues for which incurred tea samples are lacking.

The study gives a better insight into the parameters influencing the pesticide TFs. It confirmed the hypothesis that tea type and flavour influenced the pesticide transfer in the brew and that these factors need to be considered in the risk assessment.

Interestingly, infusion time and water temperature (80 – 100 °C) did not significantly influence pesticide transfer. Excluding these parameters as variables will make the risk assessment more straightforward. Indeed, infusion time and water temperature strongly differ among consumers. Similarly, water hardness, which may vary from region to region or the presence of water softeners, did not play a major role in the pesticide behaviour and can be excluded as a contributing factor.

The recommended procedure for risk assessment is to first refer to the database containing TFs for various tea types and pesticide combinations. Then, for strongly apolar pesticides (log (P) > 4.5) not yet included in the database, a default maximum TF fixed at 10 % should be used as the worst-case scenario. The model for each tea type can be used as a first approximation for pesticides with a log (P) < 4.5, not yet included in the database.

Acknowledgment

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Chapter 4

Identification of new sources of acrylamide in food, impact of the consumer cooking practices and evaluation of the risk

Outline

Acrylamide has been studied for several decades in foods known to generate high levels of this contaminant during cooking, such as French fries, chips, coffee, and biscuits. However, acrylamide can be formed in any starch- and protein-containing food subjected to heat processing. Moreover, recent studies have shown that acrylamide may also form spontaneously in foods without any cooking step, such as olives. To fill existing data gaps, the European Commission issued Recommendation (EU) 2019/1888, encouraging the collection of occurrence data for specific food categories where non-negligible acrylamide levels were suspected.

This chapter aims to address this recommendation and subsequently reassess the potential health risk to consumers using updated occurrence data. First, analytical methods were developed and validated for all matrices covered by the EU recommendation. Then, acrylamide concentrations were determined in 210 food samples collected from the Belgian market, representing as closely as possible the Belgian diet. In parallel, a pilot study was conducted to investigate the impact of domestic cooking habits on acrylamide formation. Finally, the occurrence data generated in this work and those provided by the Federal Agency for the Safety of the Food Chain (FASFC) monitoring program were combined with national consumption data to estimate acrylamide exposure and reassess the risk for the Belgian population.

This chapter is an adapted version of the following scientific article and project report:

- **Ph. Szternfeld**, V. Van Leeuw, M-L. Scippo, C. Vinkx, E. Van Hoeck & L. Joly, 2025. Characterisation of new sources of acrylamide in food marketed in Belgium. Food Anal. Methods 18, 86–98. DOI: 10.1080/19393210.2024.2440362.
- **Ph. Szternfeld**, V. Van Leeuw & M. Andjelkovic, 2020. Acrylamide in other food – Determination of acrylamide levels in some food categories and estimation of the exposure for the Belgian population. Scientific report

Contribution

Philippe Szternfeld, Laure Joly, Mirjana Andjelkovic and Els Van Hoeck acquired funding for the study. Philippe Szternfeld coordinated and supervised the project. Philippe Szternfeld and Virginie Van Leeuw developed and validated the analytical methods and performed all sample preparation and analytical measurements.

Mirjana Andjelkovic performed the dietary exposure assessment and risk assessment calculations.

Philippe Szternfeld interpreted the analytical results and drafted the manuscript. Philippe Szternfeld, Marie-Louise Scippo, Christine Vinkx, Els Van Hoeck and Laure Joly contributed to data interpretation, manuscript revision and data visualization. Philippe Szternfeld, Virginie Van Leeuw and Mirjana Andjelkovic contributed to additional scientific reporting related to the study. All authors reviewed and approved the final manuscript.

I. Characterisation of new sources of acrylamide in food marketed in Belgium

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Abstract

This study provides occurrence data for acrylamide in various foodstuffs, including those covered by the Recommendation (EU) 2019/1888, from 210 samples purchased on the Belgian market. Acrylamide detection frequencies exceeded 84 % in potato-based products other than fries, vegetable crisps, black olives, cocoa powders, coffee substitutes and cereals and snacks. Large variations in acrylamide levels were found in cereals and snacks, with no correlation between cereal type or process. Snacks containing chia didn't show higher acrylamide levels than other cereal-based snacks. Maximum levels were 4389 and 3063 µg kg^{-1} in coffee substitutes and vegetable crisps, respectively. Potato-based products contained 2 to 27 times less acrylamide when prepared in oven compared to deep fryer. Artificially oxidized 'Californian-style' black olives contained five times more acrylamide than 'Greek-style' olives. In bread, pastries, nuts, oilseeds, dried fruits and confectionaries, acrylamide detection frequency varied from 27 to 69 % and average acrylamide content was <30 µg kg^{-1}.

Keywords: Acrylamide - food - monitoring - Belgium - LC-MS/MS

1 INTRODUCTION

Acrylamide is a well-known process contaminant in carbohydrate-rich foods prepared at temperatures higher than 120 °C and low moisture content (Mottram et al. 2002; Stadler et al. 2002). It has been classified as probably carcinogenic (group 2A) by the International Agency for Research on Cancer (IARC) due to its carcinogenic effect on rodents (IARC 2010). Since its discovery in food by the University of Stockholm in 2002, intensive research has focused on understanding its formation mechanisms. Mottram et al. (2002) concluded that acrylamide is formed in food rich in amino acids such as asparagine and reducing sugars when heated at a temperature higher than 120 °C as a product of the Maillard reaction. Later, other pathways and precursors leading to acrylamide formation have been highlighted by Keramat et al. (2011).

Therefore, Commission Recommendation 2010/307/EU (European Commission 2010), recommended that European Union (EU) Member States monitor acrylamide levels in certain foods. The monitoring exercise targeted foods known to contain high acrylamide levels and/or contribute significantly to the human dietary intake. Hence, the impact of the process and the selection of raw material on acrylamide generation have been widely studied in food such as bread, potato fries and coffee (EFSA 2015). For instance, Biedermann et al. (2002) demonstrated the importance of water content in acrylamide generation. They observed that acrylamide content in wet potatoes cooked at 120 °C was < 20 µg kg^{-1}, but higher than 10 000 µg kg^{-1} for dry potato powder. Lantz et al. (2006) concluded that time and degree of roasting were the main factors influencing acrylamide levels in coffee. Even more, prolonging the roasting process could lead to a decrease in acrylamide. For instance, an average of 374 µg kg^{-1} of acrylamide was observed for light-roasting coffee but only 187 µg kg^{-1} for dark-roasting coffee (EFSA 2015; EFSA 2022b). Acrylamide is also often found in bread, but at a lower level (42 µg kg^{-1} on average) since acrylamide is particularly located in the crust and then diluted in the whole sample (Surdyk et al. 2004; EFSA 2015).

Based on European monitoring data from 2010-2013, the European Food Safety Agency (EFSA) panel for the Contaminants in the Food Chain estimated, in 2015, that the margin of exposure (MOE), compared to a BMDL (benchmark dose lower confidence llimit) of 0.17 mg kg^{-1} bw day^{-1} was below 10.000. Hence, EFSA concluded that the presence of acrylamide in food might be a concern for the human health regarding the carcinogenic effect (EFSA 2015). Since then, the Regulation (EU) 2017/2158 established mitigation measures for various foods to reduce acrylamide levels as much as possible in the final product. Examples are the selection of raw materials with low asparagine and/or reduced sugar content and optimisation of the storage condition to minimise the production of these precursors. Advice is also given regarding the cooking process. For example, for french fries, the frying temperature has to be kept between 160 and 175 °C or oven cooking has to be performed between 180 and 220 °C.

Furthermore, this Regulation also assigns benchmark levels (BMLs) for specific food categories that the food sector should be able to achieve when respecting the mitigation measures. However, it was recognized that the number of data available on the presence of acrylamide in the foodstuffs covered by Regulation (EU) 2017/2158 is insufficient (European Commission 2019). In addition, Regulation (EU) 2017/2158 does not include all the food categories that may contain significant quantities of acrylamide and which could potentially

contribute to the total dietary exposure to acrylamide. As acrylamide tends to be formed through precursors such as asparagine and reducing sugars, it can virtually concern all cereal-based and tuber food undergoing heating. For instance, recent research highlights the wide variation of acrylamide levels in corn products such as tortilla crisps, crackers and popcorn (57 to 1660 µg kg^{-1}) (Žilić et al. 2022). Root vegetables crisps other than potato-based, have also been characterized by high acrylamide content (Breitling-Utzmann and Wendler 2019; Nguyen et al. 2022; MacDonald et al. 2023). Furthermore, Becalski et al. (2011) reported that other specific food products that are not cereal-based can generate substantial amounts of acrylamide, even when the heating temperature is set at 100 °C, lower than the often quoted acrylamide formation (120 °C). Their study showed that acrylamide content of up to 332 µg kg^{-1} can be generated during the drying process of fruits such as prunes. The authors concluded that acrylamide in prunes and prune juice very likely originates from sugars and asparagine, which is present in considerable amounts in the raw material. Breitling-Utzmann et al. (2023) and Nguyen et al. (2022) showed that high acrylamide levels could be found in black olives, especially for the 'Californian' style black olives undergoing an artificial oxidized process (50 - 1100 µg kg^{-1}). In this process, olives are artificially colored by air oxidation and the use of the color fixative agent E579 (ferrous gluconate), followed by a sterilization step. The sterilization process involved temperatures ranging from 110 °C to 121 °C for up to 50 minutes, exceeding the temperature threshold for acrylamide formation (Casado and Montaño 2008).

The Recommendation (EU) 2019/1888 about the monitoring of the presence of acrylamide in certain foodstuffs includes potato-based products other than fries, potato-based composite dishes, black olives, various types of pastries and cereal-based crackers and snacks, nuts, oilseeds, dried fruits and some confectionaries (European Commission 2019). In parallel, EFSA also issued a call on 25 October 2019 to collect data at the European level on the acrylamide contents of foodstuffs based on chia seeds or chia flour and which have undergone a cooking process. This call echoes an EFSA scientific opinion of March 2019 (EFSA 2019) stating that partial substitution of wheat flour with chia flour and using chia seed in bread and cookies may lead to higher levels of acrylamide in the final product. The lack of such essential occurrence data in these less-studied foods precludes a precise estimation of acrylamide exposure for the consumers and the associated risk for the population.

The spectrum of food products addressed by Recommendation (EU) 2019/1888 is thus very large. It is essential to use adequate analytical methods for all these food categories. As reviewed by Keramat et al. (2011), most analytical methods use water as an extraction medium. However, pure water tends to form an emulsion in case of cereal-based matrices that may clog filters and instruments. This phenomenon can be avoided by adding an organic solvent such as n-propanol or Carrez I and II solutions. The QuEChERS method involves an extraction with a water and acetonitrile (ACN) mixture in the presence of NaCl, as proposed by Anastassiades et al. (2003). De Paola et al. (2017) successfully applied the QuEChERS method to dried-fruits and edible seeds for acrylamide extraction. A key advantage of QuEChERS lies in the use of ACN, which mitigates emulsion formation, while the presence of NaCl facilitates the transfer of acrylamide into the ACN phase. Furthermore, the QuEChERS is a modular method, as a common extraction is used for various food products before using a specific mix of dispersive-solid phase extraction (d-SPE) sorbents for clean-up (Anastassiades et al. 2006). This modularity potentially reduces the developmental complexity and makes analysis more straightforward, which is essential to facing the variety of food targeted by Recommendation

(EU) 2019/1888.

This study aims to fill the data gaps for specific food categories targeted by Recommendation (EU) 2019/1888. First, two analytical methods have been developed and validated to address the diversity of the food categories. The first method was dedicated to coffee substitutes and cocoa powder for beverages, while the second method was a modular QuEChERS-based method applied to all the remaining food categories. Then, occurrence data were collected by analyzing 210 food samples targeted by the Recommendation (EU) 2019/1888 and representative of the Belgian food diet. Particular attention was paid to including food items containing chia (flour or seeds) to verify the hypothesis from EFSA (2009). Furthermore, acrylamide distribution in food products was investigated to determine if a specific type of cereal, vegetable or process was responsible for the higher acrylamide content.

2 MATERIALS AND METHODS

2.1. Chemicals and reagents

Acrylamide and deuterated acrylamide (acrylamide-d3) standards were from Ehrenstorfer GmbH (Augsburg, Germany). Hexane (HPLC grade), ACN (HPLC-S grade), methanol (HPLC grade) and ethyl acetate (Pesti S) were from Biosolve (Valkenwaard, The Nederlands), formic acid 98-100 % was from Merck (Darmstadt, Germany) and dichloromethane (HyperSolve HPLCTM) was from BDH chemicals (England). Distilled water (<18 MΩ resistivity) was produced by a Millipore Milli-Q system (Millipore Corp., Bedford, M, USA). Potassium hexacyanoferrate(II) trihydrate (K4[Fe(CN)6] x 3.H2O), zinc sulfate heptahydrate (ZnSO4 x 7.H2O), magnesium sulfate and sodium chloride were purchased from Merck N.V/S.A. (Overijse, Belgium). Bulk PSA (40 µm), bulk C18 (40-70 µm) and SPE accuCAT 200 mg cartridges were from Agilent Technologies (Machelen, Belgium).

Standard stock solutions of acrylamide and acrylamide-d3 were prepared at 1 mg mL^{-1} in water/methanol (50/50 v/v). Working solutions at 10 µg mL^{-1} and 1 µg mL^{-1} were made from stock solutions in water/methanol (50/50 v/v). All solutions were stored at -20 °C. The stability of acrylamide-D3 is regularly checked with a mass spectrometer. To date, no issues have been encountered, and it is, therefore, preferred to the more expensive C13 standard.

2.2. Sampling strategy

The sampling plan was based on specific food products targeted by the Recommendation (EU) 2019/1888 (European Commission 2019). Moreover, other food products containing chia were added to the sampling plan due to the ESFA Scientific opinion on the safety of chia seeds (Salvia hispanica L.) as a novel food for extended uses under Regulation (EU) 2015/2283 (European Parliament and Council 2015; EFSA 2019). The number of samples per category of food products was based on their availability in the Belgian market and the consumption habits of the Belgian population according to the National Food Consumption Survey (FCS 2014) (Bel et al. 2016). The brand selection has been made based on the Belgian market share data from the Euromonitor database (Euromonitor 2017). Specific stores, such as bakeries, organic

stores, and restaurants, were also included to reflect Belgians' shopping habits as closely as possible. Moreover, particular care has been taken to include at least one product bearing the organic label in each food category. A total of 210 samples were purchased from Belgian retailers in 2019 and 2020. The selected food products were cereals and snacks (n=64 [11 with chia seeds]), potato-based products other than fries (n=43), pastries (n=32), bread (n=30 [8 with chia seeds]), grilled nuts and oilseeds (n=16), dried fruits and confectionaries (n=11), vegetable crisps (n=8), coffee substitutes (n=7), black olives (n=5), cocoa powders for beverage (n=5). Fifty-four (25 %) samples bore an organic label (tables B1 and 4.3).

2.3. Samples preparation and homogeneization

Samples were analyzed as consumed, in line with Regulation (EU) 2017/2158 (European Commission 2017). Samples bought as "ready-to-eat" were directly homogenized. Samples requiring a cooking process (e.g. croquettes, sweet potato fries, etc.) were prepared according to the manufacturer's instructions described on the packaging. This approach ensured that the different cooking modes were evenly represented across all samples. Moreover, twenty samples were cooked following both oven and fryer instructions to assess the effect of the cooking process. Samples were homogenized with a mill (IKA M20, IKA®-Werke GmbH & Co. KG, Staufen, Germany) in accordance with Regulation (EU) 2017/2158 and 333/2007 (European Commission 2007; European Commission 2017).

2.4. Determination of acrylamide content

The methodology for analyzing acrylamide in a broad range of food was based on the QuEChERS extraction (Anastassiades et al. 2003) common to all food categories except for the categories 'coffee substitutes' and 'chocolate powder for beverages' for which Carrez solutions I and II were added prior to the extraction step. Subsequently, a customized clean-up procedure was implemented for each food category based on d-SPE with C-18 and PSA sorbents.

2.5. Extraction and clean-up

First, 25 µL acrylamide-d3 at 1 µg mL^{-1} is added to 1.0 g of homogenized sample. Then, for all matrices except coffee and cocoa products, a QuEChERS extraction was carried out. To that end, 10 mL of Milli-Q water, 10 mL of ACN, 0.5 g of NaCl and 4 g of MgSO4 were added to the sample before shaking for 1 min by hand, followed by a centrifugation step. Then, 4 mL of supernatant were cleaned up on d-SPE, consisting of a specific amount of PSA and C18, depending on the sample's nature. Fried potato products, root tuber and vegetable-based products (such as vegetable crisps), black olives, oil seeds, nuts and food high in sugar (such as nougat, caramel or dried grapes) needed 150 mg PSA, while cereal-based products (such as rice and maize crackers), pastries and pancakes needed 300 mg PSA and 300 mg C18. Then, the supernatant was evaporated up to 500 µl under a nitrogen stream, and the volume was adjusted at 2 mL with Milli-Q water. Finally, 500 µl of the solution was filtered on a 0.2 µm PVDF auto-filtrating vial prior to LC-MS/MS analysis.

For coffee substitutes and cocoa products, extraction was performed with 10 mL of Milli-Q water and 1 mL of Carrez I and II solutions, then shaken with a vortex for 10 min. After centrifugation, 6 mL of supernatant was taken for a liquid-liquid extraction with 13 mL of

ethyl acetate. The upper layer of ethyl acetate was transferred into a new Falcon tube, and the liquid-liquid extraction was repeated with 13 mL of fresh ethyl acetate. Then, both upper phases were combined, and 1 mL of Milli-Q water was added before concentrating the solution to 1 mL under a nitrogen stream. Finally, the concentrated extract (1 mL) was loaded on a multimode SPE cartridge in a pass-through mode, and 500 µl of purified extract was collected. The eluate was then filtered on a 0.2 µm PVDF auto-filtrating vial before injection on the LC-MS/MS.

2.6. UPLC-MS/MS analysis

LC-MS analyses were performed using an Ultra Performance Liquid Chromatography (Acquity Ultra Performance LC®) system coupled to a Xevo TQ mass spectrometer (Waters, Milford, MA, USA) conducted under MassLynx® v4.2 software. Five microlitres of the samples were injected into a Hypercarb column (100 x 2,1 mm, 5 µm). The column temperature was set at 45 °C. The mobile phase was composed of Milli-Q water, 0.5 % methanol and 0.1 % formic acid. The chromatographic separation was performed at a flow rate of 0.4 mL min^{-1} with an isocratic elution mode. table 4.1 summarizes the optimized MS/MS parameters for monitoring acrylamide and acrylamide D3. Two transitions were monitored, one for quantification, and the other for the analyte's confirmation. The following source parameters were used: capillary voltage for electrospray 3.5 kV, source temperature 150 °C, desolvation temperature 550 °C, cone gas flow 80 L h^{-1} and desolvation gas flow 800 L h^{-1}.

Table 4.1: MS/MS parameters for the analysis of acrylamide and acrylamide D3

Compound	Precursor ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision energy (eV)
Acrylamide	72.1	55.0 (Q*)	20	10
	72.1	72.1 (q**)	20	2
	72.1	44.0	20	10
Acrylamide D3	75.0	58.0 (Q)	20	10

*Q quantifier ion, **q qualifier ion

2.7. Method validation

The protocol followed to validate the method is described in supplementary information Appendix B2. Briefly, the two methods used have been validated for the linearity, matrix effect, recovery, trueness, limit of detection (LOD) and limit of quantification (LOQ). Validation criteria were based on the Regulation (EU) 2017/2158 and the SANTE document (European Commission 2017; European Commission 2021).

2.8. Quality assurance and quality control

The sensitivity and stability of the LC-MS/MS system have been controlled by injecting a calibration point at LOQ level (20 µg kg^{-1}) at the beginning and the end of each chromatographic run. A S/N > 10 for both acrylamide transitions and for both injections ensured that there was no sensitivity loss. Additionally, the concentrations of each calibration level were back-calculated using the regression equations of the calibration curve and with a 1/x weighing factor. A deviation lower than ± 20 % compared to the theoretical concentration was required to ensure an accurate quantification. A procedural blank was analyzed for each batch of samples to ensure no acrylamide contamination occurred, either at the level of the extractions or at the LC-MS/MS system level. Extraction efficiencies were evaluated for each analysis batch by checking the recovery of a control sample of the same nature as the analyzed samples and fortified at 250 µg kg^{-1}.

3 RESULTS AND DISCUSSION

3.1. Method validation

All foods targeted by Recommendation (EU) 2019/1888 were first sorted into categories according to their main constituents (i.e. sugar content, water content, or fat content) and their nature (i.e. cereal or vegetable-based food). Ten categories were validated: cereal-based baby food, vegetable and tuber-based crisps, fried potato products, black olives, pancakes, pastries, oil seeds and nuts, dried fruits and confectionaries, cocoa powder for beverages, coffee substitutes (tables B2 and B3). An overview of the detailed validation results is provided in supplementary information Appendix B2. No significant matrix effects were observed for all validated food categories (table B2). Hence, the ME is not taken into account for the determination of the recovery value. All recoveries ranged from 91.7 to 106%, matching EU criteria (70 – 110 %). Precision in repeatability and reproducibility conditions were below 15 %, matching EU criteria fixed at 14.5 % and 22 %, respectively (European Commission 2017) (table B3). For all matrices, LOD and LOQ have been set at 7 and 20 µg kg^{-1}, respectively. fig. B1 represents chromatograms at a level close to the LOQ for each food category analysed in this study. MUs have been calculated from the bias and RSDRW data from control charts initially generated with validation data. Then, MU was fixed at 15 % for all food categories except for coffee substitutes and cocoa powder for beverages (20 %).

Finally, the method has been successfully evaluated by participating in 4 proficiency tests organised by the European Reference Laboratory for processing contaminants. In all cases, acceptable Z-scores (<|±2|) were obtained. Regarding blind samples, a good match was obtained between the fortification level and results, including the measurement uncertainty for the blind samples (table 4.2).

Table 4.2: Z-scores and results obtained for acrylamide in representative proficiency test materials or blind samples

Test material	Assigned value/ fortification value (μg kg^{-1})	Results obtained $\pm$ MU (μg kg^{-1})	Z-score ($\sigma = 22\,\%$)
Proficiency test:			
EURL-PAH 2017 potato chips	673	657 $\pm$ 98.6	-0.1
EURL-PC PT-2018-01 Bread	37.4	34 $\pm$ 5.1	-0.4
EURL-PC PT-2019-03 coffee	244	242 $\pm$ 48.4	0.0
EURL-PC PT-2020-05 cereal-based baby food	37	50.4 $\pm$ 7.56	0.6
Blind samples:			
Fried potato products	75	74 $\pm$ 11.1	
Pastries	150	164 $\pm$ 24.6	
Black olives	300	318 $\pm$ 47.7	
Nuts and oilseeds (roasted)	125	136 $\pm$ 20.4	
Dried fruits and confectionaries	250	254 $\pm$ 38.1	
Cocoa powder	150	142 $\pm$ 28.4	

MU: measurement uncertainty

3.2. Occurrence data

The validated methods have been applied to analyse 210 samples distributed in 10 different food categories. Thirteen potato-based products have been analyzed twice to assess if the cooking mode (oven and deep fryer coking) would have an impact on the final acrylamide content. Eventually, 223 analyses have been conducted. Results of acrylamide occurrence in each food category are presented in table 4.3, while all the individual results are shown in the supplementary information (table B1). Acrylamide has been detected in 61 % of the 223 analyses performed, with content ranging from <LOD to 4389 µg kg^{-1} in a coffee substitute sample (table 4.3). Globally, vegetable crisps, potato-based products other than fries (deep fryer cooked), and black olives reach a relatively high acrylamide level. In contrast, cocoa powder for beverages showed a wide variation in acrylamide content. Similarly, cereals and snacks showed a wide variation of acrylamide content without exceeding BML for related products, except for one sample of honey-roasted muesli (table B1). A wide variation was observed in the acrylamide content in coffee substitutes, depending on the substitutes' nature (EFSA 2015). Breads, pastries, dried fruits and confectionaries were characterized by the lowest detection frequency of acrylamide (table 4.3).

Table 4.3: Detection frequency and minimum, maximum and mean levels of acrylamide detected in the 10 studied food categories

Food category	n	Detection frequency (%)	Acrylamide content (μg kg^{-1})		
			Min	Max	Mean
Vegetable crisps	8	100	98	3063	1012
Black olives	5	100	31.9	575	239
'Californian-style'	*2*	*100*	*432*	*575*	*504*
'Greek-style'	*3*	*100*	*31.9*	*102*	*63.6*
Cocoa powder for beverage	5	100	<LOQ	141	55.9
Potato-based products other than fries	45*	98	<LOD	1503	368
Deep fryer cooked	*26**	*100*	*57.3*	*1503*	*554*
Oven cooked	*19**	*95*	*<LOD*	*377*	*113*
Coffee substitutes	7	86	<LOD	4389	930
Cereals & snacks	64	84	<LOD	353	88.2
Rice and maize crackers	*21*	*95*	*<LOD*	*259*	*104*
Wheat, maize and manioc-based snacks	*21*	*81*	*<LOD*	*337*	*111*
Honey-roasted muesli	*11*	*91*	*<LOD*	*353*	*54.6*
Cookies with chia seeds	*11*	*45*	*<LOD*	*190*	*43.3*
Nuts and oilseeds (roasted)	16	69	<LOD	155	29.5
Pastries	32	53	<LOD	32.5	10.8
Breads	30	33	<LOD	211	17.2
Dried fruits and confectionaries	11	27	<LOD	59.6	8.64

n: number of samples. The mean is calculated on the lower-bound (LB) approach, i.e. LOD and LOQ were fixed at 7 and 20 μg kg^{-1}, respectively. Results below LOQ were replaced by 0. *: 13 samples were cooked twice using two distinct methods (i.e. oven and deep fryer)

3.2.1. Food categories with relatively high concentrations of acrylamide

Vegetable crisps

Vegetable crisps were one of the most contaminated food categories, with a detection frequency of 100 %. No BML exists for vegetable crisps in Regulation (EU) 2017/2158. Potato crips are the closest regulated food category, with a BML set at 750 µg kg^{-1}. Compared to this level, three out of eight samples exceeded this level, i.e. two crisps samples containing beetroot, carrot and sweet potato (1554 and 2183 µg kg^{-1}) and one crisp based on manioc with a maximum acrylamide content of 3063 µg kg^{-1} (tables B1 and 4.3). The type of vegetables used in the crisps may be the source of the acrylamide. However, three other samples with similar compositions contained less than 250 µg kg^{-1} of acrylamide. Those results are in agreement with the study of MacDonald et al. (2023), where they found acrylamide ranging from 58.6 to 2634 µg kg^{-1}, the study of Oellig et al. (2022) from 77.3 to 3090 µg kg^{-1} and with the study of Nguyen et al. (2022), from 123 to 3606 µg kg^{-1}. The higher concentration may be due to the selection of raw materials and/or the cooking process. A recent study by Breitling-Utzmann and Wendler (2019) already demonstrated the strong link between precursor content (asparagine, reducing sugars) in the raw material, temperature, cooking time and final acrylamide levels for some vegetables (beetroot, sweet potatoes and carrots) and the higher acrylamide content generated in sweet potatoes for a similar process.

The sample with an initial acrylamide content of 2183 µg kg^{-1} was repurchased (though with a different batch number). Then, individual vegetable types (beetroots, carrots and sweet potatoes) were sorted and analyzed individually to identify the source of this high acrylamide level. The highest acrylamide contents were found in beetroot and carrot crisps (1117 and 1020 µg kg^{-1}, respectively), followed by sweet potato (548 µg kg^{-1}). These results do not confirm the observations on the higher acrylamide content generated in sweet potatoes of Utzmaan and Hankele (2019). However, these variations can result from the intra-variability of precursors inside a vegetable type depending on the season, geographical area and storage conditions (Breitling-Utzmann and Wendler 2019). Nevertheless, some producers succeeded in having acrylamide content largely below the BMDL for potato crisps set at 750 µg kg^{-1} (European Commission 2017). Since vegetable crisps have grown in popularity, it might be interesting to see if applying the mitigation measures fixed for potato-based crisps could effectively reduce their acrylamide content as well. Moreover, Annex I of Regulation (EU) 2017/2158 already fixed mitigation measures for potato-based crisps, such as the selection of variety showing fewer precursors content, stock and transportation conditions and cooking processing to limit acrylamide formation at the maximum (European Commission 2017). The same information for vegetable crisps could be investigated and subsequently implemented.

Coffee substitutes

Coffee and cereal and/or chicory coffee substitutes are included in Regulation (EU) 2017/2158 with BML ranging from 400 to 4000 µg kg^{-1} (table 4.3), depending on their type (EFSA 2015). Moreover, Recommendation (EU) 2019/1888 extends the monitoring to coffee substitutes not based on cereal or chicory as well. Seven samples were purchased on the market, including web shops. These are detailed in table B1.

Acrylamide was detected in 86 % of the samples and ranged from < LOD to 4389 µg kg^{-1}, the highest level detected in any sample in this study (table 4.3). Acrylamide was not detected in the sample mainly composed of fruits and tea leaves, while the 5 plant and/or nuts-based

samples contained acrylamide levels in the same order of magnitude as regular roasted coffee (250 µg kg^{-1}) (EFSA 2015). Higher acrylamide content was found in coffee containing roasted chicory roots as expected for this coffee type, and the highest concentration (4389 µg kg^{-1}) was found in a sample of roots and rhizome (table B1). As rhizomes and roots contain more sugar than the other parts of the plant, this may explain this high level of acrylamide (Mojska and Gielecińska 2013). The coffee substitutes reported in the literature were always based on cereals or chicory but not on other alternatives. Claeys et al. (2016) reported acrylamide levels of up to 5400 µg kg^{-1} for 84 coffee substitutes on the Belgian market between 2002 and 2013 (Claeys et al. 2016), while the concentrations reported in the EFSA opinion on acrylamide were 510 µg kg^{-1} for malt, barley and wheat coffee, 2942 µg kg^{-1} for chicory coffee (EFSA 2015). It should be noted that the EU recommendation requested data for coffee substitutes, not based on cereals and chicory (European Commission 2019). However, the presence of acrylamide in this product should not be a concern for the general Belgian population as the availability of such products on the Belgian market is very limited.

Potato-based products

French fries were among the first foods deeply studied, as they showed relatively high acrylamide concentrations. However, data is lacking for fried potato-based products other than fries. For instance, the EFSA opinion of 2015 was based on 1598 data on french fries but only 96 data for other potato fried products. Sweet potato fries have also gained in popularity in recent years. Hence, Recommendation (EU) 2019/1888 targeted these food products to gather missing data.

Therefore, potato balls, potato cubes, duchesse potatoes, croquettes, rosti and sweet potato fries were purchased and regrouped into the potato-based products category. This food category was divided depending on the cooking mode (oven or deep-fried cooking). The results show (table 4.3) that deep-frying generates a higher concentration of acrylamide (up to 25 times, compared to oven cooking) with a maximum acrylamide content of 1503 µg kg^{-1} for a potato cube sample (tables B1 and 4.3). Interestingly, when a golden color was obtained at the end of the preparation, the acrylamide content was lower than the BML for 'Other potato products from potato dough' fixed at 750 µg kg^{-1}. This color is also mentioned in Regulation (EU) 2017/2158 as a criterion for achieving acceptable acrylamide levels in fried food (European Commission 2017). However, information about stopping cooking when a golden color is reached was mentioned only on 50 % of the labels. A brown color was obtained for all samples with a concentration higher than 750 µg kg^{-1} despite the strict application of cooking instructions. More indications about the final golden color could reduce the acrylamide levels. Additionally, 13 samples were prepared both with oven and deep fryer to investigate the impact of the cooking mode on the acrylamide content, both following the manufacturer instructions. The results confirmed that deep-frying led systematically to higher acrylamide levels (table 4.4). The acrylamide content for seven of the 13 deep-fried samples was below the BML of 750 µg kg^{-1}.

For all oven-cooked samples, the acrylamide content was below the BML, below the LOQ for three samples and was not even detected in a potato ball sample (table 4.4). fig. 4.1 shows the comparison between 4 fried potato dough-based sub-categories, but the high dispersion of results for potato balls and potato cubes does not allow to draw any conclusion.

A specific sub-category was the 'sweet potato fries', a novel food category with increasing popularity. Relatively high concentrations of acrylamide were found (table B1). Currently,

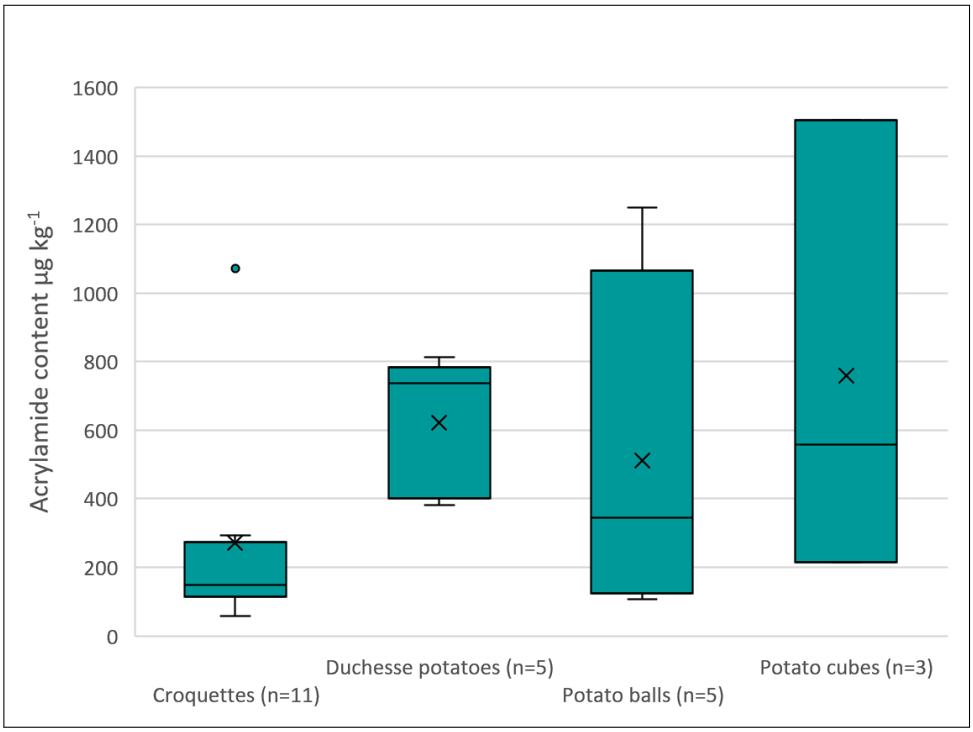

Figure 4.1: Acrylamide content in the four fried potato dough-based sub-categories. Boxes indicate 25th, 50th and 75th percentiles, whiskers represent minimum and maximum, the cross represents the average, and the horizontal line represents the median. Values <LOD and LOQ have been replaced by 0. Statistical outliers are represented by a dot

no BML applies to this product in Regulation (EU) 2017/2158, but the most closely related category is potato fries, with a BML of 500 µg kg^{-1}. All samples based on sweet potato fries exceeded this level (table B1). These results show that including sweet potato fries in Regulation (EU) 2017/2158 might be relevant, including mitigation measures.

Table 4.4: Acrylamide content in 13 samples that were cooked both with oven and deep-fryer

Samples	Acrylamide content (μg kg^{-1})		Acrylamide content ratio (deep fryer/oven)
	deep fryer	oven	
Croquettes (Brand A)	1071	199	5.4
Duchesse potatoes (Brand A)	813	34.7	23
Duchesse potatoes (Brand B)	755	27.7	27

Table 4.4 (continued)

Samples	Acrylamide content (μg kg^{-1})		Acrylamide content ratio (deep fryer/oven)
	deep fryer	oven	
Duchesse potatoes (Brand C)	737	<LOQ	N.A
Duchesse potatoes (Brand D)	419	57.7	7.3
Duchesse potatoes (Brand E)	381	<LOQ	N.A
Potato balls (Brand A)	1248	81.0	15
Potato balls (Brand A)	516	<LOQ	N.A
Potato balls (Brand B)	172	<LOD	N.A
Potato balls (Brand C)	107	54.5	2.0
Rosti (Brand A)	1199	216	5.6
Rosti (Brand B)	506	63.6	8.0
Sweet potato fries (Brand A)	981	46.7	21

LOD: 7 μg kg^{-1} LOQ: 20 μg kg^{-1} N.A: Not Applicable

Black olives

The acrylamide content in black olives ranged from 31.9 to 575 µg kg^{-1}, showing a wide variation (table 4.3, S1). Samples were divided into two sub-categories: natural black olives, also called 'Greek-style' and artificially colored (oxididized) black olives, known as 'Californian-style'. The 'Californian-style' olives are artificially colored by air oxidation and the use of the color fixative agent E579 (ferrous gluconate), followed by a sterilization step. The sterilization process involves temperatures ranging from 110 °C to 121 °C for up to 50 minutes, exceeding the temperature threshold for acrylamide formation. This additional step in the production of 'Californian-style' olives could possibly explain why the average acrylamide content (503 µg kg^{-1}) is 5 times higher than that of the 'Greek-style category' (63.9 µg kg^{-1}) (table 4.3).

The E579 agent does not contribute to acrylamide formation (Casado and Montaño 2008; Charoenprasert and Mitchell 2014) but may indicate the use of the Californian-style process and the presence of a sterilization step. Several authors have reported that this sterilization process primarily contributes to acrylamide formation in black olives (Amrein et al. 2007; Casado and Montaño 2008; Charoenprasert and Mitchell 2014; Brenes-Álvarez et al. 2024). Breitling-Utzmann et al. (2023) studied the link between acrylamide in oxidized black olives and their packaging. They found that, on average, olives packed in cans and glass jar contained more acrylamide than those packed in plastic boxes. They reported that this result may be due to the fact that plastic boxes cannot undergo intensive sterilization as cans and glass jars.

A study by Martín-Vertedor et al. (2020) demonstrated that the ripeness stage of olives significantly impacts acrylamide content in table olives, with differences of up to 39 %

depending on ripeness. Consequently, ripeness could be an important parameter to consider in processing and in future mitigation measures. Interestingly, Duedahl-Olesen (2019) conducted a study on the effects of home cooking on black olives, and observed an extremely high acrylamide formation (up to 5270 µg kg^{-1}) for 'Californian-style' black olives.

Since 'Californian-style' olives are widely consumed in Mediterranean dishes (pasta/pizzas) or appetizers, it may be relevant to regulate this kind of olives and suggest mitigation measures to reduce the acrylamide exposure associated with the sterilization process.

3.2.2. Food categories with variable concentrations of acrylamide

Cocoa powder

Acrylamide was detected in the 5 cocoa samples, with cocoa content ranging from 13 to 100 % (table 4.3). Levels of acrylamide ranged from <LOQ to 141 µg kg^{-1}, with no correlation between acrylamide and cocoa contents. The mean acrylamide content in this study was 58.0 µg kg^{-1}, which is approximately three times lower than the mean concentrations reported in the EFSA report (178 µg kg^{-1}) (EFSA 2015). It is also between 5 and 6 times lower than the mean concentrations reported in a UK study (364 and 274 µg kg^{-1} in 2020 and 2021, respectively), for which the exact nature of the samples was not available (MacDonald et al. 2023). A recent study conducted by Kruszewski and Obiedziński (2020) found the same observed and studied the origin of such discrepancies. They concluded that it might be explained by the variety and geographical origin of cocoa beans, the form of roasted cocoa beans (whole beans or nibs) and the roasting conditions (temperature, relative humidity and roasting time).

Cereals and snacks

'Cereal and snacks' are generally consumed as snacks or appetizers (table 4.3). Acrylamide has been detected in 84 % of the 64 samples, ranging from <LOD to 353 µg kg^{-1}, showing wide variability (table 4.1 and fig. 4.2). Maize and rice crackers and maize, wheat, manioc snacks have been further subdivided according to the cereal type (maize, rice, wheat, manioc) and the process involved (extrusion, puffing) to assess if there was a difference in acrylamide content depending on the cereal type or the heating process involved. For instance, crackers are usually extruded, and snacks samples were puffed maize of rice grains. fig. 4.2 illustrates the wide variation of acrylamide observed among cereals and snack products. Manioc data is not shown, as only 2 samples were analyzed, but no acrylamide was detected in both samples (table B1). Samples composed of a mix of cereals are not shown either. These results are similar to those found in a recent UK retail monitoring study where acrylamide content varied from 50 to 474 µg kg^{-1} (MacDonald et al. 2023).

In the present study, all acrylamide levels were below the BML for 'cereal crisps other than potato crisps', which is the most closely regulated food category with a BML set at 400 µg kg^{-1} (European Commission 2017). However, it is very common for parents to give rice and maize crackers or extruded corn/wheat corn as snacks to young children. For 'baby foods, processed cereal-based foods for infants and young children excluding biscuits and rusks' and 'biscuits and rusks for young children', the BMLs are specifically set at 40 and 150 µg kg^{-1}, respectively. In this case, one sample exceeded the BML, a rice waffle dedicated to young children with an acrylamide content of 41.5 µg kg^{-1}.

Nevertheless, it is essential to understand if the variation comes from the raw materials

and/or the process. A review by Žilić et al. (2022) concluded that acrylamide precursors in corn-based products strongly depended on genetic factors and environmental and storage conditions. Hence, it might be relevant to regulate this food category and apply mitigation measures based on selecting the cereal varieties and optimizing the storage conditions.

In the 'honey roasted muesli' category, the acrylamide content was below 60 µg kg^{-1} for 10 out of 11 samples, except for one outlier at 353 µg kg^{-1}. This suggests that honey does not appear to increase acrylamide content compared to other products in the cereals and snacks category. The elevated acrylamide level of the outlier may also originate from other sources, such as additional ingredients or the cooking process (table B1).

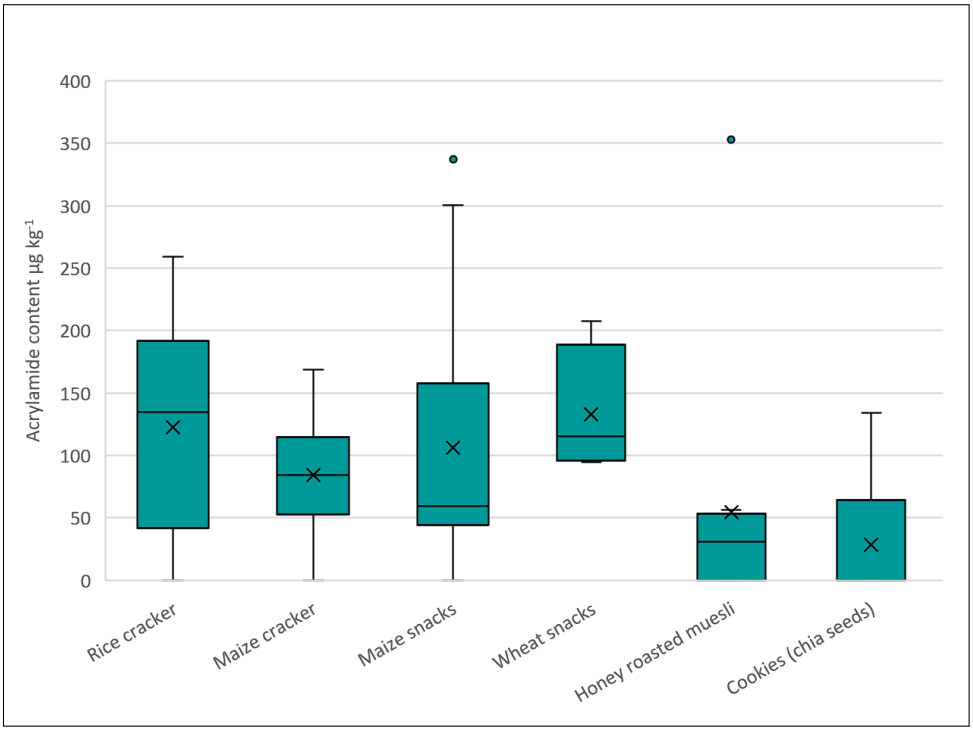

Figure 4.2: Acrylamide content in cereals and snacks food sub-categories. Boxes indicate 25th, 50th and 75th percentiles, whiskers represent minimum and maximum, the cross represents the average, and the horizontal line represents the median. Values <LOD and LOQ have been replaced by 0. Statistical outliers are represented by a dot. Rice and maize crackers were extruded and maize and wheat snacks were puffed

The acrylamide content in the eleven products containing chia seeds varied between <LOD to 190 µg kg^{-1} (tables B1 and 4.3 and fig. 4.2) with an average of 43.3 µg kg^{-1}. No link could be made between acrylamide content and percentage of chia seeds used in the cookie recipes for these eleven samples. Acrylamide contents in this study are lower than those in previous studies since Das and Srivastav (2012) found contents of 100 to 600 µg kg^{-1} for biscuits and crackers (without chia seeds), and Croft et al. (2004) reported contents of 118 to 573 µg kg^{-1} in biscuits. The EFSA opinion mentions acrylamide contents of 201 to

297 µg kg^{-1} for cookies but does not specify the presence or absence of chia seeds (EFSA 2015). However, recent studies by Mesías et al. (2023) and Hölzle et al. (2024) demonstrated that acrylamide were found in chia seeds undergoing a thermal process. Notably, roasting ground chia seeds produced up to 9 times more acrylamide than roasting whole seeds. In the present study, only whole chia seeds were included in the samples. However, a recent study by Schouten et al. (2024) studied the effect of adding 10 % nuts, dried fruits, dried seeds and black olives to biscuits on acrylamide content. They concluded that, although this addition increased acrylamide levels, accurately predicting the final content based on individually cooked ingredients was challenging. This highlights the importance of understanding how ingredients interact during the cooking process. In the present study, chia seeds were present in limited proportion in comparison with these studies (<6 % on average) (table B1), which may further mitigate acrylamide formation.

3.3. Food categories with low acrylamide content

Bread and pastries

Bread and pastries exhibited detection frequencies of 33 % and 53 %, respectively, as presented in table 4.3. The quantified concentrations ranged from values below LOD to 211 µg kg^{-1} for bread and from below LOD to 32.5 µg kg^{-1} for pastries. The low detection level in these categories was expected as acrylamide is mainly produced in the crust, which represent a small surface-to-volume ratio and then a small percentage of the total sample. Consequently, acrylamide is diluted throughout the food product. The obtained results are similar to the results previously reported by Croft et al. (2004) (i.e. < 25 µg kg^{-1} for croissants and from 25 to 50 µg kg^{-1} for bread) but lower compared to the data included in the EFSA opinion on acrylamide (i.e. 61 to 71 µg kg^{-1} for cakes and pastries) (EFSA 2015) and the UK monitoring of 2020-2021 (92 µg kg^{-1} for pastries and 63 µg kg^{-1} for bread in average) (MacDonald et al. 2023). Nevertheless, two bread samples contained higher acrylamide content, i.e. a rusk sample (211 µg kg^{-1}) and a bread containing olives (98 µg kg^{-1}). The larger surface-to-volume ratio can explain the higher acrylamide concentration in rusk. These results agree with the study of MacDonald et al. (2023), who obtained 194 µg kg^{-1} on average for similar products.

As illustrated above, the presence of olives in bread may explain higher acrylamide concentrations, since maximum acrylamide concentrations in olives were up to 575 µg kg^{-1} (table 4.3). No correlation was identified between acrylamide levels in bread and the inclusion of chia seeds or flour. Galluzzo et al. (2021) conducted an extensive investigation into the influence of chia flour on the acrylamide content of wheat bread. Their findings indicate that substituting wheat flour with chia flour does not lead to an increase in acrylamide content.

Dried fruits and confectionaries

Among eleven samples, only 2 were >LOQ, one nougat and one fudge with acrylamide contents of 25.4 and 59.6 µg kg^{-1}, respectively. No acrylamide was detected in dried fruits. Results are similar to those of the Canadian acrylamide monitoring program of 2012 and the UK national survey of 2020-2021 (Bureau of Chemical Safety 2012; MacDonald et al. 2023). However, the UK study detected a high acylamide level in one dried apricot sample (454 µg kg^{-1}). Amrein et al. (2007) found variable but quantifiable (>10 µg kg^{-1}) acrylamide content in dried fruits ranging from 19 to 146 µg kg^{-1}. De Paola et al. (2017) confirmed those results

with acrylamide content in dried prunes ranging from 14.7 to 124 µg kg^{-1}.

Nuts and oilseeds (roasted)

The detection frequency for acrylamide in the 16 samples of nuts and oilseeds (roasted) was 69 %. Acrylamide content ranged from <LOD to 155 µg kg^{-1} with an average of 29.5 µg kg^{-1} (table 4.3). The highest concentration (considered an outlier) was found in a sample of roasted oilseed (table B1). These results are in accordance with the results of De Paola et al. (2017) and the UK monitoring of 2020-2021 (MacDonald et al. 2023) since low acrylamide levels (below 100 µg kg^{-1}) were found for most samples. In their study, the highest results were found in sunflower seeds, although low concentrations were detected in other sunflower seeds. Other studies reported acrylamide concentrations that varied widely from <LOD for almonds, hazelnuts, pine nuts and walnuts and <LOQ for pistachios up to 1000 µg kg^{-1} in roasted almonds (Amrein et al. 2005; Žilić 2016; De Paola et al. 2017). Jägerstad and Skog (2005) reported an acrylamide concentration of 66 µg kg^{-1} in sunflower seeds, while a mean value of 93 µg kg^{-1} was reported in the EFSA opinion on acrylamide for roasted nuts and seeds (EFSA 2015).

4 CONCLUSION

This comprehensive study of foods on the Belgian market provided valuable additional data on acrylamide in all the food categories targeted by the Recommendation (EU) 2019/1888. Occurrence data revealed relatively elevated acrylamide levels in vegetable crisps and 'Californian' style black olives, coffee substitutes (without chicory) and deep-fried potato-based products. Adapted mitigation measures on the variety selection, condition storage of the raw materials and the process could also be implemented for these food categories. In contrast, dried fruits, confectionaries, bread, and pastries exhibited a low detection frequency of acrylamide. Lastly, this study did not confirm the hypothesis that chia flour or seeds may generate substantial amounts of acrylamide compared with traditional wheat bread.

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Disclosure statement

The authors report there are no competing interests to declare

II. Influence of the Belgian cooking habits on acrylamide formation

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1 INTRODUCTION

Since its discovery in 2002, the formation and occurrence of acrylamide have been extensively studied in specific types of food. Previous sections have addressed additional food categories for which limited data were available, despite evidence that they may contain significant amounts of acrylamide. However, most of the existing data, including those considered in the 2015 EFSA opinion (EFSA 2015), share a major limitation: they are primarily derived from ready-to-eat or industrially processed foods available on the market. This bias results from the fact that most datasets originate from national monitoring programs, which focus on products from food business operators rather than on foods prepared by consumers.

Yet, acrylamide can also be formed during home cooking practices such as toasting bread, frying croquettes, or preparing French fries, whether in an oven or a deep fryer as it has been demonstrated in the previous section. Several studies have demonstrated that relatively high acrylamide levels may arise from these domestic processes, even though such data are never included in official monitoring. For example, Jackson and Al-Taher (2005) reported acrylamide levels ranging from 55 to 2130 µg kg^{-1} French fries fried between 150 °C and 190 °C for up to ten minutes. Despite the recognized formation of acrylamide during home cooking, current exposure assessments generally fail to consider this source. The datasets used for these assessments are based on analyses of commercial products, as required under Commission Regulation (EU) 2017/2158, which establishes mitigation measures and benchmark levels for food business operators. Consequently, exposure estimates do not account for the contribution of household cooking, which may vary considerably depending on consumer habits.

Recent studies highlight the importance of this overlooked aspect. Eicher et al. (2020) analyzed 71 home-cooked rösti samples and found average acrylamide levels 16 % higher than those reported by EFSA in 2015. The authors also noted elevated sugar contents, suggesting that the potatoes had been stored in the refrigerator. Cold storage promotes the conversion of starch into reducing sugars, which are key precursors in acrylamide formation, a fact that remains largely unknown to the general public.

In the United Kingdom, public health authorities have already taken steps to address this issue through the “Go for Gold” campaign, which provides practical advice to consumers. Recommendations include targeting a golden-yellow color when cooking potato-based products and avoiding storage temperatures below 6 °C to prevent excessive sugar formation. Nevertheless, studies on home cooking remain surprisingly limited. The available evidence clearly indicates that consumer cooking practices can significantly influence acrylamide levels in foods, and thus potential dietary exposure.

The present study aims to investigate Belgian home cooking habits and their impact on acrylamide formation. Participants were invited to prepare common foods such as croquettes, sweet potato fries, and bread according to their usual cooking practices. In addition, a questionnaire was used to collect information on these practices, enabling the assessment of how typical Belgian cooking behaviors may affect acrylamide content and the associated health risks.

2 MATERIAL AND METHODS

2.1. Candidate recruitment

Participants were recruited through an e-mail invitation distributed across various departments of the Sciensano Ixelles site, including both scientific and non-scientific units. The message did not mention acrylamide analysis, contaminants, or the specific objective of the study, in order to minimize potential bias in sample preparation by participants.

Both scientific and non-scientific staff were invited to participate, with the dual purpose of reducing the likelihood that participants could guess the actual aim of the study—non-scientists being less likely to identify the link with acrylamide study—and of diversifying the participant profiles in terms of background and cooking practices habits.

Volunteers who agreed to participate were asked to select the type of food they wished to prepare (croquettes, sweet potato fries, or buns) and to indicate the cooking method they would use (deep-fryer, oven, toaster, or other).

2.2. Sample purchasing

Croquettes, sweet potato fries, and buns were purchased from Belgian retailers. Brand selection was based on Belgian market share data obtained from the Euromonitor database (Euromonitor, 2017). All samples were purchased from the same production batch to avoid variability arising from differences in raw materials or manufacturing processes. Croquettes and sweet potato fries were stored at -20 °C, while buns were kept at room temperature until distribution.

2.3. Sample distribution and questionnaire

Each participant received the selected food sample together with an anonymized questionnaire. Each questionnaire was linked to a participant only through a randomly assigned identification number. The questions focused on consumer behaviour related to the preparation and cooking of the received food items—again, without mentioning acrylamide or any other contaminants, to minimize bias during the food cooking. The questionnaire covered aspects such as cooking practices, temperature and duration of heating, respect of cooking instruction present on food packaging. Both the questionnaire and the participants' responses are available in Appendices B3 and B4. After cooking the food samples, participants were instructed to return the completed questionnaire along with a portion of the cooked food for analysis.

2.4. Sample handling and homogenization

For oven cooking, participants have been asked to sample croquettes/fries from the center of the oven but also from the four outer parts in order to get a representative cooked portion since the cooking process is not homogeneous. In case of the deep-fryer, the participants were asked to prepare the same portion as they usually do, then mixed it properly before setting aside a portion for analysis. A portion consisted of 5 – 6 croquettes, 10 – 12 sweet potato fries and one bun. Upon reception; pictures of samples were taken to make the link

between their color and acrylamide content. All samples pictures are available in Appendix B5. Samples were then homogenized using a mill (IKA M20, IKA®-Werke GmbH & Co. KG, Staufen, Germany), in accordance with the requirements of Regulation (EU) 2017/2158 and Regulation (EC) 333/2007 (European Commission, 2007; 2017). Homogenized samples were stored at -20 °C until analysis.

2.5. Sample analysis

Food samples were analysed using an in-house adapted QuEChERS extraction method, following the procedure described in Chapter 4, Section I, § 2.4–2.6. Quality control was performed as outlined in Chapter 4, Section I.

3 RESULTS AND DISCUSSION

A total of 14 participants have been recruited to study Belgian cooking practices. The table 4.5 shows the number of participants, the food they chose to cook and the cooking devices they possessed. Most participants prepared the food in the oven or purchased this kind of food at a “frietkot”, while only 5 participants used a deep-fryer at home.

Table 4.5: Overview of food items allocated to the participants in the cooking habits evaluation study and their personal cooking devices

Participant	Food			Cooking device		
	Croquettes	Sweet potatoes fries	Buns	Deep-Fryer	Oven	Toaster
1	X		X		X	X
2	X	X	X		X	X
3		X	X		X	X
4	X			X	X	
5	X				X	
6	X		X	X		X
7	X				X	
8		X	X		X	X
9	X			X	X	X
10	X	X			X	
11	X	X	X	X	X	X
12	X	X			X	
13	X			X		
14	X				X	
Total	12	6	6	5	12	7

3.1. Trends in cooking habits

While evaluating the answers to the questions related to the cooking habits, only the preparation of croquettes and sweet potato fries have been taken into account since no specifications are given for the preparation of buns, except that it can be done in the oven or with a toaster.

Respect and considerations of cooking instructions:

Participants were asked if they usually respect the cooking instructions available on the label. Five participants answered ”No” (36 %) while 64 % answered “Yes” (Appendix B4)

After questioning the respect of cooking instructions, the participants were also asked to indicate their thoughts on the labeled instructions. It has to be noted that the question was totally open in order to avoid bias. Most of the participants answered that they respect these instructions as a basis and adapted it according to their taste. Five of them clearly specified that they adapted the cooking time because the specifications on the label were insufficient to cook the food properly.

Criteria to stop the cooking:

Participants were also asked to mention what their criteria were to stop the cooking. Twelve participants out of 14 mentioned that they stopped the cooking based on the color of the food item. They used different terminology to characterize this color, but most of the time the golden color is cited which is also the preconized one in the commission Regulation (EU) 2017/2158. Four participants out of 14 mentioned also that they referred to their timer and that they respected the time mentioned on the label. Four participants referred also to non-quantitative criteria such as the crispy aspect of the food item.

3.2. Acrylamide content

3.2.1. Preparation of croquettes

Twelve participants prepared croquettes at home, of which 4 used a deep-fryer and 8 used an oven. The table 4.6 summarizes all cooking parameters that were used and the concentration of acrylamide found in the prepared food. The acrylamide content found in the food prepared by the participants showed a very wide variation ranging from <LOD up to ~500 µg kg^{-1} although cooking parameters are, in most cases, similar. Therefore, a more thorough investigation using the pictures and questionnaires was needed.

Table 4.6: Cooking parameters and acrylamide content of croquettes cooked by participants

Participant (n°)	Cooking mode	T (°C)	Time (min)	AA content (μg kg^{-1})
1	Deep-Fryer	175	6	78.7
6	Deep-Fryer	175	4.5	127
9	Deep-Fryer	175	8	<LOD
10	Deep-Fryer	180	4	295

Table 4.6 (continued)

Participant (n°)	Cooking mode	T (°C)	Time (min)	AA content (μg kg^{-1})
12	Oven*	220	16	339
13	Oven*	190	16	252
14	Oven	180	29	140
2	Oven*	220	17	500
4	Oven	220	18	173
5	Oven	220	16	288
7	Oven	220	22	52.1
11	Oven*	220	16.5	295

* Rotating heat program has been used

Croquettes – deep-fryer cooking:

Most of the participants respected the temperature indicated on the label (175 °C). Only one participant used a higher temperature of 180 °C (corresponding to the previous temperature requirements for this kind of food) and obtained the highest acrylamide content for deep-fryer cooking. Concerning the cooking time, a wide range of times have been used ranging from 4 min as written on the label to 8 min (i.e. twice the preconized time). Even if most of the participants applied a cooking time that exceeded the manufacturer's instructions, acrylamide content was in all cases much lower than the benchmark level of 750 µg kg^{-1} as mentioned in the commission Regulation (EU) 2017/2158 for potato products from potato dough category (category closest to croquette). When plotting the acrylamide content versus the cooking time, without taking into account the temperature as they are all very similar, it could be noted that there is an inverse correlation between these two parameters.

When looking to the pictures of the corresponding cooked items (Appendix B5), colors ranging from golden to dark orange have been obtained and are positively correlated to acrylamide concentration. For participant 9, acrylamide content seems to be an aberrant value as the croquettes were cooked for 8 minutes and no acrylamide was found. Nevertheless by checking the corresponding pictures, it can be seen that croquette is very pale compared to the others. This may result from a mistake in the answers or a malfunction in the temperature controller itself. This last point can be also the conclusion for other participants to a lesser extent: as it is impossible to link time cooking to acrylamide content. The explanation may be that the accuracy of the thermostat on domestic fryers is limited and may differ from model to model.

Croquettes – oven cooking:

The concentration of acrylamide detected in the croquettes prepared in the oven varied widely, but was in all cases below the benchmark level of 750 µg kg^{-1}. When looking at the cooking parameters of the participants, most of them respected the temperature indicated on

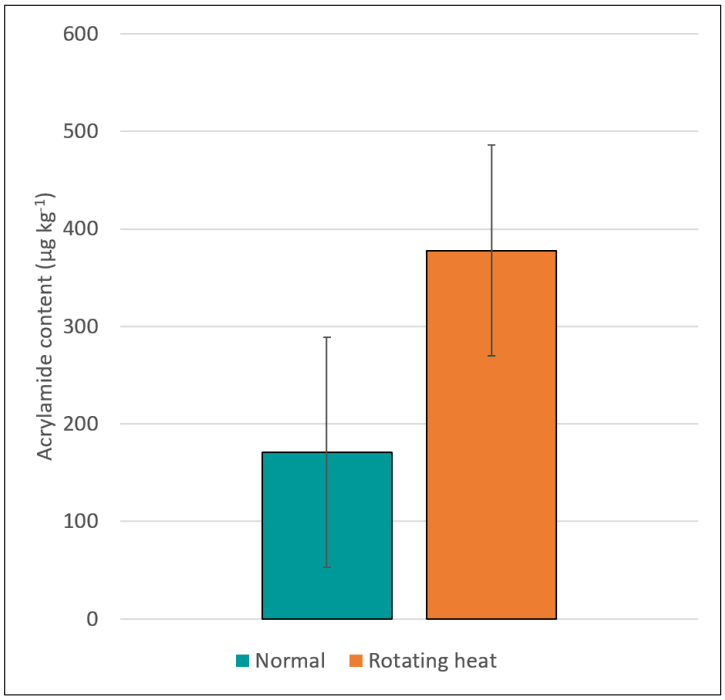

Figure 4.3: Acrylamide content in croquettes cooked by participants depending the oven program used

the label (220 °C), while two participants (i.e. participant 13 and 14), cooked the croquettes at a lower temperature, i.e. 190°C and 180°C respectively. The manufacturer Recommendation for cooking time is 16 min which has been respected by most of the participants with cooking times ranging from 16-18 min. Participants 14 and 7 exceeded the preconized time for several minutes (i.e. 29 and 22 min, respectively). Even tough, only 140 µg kg^{-1} of acrylamide has been found in the sample prepared for 29 minutes by participant 14. This could be explained by the temperature used for the preparation that is far below the 220 °C as recommended by the manufacturer. By checking more thoroughly the questionnaire of this participant, it can been seen that the criteria to stop the cooking is listed as the gold color but, the participant also indicated that in general he finds the cooking time specified on the label are too short. Finally, candidate 7 cooked croquettes during 22 min at 220 °C and only 52.1 µg kg^{-1} of acrylamide has been found. This cooking time is more than 5 min longer than the preconized cooking time ~16 min. When checking the questionnaire, participants 7 also answered that it is necessary to extend the cooking time in order to improve the 'crispy' character, although the cooking process is also stopped based on the color. Both of these participants do not have the rotating heat mode.

Comparison of the cooking modes:

The wide range of cooking temperatures used can be explained by the oven cooking program used. Half of participant used rotating heat (participants 2, 11, 12, 13) while other participants

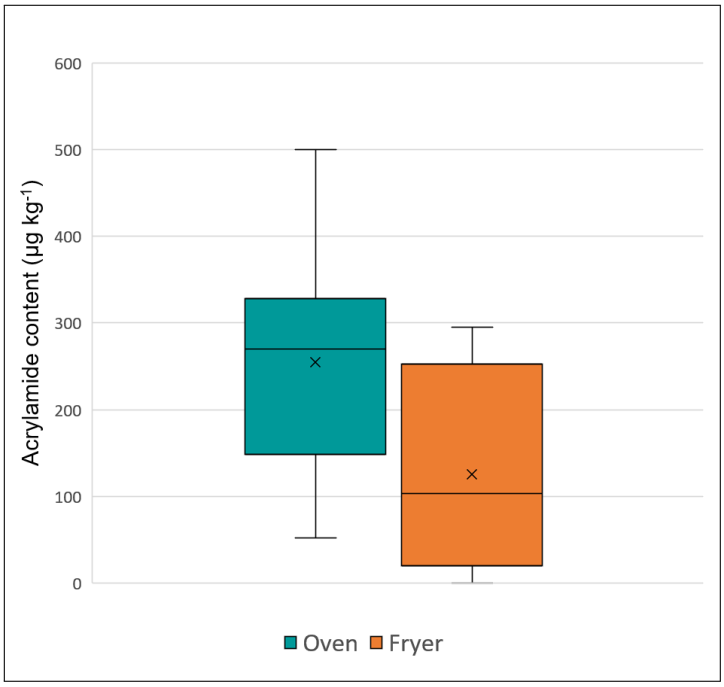

Figure 4.4: Acrylamide content in croquettes cooked by participants depending on the cooking mode

use a normal cooking program (information available in the questionnaires in Appendix B4). As the rotating heat program is very efficient, the cooking time is in general lower compared to the classical program, this point is also confirmed in the Regulation (EU) 2017/2158. It is also illustrated in fig. 4.3 where it can be seen that the average level of acrylamide is higher with rotating heat for a shorter period of time compared to the classical program. Hence it explains why there is no positive correlation between cooking time and acrylamide content. Again, a positive correlation between the color obtained ranging from gold to dark orange and acrylamide content (see pictures Appendix B5). This confirms that the cooking parameters are more linked to the device used (quality of the model, program used) than the acrylamide content. Some participants also report (information not in questionnaires) that the cooking time depends strongly of the oven used. These declarations support these results. Pictures of prepared samples in combination with the applied temperature can indicate the presence of acrylamide, regardless of the cooking time.

As the fig. 4.4 below shows, the cooking mode does not seem to have a significant impact on the acrylamide content. This may be explained by the fact that most of participants respected the preconized temperature and tend to get a golden color. This golden color coupled to a good temperature are adequate criteria to guarantee a safe acrylamide content for the consumer, regardless of the cooking time and cooking mode.

Based on these results, it can be concluded that it is challenging to find a correlation between the cooking parameters and the acrylamide content detected in the prepared food as all

participants possessed different oven models oven and use different cooking programs such as rotating heat. Nevertheless, the most important finding is that participants respect in majority the instructions on the label, especially the temperature. Even if the cooking time is not respected, most participants stop the cooking based on the golden color of croquettes. If temperature and adequate color are respected, acceptable levels of acrylamide are found. The non-respect of cooking time is not considered to be a problem and depends more on characteristics inherent to model of oven/deep-fryer used.

3.2.2. Preparation of sweet potato fries

A total of 8 samples of fries have been analyzed, 4 with a deep-fryer and 4 in oven. The table 4.9 summarizes all cooking parameters and the detected acrylamide content. Most of the participants declared that they are not familiar with sweet potato fries. Hence, it was requested to attempt several times in order to obtain the adequate color and texture (according to their own taste).

Table 4.7: Cooking parameters and acrylamide content of fries cooked by participants

Participant	Cooking mode	T (°C)	Time (min)	AA content (μg kg^{-1})
2	Deep-Fryer	170	6.5	34.5
9	Deep-Fryer	170	6.3	35.1
10	Deep-Fryer	180	2.0	134
3	Fryer (air-)	180	12.0	179
3	Oven	220	20.0	166
8	Oven	220	11.0	<LOD
12	Oven	220	17.0	76.3
11	Oven	220	16.0	59.3

Sweet potatoes fries – deep-fryer cooking:

One candidate used an air-fryer. As this cooking mode is different from classical deep-fryer, it is difficult to include it for comparison. Cooking instructions, available on the packaging, were a cooking temperature of 175 °C during 2.5-3 min. Fifty percent of the participants used a cooking temperature of 170 °C, the others applied 180 °C. Nobody followed the Recommendation of the cooking temperature. This could be explained by the absence of this graduation on devices. Nevertheless, only low acrylamide concentrations were determined, even for participants that applied very long cooking times. Furthermore, none of the results exceeded the benchmark level of 500 µg kg^{-1} as stated in the Regulation (EU) 2017/2158 for French fries, which was considered to be the closest food category to sweet potato fries. Plotting the cooking time versus acrylamide content has not been judged necessary as it will result in the same observations as discussed for the croquettes.

A good correlation was found between the color of the fries and its acrylamide content. For instance, pictures of candidate 2 are very light and the lowest acrylamide content (34.5 µg

kg^{-1}) was detected while, the fries of participants 3 were slightly burned on the extremities and they contained the highest amount of acrylamide.

Sweet potatoes fries – oven cooking:

For the preparation of sweet potatoes in an oven, the same order of magnitude of acrylamide content was found as for the deep-fryer. The preconized temperature was 220 °C and the cooking time 15-20 min. All participants respected indications for temperature and time, except the candidate 8 who baked fries during only 11 min. No acrylamide was detected in this food item. By checking the questionnaire, the mode used for the preparation was very specific, i.e. "turbo pizza" specifically designed to get food brown and crusty. This description does not seem to link to the absence of acrylamide. By checking the picture of this cooked sample (Appendix B5), it can be seen that the sample is lightly orange, pale and absence of burned parts which could indicate that no acrylamide has been generated during the short cooking time.

Furthermore, by checking the questionnaire more thoroughly, the participant declared that the taste was good but that the fries were not crusty at all, which is coherent with the absence of acrylamide. Therefore, it can be concluded that it was logical that no acrylamide was found. When comparing the pictures it can be seen that the most colored and burned fries (candidate 3, oven and deep-fryer) are linked to the highest acrylamide content. This confirms that the color is a good indicator of the acrylamide content if we consider samples cooked at the same temperature.

Sweet potatoes fries – comparison of cooking mode:

fig. 4.5 shows that there is no significant differences between cooking in the oven and in the deep-fryer. No link was found between the acrylamide content and the cooking parameters as they reflected more the quality of the equipment used during the cooking. Furthermore, acrylamide concentration was low and in the same order of magnitude, regardless of the technique used. Therefore it was not relevant to continue further the interpretation of these results.

The conclusion is similar to the evaluation of the croquettes. When consumers respect the golden colors and an adequate cooking temperature, safe acrylamide content will be generated. It is not relevant to make a link between the cooking time and acrylamide content. Cooking time is more reliant on cooking model devices, cooking programs and others non-controllable parameters.

3.2.3. Preparation of Buns

A total of 4 buns have been analyzed by participants since 2 participants were not able to cook the samples as they were too thick to enter in their toaster. Three of them have been cooked with a toaster, and 1 with oven in grill mode. No correlation can be made between cooking parameters and acrylamide content as it depends strongly of the power of the device, the design of the device such as position and number of heating resistances. However, this food group enables to have an overview of the acrylamide concentration that may be generated during buns cooking.

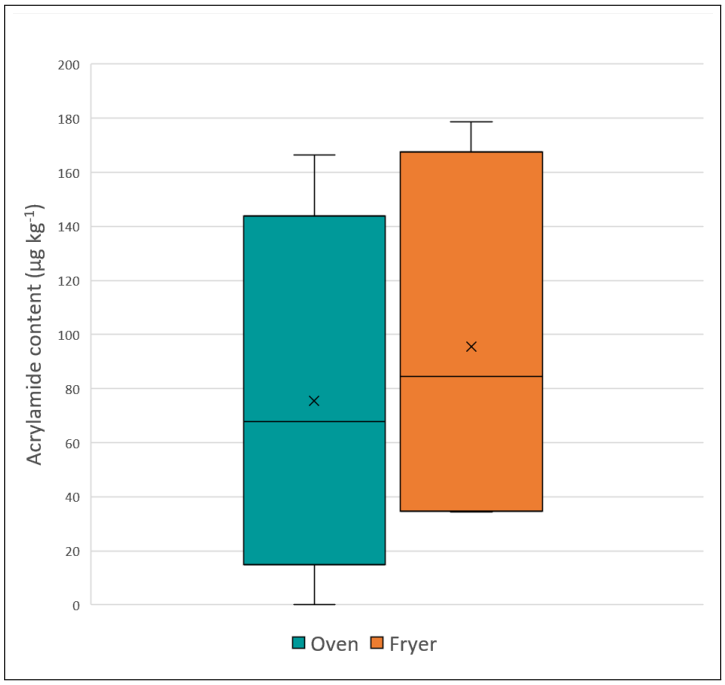

Figure 4.5: Acrylamide content in sweet potatoes fries cooked by participants depending on the cooking mode

Table 4.8: Cooking parameters and acrylamide content of buns cooked by participants

Participant (n°)	Cooking mode	Cooking program	Time (min)	AA content (μg kg^{-1})
7	Oven	grill	4	52.1
8	Toaster	Not mentioned	2	43.0
10	Toaster	4 on a scale between 1–8	1	<LOQ
13	Toaster	4 on a scale between 1–6	–	24.9

As the table 4.10 shows, the maximal concentration found in buns was 52.1 µg kg^{-1} after 4 min of cooking in an oven with grill mode. This value is slightly higher than the benchmark level (50 µg kg^{-1}) for the closest regulated food category (wheat based bread). Nevertheless, attention must be paid before drawing conclusion as this benchmark level doesn't concern bread samples that follow a toasting process. Values obtained for this food group are very low considering the aggressive cooking mode (toaster/grill mode) as they reach a very high temperature (~300 °C) and generally burn the surface of the food item. However, it can be assumed that the burned surface is very thin in comparison with the whole volume of the bun. The acrylamide content generated at this specific location is somewhat diluted in the

total food item.

Comparison between samples pictures and acrylamide content can be made. For example, participant 7 and 8 obtained the highest acrylamide concentration and pictures shown that these samples are clearly overcooked. Sesame seeds on the tops are roasted and the inner sides are brown. On contrary, the sample of participant 10 that has been toasted during only one minute does not show burning trace, nor brown color on its inner or outer sides, which is also reflected by its acrylamide content (i.e. < LOQ). Sample 13 is barely above the LOQ level with 24.8 µg kg^{-1} of acrylamide. When checking the picture, the outside surface does not seem burned, nor the sesame seeds, but the inside is clearly burned on a wide but distinct zone. Hence the toasting is not homogeneous and very local which can be explained by the design of the toaster (position of the heating resistance) and explained also why the acrylamide content is low.

Although only 4 participants participated to this small study, we can conclude that no abnormal acrylamide content have been generated during the cooking of buns. The toasting is not always homogeneous and strongly depends of course of the design of the toaster (positions and number of heating resistance). But even If the food item is burned at some local points, it concerns a very thin surface in comparison of the entire volume of the buns in the same way that has been observed for pastries.

4 Conclusion

Although the number of subjects in the study was too low to extrapolate to the entire Belgian population, these results are still very interesting and informative.

It can be concluded that low concentrations of acrylamide were generated and were in general far below benchmark levels for regulated food categories closest to food items studied (i.e. croquette, sweet potatoes and buns). Most participants respected the preconized temperature and generally stopped the cooking when a « golden color » is reached. They declare that this golden color is the most suitable for their taste, and luckily is also the preconized color in the Regulation (EU) 2017/2158.

This study highlights that the cooking time parameters in “domestic” conditions cannot be linked to acrylamide content, but reflects more the quality of model cooking device, the cooking program used, the position in the oven, etc... If temperature and color parameters are respected together by consumers, safe acrylamide level should be reached no matter the cooking time followed and variation in other parameters.

III. Updated dietary exposure assessment of acrylamide and associated risk characterisation for the Belgian population

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1 INTRODUCTION

According to the 2015 EFSA scientific opinion, acrylamide is considered both genotoxic and neurotoxic to humans (EFSA 2015). This conclusion was confirmed in the 2022 EFSA reassessment, which incorporated more recent studies conducted both in vitro and in vivo (EFSA 2022b). These studies expanded the toxicological database and provided additional evidence of chromosomal damage and DNA strand breaks in animal models as well as in human cell lines, including monocytes (Xiao et al. 2016), lymphocytes (Zamani et al. 2018; Hansen et al. 2018), and hepatic cells (Mandon et al. 2019).

Using a $BMDL_{10}$ of 0.17 mg kg^{-1} body weight day^{-1} for neoplastic effects, EFSA reported margin of exposure (MOE) values well below the reference value of 10,000 in 2015, indicating a potential health concern related to dietary acrylamide exposure. In contrast, risk characterisation for neurotoxicity, based on a $BMDL_{10}$ of 0.43 mg kg^{-1} body weight day^{-1}, resulted in MOE values above the threshold of concern, suggesting a low risk for neurotoxic effects (EFSA 2015). Similar conclusions were reported by Claeys et al. (2016), who combined Belgian occurrence data with national food consumption data and observed MOE values for neoplastic effects in the same range as those reported by EFSA in 2015, confirming the public health concern associated with acrylamide intake. Belgium is not an isolated case, as comparable conclusions have been reported across different population groups in several EU Member States, with MOE values consistently below 10,000, indicating a concern for public health regarding neoplastic effects (EFSA 2015; Costa et al. 2022; MacDonald et al. 2023).

Following the concerns raised in 2015, the European Commission adopted Regulation (EU) 2017/2158, establishing mitigation measures and benchmark levels for acrylamide in certain food categories known to contain substantial levels of this contaminant. Later, the European Commission issued Recommendation (EU) 2019/1888, calling for the collection of occurrence data on specific food products for which limited data were available, but which may contain significant acrylamide concentrations and contribute substantially to total dietary exposure (European Commission 2019). In parallel, an EFSA call issued on 25 October 2019 requested the collection of European-level data on acrylamide concentrations in thermally processed foods containing chia seeds or chia flour, which may present elevated acrylamide levels (EFSA 2019).

Consequently, the risk assessment performed in 2015 may have underestimated consumer exposure to acrylamide due to incomplete occurrence data for certain food categories. Conversely, overall exposure may have decreased since the entry into force of Regulation (EU) 2017/2158, which led to the implementation of monitoring programmes and mitigation measures. These uncertainties highlight the need for an updated risk assessment.

This section aims to address existing data gaps by re-evaluating the risk using more recent monitoring data generated after the implementation of mitigation measures under Regulation (EU) 2017/2158, as defined in Annex II, together with additional occurrence data for emerging food sources identified in Recommendation (EU) 2019/1888 and foods containing chia seeds. These occurrence data will be combined with data from the Belgian National Food Consumption Survey conducted in 2014 (FCS 2014). This approach enables an updated and refined risk assessment across population subgroups defined by age and dietary habits, addressing both genotoxic and neurotoxic effects. The results will provide updated

evidence to support competent authorities in implementing and evaluating risk management measures.

2 MATERIALS AND METHODS

2.1. Exposure assessment

Dietary exposure assessment was performed by combining acrylamide occurrence data from the present study and those obtained through FASFC with individual food consumption data from the Belgian Food Consumption Survey 2014 (FCS2014). For each food category, measured acrylamide concentrations were linked to the corresponding food consumption records. Individual daily intakes were subsequently calculated and modelled using SPADE (Statistical Program to Assess Dietary Exposure) in order to estimate habitual exposure distributions while accounting for within-person and between-person variability.

2.1.1. Occurence data

Data sources

Occurrence data used for the exposure assessment were from two datasets: (i) from the « Acrylamide in Other Food » project described in Chapter 4, Section I - Characterisation of new sources of acrylamide in food marketed in Belgium and (ii) from the monitoring programme from the Federal Agency for the Safety of the Food Chain (FASFC). In order to perform the intake and the further risk assessment, the data on dietary intake and analytical concentration levels had to be combined.

A total of 217 analytical results for acrylamide from the “Acrylamide in Other Foods” project were included in the exposure assessment. Each food item with a measured acrylamide concentration was first aggregated into product groups and then manually linked to the corresponding food entry in the Belgian Food Consumption Survey 2014 (FCS2014).

The FASFC kindly provided acrylamide data from the monitoring program for the period 2016-2020. The 1128 FASFC occurrence data was refined to ensure the completeness and their applicability to this study: (i) only data from 2018 were taken into account when the regulation (EU) 2017/2158 came into force, in order to avoid any high occurrence level or bias caused by the application of benchmark levels, (ii) data “<LOQ” obtained with a LOQ higher than this study (20 µg kg^{-1}) were not considered (babyfood, insects, food supplements), (iii) the food groups that could not be matched to food consumption were not considered, (iv) foods with no consumption data were not considered. After applying these criteria, 447 acrylamide data was selected from the following food groups: cereal bars, biscuits, soluble coffee and substitutes, cereals, chips, potato fries, coffee (grains) and bread.

Data analysis and validation, data validation and left-censoring management

The data analysis was performed following the principles of EFSA data analysis. The input data sets were cleaned, refined and validated prior to exposure assessment analyses. The left censored data were treated following international guidelines (Authority 2010; IPCS 2011) and the substitution method was used. As the proportion of left-censored results was low,

the uncertainty associated with their treatment was considered limited. Therefore, only the upper-bound scenario was retained, whereby occurrence values reported below the LOD or LOQ were replaced by their respective LOD or LOQ values.

Food categorization

In accordance with the project objective, which aimed to evaluate additional food products potentially containing acrylamide, food groups were established based on stakeholder input and the Recommendation (EU) 2019/1888. The sampling strategy was considered selective, as food targets were predefined prior to exposure assessment.

For exposure estimation, analysed products were categorised into "acrofood_groups" according to their description, consumption patterns, and potential for acrylamide formation. Either mean or maximum concentration values were assigned to each group. In total, 38 food groups were included, derived from two datasets: the Acrofood project and the national food monitoring program. The food grouping and categorization are available in appendix B6.

2.1.2. Food consumption

The intake and risk assessments were performed for the Belgian population aged 3-64 years (children, adolescents and adults) using the FCS2014 food consumption database (n=3096; 1529 men and 1567 women). The objectives, concept and methodology of the food consumption survey have been described elsewhere by Bel et al. (2016). Dietary assessment in adolescents and adults (> 10 years) was performed by the 24-h dietary recall method, carried out on two non-consecutive days, using GloboDiet© (former EPIC-Soft), a computerised 24-h recall program. Dietary assessment in children (3 to 9 years old) was done using two self-administered non-consecutive one-day food diaries followed by a GloboDiet completion interview with a proxy respondent. Pre-defined coded lists of foods, recipes, facets and descriptors are used in Globodiet©. Facets and descriptors describe foods and recipes in more detail. Facets characterize different aspects of the dietary item such as the cooking method used, brand name and preservation method. Descriptors are pre-defined answers for the facets, e.g. grilled, fried or boiled for the facet 'cooking method' (Crispim et al. 2014).

2.1.3. Intake assessment

To assess the long-term average intake from these short-term measurements, the data required statistical modelling in order to take into account between-person and within-person variations. The daily habitual intake distributions were estimated by the SPADE software (Dekkers et al. 2014). Uncertainty in the habitual intake distribution was quantified with a ready-for-use bootstrap (n=1000), which provided 95 % confidence intervals (Dekkers et al. 2014). The 2-part model for episodical-consumed food components was used because acrylamide was not consumed daily by all of the subjects. To ensure representative results for the Belgian population and for the different seasons and interview days (week versus weekend days), weighting factors were used for age, sex, province, season and day of the week.

Table 4.9: Consumption per consumption event (g or ml day^{-1}) of food groups analysed in the scope of the study; as reported in FCS 2014

Food Groups	Age classes			Average consumption
	3–9 years	10–17 years	18–64 years	per food group
appetizers	33.0	44.1	58.8	45.3
bakery ware	58.6	71.9	70.2	66.9
beignet	62.2	88.8	84.3	78.4
bread	64.5	81.4	80.2	75.4
bread (white and brown)	51.8	72.5	73.9	66.1
bread, soft	51.2	67.0	74.1	64.1
bread, sprecial	59.6	186	218	155
cacao powder	8.59	12.5	14.0	11.7
chia products (similar)	26.8	37.2	30.8	31.6
chicory coffee	50.0	225	225	167
chips (ns)	19.5	25.0	16.7	20.4
chips, corn based	25.6	43.0	52.6	40.4
chips, potato based	28.0	24.1	53.6	35.2
coffee			72.2	72.2
coffee substitutes			166	166
crackers	14.8	19.4	19.7	17.9
croquettes	57.6	121	133	104
cruesli bar	25.0	29.8	29.5	28.1
dry fruits	51.3	71.7	66.1	63.0
gingerbread	38.4	27.7	33.74	33.4
muesli	27.5	41.1	36.7	35.1
muesli, cruesly type	47.6	67.3	70.7	61.9
nuts	17.1	37.9	34.1	29.7
olives, black	10.1	21.8	16.9	16.3
other	6.50	27.6	34.4	22.8
pancakes	106	152	105	121
potato balls	98.2	77.0	194	123
potato dishes	10.0		10.0	10.0

Table 4.9 (continued)

Food Groups	Age classes			Average consumption
	3–9 years	10–17 years	18–64 years	per food group
potato fries	68.6	128	135	111
rosti	65.9	74.0	102	80.6
rusks (cracotte)	10.5	35.0		22.8
shrimp crackers	25.0			25.0
snacks	25.4	32.5	33.7	30.5
speculaas	14.0	95.4	14.0	41.1
sweet potato	66.8	83.8	111.6	87.4
sweets	14.4	29.5	29.8	24.5

For the exposure assessment, some food items required specific consideration. As instant coffee was not listed separately in FCS2014, it was described as "prepared from powder or flakes using descriptors (facets 0202). Since analyses were performed on dry products, dilution factors were applied as recommended by EFSA (EFSA 2015). Specifically, dilution factors of 0.05 and 0.02 were used for 'coffee substitutes (solids) and instant coffee, respectively. For coffee drinks, factors of 0.05 ('café americano') and 0.04 ('coffee with milk') were applied. For cocoa powder, a dilution factor of 0.028 was used to estimate acrylamide levels in the corresponding beverage.

In defining the exposure, potential consumption patterns of the selected food items were considered, including both average and high consumers. Within the project scope, the acrylamide source (baking, frying, or microwave preparation) and contamination level were also taken into account for the food group "potato-based products, particularly for ready-to-eat dishes such as hachis parmentier and gratinated potato dishes.

Exposure was assessed under four scenarios: two baseline and two simulated exposure scenarios.

Baseline exposure scenarios

Scenario 1: Exposure scenario using the maximum analytical concentration (MAC)

Firstly, a maximum analytical concentration exposure assessment scenario was performed using food consumption data combined with the maximum acrylamide content. This scenario is considered to be the most conservative scenario as it assumes that the consumer will be continuously (over a lifetime) exposed to these contaminants present in foods at the maximum observed level. The individual intake of acrylamide was estimated using the following eq. (4.1):

$$Y_i = \sum_{k=1}^{n} \frac{X_{k,i}\, C_k}{\mathrm{bw}_i} \tag{4.1}$$

where: Yi is the daily acrylamide intake of a given individual i (ng kg^{-1} bw day^{-1}); n is the number of food items containing the acrylamide, bwi is the measured body weight of a given individual i (kg); Xk,i is the amount of the food k consumed on that day (g day^{-1}); Ck is the detected amount of acrylamide in the food item k (ng g^{-1}).

Scenario 2 : Exposure scenario using mean analytical concentrations (MeAC)

Secondly, a mean analytical concentration (MeAC) exposure assessment scenario was performed using food consumption data combined with the mean acrylamide content for each food category. This scenario is considered to be a more realistic scenario for the chronic intake of acrylamide. The equation as described above has been used. Only the upper bound was evaluated as a relatively low number (all data taken into account) of data reported as <LOQ.

Simulated exposure scenarios

Scenario 3 : Exposure scenario using the maximum analytical concentration for crisps made with root and tuber vegetables other than potatoes (simMAC)

In addition to the baseline exposure scenario, a specific scenario was designed in order to simulate the consumption of crisps made from vegetables and tuber vegetables over potato chips. Hereby the products like vegetable chips, chips from mixed vegetables (red beat, carrots and parsley) as well as manioc also known as cassava or yucca which is classified as a tuber vegetable. For scenario 3 the consumption events reported as “Crisps n.s” and “Potato crisps” were considered as “crisps made with root and tuber vegetables other than potatoes” and coupled with the max analytical concentration of the food group.

Scenario 4 : Exposure scenario using the mean analytical concentration for crisps made with root and tuber vegetables other than potatoes (simMeAC)

In scenario 4 the mean analytical concentration for the group “crisps made with root and tuber vegetables other than potatoes” was coupled with the consumption events reported as “Crisps n.s” and “Potato crisps”.

Sources of uncertainty

Several sources of uncertainty may influence the dietary exposure assessment. First, some of the analysed food products were consumed only occasionally. Consequently, products for which consumption data were not available on both survey days could not be included in the chronic exposure assessment, potentially leading to a slight underestimation of exposure from these specific food items.

Regarding occurrence data, only the upper-bound approach was considered. Values reported below the LOQ were therefore replaced by the corresponding LOQ value (20 µg kg^{-1}). This approach may lead to a slight overestimation of exposure. However, the impact of this assumption is expected to be limited due to the relatively low proportion of left-censored results.

Uncertainties are also associated with food consumption data. As with any dietary survey, under-reporting or over-reporting of food intake, misclassification of consumed foods, and inaccuracies in portion size estimation may affect exposure estimates. In addition, the estimation of the contribution of individual food categories to total acrylamide exposure was based on food group calculations using only the first 24-hour dietary recall, which may

introduce additional variability.

Overall, these uncertainties may influence the magnitude of the estimated dietary exposure. However, they are unlikely to substantially affect the overall conclusions of the study, as the different exposure scenarios consistently indicated similar trends in acrylamide exposure and risk characterization.

2.2. Risk assessment

The risk characterization was performed using the Margin of Exposure approach (MOE) (EFSA 2005). The CONTAM Panel performed benchmark dose (BMD) analyses on data for neurotoxicity and on the tumour incidences induced by acrylamide in experimental animals. They selected the value of 0.43 mg kg^{-1} bw day^{-1} as the reference point (RP) for non-neoplastic effects. This RP was derived as the lowest $BMDL_{10}$ from the data on incidences of peripheral nerve (sciatic) axonal degeneration in male F344 rats exposed to acrylamide in drinking water for two years. For neoplastic effects, they selected as a reference point the value of 0.17 mg kg^{-1} bw day^{-1}. This RP was derived as the lowest $BMDL_{10}$ from data on incidences of Harderian gland adenomas and adenocarcinomas in male B6C3F1 mice exposed to acrylamide for two years. Next, the MOE can be calculated using the following eq. (4.2).

$$\text{MOE} = \frac{\text{BMDL}_{10}}{\text{Estimated Exposure Dose}} \tag{4.2}$$

According to the EFSA Scientific Committee, for substances that are both genotoxic and carcinogenic, an MOE of 10 000 or higher, based on a $BMDL_{10}$ from an animal study, would be of low concern from a public health point of view (EFSA 2005). A MOE of 100 is usually considered sufficient for non-genotoxic compounds to conclude that there is no health concern. For the risk assessment, both MAC and MeAC scenarios were considered (EFSA 2005).

3 RESULTS AND DISCUSSION

3.1. Exposure assessment

3.1.1. Occurence data

table 4.10 summarizes the acrylamide occurrence data used in this study. For each food group, the data sources are specified, together with the mean and maximum acrylamide concentrations, as well as the number of samples analyzed. These data were combined with food consumption survey data (table 4.9) to estimate dietary exposure to acrylamide in the Belgian population and to perform the subsequent risk assessment.

Table 4.10: Overview of the assigned acrylamide values for MAC (maximum concentration) and MeAC (mean concentration) in analysed food groups

Food groups	Data source	Mean (μg kg^{-1})	Max (μg kg^{-1})	Number of samples
Apetizers	project	20.9	31.9	3
Bakery ware	project	16.3	32.5	14
Beignet	project	12.0	31.3	10
Bread	project	18.2	98.0	19
Bread (white–brown)	monitoring	18.4	76.8	36
Bread, soft	project	15.4	22.9	5
Bread, special	project	13.5	20	4
Cacao	project	60.0	141	5
Chia seed products	project	38.6	211	11
Chips, potato	monitoring	529	2648	38
Chips, corn	project	132	337	13
Chips-like products	project	162	190	2
Chips, vegetable	monitoring/project	1394	3063	10
Coffee substitute (chicory)	monitoring	2702	4994	25
Coffee	monitoring	475	1085	72
Crackers	monitoring/project	104	259	22
Cracotte	monitoring	158	723	96
Small potato croquettes	project	656	1503	9
Croquettes	project	385	1071	16
Muesli (Cruesli type)	project	31.4	56.5	10
Cruesli bars	monitoring/project	153	353	7
Prepared dishes	project	33.1	102.9	6
Dry fruits	project	7.00	7.00	6
Potato fries	monitoring	341	1016	94
Gingerbread	monitoring	336	641	16
Muesli	monitoring	140	452	29
Nuts	project	34.6	155	11
Olives	project	239	575	5
Other food	project	7.00	7.00	2

Table 4.10 (continued)

Food groups	Data source	Mean (μg kg^{-1})	Max (μg kg^{-1})	Number of samples
Pancakes	project	19.9	28.2	5
Rosti	project	492	1199	4
Snack nuts	project	29.5	75.6	5
Snacks	project	115	207	5
Speculaas	monitoring	388	2401	12
Coffee substitutes, other	project	931	4389	7
Sweet potato	project	737	981	3
Sweets	project	23.8	59.6	5

3.1.2. Intake assessment

Baseline exposure scenarios

Scenario 1: Exposure using the maximum analytical concentration (MAC)

Children are the most exposed groups, followed by the adolescents while the adults are the least exposed. This trends was also observed during the first risk assessment perform by EFSA and for all EU countries (EFSA 2015). The mean exposure levels ranged from 0.58 (Adults) to 0.89 µg kg^{-1} bw day^{-1} (Children), while the P95 varies from 1.75 to 2.51 µg kg^{-1} bw day^{-1} table 4.11. This is in the range of the median exposure levels calculated by EFSA in 2015 with 0.5 (adults) to 1.2 µg kg^{-1} bw day^{-1} (children) for the mean exposure and from 1.0 (adults) to 2.2 (children) µg kg^{-1} bw day^{-1} for the P95 exposure (EFSA 2015). P95 exposure estimates are higher than those estimated by EFSA. However, P95 estimated exposure for children is below the highest P95 estimated by EFSA (3.2 µg kg^{-1} bw day^{-1}) (EFSA 2015).

Table 4.11: Estimated exposure to acrylamide (μg kg^{-1} bw day^{-1}) in the Belgian population using the maximum analytical concentration (MAC)

Age (years)	N	Mean	P50	P95	P97.5	P99
3–9	737	0.89	0.62	2.51	3.26	4.28
10–17	725	0.80	0.57	2.32	2.95	3.83
18–64	922	0.58	0.39	1.75	2.25	2.98

Scenario 2: Exposure Mean analytical concentrations scenario (MeAC)

Similarly to the intake assessment using the MAC, the children are also the most exposed group when the MeAC is used. The mean exposure levels ranged from 0.17 to 0.25 µg kg^{-1} bw day^{-1}, and the 95th percentile exposure level from 0.52 to 0.72 µg kg^{-1} bw day^{-1} (table 4.12).

The MeAC exposure of the all age groups is lower than what was estimated by EFSA (EFSA 2015).

Table 4.12: Estimated exposure to acrylamide (μg kg^{-1} bw day^{-1}) in the Belgian population using the mean analytical concentration (MeAC)

Age (years)	N	Mean	P50	P95	P97.5	P99
3–9	977	0.25	0.17	0.72	0.92	1.25
10–17	918	0.23	0.16	0.69	0.89	1.17
18–64	1201	0.17	0.11	0.52	0.68	0.92

On the basis of monitoring data for period 2002-2007, Clayes et al (2016) estimated the acrylamide average intake (middle bound) using a probabilistic approach to be 0.35 µg kg^{-1} b.w. day^{-1}, and for high consumers (P97.5) of 1.58 µg kg^{-1} b.w. day^{-1}. Taking into account that consumption patterns slightly changed, that some food commodities were not included in the monitoring program and more importantly that various mitigation measures were implemented, it is possible to conclude that overall acrylamide exposure estimated in the project is on the lower level than what was previously reported.

Simulated exposure scenarios

Scenario 3 : Exposure scenario using the maximum analytical concentration for crisps made with root and tuber vegetables other than potatoes (simMAC)

Scenario 3 simulated the situation where all potato chips (crisps) were root and tuber chips similar to those either made out of one vegetable or mixed vegetable chips and of different available brands. The maximum scenario was evaluated for high consumption and presented in table 4.13.

Table 4.13: Estimated exposure to acrylamide (μg kg^{-1} bw day^{-1}) in the Belgian population using the maximum analytical concentration and simulated vegetable chips consumption (simMAC)

Age (years)	N	Mean	P50	P95	P97.5	P99	% difference to baseline Mean / P95
3–9	977	0.94	0.65	2.67	3.46	4.57	5.61 / 6.21
10–17	918	0.85	0.59	2.46	3.14	4.08	5.41 / 5.97
18–64	1201	0.61	0.40	1.84	2.38	3.16	4.74 / 5.40

As shown in table 4.13, compared to the baseline scenario where all the data available on potato chips (crisps) were considered, this scenario resulted in an increase in the mean and 95th percentile exposure levels up to 4 to 6 %, depending on age group.

Scenario 4 : Exposure scenario using the mean analytical concentration for crisps made with root and tuber vegetables other than potatoes (simMeAC)

Scenario 4 simulated the situation where mean acrylamide concentration of all vegetables and manioc chips analysed in the project was assigned to the consumption data. This mean concentration scenario was evaluated and population percentile was presented in table 4.14.

Table 4.14: Estimated exposure to acrylamide (μg kg^{-1} bw day^{-1}) in the Belgian population using the mean analytical concentration and simulated consumption of root and tuber vegetable chips (exposure scenario 4)

Age (years)	N	Mean	P50	P95	P97.5	P99	% difference to baseline Mean / P95
3–9	977	0.32	0.21	0.95	1.23	1.69	39.56 / 44.87
10–17	918	0.29	0.19	0.91	1.18	1.58	37.39 / 43.22
18–64	1201	0.20	0.13	0.66	0.86	1.19	29.87 / 35.16

Similarly to scenario 3, the results showed in table 4.14 are illustrating an increase in exposure. This difference may be explained by high and varying acrylamide values in different root and tuber vegetable crisps. The average concentration of root and tuber vegetable crisps was 2.5 times higher than average concentration of potato crisps.

Identification of the main contributors

The percentage contribution of the different food categories to the exposure were calculated for each age class based on the food groups defined in the project for the intake assessment with the mean or maximum concentration levels. The results are illustrated in fig. 4.6 and fig. 4.7.

The main contributors for the acrylamide exposure are potato fries, potato chips, bread (white and brown), more special bread, cacao and bakery ware. These food groups each contribute at least by 5 % to the acrylamide exposure of any of three age classes for both MAC and MeAC scenarios. Potato fries are clearly the main contributor to acrylamide exposure of adolescents. On the other hand, potato fries and bread (white and brown bread) similarly contribute to the acrylamide exposure of adults whereas potato fries and cacao have a similar contribution to acrylamide exposure of children.

For children (3-9 year) the main contributor to the total average exposure was potato fries, representing around 14 % of the total exposure. Chips, potato based contribute to 12 % of the total exposure. This is followed by bread products (9.33 %) and pancakes (7.85 %). Food groups like “Bakery ware”, “Cracotte”, “Croquettes”, “Crackers” and “Prepared dishes (potato based) are contributors representing 5-2 % of the total exposure (in descending order). Other products (Muesli (Cruesli type), Gingerbread, Chia seed products, Nuts, Bread, special, Snacks, Beignet and Dry fruits) did not represent more than 2 % of the total exposure. These products also did not contain acrylamide or are less consumed. The acrylamide exposure patterns for adolescents is represented mostly by potato fries (24 %) and potato chips (16 %).

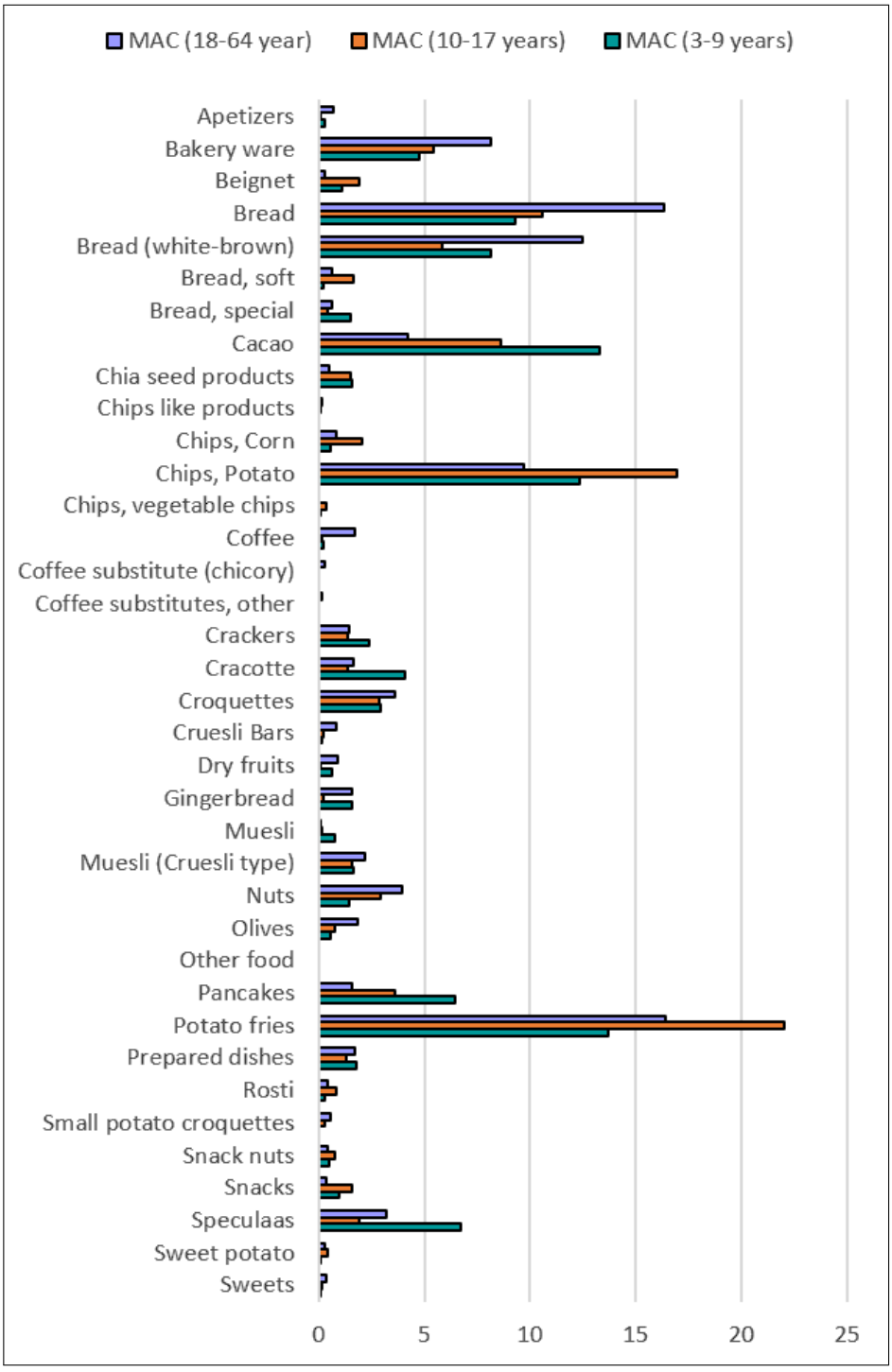

Figure 4.6: Relative contribution of the main food groups to the daily intake of acrylamide expressed for three age classes and based on MAC

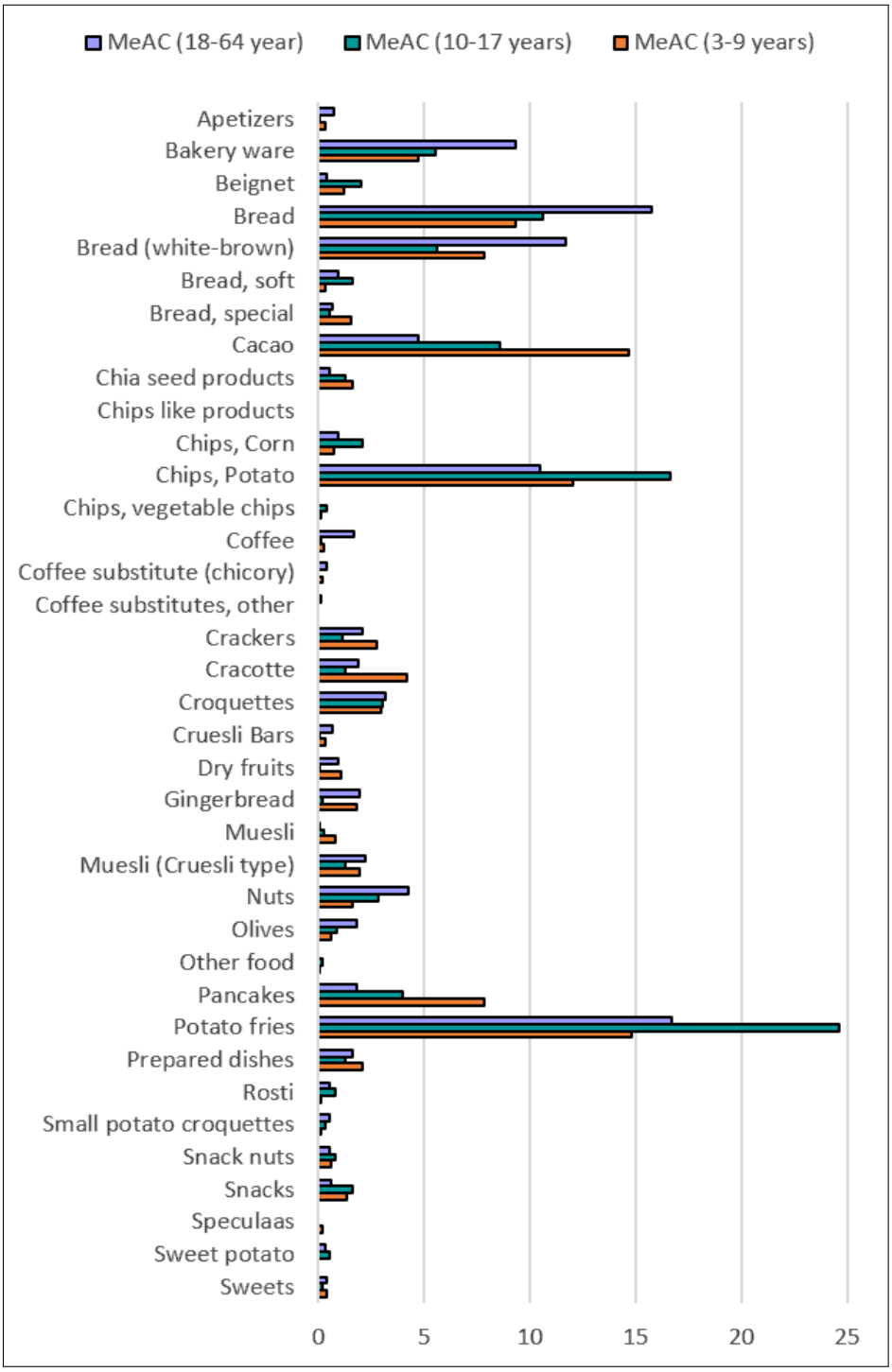

Figure 4.7: Relative contribution of the main food groups to the daily intake of acrylamide expressed for three age classes and based on MeAC

Comparing the main contributors to acrylamide exposure with those reported in other countries remains challenging due to differences in dietary habits, food categorisation, and the range of analysed food products. The only recent assessments conducted after the implementation of Regulation (EU) 2017/2158 is the French total diet study (TDS) performed by ANSES in 2026, based on 312 samples collected between May 2021 and August 2022 (Anses 2026). However, some food categories analysed in that study were not included in the present work and are not specifically targeted by Regulation (EU) 2017/2158 or Recommendation (EU) 2019/1888 (e.g. fish and seafood products, alcoholic beverages, pasta, spices and herbs), and the food grouping used also differed.

Despite these methodological differences, the three main contributors identified in both studies appear broadly similar when food categories are grouped in a comparable way. Potato-based products (e.g. crisps, fries and croquettes) were the primary contributors to acrylamide exposure, accounting for approximately 47–68 % of total exposure in the French study and 30–46 % in the present study. Bread represented the second most important contributor, accounting for 8.1–12 % of exposure in adults and 4.1–5.3 % in children in the French study, compared with 12–20 % across all age categories in the present study. Sweet bakery products, including pastries, were ranked third, contributing 6.6–9.1 % of exposure in children and 3.7–6 % in adults in the French study, while similar contributions were observed in the present study (6–12 %) (Anses 2026). The consistency of these findings suggests that similar patterns of acrylamide exposure may be observed across countries.

3.2. Risk assessment

3.2.1. Neurtoxic effect

In order to calculate the MOE for neurotoxic effects, the $BMDL_{10}$ value of 0.43 mg kg^{-1} bw day^{-1} was used. As all MOE values are above 100, exposure to acrylamide can be considered of low concern with respect to neurotoxic effects (table 4.15). These results are consistent with those reported in the recent French Total Diet Study (Anses 2026), which showed MOE values ranging from 250 to 814 for children and from 489 to 1863 for adults, also above the value of 100 considered protective for neurotoxicity. Similar ranges were reported by the first risk assessment performed by EFSA (2015), with MOE values between 179 and 538 for children and between 307 and 1075 for adults. However, EFSA also highlighted that MOE values for high consumers (95th percentile), particularly among children, may approach levels that could raise concern. Similarly, the results of the present study suggest a potential concern for children with high acrylamide exposure (P95).

Table 4.15: Margin of exposure (MOE) values for neurotoxic effects of acrylamide estimated for the Belgian population

Age group	MAC		MeAC	
	Mean exposure	P95 exposure	Mean exposure	P95 exposure
3–9 years	486	171	1744	600

Table 4.15 (continued)

Age group	MAC		MeAC	
	Mean exposure	P95 exposure	Mean exposure	P95 exposure
10–17 years	536	185	1860	623
18–64 years	741	246	2569	825

3.2.2. Neoplastic effect

For neoplastic effects, the $BMDL_{10}$ value of 0.17 mg kg^{-1} bw day^{-1} was used in combination with the dietary intake estimates. According to EFSA, an MOE of 10,000 or higher for neoplastic effects is considered of low public health concern (EFSA 2015). In the present study, all calculated MOE values were substantially lower than 10,000 (table 4.16), indicating a potential health concern related to the neoplastic effects of acrylamide.

These results are consistent with those reported by EFSA (2015), which estimated MOE values ranging from 70 to 2125 for children and from 121 to 425 for adults. Similarly, the recent French Total Diet Study (Anses 2026) reported MOE values for carcinogenicity ranging from 99 to 322 for children and from 193 to 737 for adults, which are of the same order of magnitude as those obtained in the present study. These comparable results suggest that acrylamide exposure levels may be relatively similar across European populations, despite some differences in dietary habits between countries.

Current exposure estimates reported in the present study are approximately 0.5–1.0 µg kg^{-1} bw day^{-1} on average, which is about 15–60 times higher than the exposure level corresponding to an MOE of 10,000. Achieving such an MOE therefore appears challenging in practice. Indeed, the main contributors to dietary acrylamide exposure are commonly consumed foods such as potato-based and cereal-based products (figs. 4.6 and 4.7) (EFSA 2015; Anses 2026). Because these foods are eaten frequently, even moderate acrylamide concentrations can significantly contribute to total dietary exposure. In addition, some specific food groups identified in the present study, such as vegetable crisps and cocoa powder, may further contribute to overall exposure and should therefore be considered when evaluating population risk and implementing mitigation strategies. Consequently, further reductions in acrylamide formation across multiple food categories remain necessary to significantly decrease population exposure.

Table 4.16: Margin of exposure (MOE) values for neoplastic effects of acrylamide estimated for the Belgian population based on MAC and MeAC exposure scenario

Age group	MAC		MeAC	
	Mean exposure	P95 exposure	Mean exposure	P95 exposure
3–9 years	192	237	690	237
10–17 years	212	246	735	246
18–64 years	293	326	1015	326

An additional source of uncertainty relates to the toxicological reference point used by EFSA for the derivation of the MOE approach. The benchmark dose selected for neoplastic effects was based on tumour formation in the Harderian gland, an organ present in rodents but absent in humans (EFSA 2015). The relevance of this endpoint for human risk assessment has therefore been questioned by different studies (as cited by Powers et al. 2021). However, EFSA re-evaluated the genotoxicity of acrylamide in 2022 and concluded that evidence from more recent studies, including investigations conducted in human cell lines, confirms the genotoxic potential of acrylamide for humans (EFSA 2022). Consequently, although the use of the Harderian gland endpoint remains a source of uncertainty when extrapolating animal toxicologic data to humans, it is unlikely to modify the overall conclusion that acrylamide exposure through food may represent a health concern for consumer safety.

4 CONCLUSIONS AND RECOMMENDATIONS

By incorporating more recent monitoring data and previously missing food categories, as requested by Commission Recommendation (EU) 2019/1888 (European Commission 2019) and the EFSA call for data in 2019 (EFSA 2019), it was possible to re-evaluate the dietary exposure to acrylamide in the Belgian population.

Overall, the estimated exposure to acrylamide was lower than the intake assessment reported by EFSA in its 2015 opinion (EFSA 2015). The main contributing food categories were subsequently identified. Across all age groups, dietary exposure originated predominantly from grain and grain-based products. This finding highlights that the food products analysed within the present project—such as bakery wares, pancakes, various types of bread, and potato croquettes—represent important sources of acrylamide exposure. Notably, this lower exposure estimate was obtained despite the inclusion of additional acrylamide-containing food products that were not considered in the EFSA 2015 assessment. Nevertheless, the calculated margins of exposure (MOEs) for neoplastic effects remained below the reference value of 10 000.

Regarding neurotoxic effects, all calculated MOEs were well above 100, indicating that dietary exposure to acrylamide does not raise concerns for neurotoxicity. In contrast, for

neoplastic effects, nearly all MOEs were substantially below 10 000. Therefore, although available human studies have not conclusively demonstrated acrylamide to be a human carcinogen, the MOEs derived from current levels of dietary exposure indicate a potential concern with respect to neoplastic effects.

Chapter 5

PAHs contamination of spices and dried herbs available on Belgian retail shops and evaluation of the risk for the consumer

Outline

Polycyclic aromatic hydrocarbons (PAHs) have been investigated in food for several decades. In recent years, increasing concern has emerged following the detection of these contaminants in spices and dried herbs. However, there was a lack of reliable analytical methods suitable to analyse the wide variety of spices and herbs available on the markets. Consequently, assessing the potential health risk for consumers remains challenging in the absence of a robust analytical approach. This chapter presents the development and validation of an analytical method for the quantification of PAHs in spices and dried herbs. The method was then applied to the analysis of 120 commercial samples. Finally, the measured PAH concentrations were combined with consumption data to estimate dietary exposure and assess the potential health risk to consumers.

This chapter is an adapted version of the two following scientific articles:

- **Ph. Szternfeld**, J. Marchi, S.V. Malysheva, S.V. & L. Joly, 2019. Modular Method for the Determination of Polycyclic Aromatic Hydrocarbons in Spices and Dried Herbs by Gas Chromatography–Tandem Mass Spectrometry. Food Anal. Methods 12, 2383–2391. DOI: 10.1007/s12161-019-01579-4.
- **Ph. Szternfeld**, A. Marakis, M-L. Scippo, E. Van Hoeck & L. Joly, 2022. Polycyclic aromatic hydrocarbons in spices and dried herbs and associated risk for the Belgian population. Food Additives % Contaminants: Part B, 15:4, 292-300, DOI: 10.1080/19393210.2022.2106518.

Contribution

Article 1 (method development): Philippe Szternfeld conceived and supervised the project. Philippe Szternfeld and Jessica Marchi developed and validated the analytical methods. Philippe Szternfeld drafted the manuscript. Philippe Szternfeld, Svetlana V. Malysheva, and Laure Joly contributed to manuscript revision and data visualization. All authors approved the final version. Draft version

Article 2 (occurrence and risk assessment): Philippe Szternfeld conceived and supervised the project. Philippe Szternfeld and Alexios Marakis performed the sample analyses. Philippe Szternfeld conducted the exposure and risk assessment. Philippe Szternfeld drafted the manuscript. Philippe Szternfeld, Els Van Hoeck, and Laure Joly contributed to manuscript revision and data visualization. All authors approved the final version.

I. Modular Method for the Determination of Polycyclic Aromatic Hydrocarbons in Spices and Dried Herbs by Gas Chromatography-Tandem Mass Spectrometry

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Abstract

Polycyclic Aromatic Hydrocarbons (PAHs) belong to a very broad class of environmental contaminants that shows genotoxic and carcinogenic effects. Since more than 15 years, large quantities of PAHs has been found in spices and dried herbs mainly due to poor drying practices. In 2015, European Commission fixed maximal residue limit (MRL) for these matrices at 10 µg kg^{-1} for the Benzo[a]Pyrene (BaP) and at 50 µg kg^{-1} for the sum of the four following PAHs (PAH4): Benzo[a]Anthracene (BaA), Chrysene (CHR), Benzo[b]fluoranthene (BbF) and BaP. This paper describes a method for quantification of PAHs in the wide matrix group of spices and dried herbs by gas chromatography-tandem mass spectrometry (GC-MS/MS). The design of this method was drawn up using basic material and equipment available in most laboratories and a modular clean up fitting for each category of this very diverse matrix group. The clean-up strategy consists of dividing this matrix group into three subclasses: regular spices, highly pigmented matrices and complex mix of spices/fatty spices. Depending on the subclass, SPE sorbent is adapted to remove maximum of co-extracts and obtain clean samples. This method has a limit of quantification of 0.5 µg kg^{-1} for each PAH and the validation criteria fully satisfied the Commission Regulation (EU) N° 836/2011. The quantification performed by isotopic dilution allowed the complete correction of matrix effect and led to very good validation data with a mean recovery close to 100 % and a RSD below 10 % for each PAH.

Keywords: PAHs – Spices – Dried herbs – Gas chromatography – Mass spectrometry

1 INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are a large class of organic compounds that is composed of two or more fused aromatic rings of carbon and hydrogen atoms. Human exposure routes to PAHs are various but it is assessed than the most important source of exposure for a non-smoker is food. Food can be contaminated by environmental pollution as well as during processing like grilling, roasting, drying or smoking (EFSA 2008).

In 2002, the European Scientific Committee on Food assessed the toxicity of many PAHs and identified 15 of them possessing genotoxic or carcinogenic effects. Further in 2008, the European Food Safety Authority Panel on Contaminants in the Food Chain (EFSA CONTAM Panel) concluded that a system based on the monitoring of 4 PAHs (benzo[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene (BaP)) is a good indicator of general PAHs contamination of food (EFSA 2008). In 2011, based on this conclusion, the European Commission established maximal residue limits (MRLs) for the sum of these 4 compounds in different food matrices (European Commission 2011a).

During the last 15 years, large quantities of PAHs has been found in dried herbs and spices due to bad drying practices (EFSA 2008; European Commission 2015). Few tests performed in our laboratory have also revealed such large quantity of PAHs, especially in smoked spices. In 2015, the European Commission has fixed MRLs for those food matrices with a limit of 10 $\mu g\ kg^{-1}$ for BaP and 50 $\mu g\ kg^{-1}$ for the sum of 4 PAHs (European Commission 2015). Hence, validated methods for the quantification of these compounds are required to investigate the compliance of spices and herbs available on the market.

Although this regulation has been adopted since 3 years, very few specific methods designed for spices and herbs have been found in the literature. Recent works have considered modern extraction method such as QuEChERS extraction which has been originally developed for the analysis of pesticides in food (Anastassiades et al. 2003). Urban and Lesueur used it to determine PAHs in curry powder. In this method solvent and time consumption was reduced, but obtained extracts can be dirty depending on the matrix and quickly lead to instrumentation maintenance (Urban and Lesueur 2017). In other cases, methods are adapted from the analysis of botanical food supplements (Windal et al. 2008; Rozentale et al. 2018). As for extraction methods dedicated to food matrices other than spices and herbs (e.g., meat, fish), Pressurised Liquid Extraction (PLE) has been successfully applied in the field of PAHs analysis since many years (Veyrand et al. 2007; Vazquez-Roig and Picó 2015; Duedahl-Olesen et al. 2015), while other researchers used Soxhlet extraction (Veyrand et al. 2007; Janska et al. 2008; Yoshimine et al. 2012). However, these “traditional” extraction devices are often expensive and time- and solvent-consuming.

After extraction, clean-up procedure has to be seriously considered as it is the most critical and difficult part in the analysis of PAHs in spices and dried herbs. This food group is very heterogeneous and contains a lot of compounds with similar chemical properties like PAHs which make it very difficult to eliminate matrix interferences during sample preparation. This critical point has to be taken into account in establishing an efficient purification strategy that allows a low limit of detection (LOD) and extracts cleaned enough to avoid laborious instrument maintenance. Many laboratories use Gel Permeation Chromatography followed or preceded by solid-phase extraction (SPE) (Janska et al. 2008; Hassan and Farahani 2011;

Rozentale et al. 2018). The disadvantages of this technique are lengthy run time and high solvent consumption (usually 45-65 min at a flow of 5 ml^{-1}). Others perform a first purification step during PLE extraction. Veyrand et al., for instance, add florisil in PLE tubes in order to remove the majority of co-extracts (Ziegenhals et al. 2007; Veyrand et al. 2007). Other authors use various dispersive solid phase extraction sorbents (d-SPE) after a QuEChERS extraction (Urban and Lesueur 2017), but it does not have the flexibility of an SPE cartridge which can be used as a chromatographic device in order to separate PAHs from matrix components more efficiently than a d-SPE.

This work focused on development and validation of a method dedicated to the quantification of four PAHs, Benzo[a]Anthracene, Chrysene, Benzo[b]fluoranthene, Benzo[a]Pyrene, which, according to the current legislation (European Commission 2011a), have to be monitored: in spices and dried herbs. Specific attention was paid to make the method modular in order to be easily adaptable to the large variety of spices and herbs that can be found on the market. Furthermore, this method has to be suitable for conventional equipment and remain transferable to other laboratories for a low cost and with a good compromise between experimental time and extract cleanliness.

2 MATERIALS AND METHODS

2.1. Chemicals and reagents

Mix of 15+1 EU PAHs (10 ng μl^{-1} in cyclohexane) and isotopically-labelled (deuterated D12) mix PAHs (10 ng μl^{-1} in cyclohexane) have been purchased from Dr Ehrenstorfer Gmbh (Ausburg, Germany). Hexane (HPLC grade) was from Biosolve (Valkenwaard, The Netherlands) and dichloromethane (HiperSolve HPLC^{TM} was from BDH Chemicals (England). Bond Elut SI cartridges (500 mg, 3 ml) were purchased from Agilent Technologies (Machelen, Belgium). Florisil, sorbent for chromatography (60-100 mesh) was purchased from Fluka (Sigma Aldrich, Overijse, Belgium). Empty SPE cartridges (6 ml) were purchased from Biotage Isolute® (Sopachem, Gent, Belgium). SupelMIP^{TM} SPE cartridge were from Supelco (Sigma Aldrich, Overijse, Belgium).

Working solutions of PAHs mix at 1 µg ml^{-1} and 0.1 µg ml^{-1} in cyclohexane were made from the commercial solution at 10 µg ml^{-1} and stored at -20 °C. Working solutions of isotopically-labelled PAHs (IL-IS) were prepared in hexane at concentration of 1 µg ml^{-1} from the commercial solution at 10 µg ml^{-1} and stored at -20 °C.

2.2. Sample extraction

The EU Regulation 836/2011 (European Commission 2011b) requests to avoid the use of polypropylene or PTFE on which PAHs may be adsorbed. Several adsorption tests with spiked samples and natural contaminated samples have been conducted and no difference between glass tube and Falcon polypropylene tubes has been noticed. Hence, polypropylene Falcon tubes were used.

The first step of the procedure is common for every type of spices and dried herbs. One

gram of homogenized sample is weighed in a Falcon tube of 50 ml. Then twenty-five µl of IL-IS PAHs (1 µg ml^{-1}) is spiked and the sample is let during 20 min for equilibration. The extraction begins by adding 20 ml of extraction solution [hexane/dichloromethane (3:1, v/v)]. The sample is shaken by hand for even distribution of the matrix in the extraction solution and the agitation is continued with Ultraturrax® during 1 min. The sample is centrifuged at 4000 rpm during 5 min at 20 °C. The totality of the supernatant is transferred into a concentration tube and concentrated under a stream of nitrogen to a volume of approximately 1 ml.

2.3. Sample purification

Solid phase extraction is used essentially for extract clean-up. Sorbent choice depends on the matrix subgroup analysed. Three different groups have been defined depending on their nature: regular spices (e.g. pepper, garlic), herbs/spices containing large amount of pigments (e.g. parsley, oregano) and complex spices (e.g. curry) which is a very heterogeneous group composed of a mix of different spices and may contain fat (about 10 %).

2.3.1. Purification of regular spices

A commercial silica cartridge of 500 mg used as a pass-through filter is first conditioned with 5 ml of the extraction solution. The sample is then loaded onto the cartridge and directly eluted with 6 ml of the extraction solution. The eluate is evaporated under a nitrogen stream and reconstituted with 200 µl of hexane before injection on the GC-MS/MS system.

2.3.2. Purification of pigmented spices and dried herbs

An empty cartridge of 6 ml is first filled with glass fibre and 1.5 g of florisil sorbent. The cartridge is then conditioned with 6 ml of the extraction solution before adding the sample. The cartridge is then washed with another 6 ml of the extraction solution. Eluate is evaporated under a nitrogen stream and reconstituted with 200 µl of hexane before injection on the GC-MS/MS system.

2.3.3. Purification of complex spices

First, the clean-up procedure for regular spices is followed. Then, an extra purification step with the SupelMIP^{TM} PAHs SPE cartridge is done following the procedure supplied with the cartridges by Supelco: the cartridge is first conditioned with the extraction solution. The entire sample is loaded on the cartridge. A washing step is performed with 3 ml of hexane. PAHs are finally eluted with 3 ml of ethyl acetate. The sample is evaporated under a nitrogen stream until dryness, reconstituted with 200 µl of hexane and injected on the GC-MS/MS system. illustrates the sample preparation for each matrix subgroup.

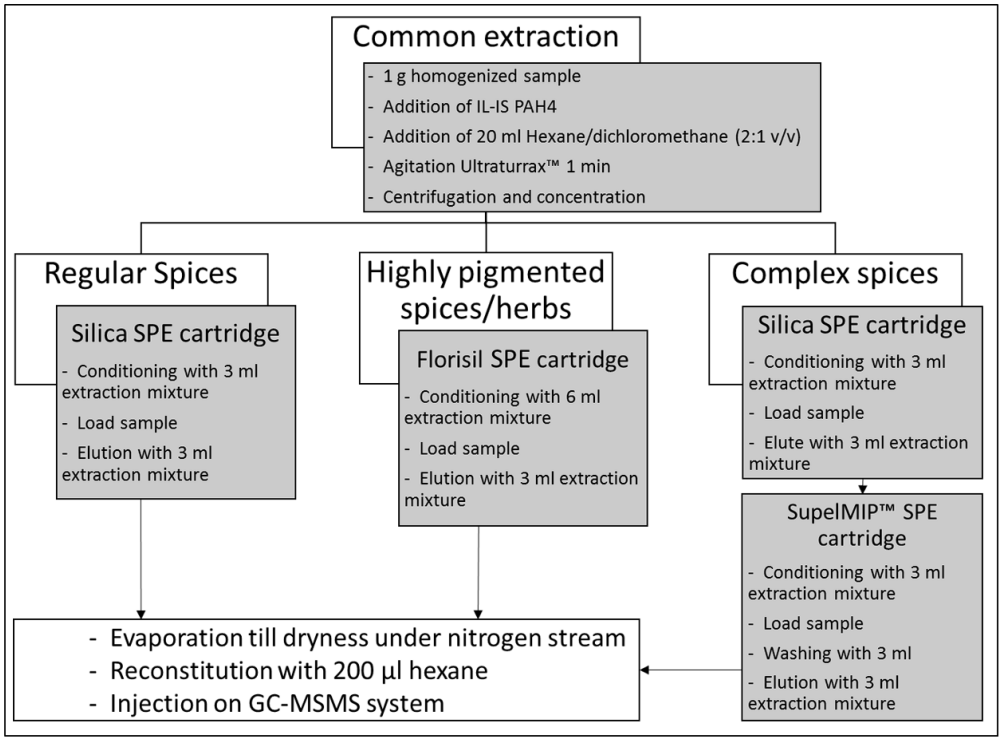

Figure 5.1: Flow chart of sample preparation procedure

2.4. GC-MS/MS analysis

GC-MS analyses were performed on an Agilent 7890B GC system (Agilent Technologies, Amstelveen, Netherlands) coupled to an Agilent 7000C GC/MS Triple Quad mass spectrometer (Agilent Technologies) equipped with an electronic impact source. The chromatographic column used was the J&W GC column Select PAH 30 m x 0.25 mm x 0.15 µm. Two µl of sample were injected on the column in a Splitless mode at 300 °C, during 1 min at a flow of 50 ml min^{-1}. The oven temperature gradient is based on the application note from Agilent (Provoost, 2010). Initial condition has been fixed at 70 °C (0.4 min), then 85 °C min^{-1}, 180 °C, 3 °C min^{-1}, 230 °C (7 min), 28 °C min^{-1}, 280 °C (10 min), 14 °C min^{-1}, 325 °C (3 min). The maximal temperature has been fixed at 325 °C instead of 350 °C required by Agilent because an HP-5ms column which is installed in the same oven, does not tolerate temperature higher than 325 °C. Despite this fact, this run allows to perform the determination of the four target PAHs with a good sensitivity, a good resolution and an acceptable run time (45 min). table 5.1 summarizes optimized MS parameters used to monitor PAHs and their respective isotopically labelled internal standards. Two transitions were monitored, one is used for quantification (Q), while the other is used for confirmation (q) of the analyte. The following source parameters were used: source temperature 280 °C and electron energy fixed at 70 eV.

Table 5.1: MS/MS parameters for the analysis of PAHs and their isotopic-labelled analogues

Compound	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)
Benzo[a]anthracene	228.1	226.1 (Q)	35
	113.1	112.1 (q)	12
Benzo[a]anthracene D12	240.2	223.6	30
Chrysene	228.1	226.1 (Q)	35
	114.1	101.1 (q)	10
Chrysene D12	240.2	223.6	30
Benzo[b]fluoranthene	252.1	250.1 (Q)	40
	126.0	113.1 (q)	12
Benzo[b]fluoranthene D12	264.2	260.2	35
Benzo[a]pyrene	252.1	250.1 (Q)	40
	126.0	113.1 (q1)	15
	125.0	124.0 (q2)	12
Benzo[a]pyrene D12	264.2	260.2	35

3 RESULTS AND DISCUSSION

3.1. Method development

3.1.1. Extraction

Van der Lee et al have demonstrated than even if the use of a single solvent gave good extraction recovery during the development, it can lead to underestimation of PAHs content in naturally contaminated samples (Van der Lee et al. 2015). Two different extraction mixtures of solvent composed of a non-polar solvent and a polar (rather polar) solvent have been

tested: hexane/acetone (1:1; v/v) and hexane/dichloromethane (1:1; v/v). Although no significant difference in extraction recovery has been noticed between these two mixtures, the mixture hexane/acetone co-extracted many pigments and other compounds from the matrix which were very difficult to remove during further purification steps. This issue was less pronounced with the mixture hexane/dichloromethane, but the extract cleanliness was still not satisfactory. Consequently, a compromise has been found by choosing a mixture hexane/dichloromethane (3:1; v/v) without affecting extraction recovery. For agitation, Ultraturrax® has been selected as mechanical shaker. This tool has the advantage of requiring very short extraction time (only 1 min) to produce very good extraction results in comparison with other equipment like PLE ($\sim$24 min) (Hitzel et al. 2013) or sonication (20 min) (Rozentale et al. 2018). Moreover, Ultraturrax® enables to obtain higher recoveries of incurred contaminants than ultrasonic sonication and this device was already used in our lab since many years for the extraction of pesticides in food (Hanot et al. 2015).

3.1.2. Purification

To remove most co-extracts, two different liquid/liquid extractions have been tested: one with a mixture of hexane/acetone/water (1:1:2; v/v/v) and another with hexane/dichloromethane/water (1:1:2; v/v/v). In the first case, co-extracts were not transferred in the aqueous phase, probably due to acetone which is too polar to allow the transfer in water. In the case of hexane/dichloromethane, even if many co-extracts were transferred into the aqueous phase, this liquid/liquid purification was not robust enough and sometimes led to formation of three phases. Consequently, this strategy has been rejected and dispersive SPE (d-SPE) has been investigated for potential advantages it offers (fast, cost-effective, variety of sorbent available). C18, florisil, aluminium oxide and silica sorbents were tested but they were not efficient enough to allow injection on the chromatographic system. Solvents used during extraction were not suitable to permit the retention of pigments and other matrix compounds on the utilized sorbent. Hence, it has been decided to exploit the advantage of the SPE cartridge which can be used as a chromatographic device by adapting the separation speed between PAH and co-extracts. Sorbent used during SPE extraction should be adapted depending on the kind of matrix. Three different kinds of sorbent were compared: silica, alumina oxide and florisil. Commercial silica SPE cartridge of 500 mg used as filter was sufficient to obtain clean extracts for regular spices like pepper, garlic, etc… However, silica was not selective enough for the separation of PAHs and photosynthetic pigments present in dried herbs commodities. A combination of silica and alumina oxide gave very clean extract but PAHs seemed to be trapped on alumina oxide sorbent, leading to low recoveries. Finally, home-made florisil cartridge was the most efficient sorbent to separate these pigments from PAHs and obtain very clean extract for dried herbs commodities containing large amount of photosynthetic pigments.

More complex spices like curry are very heterogeneous matrices and can be frequently very fatty (14 g of fat /100 g of curry). In case of these matrices, neither commercial silica cartridge nor florisil home-made cartridge were adequate to remove all co-extracts and fat extracted with hexane/dichloromethane. Cartridges with an SPE sorbent produced by Supelco (SupelMIP^{TM}) and initially designed to extract PAHs from oil have been applied to three very different kinds of curry and were able to remove not only fat matter but also most co-extracts.

3.1.3. GC-MS/MS analysis

The developed GC-MS/MS method is sensitive and its LOD and LOQ satisfy the requirements of the European regulation (EFSA 2008; European Commission 2011b). For the separation, two columns of different length (15 m and 30 m) specifically designed for the separation of PAHs have been tested. The 15 m column allows a shorter run (30 min against 45 min for the 30 m column) but leads to a lack of resolution between BbF (included in the regulation) and benzo[j]fluoranthene (BjF) (not included in the regulation) over time. Hence, the decision has been taken to use the 30 m column which allows the determination of the 4 regulated PAHs with a very good resolution.

3.2. Method validation

The method was validated according to the Commission Regulation (EU) 836/2011. As required by the guideline, the limit of quantification/detection, the trueness (t), the within-day repeatability (r), day-to-day reproducibility (RW) and expanded measurement uncertainty (U) have been evaluated. For other parameters not covered by this regulation such as linearity and matrix effect, criteria have been based on the SANTE document used in pesticide field (SANTE/11813/2017 2017). In every case, it was impossible to find completely blank (PAH-free) samples for the validation. Consequently, for every matrix, a triplicate of non-spiked samples has been analysed. The high robustness of the method enables to average and subtract obtained concentrations from each value of spiked samples in order to compensate the overestimation caused by natural contamination without increasing standard deviations.

3.2.1. Linearity

The linearity of the detector response was investigated at 6 different concentrations for each PAH standard: 0.5 µg kg^{-1}, 2 µg kg^{-1}, 5 µg kg^{-1}, 15 µg kg^{-1}, 25 µg kg^{-1}, 50 µg kg^{-1}. Measurements were performed in triplicate. A Mandel's fitting test was used to determine whether the residual variances, resulting from the linear and the quadratic calibration function, significantly differ. As the test revealed no significant difference between these regression models, the linear model has been chosen for the ease of use. All coefficients of determination r2 for the linear regression were greater than 0.999.

3.2.2. Matrix effect

The matrix effect has been tested by comparing slope of a matrix-matched calibration curve to a calibration curve prepared in neat solvent. The SANTE document (SANTE/11813/2017 2017) stipulates that if the difference between slopes differs less than |± 20 %|, it can be concluded that there is no significant matrix effect. Calculations were performed in two ways, without and with correction of PAHs signal using isotopic labelled internal standards (IL-IS PAHs). table 5.2 shows difference between slopes of calibration curves in neat solvent and in matrix. These data unravel presence of a negative matrix effect for every PAH except for BaA based on the criteria |± 20 %|. Matrix effect is fully compensated by using corresponding deuterated IS. Eventually, for the quantification purpose, a calibration curve in solvent and response correction using IL-IS PAHs was used. Investment in deuterated standards is compensated by the time saved with making a calibration curve in solvent.

Table 5.2: Characterisation of matrix effect

PAHs	Difference (%) between slopes (matrix / solvent)	
	without IL-IS	with IL-IS
BaA	-16	0.9
CHR	-23	0.7
BbF	-32	0.9
BaP	-44	1.5

3.2.3. Detection and quantification limits

Recently, the four European Reference Laboratories for PAHs, mycotoxins, heavy metals and dioxins prepared together a guideline for an accurate determination of the LOD/LOQ (Wenzl et al. 2016). The document stipulates that it is not required to have an accurate determination of LOD/LOQ, if MRLs are far above the estimated LOD/LOQ of the analytical method. As BaP MRL is fixed at 10 µg kg^{-1} (European Commission 2015), it has been assumed that this value is far enough from LOQ demanded by the Regulation (EU) 836/2011 (0.9 µg kg^{-1}). Consequently, LOD and LOQ have been determined by a classical approach using a pseudo blank sample (naturally contaminated) close to the expected LOQ inducing a peak with a signal-to-noise (S/N) ratio of 3 and 10, respectively. fig. 5.2 shows chromatograms obtained for naturally contaminated samples at levels close to the expected LOQ (0.5 µg kg^{-1}) and a S/N of approximately 20 has been obtained for each PAH. For practical reason, LOD and LOQ have been set at 0.25 µg kg^{-1} and 0.5 µg kg^{-1} respectively and are in the same order of magnitude as those of Urban and Lesueur (Urban and Lesueur 2017). These values correspond to 5 pg of PAH injected on the GC-column instead of 1 pg obtained by Urban and Lesueur in curry powder with QuEChERS extraction and EMR-lipid dSPE. The specific clean-up described in this paper has the advantage to reach the same range of LOQ by injecting 5 times more analyte/matrix on the GC-column without losing the chromatography quality (loss of sensitivity/resolution/retention time shift) (Urban and Lesueur 2017).

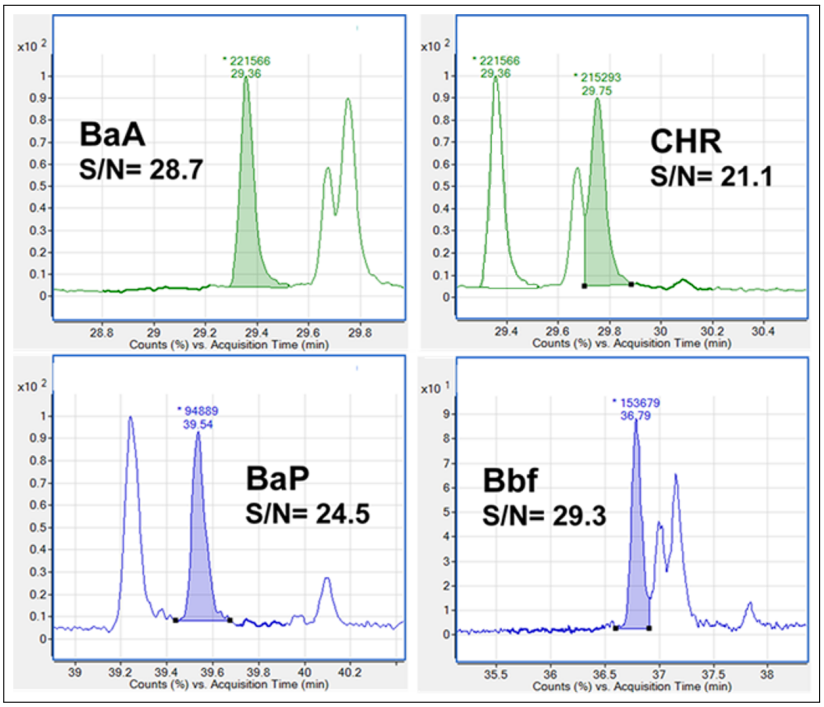

Figure 5.2: Ion chromatograms of contaminated sample and the signal-to-noise (S/N) ratio for BaA at 0.5 µg kg^{-1} in oregano, CHR at 0.6 µg kg^{-1} in curry, BbF at 0.4 µg kg^{-1} in curry and BaP at 0.5 µg kg^{-1} in curry

3.2.4. Precision and trueness

Firstly, a primary validation was performed in black pepper as representative matrix for the group of regular spices. To evaluate the precision, pseudo blank samples of pepper have been spiked at three concentration levels, namely at 0.5 x MRL (5 µg kg^{-1}), at MRL (10 µg kg^{-1}) and at 2 x MRL (20 µg kg^{-1}) during three different days with three different operators. The relative standard deviations for the repeatability (RSDr) and the reproducibility (RSDRW) were calculated for the 3 levels as described by ISO 5725-2 guidelines (ISO 5725 2019) and ranged from 3.2 % to 11.4 %. All RSDr and RSDRW values were well below the maximum demanded by the Regulation (EU) 836/2011, which are fixed at 14.5 % and 22 % respectively. Difference between RSDr and RSDRW is very low even if three different analysts performed the 3 different days of the reproducibility. To assess the trueness, recoveries from spiked samples have been checked with and without the correction by IL-IS. The yield was in the range of 60 – 80 % while corrected recoveries were all close to 100 % thanks to the use of IL-IS PAHs. A summary of results for the lowest spiking level are shown in table 5.4. Furthermore, secondary validations have been performed for the groups of pigmented spices and complex spices at the lowest level (0.5 x MRL, 5 µg kg^{-1}) in triplicate and at three different days. Three different kinds of matrices have been chosen in each group to obtain the worst deviation intra group. Oregano, parsley and basil have been used as representative for pigmented spices group and three very different curry have been used as representative for the complex spices group. Thanks to

the IL-IS standards, all recoveries are close to 100 % (from 93 to 110 %). RSDr and RSDRW were lower than 10 % even if 3 different matrices for each group were used for each day of the validation. All the RSDs are well below the maximum demanded by the Regulation (EU) 836/2011 table 5.3 14.5 and 22 % respectively, which demonstrate the method suitability for various types of spices.

These extensive validation data demonstrate the added value of having a generic extraction step followed by a specific clean-up adapted for each group of spices. Finally, the method has been successfully tested during a European Proficiency Test on PAHs in smoked pepper. Z-scores close to 0.0 were obtained for each PAH which proved efficiency of the developed analytical method table 5.4.

3.2.5. Measurement uncertainty

To assess the reliability of results given by this analytical method, expanded uncertainty (U) has been calculated from the relative standard uncertainty (u) with the coverage factor for 95 % of confidence (k=2) following eq. (5.1) and eq. (5.2) (SANTE/11813/2017 2017).

$$U = k * u \tag{5.1}$$

measurement uncertainty for individual PAH

The relative standard uncertainty of each individual PAH is calculated using both the RSD_r, RSD_{rw} and the laboratory bias based on the data generated during the validation following eq. (5.2).

$$u = \sqrt{u(\text{RSD}_{\text{rw}})^2 + u(\text{bias})^2} \tag{5.2}$$

This level was selected because analytical variability generally increases at lower concentration levels. Therefore, the resulting uncertainty can be considered a conservative estimation. This approach minimizes the risk of underestimating measurement uncertainty during routine monitoring, particularly as validation studies are performed under controlled conditions that may not fully reflect the variability encountered during long-term routine use and may therefore lead to artificially optimistic uncertainty estimates.

measurement uncertainty for PAH4

In the case of PAH4, the calculation of the combined uncertainty (u) of the sum of parameters is calculated as the square root of the sum of squares of each individual PAH following eq. (5.3) (European Commission 2022).

$$u_{\text{sum(abs)}} = \sqrt{\sum_{i=1}^{n} u_{i(\text{abs})}^2} \tag{5.3}$$

The expanded uncertainty for each PAH at low level is given in the (table 5.3). Recovery, precision and expanded uncertainty data for the 4 PAHs at 0.5 x MRL level (5.0 µg kg^{-1}) in regular spices, pigmented spices and complex spices.

Table 5.3: Recovery, precision and expanded uncertainty data for the 4 PAHs at 0.5 x MRL level (5.0 µg kg^{-1}) in regular spices, pigmented spices and complex spices

PAH	Regular spices (n=9)				Pigmented spices (n=9)				Complex spices (n=9)			
	Average recovery (%)	RSD_r (%)	RSD_{rw} (%)	U (%)	Average recovery (%)	RSD_r (%)	RSD_{rw} (%)	U (%)	Average recovery (%)	RSD_r (%)	RSD_{rw} (%)	U (%)
BaA	100	4.5	8.8	17.4	100	3.5	3.7	7.4	93	5.4	5.4	17.7
CHR	104	6.3	6.3	14.9	100	4.2	4.2	8.4	110	2.5	3.4	21.1
BbF	101	6.1	9.7	19.5	95	4.0	4.0	12.8	96	7.7	7.7	17.4
BaP	96	3.2	6.6	14.9	97	3.0	3.0	8.5	93	5.0	5.0	17.1

Table 5.4: Assigned values and result obtained for the inter-laboratory test 2016: Four marker PAHs in smoked pepper

PAHs	Assigned value $\mu g\ kg^{-1}$	Result obtained $\mu g\ kg^{-1}$	Z-scores
BaA	34.22 ± 1.03	31.65 ± 2.75	-0.4
CHR	39.84 ± 1.17	38.92 ± 2.90	-0.1
BbF	17.16 ± 0.64	17.42 ± 1.70	0.1
BaP	14.40 ± 0.52	13.67 ± 1.01	-0.3
ΣPAH4	105.62 ± 2.13	101.65 ± 6.07	-0.3

4 CONCLUSION

A GC-MS/MS method to quantify the four regulated PAHs (BaA, CHR, BbF and BaP) has been developed and successfully validated in various types of spices and dried herbs. This method has been applied to the European Proficiency test of 2016 on a naturally contaminated smoked pepper sample. Z-scores were all close to 0, which confirmed accuracy of the method. Extraction and purification steps have been specifically designed to be easily adapted to all spices and dried herbs available on the market. Furthermore, this method does not require the use of sophisticated equipment traditionally used in this field of analysis such as GPC or ASE, Soxhlet or bulky laboratory material which can be time and solvent consuming. Hence, it can easily be used with conventional equipment available in most laboratories. The use of internal standards for each PAH allows to reach good recoveries and compensates the matrix effect making the preparation of the calibration solutions simpler.

The method validation successfully met the criteria of the European Regulation 836/2011 with mean recoveries close to 100 % and RSD largely below 22 % for both intra-day repeatability and inter-day-reproducibility. Finally, the LOD and LOQ were set at 0.25 $\mu g\ kg^{-1}$ and 0.5 $\mu g\ kg^{-1}$.

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Compliance with ethics Requirements
Philippe Szternfeld declares that he has no conflict of interest. Jessica Marchi declares that she

has no conflict of interest. Svetlana V. Malysheva declares that she has no conflict of interest. Laure Joly declares that she has no conflict of interest.

II. Occurrence of Polycyclic Aromatic Hydrocarbons in spices and dried herbs and associated risk for the Belgian population

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Abstract

A total of 120 spices and herbs bought on the Belgian market have been analysed for occurrence of the four EU regulated polycyclic aromatic hydrocarbons (ΣPAH4). Samples were sorted in three groups according to their use: traditional spices, exotic spices and herbs. Benzo[a]pyrene has been detected in 56 % of samples, while ΣPAH4 were found in 96 % of samples. ΣPAH4 were evenly distributed among the 3 groups with a median content of 7.2 (traditional), 5.5 (exotic) and 6.0 µg kg^{-1} (herbs). According to the EU legislation, three samples were exceeding maximal limits, with a maximum ΣPAH4 content of 164 µg kg^{-1}. The ΣPAH4 dietary intake has been estimated at 1.4 and 27.8 ng day^{-1} for average and high consumption respectively. The risk for the consumer was evaluated by using the margin of exposure (MOE). In all cases, MOE were > 20 000, indicating a low concern for the population.

Keywords: PAH - spices - dried herbs - GC-MS/MS - monitoring - Belgium - risk assessment

1 INTRODUCTION

Spices and herbs are widely used as flavourings, colourants, and aromas for dishes. Besides their organoleptic qualities, they are also used as preservatives and possess some health-beneficial properties such as antioxidant, antimicrobial, hepatoprotective, or anti-inflammatory effects (Kurian 2012). As the global market of spices and herbs has been expanding continuously for two decades and considering their process of production, questions may arise regarding consumer safety (Businesscoot 2021; CBI 2022). Indeed, the presence of pesticide residues, mycotoxins and heavy metals has already been reported in the literature (Mubeen et al. 2009; Yogendrarajah et al. 2014; Reinholds et al. 2016; Reinholds et al. 2017) and in the Rapid Alert System of Food and Feed (RASFF) of the European Commission with 112 notifications for pesticides residues, 73 for mycotoxins and 6 for heavy metals for the 2020-2022 period (RASFF 2022).

Additionally, polycyclic aromatic hydrocarbons (PAHs) have also been found in large quantities in spices and dried herbs in studies over the last 15 years (EFSA 2008). PAHs are a large class of organic compounds composed of two or more fused aromatic rings (Harvey 1991). Food is considered the main source of exposure to PAHs for a non-smoker. The contamination may occur via food processing (e.g. grilling, roasting, drying or smoking). However, environmental pollution cannot be excluded for plant products such as spice and herbs. Indeed, PAHs, produced in industrial areas or originated from heavy motor traffic, may be bound to fine particles and be adsorbed on the waxy foliar surface via atmosphere deposition (Ignesti et al. 1992; Howsam and Jones 1998; Phillips 1999). PAHs can also contaminate the soil and surface water and then be translocated in the plant before the harvest (Scientific Committee on Food 2002).

The primary concern of PAHs is their carcinogenic and mutagenic properties (Phillips 1999). In 2002, the European Scientific Committee on Food assessed the toxicity of many PAHs, thereby identifying 15 PAHs as genotoxic or carcinogenic (Scientific Committee on Food 2002). Afterwards, the CONTAM Panel of the European Food Safety Authority (EFSA) concluded in 2008 that monitoring of 4 PAHs (i.e. benzo[a]anthracene (BaA), chrysene (CHR), benzo[b]fluoranthene (BbF), and benzo[a]pyrene (BaP)) would be suitable for evaluating the overall occurrence of PAHs in food (EFSA 2008). As a result, the European Commission established maximum levels (MLs) for the sum of these four PAHs and the individual occurrence of BaP in different food matrices (European Commission 2011a). Specifically for spices and herbs, the MLs were set at 50.0 µg kg^{-1} for the sum of the four regulated PAHs (ΣPAH4) and 10.0 µg kg^{-1} for BaP (European Commission 2015). Since the establishment of these MLs, twelve severe alert notifications have been reported by RASFF in the period 2020-2022 (RASFF 2022). Although the Regulation (EU) No 2015/1933 entered into force seven years ago, only a few specific methods dedicated explicitly to PAHs in herbs and spices can be found in the literature. One reason may be the analytical challenge posed by the broad and heterogeneous group of spices and herbs. This food group contains many compounds similar to PAHs that are the major obstacle hampering correct quantification (Szternfeld et al., 2019). HPLC-UV/FLD analysis, in particular, may lead to overestimating PAHs content as the lack of specificity inherent to this technique avoids the distinction between PAHs signals and matrix interferences. The use of GC-MS/MS efficiently solves this problem (Bratinova et al. 2016). However, if the clean-up is not intensive enough, the matrix may compromise

the adequate separation of the PAHs isomers. This is the major concern during GC-MS/MS analysis. Nevertheless, some methods were already reported.

Rozentale et al. (2018) developed a method based on extraction with a dichloromethane (DCM)/hexane mixture, followed by gel permeation chromatography and purification on silica. Even though such technique can be time and solvent consuming, it allowed them to analyse a variety of spices and herbs matching the analytical EU criteria (European Commission 2011b). Urban and Lesueur (2017) considered the use of a modern method such as QuEChERS extraction, initially designed for pesticides analysis in food (Payá et al. 2007), followed by a Captiva Enhanced Matrix Removal-lipid clean-up (sorbent design to remove lipids based on size exclusion and hydrophobic interaction). With this method, time and solvent consumption are drastically reduced. Although the obtained extract was dirtier, necessitating more frequent instrument maintenance, Urban and Lesueur (2017) successfully used it to quantify PAHs in curry powder.

More recently, Szternfeld et al. (2019) proposed a GC-MS/MS method capable of facing the wide variety of spices and herbs available on the market. The method is based on extraction with DCM and hexane followed by a specific Solid Phase Extraction (SPE) clean-up depending on the spices and herbs considered. This modular method allows very low limits of quantifications (LOQs), matching all criteria of the Regulation in force (European Commission 2011b). However, the use of DCM is forbidden in some EU countries. Therefore, the European reference Laboratory for processing contaminants (EURL-PC) initiated, in 2020, an inter-laboratory validation of an analytical method adapted from Szternfeld et al. (2019), replacing the use of DCM with ethyl acetate and different SPE sorbent (Sina Wilde and Duedahl-Olesen 2021). Even if more dirty extracts are obtained, this method allows the analysis of a wide variety of matrices similar to spices and herbs with acceptable LOQs for the four regulated PAHs.

Nevertheless, the number of studies dealing with PAHs monitoring in spices and herbs are still limited. Zelinkova and Wenzl (2015) have studied PAHs content in plant and herbal food supplements that are of the same nature and following a drying process very similar to spices and herbs. Their main findings illustrated non-systematic ΣPAH4 contamination originating from environmental pollution and processing techniques (mainly due to bad drying practices). Moreover, high ΣPAH4 content was found in some samples, which could remarkably increase the daily exposure to these substances, stressing the need to continue to monitor this kind of food matrices (Zelinkova and Wenzl 2015). A study of (Bogdanović et al. 2019), quantifying PAHs in various spices and herbs in a limited number of samples (20), concluded that the PAHs content is much higher in these matrices. A recent study by Rozentale et al. (2018), focusing on six spices and herbs (thyme, pepper, nutmeg, basil, oregano, paprika), confirmed through the analysis of 150 samples the non-systemic contamination observed by Zelinkova and Wenzl (2015). Furthermore, they recommended collecting data to properly manage the risks associated with the presence of PAHs in spices and herbs.

Furthermore, as spices and herbs consumption is continuously increasing over the years (Businesscoot 2021), a deeper investigation is needed on the occurrence of ΣPAH4 in spices and herbs. In this contribution, an extensive study on the occurrence of the four regulated PAHs (i.e. BaA, Chr, BbF, and BaP) in 120 spices and dried herbs available on the Belgian market is presented. The compliance of samples compared to maximal limits from the Regulation (EU) No 2015/1933 and the potential risk for the Belgian population have been assessed.

2 MATERIALS AND METHODS

2.1. Chemicals and Reagents

A mixture of 4 regulated EU PAHs : BaA, CHR, BbF and BaP (10 ng μl^{-1} in cyclohexane) and a mixture of isotopically-labelled (deuterated) standards (IL-IS): BaA D12, CHR D12, BbF D12, BaP D12 were purchased from Dr. Ehrenstorfer Gmbh (Ausburg, Germany). Hexane (HPLC grade) was from Biosolve (Valkenwaard, The Netherlands), while DCM (HiperSolve HPLC^{TM} was supplied by BDH Chemicals (England). Bond Elut SI (silica) cartridges (500 mg, 3 ml) were purchased from Agilent Technologies (Machelen, Belgium). Empty SPE cartridges (6 ml) of Biotage Isolute® (Sopachem, Gent, Belgium) were filled with Florisil (60-100 mesh) from Fluka (Sigma Aldrich, Overijse, Belgium) to prepare in-house SPE cartridges. SupelMIP^{TM} SPE cartridges were supplied by Supelco (Sigma Aldrich, Overijse, Belgium).

Working solutions of PAHs were prepared from the commercial solution at 10 µg ml^{-1} in cyclohexane, resulting in a concentration of 1 µg ml^{-1} and 0.1 µg ml^{-1}. Working solutions of IL-IS PAHs were prepared by diluting the commercial solution at 10 µg ml^{-1} in hexane to obtain a concentration of 1 µg ml^{-1}. All solutions were stored at -20 °C.

2.2. Sampling strategy

120 Samples were selected in 2019 based on the representativeness of the Belgian market and the habits of consumers. The choice of brands was based on Euromonitor's Belgian market share data (Euromonitor Grocery universe 2017). Organic stores were also included to reflect as much as possible Belgians' shopping habits. The availability of food products on the Belgian market was also considered a parameter to modulate the number of samples per type of spices or herbs.

Samples were milled to a fine powder and subsequently homogenised in accordance with Regulation (EU) No 836/2011 (European Commission 2011b) using a mill (IKA M20, IKA®-Werke GmbH & Co. KG, Staufen, Germany) suitable for dry grinding and powder matrices.

2.3. Determination of PAHs content

2.3.1. Extraction and purification

Afterwards, the PAHs were extracted using the procedure described by Szternfeld et al. (2019). Briefly, 1.0 g of homogenised sample was weighed, and the internal standards were added (IL-IS PAHs). After equilibrating for 20 min, the PAHs were extracted with 20 ml of a mixture of hexane/DCM (3/1, v/v) using an ULTRA-TURRAX® (Ultra Turrax, IKA, Staufen, Germany). After centrifugation of 5 min at 3900 rpm, the supernatant was concentrated to approximately 1 ml and further purified using SPE. Different SPE sorbents were used, depending on the spices or herbs. Traditional spices were purified with a silica SPE cartridge, while herbs and pigmented spices were purified using a florisil sorbent. Purification of complex spices or fatty spices were first purified with silica, followed by a clean-up using SupelMIP^{TM} PAHs SPE, which is specifically designed for the purification of PAHs in oil. After the purification step, the extracts were concentrated under a nitrogen stream to a volume of 200 µl before

analysis by GC-MS/MS.

2.3.2. GC-MS/MS analysis

GC-MS/MS analyses were performed on an Agilent 7890B GC system coupled with an Agilent 7000C triple quad mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) with an electronic impact source. The chromatographic column was the J&W GC column Select PAH (30 m x 0.25 mm I.D. x 0.15 µm df), and the liner inlet was a single taper (4 mm I.D) containing wool (Agilent Technologies). Injections were performed in splitless mode at 300 °C with an injection volume of 2 µl. Helium (≥ 99.9999 %) was used as carrier gas at a flow rate of 1.2 ml min^{-1}. The oven temperature was programmed according to an Agilent application note (Provoost 2010). The initial temperature was set at 70 °C (held for 0.4 min) and increased at a rate of 85 °C min^{-1} to reach 180 °C, followed by an increase with 3 °C min^{-1} to 230 °C (held for 7 min) and up to 280°C (held for 10 min) with a speed of 28 °C min^{-1}. Finally, the temperature increased with 14 °C min^{-1} up to 325 °C, held for 3 min. The following source parameters were used: temperature of 280 °C and electron energy fixed at 70 eV. Data acquisition was performed in MRM mode, and 2 transitions were selected for each PAH. The results were interpreted using the Mass Hunter software from Agilent (Agilent Technologies). Two criteria were used for the correct identification of the PAHs: (i) retention time difference between the native compound, and its IL-IS should not differ more than 0.1 min; (ii) ion ratio of the two most abundant product ions (i.e. 2 MRM transitions) should be within the range of 30 % compared to the closest calibration standard. Finally, relative areas of analyte and corresponding internal standards were used for the quantification.

2.3.3. Quality control and quality assurance

The sensitivity and stability of the GC-MS/MS system have been controlled by injecting a calibration point at LOQ level (0.5 µg kg^{-1}) at the beginning and the end of each analysis batch. A signal-to-noise ratio > 3 for all individual PAH for both injections ensures there was no sensitivity loss. Additionally, the concentrations of each calibration level were back-calculated using the regression equations of the calibration curve. The deviation should be lower than ±20 %, illustrating accurate PAHs quantification. A procedural blank was analysed for each batch of samples to ensure that no PAH contamination occurred, either at the level of the extractions or at the GC-MS/MS system level. Extraction efficiencies were evaluated for each analysis batch by checking the recovery of a control sample fortified at 10.0 µg kg^{-1}. Results were plotted on a control chart to ensure no method deviation occurred. The method has been validated in accordance with criteria laid down in Regulation (EU) No 836/2011 (European Commission 2011b; Szternfeld et al. 2019). Finally, the developed methodology was applied successfully in two proficiency tests (i.e. EURL PC-PT-2016 on smoked pepper and EURL-PC-PT-2019 on herbal food supplements) (Bratinova et al. 2016; Duedahl-Olesen 2019). Acceptable z-scores (|z-score|<2) were obtained for all individual regulated PAHs demonstrating that this method is fit-for-purpose.

2.4. Dietary exposure

2.4.1. Consumption data

The Food Additives Intake Model (FAIM) is a tool for estimating chronic dietary exposure to food additives. In the first version of the FAIM template, summary statistics from the

Comprehensive Database to estimate chronic dietary exposure in different population groups and EU countries were included (EFSA 2011). Although the FAIM tool was initially developed for food additives, it contains valuable information regarding the consumption of spices and herbs. Indeed, the consumption records in the FAIM tool are codified according to the food categories as presented in Annex II, Part D, of Regulation (EC) No 1333/2008 (European Commission 2011c). One of these categories is dedicated explicitly to herbs and spices (i.e. category 12.2.1). The 1st version of this tool contained the Belgian consumption data of 2004 for this specific category for both the mean of the total population (i.e. 0.0017 g kg^{-1} body weight (bw) day^{-1}) and the high level for consumers only (i.e. 0.03412 g kg^{-1} bw day^{-1}). Hence, consumption data contained in this tool were then combined with the average concentration data for ΣPAH4 obtained in this study to evaluate the exposure of the Belgian population.

2.4.2. Treatment of left-censored data

The values reported as "< LOQ (0.5 µg kg^{-1}) have been replaced by LOQ/2 (i.e. 0.25 µg kg^{-1}) to perform medium bound calculations. Afterwards, the exposure due to spices and herbs was added to the overall exposure determined by EFSA in 2008 (EFSA 2008). The uncertainty associated with the treatment of left-censored data is expected to be negligible, as PAHs were quantified in 93 % of the analysed samples (112 out of 120).

2.4.3. Exposure scenario

Only the average concentration data were considered. No worst-case scenario using the maximal level found for the ΣPAH4 has been assessed. The reason is that consumption data were not detailed for each spices and herbs. Hence if the maximum concentration would be used instead, it would be applied for spices and herbs for which very low concentration have been observed and would lead to abnormally high and unrealistic dietary exposures.

2.5. Risk assessment

Risk assessment was based on the margin of exposure (MOE) approach as proposed by EFSA for risks associated with genotoxic and carcinogenic compounds in food and feed such as PAHs (EFSA 2005). The MOE is the ratio between a defined point on the dose-response curve for the adverse effect of ΣPAH4 and the exposure to ΣPAH4 (eq. (5.4)). This specific point refers to the benchmark dose (BMD) for which a low but measurable response is seen (in this case, 10 % incidence above the population control) (BMD_{10}). Then a BMD_{10} lower limit ($BMDL_{10}$) corresponding to the lower limit of the one-sided 95 % confidence interval of the BMD_{10} has been defined. This lower bound allows considering uncertainty derived from a given study. If the $BMDL_{10}$ is derived from an animal carcinogenicity study, the EFSA Scientific Committee suggests that a MOE of 10000 or higher would indicate a low concern for the public health (EFSA 2005).

$$\text{MOE} = \frac{\text{BMDL}_{10}\ \Sigma\text{PAH4}}{\Sigma\text{PAH4 exposure}} \tag{5.4}$$

Where $BMDL_{10}$ is the benchmark dose lower confidence level in mg kg^{-1} body weight (bw) day^{-1} and ΣPAH4 exposure is the total amount of PAH4 ingested by the consumer in mg kg^{-1} bw day^{-1}. In the case of ΣPAH4, the $BMDL_{10}$ is 0.34 mg kg^{-1} bw day^{-1}.

3 RESULTS AND DISCUSSION

3.1. Sampling

In total, 120 herbs and spices were purchased in 2019 in Belgian supermarkets (75 %) and organic stores and groceries (25 %). Spices and herbs have been grouped depending on their nature in 3 different categories to facilitate data treatment and risk assessment: (i) traditional spices, (ii) exotic spices, (iii) dried herbs (table C1). Spices commonly or daily used in Belgian dishes have been classified as traditional spices. Other spices primarily used in Asian or North African cooking have been classified as exotic spices. As a result, 27 samples were categorised in the group traditional spices, 38 samples were allocated to the group exotic spices, 55 samples were considered dried herbs (including 9 samples of mixed herbs).

3.2. Occurence data of PAHs in herbs & spices

The results of the occurrence data (BaP and ΣPAH4) are presented in fig. 5.3, while all the individual results of the four PAHs are shown in the Appendix C, table C1. Overall, the ΣPAH4 have been detected in 93 % of the samples, while BaP was present in only 56 % of samples (table C1). This can be explained by the heavier 5-ring structure of BaP, which requires a higher temperature and longer processing time to be formed, compared to other PAHs (BaP, CHR) with a 4-ring system (Harvey 1991). Moreover, BaP and the ΣPAH4 were similarly distributed in each group (fig. 5.3 B), despite the relatively high number of statistical outliers in the exotic and dried herbs groups (fig. 5.3 A). The median BaP levels were <LOQ for each group, while the medians for the ΣPAH4 were 7.2 µg kg^{-1} (classic spices), 5.5 µg kg^{-1} (exotic spices) and 6.0 µg kg^{-1} (dried herbs) (fig. 5.3 B). No difference in PAHs contamination has been noticed between supermarkets, organic stores, and groceries samples. The highest outlier of the exotic group was the smoked paprika sample with a concentration for the ΣPAH4 of 164.0 µg kg^{-1}. This high concentration could originate from the extra smoking step performed with oak wood. Similar results were also observed in other studies. Berki et al. (2020) reported a ΣPAH4 content ranging from 115.0 µg kg^{-1} to 3476.0 µg kg^{-1} for smoked paprika, which was smoked using the same type of wood. Also, Coleto et al. (2021) reported an average concentration for ΣPAH4 of 780.0 µg kg^{-1} for Spanish smoked paprika. These values are much higher than this study and exceeded the MLs set in Regulation (EU) No 2015/1933 for the ΣPAH4 (European Commission 2015). These huge contents seemed to be linked to the traditional smoking process, resulting in direct contact of smoke with the spices.

An overview of the results of the sub-categories with at least 4 samples, is presented in table 5.5. The three highest outliers of the category of the dried herbs correspond to 2 samples of laurel and 1 sample of thyme with concentrations of ΣPAH4 of 154.0 µg kg^{-1}, 58.2 µg kg^{-1} and 126.0 µg kg^{-1}, respectively. The higher concentration in laurel can be explained by their large and waxy foliar surfaces on which PAHs can readily be adsorbed (Phillips 1999; Zelinkova and Wenzl 2015). However, it can also originate from the processing method applied for drying. In some regions, in summer, herbs are dried by being exposed to the sun or using a solar dryer. However, a dryer using wood burning is typically applied in winter to overcome the high humidity. As a result, an increase in the PAH content in the final product could be observed (Coleto et al. 2021). Similarly, the higher level of ΣPAH4 observed in thyme

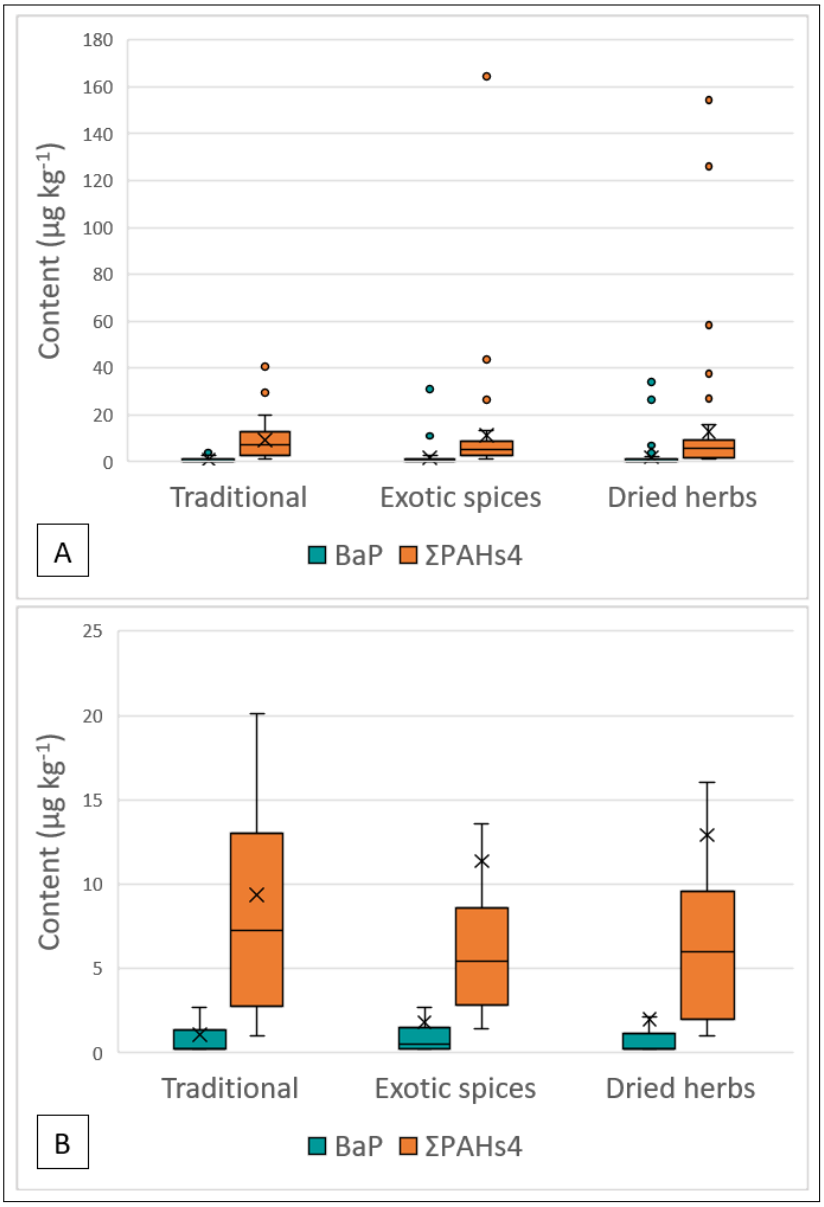

Figure 5.3: A) BaP and ΣPAH4 concentrations (µg kg^{-1}) for the three spices and dried herbs groups. Boxes indicate 25th, 50th, and 75th percentiles and whiskers (error bars) represent minimum and maximum. Values <LOQ (0.5 µg kg^{-1}) have been replaced with 1/2 LOQ. Dots represent statistical outliers. B) Zoom-in of fig. 5.3 A. Statistical outliers are not shown

could be explained. Unfortunately, information on the drying processes or the harvest period was not available on the labels of the products. Relatively higher ΣPAH4 concentrations have been found in "bouquet garni" compared to other herbs included in the group "dried herbs" (table C1). Interestingly, the composition of "bouquet garni" can vary significantly, but at least 50 % is constituted of laurel and thyme, which can explain the concentration found.

Only limited studies related to PAHs in spices and herbs have been reported. One of the studies, performed by Lee et al. (2018) reported the same PAHs content in black pepper, with an average content of 1.9 and 16.3 µg kg^{-1} for the BaP and ΣPAH4, respectively. Another study Rozentale et al. (2018), focussed on samples produced between 2010 and 2015. They reported comparable results for thyme when excluding the outlier at 126.0 µg kg^{-1} without being able to identify the origin of such contamination. Regarding pepper, they found a similar mean concentration for the ΣPAH4 content (i.e. 13.2 µg kg^{-1}). Paprika, basil, turmeric, oregano and curry were less contaminated and showed very similar levels with a concentration for ΣPAH4 ranging between 4.3 µg kg^{-1} and 6.4 µg kg^{-1} on average. Except for basil for which they found ΣPAH4 content twice higher than this study (11.6 µg kg^{-1}), Rozentale et al. (2018) found similar results for paprika and oregano with respectively 8.0 µg kg^{-1} and 4.6 µg kg^{-1}. Finally, rosemary and ginger showed the lowest average contamination levels in ΣPAH4 with 1.4 µg kg^{-1} and 2.9 µg kg^{-1}, respectively. No BaP was detected in rosemary samples. Only one sample contained a level close to the LOQ for ginger (0.5 µg kg^{-1}) (table 5.5, table C1). A recent study of Bogdanović et al. (2019) reported the same ΣPAH4 content (1.2 µg kg^{-1}) in rosemary herbs sampled during the 2017-2018 period. Our results agreed with the general observations made by Rozentale et al. (2018) and Zelinkova and Wenzl (2015). Indeed, although some trends can be noticed regarding concentrations of ΣPAH4 in spices and dried herbs, no unusual contamination has been noticed for specific products. The non-systematic ΣPAH4 contamination observed in this study may originate from environmental pollution or the drying process.

Finally, regarding samples compliance according to Regulation (EU) No 2015/1933, three samples (2.5 %) exceeded the ML for PAHs in spices and dried herbs set at 10.0 and 50.0 µg kg^{-1} for BaP and ΣPAH4, respectively (European Commission 2015). A smoked paprika, which also showed the highest concentration for both BaP (31.0 µg kg^{-1}) and ΣPAH4 (164.0 µg kg^{-1}), exceeded the ML for both limits, while a laurel exceeded the ML for ΣPAH4 (58.6 µg kg^{-1}) and the ras-el-hanout spices exceeded the ML for BaP (11.0 µg kg^{-1}). Interestingly, the results of this study were very similar to Rozentale et al. (2018) performed with samples produced prior to the Regulation (EU) No 2015/1933.

3.3. Dietary exposure

In 2008, EFSA evaluated the dietary exposure to ΣPAH4, but spices and herbs were not considered since EFSA stated that the impact on the overall exposure would be limited. EFSA determined the exposure originating from different food categories, resulting in an overall exposure to ΣPAH4 of 1158.0 ng day^{-1} (for the mean consumption level) (EFSA 2008). However, since the consumption of spices and herbs continuously increases (Businesscoot 2021), the additional exposure to ΣPAH4 also gains importance. Since the FAIM model contained specific consumption values for spices and herbs, these data were combined with the average contamination level of all samples, as ΣPAH4 were evenly distributed among the 3 groups of spices and dried herbs (fig. 5.3). In accordance with the EFSA opinion of 2008, a

Table 5.5: Min, max and average BaP and ΣPAHs4 content for spices and herbs for which sample number was > 4

Spices/herbs	Nb	BaP (µg kg^{-1})		ΣPAHs4 (µg kg^{-1})	
		min - max	average	min - max	average
Laurel	4	<LOQ – 34.0	13.0	8.8 – 154	73.9
Thyme	6	<LOQ – 26.4	6.6	6.1 – 126	39.0
Pepper	15	<LOQ – 4.8	1.4	1.3 – 40.7	12.3
Paprika	7	<LOQ – 2.0	1.1	3.0 – 9.1	6.4
Basil	8	<LOQ – 0.8	<LOQ	1.0 – 9.8	5.7
Tumeric	5	<LOQ – 2.0	0.72	1.5 – 13.6	5.0
Origano	5	<LOQ – 1.3	<LOQ	1.2 – 11.5	4.7
Curry	6	<LOQ – 1.0	0.55	1.7 – 7.0	4.3
Tarragon	5	<LOQ – 0.6	<LOQ	1.3 – 6.8	3.8
Ginger	5	<LOQ – 0.5	<LOQ	1.4 – 6.5	2.9
Rosemary	4	<LOQ - <LOQ	<LOQ	<LOQ – 1.5	1.4

body weight of 70 kg was applied, and an average-bound scenario was considered. Hence, the ΣPAH4 intake for spices and herbs was estimated at 1.4 ng day^{-1} for the mean population and 27.8 ng day^{-1} for the highly exposed population (P97.5). Next, the specific exposure through spices and herbs was combined with the overall exposure determined by EFSA (EFSA 2008). As a result, the total dietary exposure for ΣPAH4 was 1159.0 ng day^{-1} and 1186.0 ng day^{-1} for average consumption and high consumption (P97.5), respectively (table 5.6).

3.4. Risk assessment

Regarding the risk for the Belgian consumer, EFSA suggests that a MOE ≥ 10 000 would be a low health concern (if based on the $BMDL_{10}$ from animal study). This MOE only indicates the level of concern and must not be used to quantify the risk (EFSA 2005). The MOE values were calculated for the different scenarios and are presented in table 5.6. Since all MOE values are higher than 20 000, it can be assumed that the exposure to PAHs does not pose a health risk for the Belgian population, considering average contaminations of samples. The impact of the spices and herbs consumption and consumption scenario on the MOE is very low when compared with EFSA data from 2008 (that excluded this food category) (EFSA 2008). Indeed, the contribution of spices and herbs resulted in a MOE decrease of only 0.12 % (average consumption) and 2.34 % (high consumption) (table 5.6). However, the consumption data used for the exposure assessment originates from the National Consumption Survey (FCS) of 2004. A more recent National FCS is available, but these data are not yet included in the FAIM tool, nor are these data easily translated in specific consumption of herbs and spices. However, it can be assumed that the spices and herbs consumption coming from the National FCS of 2004 is underestimated. Indeed, market studies have shown that the consumption of spices and herbs has increased over the years. In France, an increase of 30 % was observed from 2009 to 2018. Furthermore, the global spices market is expected to grow at 3-4 % per

Table 5.6: Exposure to ΣPAH4 according to different consumption scenarios, associated MOE, and impact of each scenario on MOE

Food categories	Exposure ΣPAH4 (ng day^{-1})	MOE	decrease of MOE (%)
EFSA 2008 (spices & herbs excluded)	1158.0	20553	-
EFSA 2008 + spices & herbs median consumption	1159.0	20540	-0,06
EFSA 2008 + spices & herbs high consumption	1172.0	20300	-1.23

year between 2020 and 2025. Moreover, Belgium is considered the second-largest spices and herbs consumer in Europe (Businesscoot 2021). Nevertheless, even if the consumption is underestimated, it can be assumed that the risk will remain low. Indeed, to consider spices and herbs as a concern for the consumer (MOE ≤ 10 000), spices and herbs consumption should be at least between 85 (high consumption) and 854 (average consumption) times higher than the consumption level recorded in 2004, which seems highly unrealistic.

4 CONCLUSION

The extensive study of spices and herbs available on the Belgian market enabled to characterise the impact of PAHs contamination of spices and herbs on the Belgian consumer safety.

Occurrence data of 120 samples showed a similar distribution among the 3 groups studied (traditional spices, exotic spices and dried herbs), even if high variability for some categories, particularly in laurel and thyme has been noticed. Our findings confirmed observations from Rozentale et al. (2018) and Zelinkova and Wenzl (2015) regarding heterogenous ΣPAH4 contaminations among samples that may originate from environmental pollution or the drying process as well.

For all consumptions scenario studied, calculated MOE were above 20 000. Therefore, it can be concluded that the exposure of the Belgian population to PAHs through spices does not pose a potential health risk.

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Disclosure statement
The authors report there are no competing interests to declare

Chapter 6

General discussion

The studies presented in this thesis addressed multiple aspects of chemical contaminants in food, ranging from analytical method development to occurrence investigations and exposure assessment. Although each study focused on specific contaminants and food matrices, their combined interpretation provides broader insights into the analytical challenges and risk assessment considerations associated with food contamination.

The purpose of this general discussion is therefore to integrate these insights across the different chapters and to examine their scientific and regulatory implications within a unified framework. To facilitate this integration, the discussion is structured around four main themes: (i) the development of adaptable analytical strategies for contaminant analysis, (ii) the occurrence of contaminants in food based on market surveys, (iii) the impact of consumer practices on exposure, and (iv) the implications of these findings for food safety risk assessment.

1 Design of adaptable analytical strategies for contaminants in food

The initial and critical step in conducting reliable risk assessment involved developing analytical methods that were robust, accurate and sensitive enough to minimize left-censoring. These methods also needed to be compatible with a diverse range of molecules, as seen in the analysis of 55 pesticide residues in tea. For contaminants such as acrylamide and PAHs, the methods required modularity to allow adaptation to the broad spectrum of food analysed in this thesis. Lastly, the analytical methods were design to be modern, fast and that didn't require complex and expensive instrumentation. In 2003, Anastassiades et al. (2003) introduced the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, and Safe), which served as the basis for the methodological strategies employed in this work. Although initially designed for pesticide analysis in fruits and vegetables, this study demonstrated that the QuEChERS principle could be effectively adapted to a wider range of matrices and other contaminants.

1.1. Applying the QuEChERS method to pesticide transfer assessment in tea

The QuEChERS method was subsequently adapted to determine pesticide residues in tea brew in order to calculate transfer factors (TFs) from dried tea leaves to the brew. The main advantage of this method is the use of acetonitrile (ACN) as an extraction solvent that is suitable for both LC and GC amenable pesticides, avoiding performing two extractions in parallel. However, the original design of the method was not suited to the specific requirements of this application. Dilution levels in the standard protocol resulted in limit of quantification (LOQ) too high for accurate determination of TFs of non-polar pesticides such as the pyrethroid class (Log P>7). Samples, ACN volume and salt amounts have been doubled, following an evaporation step before reconstitution of the sample extract in 500 µl of water (LC-MS/MS analysis) or diisopropyl ether (GC-MS/MS analysis). The substitution of ACN with diisopripyl ether improved both chromatographic peak quality and LOQ (0.75 ng mL^{-1}).

These slight modifications enabled the determination of 55 pesticide transfer factors at a minimum of 0.5 %, demonstrating the method's capacity for determining accurate TFs for pesticides exhibiting a wide range of polarity (log P from -0.68 up to 7.5).

1.2. Applying the QuEChERS method to acrylamide analysis in heterogeneous foods

Although originally developed for the analysis of pesticides, the application of the QuEChERS method to other classes of contaminants, such as acrylamide, was investigated to see if it was possible to benefit from the same advantages. Indeed, a review of the literature revealed that most methods for the determination of acrylamide use water as the extraction medium (Keramat et al. 2011), a logical choice since it allows optimal recovery for very polar compounds such as acrylamide (log P= -0.78) (International Chemical Safety Cards 2024). However, the use of water for extraction poses challenges in the purification steps. First, concentrating water is very time consuming. A concentration step was necessary to achieve an acceptable LOQ (≤20 µg kg^{-1}). Second, for cereal-based commodities, the use of water tends to form emulsions, causing clogging of filters and solid phase extraction (SPE) cartridges. This option was not acceptable as cereal-based matrices accounted for 55 % of the samples selected for the study. A literature review showed that the QuEChERS extraction method is a suitable approach despite the high polarity of acrylamide. The salting-out effect induced by the presence of NaCl improves polar compound recovery in ACN such as acrylamide (Anastassiades et al. 2003). Furthermore, this strategy has been increasingly applied to the analysis of acrylamide (Sebastià et al. 2023). It was therefore decided to explore the potential of the QuEChERS method. However, it was first necessary to define a strategy to adapt the method to a wide diversity of foods, as specified in the EU Recommendation 2019/1888 (European Commission 2019), and how to validate it in an efficient way without losing a lot of time in the laboratory. This EU Recommendation covers 25 food categories, some of which are characterized by their heterogeneity (e.g. fudge, caramel, nougat, pastry). To meet this challenge, it was decided to group foods according to their nature (e.g. cereals, vegetables) and composition (e.g. water content, fat content, sugar content). The idea was then to perform a common extraction for all food groups and then apply a specific cleanup step based on d-SPE sorbents depending on the food being analysed.

The methods were then validated by mixing different foodstuffs belonging to the same food category to take into account the diversity of the group and to obtain a more realistic uncertainty. The strategy proved to be successful, as validations were carried out on 10 food subgroups, with recoveries ranging from 91.7 to 106 %, in line with EU criteria (70 - 110 %) of EU Regulation 2017/2158 (European Commission 2017). Precision in repeatability and reproducibility conditions was less than 15 %, in line with EU criteria set at 14.5 % and 22 %, respectively. For all matrices, the limit of detection (LOD) and LOQ were set at 7 and 20 µg kg^{-1}, respectively. Although the LOQ of the present study is lower than that of (Breitling-Utzmann and Wendler 2019) (30 µg kg^{-1}), but higher than that of De Paola et al. (2017) (5 µg kg^{-1}), comparing the LOQ between studies is challenging due to different approaches to its determination. For example, while De Paola et al. (2017) relied on the calibration signal, the present study determined the LOQ at the lowest fortification level in accordance with the validation criteria of EU Regulation 2017/2158.

Unfortunately, it was not possible to apply QuEChERS to the analysis of acrylamide in coffee

and cocoa powder, as this resulted in unacceptable background noise and interference in the chromatogram, preventing accurate quantification. Therefore, a water-based extraction followed by a purification step using a multimode SPE was used for these two groups. Ultimately, the chosen strategy proved to be successful, as these methods have been validated for a wide variety of foods and allow the analysis of acrylamide in 226 samples in a limited amount of time.

1.3. Adapting the QuEChERS concept to PAH determination in spices and herbs

Prior to this study, the QuEChERS method had already been applied to the quantification of PAHs in food (Urban and Lesueur 2017). However, the use of acetonitrile (ACN) for PAH extraction presents several challenges. It often results in complex, difficult-to-clean extracts, which can negatively impact the limit of quantification (LOQ), set at a minimum of 0.9 µg kg^{-1} according to the Regulation (EU) 836/2011 (European Commission 2011b). Additionally, ACN may lead to broader chromatographic peaks, compromising resolution—an essential factor in PAH analysis due to the presence of numerous isomers prone to co-elution. Despite the lower sensitivity of GC-MS/MS compared to LC coupled to fluorescence/UV detectors, its use was deemed necessary due to its superior specificity, which was crucial for mitigating interferences encountered during the analysis of spices and herbs.

The novelty and challenge of the PAHs method developed in this thesis lay in replacing ACN with an alternative solvent mixture capable of achieving high extraction recoveries while minimizing the co-extraction of matrix components. Two solvent mixtures were evaluated: acetone/hexane and dichloromethane (DCM)/hexane. Although the acetone/hexane mixture provided excellent recoveries, it resulted in excessively dirty extracts that were impossible to purify, particularly for dried herbs containing high levels of pigments. Endly, the DCM/hexane mixture was selected despite the higher toxicity of DCM compared to acetone, as it offered the best compromise between extraction efficiency and ease of subsequent clean-up. For the clean-up step, a strategy similar to that used for acrylamide analysis in food was adopted. A general extraction procedure was followed by matrix-specific purification approaches. The analysed spices and herbs were classified into three categories based on their composition: regular spices, dried herbs, and complex spice mixtures.

Another critical challenge of the method was the optimization of the elution volume and solvent composition in combination with an appropriate sorbent for SPE. Complete retention of co-extracted components or PAHs was not achievable with any of the tested SPE sorbents, including silica, Florisil, and aluminum oxide. Consequently, the SPE cartridge was employed as a chromatographic device, leveraging differential migration speeds to achieve separation between PAHs and co-extracts. This approach was particularly crucial for herb samples, which contain photosynthetic pigments (e.g., chlorophyll, carotenoids, and xanthophylls) that share structural similarities with PAHs, such as low polarity and aromatic cycles (Fleming 1967; Jackson et al. 2008). Due to these characteristics, dispersive SPE (dSPE) was not a viable option for clean-up. However, the use of SPE remained less time- and solvent-consuming than Soxhlet extraction, gel permeation chromatography (GPC), or preparative HPLC, and did not require complex instrumentation. Hence it was still acceptable for the performance criteria of the method fixed initially.

Due to its originality, the developed method was presented within the NRLs-EURL networks,

where it was well received. To facilitate broader adoption, and for the purpose of interlaboratory validation the method was adapted by replacing DCM with ethyl acetate, since DCM is not authorized in all European countries. However, this substitution proved ineffective, leading to dirtier extracts in some cases and a potentially higher LOQ for some laboratories (Duedahl-Olesen 2020; Wilde and Duedahl-Olesen 2022). These findings confirmed that DCM was the most suitable solvent to meet the primary objective of this thesis: to develop a simple, modern and modular method with a satisfactory LOQ, enabling the analysis of 120 diverse spice and herb samples within a short timeframe.

2 Occurrence of contaminants in food: insights from market surveys

Once the analytical methods were developed, it became possible to address the next stage of this thesis: filling the data gap regarding the occurrence of pesticides in tea, acrylamide in less studied food (i.e. croquettes, röstis, black olives, vegetables-based crisps, …), and PAHs in spices and herbs. A general key challenge was ensuring that the food samples purchased were as representative as possible of the food consumed by the Belgian population, considering all types of consumers, including those purchasing from organic stores. To achieve this, existing databases were used when available. In cases where no data were accessible, products from major brands and suppliers were selected. The reason behind this approach is that the more frequently a brand appears on the market, the higher the likelihood that it is purchased by consumers. As will be discussed in the following section, some results were particularly surprising. However, some of those highlighted the importance of contextualizing findings to avoid overly alarming interpretations.

2.1. pesticides residues in tea and herbal tea

The analysis of the 55 tea samples (as dried leaves) raised some concern. For instance, results revealed that 38.2 % (21/55) of tea samples contained pesticides that exceeded the EU MRL (European Commission 2005), considering the measurement uncertainty of 50 %. These results were similar to a recent study by Heshmati et al. (2021), who found that 40 % of tea samples were positive for at least one pesticide residue. Similarities for pesticides in tea studies make sense as the two biggest tea-producer countries are China and India (Largest tea producing countries worldwide 2024 - Statistica). The primary concern was the high detection rate of EU prohibited pesticides in tea, with 17 different banned substances identified. Among them, anthraquinone, carbendazim, and biphenyl were the most frequently detected, with 16, 12, and 9 occurrences, respectively. However, a literature review and discussions with the Belgian Food Safety Agency highlight the need to put these figures into perspective: the presence of anthraquinone and biphenyl does not necessarily indicate the illegal use of plant protection products but may instead originate from environmental contamination or the drying process (EFSA 2012b; Berkvens et al. 2016; Anggraini et al. 2020). Moreover, it is not possible to precisely determine the source of contamination. As a result, a certain level of tolerance is applied to these substances, given the uncertainty regarding their origin.

In the case of propargite, the European Commission banned this pesticide due to its

carcinogenic properties. However, a Maximum Residue Limit (MRL) of 10 mg kg^{-1} was established (Reg. (EU) No 310/2011) for India, where it was regularly used to combat red spider mites, a significant agricultural pest that causes severe crop damage (Seenivasan et al. 2015). This tolerance was implemented to ensure a stable tea supply to the European market. Nonetheless, despite the relatively high MRLs established for propargite, the European Commission conducted thorough risk assessments to confirm that these levels do not pose a health risk to consumers (EFSA 2018).

Finally, the tea study revealed a high number of pesticide residues in individual samples, with up to 15 different pesticides detected in a single sample, and pesticide contamination in 57 % (8/14) of organic tea samples. It is important to highlight that many teas sold in Belgium and other Western countries are frequently blended with herbs, fruits, and flavourings or essential oils to enhance their taste (38 % of the analysed tea-based samples). Additionally, recent studies have demonstrated that essential oils may contain various pesticide residues, including banned substances such as biphenyl and carbendazim—both of which were frequently detected in our study—even in organic products (Fillatre et al. 2016; Fillâtre et al. 2017). Unfortunately, because some samples are ground into powder and homogenized prior to analysis, it is impossible to separate their individual components to determine specific contamination sources (tea, fruits, or aromatic oils). Moreover, as these ingredients are in close contact, potential pesticide transfer between components cannot be ruled out. Consequently, it remains challenging to identify the exact origin of the detected pesticides, as contamination may stem from these additional ingredients. This also raises concerns about the reliability of organic certification labels and consumer trust in such products.

Hence, this study on pesticide residues in tea revealed that the issue is more complex than initially anticipated. Several factors contribute to this complexity, including import tolerances, pesticides that may not be classified as plant protection products but rather as environmental contaminants—some of which may not even be of anthropogenic origin. Additionally, the issue extends beyond tea cultivation itself to encompass composite food products that contain multiple ingredients.

2.2. acrylamide in non-regulated food

Regarding acrylamide, the objective of this study was to analyse food products that are not yet covered by Regulation (EU) 2017/2158 due to insufficient data: vegetables crisps, black olives, cocoa powder for beverage, potato-based products other than fries, coffee substitutes (not based on cereals or chicory), rice, maize, wheat-based crackers, nuts and oilseeds, pastries, special breads, dried fruits and confectionaries. This also presented a challenge, as unlike pesticides, the absence of Maximum Levels (MLs) or Benchmark Levels (BMLs) made it difficult to determine whether the detected acrylamide concentrations were acceptable or excessively high. To address this issue, existing BMLs from comparable products, based on their composition and/or processing methods, were used as reference points.

For instance, by using this approach, acrylamide content in root vegetable crisps was compared to the BML established for potato crisps in Regulation (EU) 2017/2158, set at 750 µg kg^{-1} (European Commission 2017). Based on this threshold, it can be said that relatively high acrylamide levels were observed. Specifically, 3 out of the 8 analysed samples exceeded this BML, with concentrations of 1554, 2183, and 3063 µg kg^{-1}. Interestingly, three other samples of crisps with the same composition exhibited acrylamide levels below 250 µg kg^{-1}. These

results are consistent with previous findings by MacDonald et al. (2023) and Nguyen et al. (2022), who reported acrylamide concentrations ranging from 58.6 to 2634 µg kg^{-1} and 123 to 3606 µg kg^{-1}, respectively. These findings indicate that, although high acrylamide levels are present in some samples, significantly lower concentrations can be achieved for similar products.

Another challenge of the study was to understand the wide variation of acrylamide content among similar products. For instance, cereal-based snacks and crackers exhibited generally acceptable acrylamide levels. However, their concentrations varied a lot, ranging from <LOD to 353 µg kg^{-1}. Due to the lack of precise information on the manufacturing processes, identifying the source of this variation was not possible. However, based on the information available on product packaging, no significant differences were observed concerning the type of cereal used (maize, wheat, manioc, or rice) or the processing method applied (puffing, extrusion). Interestingly, the Federal Agency for the Safety of the Food Chain (FASFC) has reported similar findings through discussions with food operators, highlighting substantial variations in acrylamide content within this product category, even when the production process is strictly controlled, and the cereals originate from the same batch. However, food operators were unable to identify the source of these variations as discussed during the intermediate “Acrofood” project meeting (V. Vromman, personal communication, December 2020).

In contrast, strong evidence exists to explain the wide variation observed in acrylamide concentrations in black olives (31.9 to 575 µg kg^{-1}). Notably, a significant acrylamide content was detected in ’Californian-style’ black olives (504 µg kg^{-1}), which was approximately five times higher than that found in ’Greek-style’ black olives (63.6 µg kg^{-1}). Similar findings were reported by Breitling-Utzmann et al. (2023), who observed acrylamide levels reaching up to 1500 µg kg^{-1}. This difference may be attributed to the additional sterilization step used in the processing of ’Californian-style’ olives. Interestingly the European Reference Laboratory for Processing Contaminants (EURL-PC) performed an extra home cooking experiment on ‘Californian-style’ black olives and observed acrylamide content reaching up to 5270 µg kg^{-1} (Duedahl-Olesen 2019). These findings highlighted two key points. Firstly, acrylamide can be found in non-starchy food. Secondly, taking into account the consumer behaviour might be valuable to better estimate its exposure to contaminants.

Potato dough-based products have also been found to contain high and highly variable acrylamide levels. A comparative study on different cooking methods, conducted by strictly following the instructions provided on the food packaging, revealed that samples cooked using a deep fryer were up to 25 times more contaminated than those cooked in an oven. A Benchmark Level (BML) of 750 µg kg^{-1} has already been established in EU for this food category. Samples cooked in a deep fryer consistently exceeded this limit, with a maximum acrylamide content of 1503 µg kg^{-1}, whereas those cooked in an oven remained below, with a maximum acrylamide content of 377 µg kg^{-1}. This significant difference between cooking methods highlights the importance of considering consumer practices in exposure assessments, as home cooking may contribute substantially to dietary acrylamide intake.

2.3. PAHs in spices and dried herbs

In contrast to pesticides and acrylamide, the analysis of PAHs in spices and dried herbs did not raise concerns. PAHs were detected in 93 % of the samples; however, only 3

out of 120 samples exceeded the EU maximum levels (European Commission 2023) and no unusual contamination trends were observed on average. Although occasional outliers were identified, particularly in dried herbs and exotic spices, the overall PAH distribution was comparable across the three sample categories: common spices, herbs, and exotic spices. The findings of this study confirm those of (Zelinkova and Wenzl 2015; Rozentale et al. 2018), suggesting that a non-systematic PAH contamination may originate from environmental pollution or the drying process, without it being possible to distinguish each other. Once again, as observed with certain pesticides, it appears that contaminants cannot always be strictly categorized, as they can originate from multiple sources, such as from environmental pollutants (whether natural or anthropogenic) to process contaminants. This complexity further complicates the management of such contamination issues. However, this phenomenon is relatively limited in the case of PAHs compared to certain pesticides such as biphenyl and anthraquinone, which frequently exceed EU maximum residue limits (MRLs). This is not the case for PAHs in spices and dried herbs.

Nonetheless, there are cases where the source of contamination is less ambiguous. For example, the highest PAH contamination was observed in a smoked paprika sample, with a concentration of 164 µg kg^{-1} for the sum of the four regulated PAHs (ΣPAH4), exceeding the EU limit (50 µg kg^{-1}) by more than threefold. This elevated concentration is likely due to the additional smoking step using oak wood, as indicated on the product label. However, even if it is higher than the EU MLs these values remain lower than those reported in other studies, such as (Berki et al. 2020), which quantified ΣPAH4 up to 3476 µg kg^{-1}.

The main limitation of this study was the lack of detailed information on the country of origin and processing conditions, which restricted further investigation into the sources of elevated PAH concentrations. However, this limitation had minimal impact, as average PAH concentrations remained low.

3 Consideration of the food preparation process and the potential to involve the consumer practices in food safety research

The third objective of this thesis was to actively include the consumer practices in the research process and to address the current lack of data on transfer factors (TFs) for pesticides in tea infusions. While food safety assessments and regulatory frameworks typically emphasize industrial processing and contamination of raw materials, they often neglect certain food preparation steps and the influence of consumer practices on chemical exposure. Yet, these aspects are crucial for accurate risk assessment. This thesis seeks to address this gap by incorporating consumer-related variables into the evaluation of food contaminants. For instance, acrylamide formation was investigated not only through different domestic cooking methods (e.g., baking, frying), but also through a pilot study exploring how consumers prepare their food and the criteria they use to determine when to stop the cooking process. In parallel, the transfer of pesticides into the brew was assessed in relation to various preparation parameters. These examples illustrate how consumers can significantly influence their own

exposure to chemical contaminants through their food handling and preparation habits, highlighting the relevance of considering consumer practices when interpreting exposure to food contaminants.

3.1. Pesticide transfer in tea infusions and impact of consumer habits

Although it is well known that many teas exceed the EU maximum residue limits (MRLs) for pesticides, there is a significant lack of data on TFs in 'European database' from tea leaves to tea infusions with only 4 pesticides (hexythiazox, spiromesifen, fenazaquin and glyphosate) (Scholz et al. 2018; BfR 2019). Yet, such data are essential for conducting accurate and relevant risk assessments. While additional data can be found in the scientific literature, they predominantly originate from studies conducted in China, the world's largest tea producer (Food and Agriculture Organization of the United nation 2014). However, preparation habits in China do not reflect Western, and specifically Belgian, consumption practices. For instance, tea leaves are not rinsed prior to infusion in most Western countries, unlike in certain Chinese protocols. Moreover, many existing studies have focused exclusively on unflavoured black or green teas (Wang et al. 2019; Fan et al. 2022), whereas the Belgian market offers a wide variety of teas, often blended with fruit flavours. Additionally, most studies have limited their scope to specific pesticide classes, such as organochlorines or pyrethroids (Xiao et al. 2017; Witczak et al. 2018). The brewing protocols employed in these studies are also frequently unrealistic—either using tea-to-water ratios that do not reflect typical consumer behaviour, or relying on ISO 3103 guidelines, which doesn't reflect real practices (ISO 3103 2019).

This study was therefore designed to address these limitations by generating TF data under conditions that better reflect Belgian consumer practices. In addition to standard black and green teas, the study included three of the most commonly consumed herbal teas in Belgium (mint, chamomile, and linden) as well as three flavoured teas (orange, lemon, and mint), to reflect the diversity of the national market. The list of target pesticides was also expanded to include all residues detected in teas during the Belgian monitoring programmes over the past 10 years, covering a total of 54 different pesticide residues. Moreover, several infusion parameters were varied to simulate the range of preparation habits observed among consumers. Direct involvement of consumers was considered impractical due to the wide range of parameters to be tested. Therefore, simulated consumer profiles were created by varying key brewing conditions within realistic extremes—such as infusion time (2–10 minutes), water temperature (80 - 100 °C), and water hardness (very soft - very hard).

The possibility of studying a wide range of infusion parameters was made possible by the use of the spiked tea approach, one of the innovative aspects of this study. This method also enabled the systematic assessment of the influence of each individual parameter on transfer factors. Rather than relying on voluntarily contaminated experimental tea fields, as in previous studies (Gupta and Shanker 2009; Wang et al. 2019) which provide relevant but time-consuming and costly data, fortified tea allowed for a more flexible and efficient experimental design. Some studies have alternatively relied on naturally contaminated teas available on the market, as in our initial investigation into the presence of pesticides, heavy metals, and pharmaceutical residues in tea (Chen, Wang, et al. 2015; Chen, Pan, et al. 2015; Szternfeld et al. 2022). However, this approach presents major limitations, as it does not guarantee the presence of all targeted pesticides, nor their occurrence at concentrations

sufficient to reliably determine transfer factors, particularly for highly non-polar compounds such as pyrethroids. This approach has proven successful, as it yielded similar TFs determined from experimental tea field or market-acquired teas, making further studies simpler.

Simultaneously, the goal was to develop a practical toolbox applicable to the daily monitoring of the Belgian Food Safety Agency for the Food Chain (FASFC). This toolbox should be used as an effective means to assess the risk and take appropriate actions in case of MRL exceedance. This goal was achieved as a ”toolbox” has been created, containing a database including over 260 consolidated average TFs data for 108 pesticides across various tea types (black teas, green teas, flavoured teas, and herbal teas) available on the Belgian market. FASFC has already effectively employed this database for tea samples exceeding EU MRLs. Moreover, 4 predictive models have been created (for black, green, herbal, and flavoured teas) and added to this ”toolbox”. They have been elaborated based on the physicochemical parameters of pesticides (log (P), log (S), log (PV), log (Henry)) and are intended for use in scenarios where a pesticide is detected and no existing TF is available.

The most critical parameter affecting pesticide TFs was the tea type. A significant difference ($P < 0.01$, 99 % confidence interval) was observed between black and green teas for 29 out of 54 pesticides. This was followed by the presence of flavourings, with significant differences for orange (21/54), lemon (8/54), and mint (3/54) flavours in comparison with pure tea. On average, flavoured teas exhibited lower TFs compared to their unflavoured counterparts. Significant differences in TFs were also found when comparing black and green teas to herbal teas, for 15/54 and 23/54 pesticides respectively ($P < 0.01$, 99 % confidence interval). On average, herbal teas were associated with higher TFs. Surprisingly, parameters typically influenced by consumers practices, such as infusion time, water temperature, and even region-specific water hardness, did not significantly affect TFs ($P < 0.01$, 99 % confidence interval). These results suggest that pesticide migration is governed more by the compound’s affinity for the tea matrix than by extraction conditions. Consequently, consumers across different regions, regardless of local water hardness or preparation habits, are likely exposed to comparable levels of pesticide residues when brewing tea. These findings are particularly relevant for subsequent risk assessment. In cases of non-compliant samples, the evaluation becomes more straightforward, as the transfer factor is not influenced by consumer behaviour but rather determined by the intrinsic characteristics of the product for which all information is available for competent authorities.

The data generated in this experiment on TF were used to develop four predictive models, one for each tea type. All models were restricted to the range $\log(P) > 4.5$ and $\log(S) > -2.0$, beyond which TF values asymptotically approach 0 %, hence providing no relevant information. In all four models, the most influential variable was log(P). Log (VP) also contributed significantly in the models for green and flavoured green teas. In contrast to the findings of (Wang et al. 2019), log(S) was not a significant predictor in any of the models. This discrepancy may be attributed to the strong correlation between log(S) and log(P), as previously reported by (Hughes et al. 2008), suggesting that both variables give nearly the same information. On average, a good agreement was observed between predicted and observed TF values. The highest predictive performance was obtained for green and herbal teas, with pseudo R-squared values of 0.84 and 0.86, respectively, while lower values were observed for black and flavoured green teas (pseudo R-squared = 0.66 for both).

Although predictive models demonstrated a good overall correlation between observed transfer factors (TFs) and log P values, they also showed limitations. Certain pesticides, such

as epoxiconazole and flutolanil predicted TFs were 1.6 and 1.7 times lower than the observed TFs. The maximum deviation was observed for anthraquinone in herbal tea with a predicted TF 2.4 times lower than the observed TF. This phenomenon may originate from the large TF variation for pesticides with a comparable log (P) and/or This aligns with the findings of the most recent study found in the literature (Wang et al. 2019), which reached a similar conclusion: while modelling provides good average predictions, substantial deviations (up to 3.6 times) are observed for individual pesticides. The modelling showed that a case-by-case approach is more suitable to get more reliable pesticide TFs. However, predictive models can be used as a first approximation while keeping in mind that they have limitations. It should be possible to enhance predictions by incorporating additional data into the models, particularly for pesticides with a log P between 1-3 for which TFs data were limited.

3.2. Impact of consumer cooking practices on acrylamide formation

Unlike pesticide residues in tea, acrylamide levels in food are highly dependent on preparation methods, as it is well established that both cooking time and temperature significantly influence acrylamide formation. These parameters are directly controlled by consumers in a home setting. However, current risk assessments, such as those conducted by the European Food Safety Authority (EFSA), do not account for acrylamide exposure resulting from domestic cooking, as they primarily rely on data from food cooked by business operators (EFSA 2015). This may lead to an underestimation of total dietary exposure. Previous studies have highlighted this issue, with some reporting higher acrylamide concentrations (16 %) in home-cooked röstis (Eicher et al. 2020) than those estimated by EFSA.

To address this gap, this study investigated the impact of real home cooking practices on acrylamide formation. Controlled laboratory experiments within the project demonstrated that deep-frying potato-based products produced acrylamide levels 5 to 25 times higher than baking under identical conditions. However, these results may not reflect typical consumer behaviour, as cooking instructions were strictly followed—sometimes resulting in burnt products—an outcome that likely deviates from how people cook in everyday life.

Unlike pesticide extraction in tea, simulating consumer cooking behaviour in a laboratory setting proved challenging due to the many variables involved. Given the known influence of temperature, cooking method, and duration on acrylamide formation, a pilot study involving 15 Belgian participants was conducted. Participants were recruited from Sciensano and were asked to prepare commonly consumed potato products in their own kitchens without being informed of the study's true objective. Additional information was collected via questionnaires to assess their cooking practices, including how they determine when food is sufficiently cooked.

Even though 36 % of participants admitted to not following the cooking time instructions indicated on food packaging, acrylamide levels in home-cooked foods were generally lower than those observed under controlled laboratory conditions. For example, croquettes prepared at home reached a maximum acrylamide concentration of 500 µg kg^{-1}—the established benchmark level—while sweet potato fries showed maximum levels of 179 µg kg^{-1} for deep-frying and 166 µg kg^{-1} for oven baking. Notably, no significant difference was observed between frying and baking in the home environment, in contrast with laboratory results where deep-frying consistently led to higher acrylamide formation.

MacDonald et al. (2023) in their total diet studies analysed acrylamide in various potato-based products (e.g. croquettes, röstis) after cooking and reported concentrations ranging from <30 µg kg^{-1} to 409 µg kg^{-1} (mean: 163 µg kg^{-1}).

This apparent discrepancy between cooking time and acrylamide content observed in the present study is likely since participants relied on sensory indicators such as food colour and texture to determine when cooking was complete. Most participants reported stopping the cooking process when a golden-brown colour was achieved, a visual cue that aligns with personal taste preferences. Interestingly, this same colour is recommended in EU Regulation 2017/2158 as a practical means of minimizing acrylamide formation. Nevertheless, only 50 % of the sampled product labels referenced this visual guidance, highlighting a missed opportunity for risk communication at the consumer level.

Furthermore, no clear relationship could be established between acrylamide concentrations and the specific cooking times or temperatures used by participants, despite all food items being sourced from the same production batch. This suggests that variations in cooking equipment—such as oven size, model, age, or presence of convection fans—may have played a more substantial role in influencing acrylamide formation than individual cooking parameters alone.

Overall, these findings highlight the importance of sensory indicators on consumers behaviour and suggest that promoting visual cues, such as the golden-brown coloration, may be a more effective risk reduction strategy than relying solely on standardized cooking instructions based on time and temperature.

Although informative, this pilot study has several inherent limitations. The sample size was small (n = 15), and all participants were recruited from Sciensano, a public health institution. This recruitment strategy may have introduced bias, as participants could have had greater awareness of food safety issues or demonstrated behaviours that are not representative of the general Belgian population. As a result, the generalizability of the findings is limited. Nevertheless, the study provides valuable preliminary insights into how real-life cooking behaviours may influence contaminant formation and demonstrates the feasibility of integrating consumer practices into food safety research under realistic conditions.

4 Evaluation of the risk for the Belgian consumer

The fourth objective of this thesis, and the overarching goal of this work, was to assess the risk for the Belgian consumer linked to the presence of various chemical contaminants in food. Appropriate analytical methods have been developed, and recent occurrence data as well as information on the consumer's influence on contaminant levels have been gathered in this study, making it possible to carry out a more accurate risk assessment and thus fully address this final objective.

Each contaminant requires a specific risk assessment approach, depending on its toxicological profile. For instance, in the case of pesticides, the evaluation was based on the comparison with the Acute Reference Dose (ARfD) and/or the Acceptable Daily Intake (ADI). In contrast, for compounds with carcinogenic potential, such as acrylamide and PAHs, the Margin of Exposure (MOE) approach was applied, using a threshold of 10,000 as recommended by

EFSA (EFSA 2005).

Besides differences in toxicological approaches, exposure assessment was also performed differently depending on the contaminant considered. For pesticides in tea and herbal teas, exposure was estimated from concentrations measured directly in the infusion after applying experimentally determined transfer factors from the dry material to the brew. In contrast, exposure to PAHs and acrylamide was estimated by combining occurrence data with food consumption data. Different assumptions and scenarios were also required depending on data availability and the food categories investigated. Furthermore, the treatment of left-censored data was not identical across chapters. For PAHs, a middle-bound (MB) approach was applied, replacing results below the LOQ by one-half of the LOQ, whereas for acrylamide an upper-bound (UB) approach was used, replacing non-quantified results by the LOQ value. Although UB approaches generally lead to higher exposure estimates than MB approaches, the impact on calculated exposure is limited as the proportion of left-censored data was relatively low in both studies.

As a result, exposure estimates cannot be directly compared between contaminant groups. However, the approach used in each chapter was selected to provide the most realistic exposure estimate possible based on the available data. While methodological differences related to exposure modelling, treatment of left-censored data, and toxicological reference values may affect the numerical values obtained, they are unlikely to modify the overall conclusions of the risk assessments presented in this thesis. Indeed, the resulting risk characterization remained consistently either clearly below or above the relevant health-based thresholds, leading to the same overall interpretation regarding the level of concern for consumers.

4.1. Polycyclic aromatic hydrocarbons

EFSA performed a risk assessment related to PAHs for the European population in 2008 and concluded to a low concern due to PAHs exposure though food consumption (MOE was above 20 000). The reevaluation of the risk after including spices and herbs in the PAHs diet exposure (Chapter 5) led only to a minor decrease of the MOE (-0.06 and -1.23 % for median and high consumption, respectively). MOE were in both cases still above 20.000, indicating that adding consumption of spices and herbs to the PAHs intake through food do not pose a health risk for the Belgian population. This is good news as Belgium is considered Europe's second largest spices and herbs consumer (Businesscoot 2021).

The challenge and limitation of this study was finding accurate consumption data for this food category. Indeed, no database explicating the consumption of all spices and herbs exists. Therefore, consumption estimates were based on data from the 2004 Belgian food consumption survey (EFSA 2011). More recent consumption data from the 2014 survey could not be accessed during the period in which the study was conducted. Given the increasing popularity and market growth of spices and herbs in Belgium over the last two decades (Businesscoot 2021), the use of older consumption data may have resulted in some degree of exposure underestimation. However, the magnitude of this potential underestimation remains difficult to quantify.

Furthermore, spices and herbs consumption may be challenging to estimate, even for the consumer. Usually, quantities are referred to as "teaspoon" or "according to the taste" and not in grams. However, the impact of these two issues should be very limited on the risk

assessment, as PAHs distribution were similar in all groups, and only a highly unrealistic consumption will lead to an MOE <10.000. Despite these limitations, the inclusion of spices and herbs did not substantially alter the overall MOE values, which remained well above the level of concern.

Although the present risk assessment relied on EFSA reference values, similar MOE-based approaches have been adopted internationally, notably by JECFA. JECFA in 2006 used a $BMDL_{10}$ of 100 µg kg^{-1} bw per day derived from benzo[a]pyrene-induced tumours and reported MOE values of approximately 25,000 and 10,000 for average and high dietary exposures, respectively. Based on these results, JECFA concluded that dietary exposure to PAHs was of low concern for human health (Joint FAO/WHO 2006).

In contrast, EFSA considered that benzo[a]pyrene alone was not an adequate marker of PAH occurrence in food, as other carcinogenic PAHs may be present without detectable levels of BaP. Therefore, EFSA recommended the use of PAH4 as a more appropriate indicator for risk assessment purposes (EFSA 2008). Nevertheless, despite these methodological differences, both organizations reached similar conclusions regarding the relatively low health concern associated with dietary exposure to PAHs in the general population.

Alternative approaches have also been proposed in the scientific literature, notably the use of toxic equivalency factors (TEFs) to express PAH mixtures as benzo[a]pyrene equivalents (Nisbet and LaGoy 1992). However, regulatory risk assessments conducted by EFSA and JECFA have generally favoured the MOE approach for the characterization of dietary risks associated with PAHs, as uncertainties remain regarding the applicability of TEFs to oral dietary exposure. Indeed, many of the toxicological data used to derive TEFs originate from experimental studies that do not necessarily reflect human dietary exposure conditions (EFSA 2008).

4.2. Pesticides in tea and herbal teas

The same issue has been identified with the presence of some forbidden pesticides in tea. During this study, anthraquinone and biphenyl, two forbidden pesticides, were detected 16 and 9 times respectively in the 55 teas and herbal teas samples. Anthraquinone is a derivative of the PAH anthracene. It can be due to its use as plant protection products (PPP) in seeds as bird repellent. However, anthraquinone can enter in food via multiple pathways. It can be considered a processing contaminant issued from the drying process of tea leaves, but also an environmental pollutant as it is used in the textile industry as pigment or catalyst for paper production. Hence its detection in tea may not always be due to the non-respect of PPP use and the origin of contamination source cannot be identified easily. Hence, borders between environmental pollutants/processing contaminants or man-made action can be blurred and this facet needs to be taken into consideration when applying the EU regulation. It is why a certain tolerance is applicable in case of MRL exceedance (Authority 2010).

Regarding risk assessment for pesticides in tea and herbal tea leaves, even if occurrence data in tea were alarming regarding forbidden pesticide detection or MRL exceedance, the worst-case scenario that has been performed revealed that the risks were extremely limited for both adults and children and for acute and chronic effects (acute hazard index and hazard quotient < 1 % in every case). Results were in accordance with recent studies (Chen, Pan, et al. 2015; Fan et al. 2022) where HQ were also <1 %. This can be explained by the fact that pesticides with the lowest ADI/ARfD values are also those with a low affinity (i.e. pyrethroids) with water and were not transferred into the brew (TF ~1 %). In comparison,

neonicotinoids, more polar, were more transferred in water (TF ~40-100 %). These results showed that MRL exceedance is not always a synonym of risk for consumer health. These MRLs do not correspond to safety thresholds but are linked to good agricultural practices to keep pesticide usage low.

The challenge that has been faced during the risk assessment was that no ADI or ARfD was available for the pesticides anthraquinone and biphenyl. Only NOAELs were available for oral administration (Anthraquinone - Registration Dossier - ECHA 2022). A common way to approximate the ADI is to divide the NAOEL by a 100-fold safety factor (Chemicals Regulation Directorate 2013). Hence, the same protocol has been followed to evaluate the risk.

It is also important to note that the exposure and risk assessments based on ADIs and ARfDs performed in this thesis were conducted for population groups defined according to age and body weight and did not specifically address sensitive life stages such as pregnancy, fetal development, or women of childbearing age. ADIs and ARfDs are generally derived from toxicological studies, including reproductive and developmental toxicity studies when available, and incorporate uncertainty factors to take into account interspecies differences and human variability. Consequently, these health-based guidance values are expected to be protective for the majority of the population, including potentially sensitive individuals (Toxicological reference value | EFSA 2026) .

However, this approach remains a generic assessment and does not specifically evaluate exposure during critical windows of development. Therefore, although the risk assessments performed in this thesis are considered protective, some uncertainty remains regarding the potential effects of exposure during sensitive life stages. This is particularly relevant for toxicological endpoints that may depend not only on the level of exposure but also on the timing of exposure, such as developmental or endocrine disruption effects.

4.3. Acrylamide in food

In contrast to pesticides in tea and PAHs, health concern has been identified for acrylamide in food. The Margins of Exposure (MOEs) calculated during the risk reassessment, including newly considered food products, were all well below the threshold of 10,000 (ranging from 192 to 1015) for neoplastic effects, across all population groups and all consumption scenarios. The three main contributors to dietary acrylamide exposure were French fries (~20 %), followed by potato crisps (~13 %), and bread (~12 %).

The MOE values obtained in this thesis were similar to those reported in the recent French Total Diet Study for both children and adults (Anses 2026), suggesting that the situation may be comparable across other EU Member States.

Despite these notably low MOE values, it is important to stress that the MOE approach only indicates a potential health concern and does not allow for quantitative risk estimation (EFSA 2005). Acrylamide has been classified by the International Agency for Research on Cancer (IARC) as probably carcinogenic to humans (Group 2A). While evidence of carcinogenicity is clear in rodent models, it remains limited in humans. Furthermore, some studies (as cited by Powers et al. 2021) have questioned the relevance of EFSA's assessment, noting that the mutagenicity tests used were based on effects in Harderian glands (pigmented lacrimal gland) present in rats but absent in humans (EFSA 2015; Miedel et al. 2015). Another study has also argued that the levels of acrylamide typically found in the environment are unlikely to be sufficient to induce carcinogenesis in humans (Başaran et al. 2023). However in 2022,

EFSA re-assessed the genotoxicity of acrylamide by reviewing more recent toxicological studies (EFSA 2022b). These studies reported on the ability of acrylamide to induce DNA strand breaks in several human cell lines (both in vitro and in vivo): lymphocytes (Zamani et al. 2018; Hansen et al. 2018), liver cells (Mandon et al. 2019) or monocytes (Xiao et al. 2016).

Regarding the neurotoxic effects of acrylamide, all MOE values calculated for the Belgian population were above the reference threshold of 125 (ranging from 171 to 2569), suggesting no health concern in this context.

In contrast to pesticides, for which numerous maximum residue level (MRL) exceedances were observed without indicating any significant health concern for consumers, all calculated margins of exposure (MOEs) for acrylamide in food were well below the threshold of 10,000, despite only a few exceedances of benchmark levels (BMLs) of the EU Regulation 2017/2158. This can be explained by the fact that BMLs for acrylamide are not based on toxicological thresholds, but rather on what is achievable by food producers when applying mitigation strategies and good manufacturing practices, as outlined in Regulation (EU) 2017/2158. Furthermore, this regulation acknowledges that due to seasonal and geographical variations, it may not always be feasible to comply strictly with the established BMLs. However, food business operators must be able to demonstrate that all reasonable efforts have been made to reduce acrylamide levels to as low as reasonably achievable.

As was also observed for polycyclic aromatic hydrocarbons (PAHs), one of the main challenges in risk assessment for acrylamide lies in the limited availability of accurate food consumption data for certain emerging food categories. For instance, vegetable chips were not included in the Food Consumption Survey (FCS) 2014, even though their popularity has increased significantly in recent years. A similar issue was encountered for black olives, where this study revealed a substantial difference in acrylamide levels between Californian-style and Greek-style olives. However, FCS 2014 does not distinguish between these olive types, which limits the accuracy of exposure estimates. Nevertheless, the impact of this uncertainty on the overall MOE is likely minimal, as a reduction in acrylamide intake ranging from 9.85- to 52.1-fold would be required to reach the critical threshold of 10,000. Such reductions would imply unrealistic consumption scenarios, further reinforcing that the current exposure, while not negligible, may not pose an immediate health concern, especially in light of dietary patterns observed in the studied population.

Lastly, while the present study focused on specific food categories targeted by Commission Recommendation (EU) 2019/1888, other foods might be considered for further investigation, particularly legumes, pulses, and protein-rich ingredients used in the production of plant-based meat alternatives (e.g., tofu, soy-based products, and corn-based products), for which limited data are currently available. This is especially relevant given the growing popularity of plant-based diets and meat substitutes. For example, Delgado-Andrade et al. (2025) reported acrylamide concentrations of up to 150 µg kg^{-1} in stewed lentils. Regarding meat substitutes, Abdullajeva et al. (2024) and Pospiech et al. (2024) investigated acrylamide formation in plant-based meat alternatives after pan-frying and reported concentrations of up to 119 µg kg^{-1} and 69.2 µg kg^{-1}, respectively. Similarly, Goerke et al. (2019) found acrylamide levels of up to 100 µg kg^{-1} in meat substitute products such as fried tofu and seitan. The authors concluded that the consumption of meat substitutes could substantially increase dietary acrylamide exposure among vegan consumers.

These emerging food categories should be considered a priority for future monitoring and

occurrence studies. Furthermore, their contribution to dietary acrylamide exposure should be assessed using up-to-date food consumption data, such as those provided by the most recent national food consumption surveys (e.g., the 2026 survey), which better reflect current consumption patterns of plant-based products.

Although the present work relied primarily on the EFSA risk assessment framework, it is important to note that other international organizations have sometimes selected different toxicological reference points. For example, in addition to the $BMDL_{10}$ of 0.17 mg kg^{-1} bw per day derived from the induction of Harderian gland tumours in mice, JECFA also considered a $BMDL_{10}$ of 0.31 mg kg^{-1} bw per day based on the induction of mammary tumours in rats. As a result, JECFA generally obtained somewhat higher MOE values for a given exposure level (Joint FAO/WHO 2011). Nevertheless, despite these methodological differences, both organizations concluded that current dietary exposure to acrylamide may raise a health concern and that mitigation measures should be pursued.

Similar observations have been made in the U.S, where the Food and Drug Administration estimated an average dietary acrylamide intake of approximately 0.36 µg kg^{-1} bw per day based on Total Diet Study data (Abt et al. 2019). Applying the $BMDL_{10}$ of 0.17 mg kg^{-1} bw per day used by EFSA would result in MOE values of the same order of magnitude as those obtained in the present study. Altogether, these observations indicate that acrylamide exposure and its potential carcinogenicity are not restricted to a European issue but rather constitute a global food safety concern.

The findings of the present thesis are consistent with these international evaluations. Although the calculated MOE values depend on the selected toxicological endpoint and the exposure data used, they remain within the same order of magnitude as those reported by international risk assessment bodies and lead to similar conclusions regarding the need to minimize dietary acrylamide exposure.

Chapter 7

Conclusions, Recommendations, Limitations and perspectives

1 CONCLUSIONS AND RECOMMENDATIONS

It has been demonstrated throughout this work that a robust risk assessment relies on several essential pillars. First, reliable analytical methods are required to generate occurrence data, with sufficiently low limits of quantification to minimize left-censoring and with adequate adaptability to address the wide diversity of food matrices available on the market.

Second, comprehensive and representative occurrence data constitute the foundation of any exposure assessment. However, occurrence alone is not always sufficient. When relevant, processing and transfer factors must also be considered in order to account for the fate of contaminants between raw materials and consumed products.

Third, up-to-date and detailed food consumption data are indispensable. In a rapidly evolving food landscape, where new products and dietary trends continuously emerge, exposure assessments must capture these changes to remain relevant.

Finally, accurate toxicological reference values are necessary to translate exposure into meaningful risk assessments and to ensure that consumer protection decisions are scientifically relevant.

It is within this framework that the present thesis contributes to addressing several identified gaps and supports the refinement of key components of risk assessment. Through the development of adaptable analytical methodologies, the generation of new occurrence data for previously underexplored food categories, and the integration of consumer practices into exposure evaluation, this work contributes to a more accurate and comprehensive assessment of selected chemical contaminants in food: pesticides in tea, PAHs in spices and herbs, and acrylamide in less-studied food products.

The main contributions of this thesis can therefore be structured around three complementary dimensions: the development of analytical methodologies, the generation of occurrence data integrating consumer practices, and the refinement of exposure and risk assessment approaches. Based on these findings, several methodological, regulatory, and risk assessment recommendations can also be formulated.

1.1. Methodological Contributions

A first major contribution of this thesis lies in the development of robust and adaptable analytical methods suitable for large-scale occurrence studies characterized by a wide variety of food matrices.

For PAHs in spices and herbs, a common extraction procedure combined with matrix-adapted clean-up steps enabled efficient analysis across diverse spice and herb matrices while maintaining satisfactory analytical performance, allowing the rapid analysis of a large number of heterogenous samples. The originality and relevance of this approach were acknowledged within the NRLs–EURL networks and served as a basis for interlaboratory validation initiatives to address the lack of analytical methods for these particularly challenging matrices.

For pesticides in tea, the suitability of a spiked-tea approach for determining TFs was demonstrated. This strategy provides a practical and time-efficient alternative to field trials and avoids relying on the uncertain occurrence of contaminated products available

on the market. The study of TFs enabled the development of four predictive models that can be used when experimental transfer factors are unavailable. Although these models showed good overall performance, the results highlighted the limitations of purely theoretical approaches and confirmed the importance of experimental determination for accurate transfer assessment.

Regarding acrylamide in food, an original validation strategy based on food grouping was developed. In contrast to pesticide analysis, for which grouping guidelines are available in the SANTE guidance document, no equivalent framework exists for processing contaminants such as acrylamide. In this thesis, foods were classified according to their nature and composition in order to apply a common extraction followed by matrix-adapted clean-up. This approach enabled method validation across heterogeneous food categories while realistically accounting for within-group variability. The strategy was presented within the EURL-PC and NRLs-PC networks and may serve as a basis for future guidance on food grouping for processing contaminants. Together, these methodological developments provide adaptable tools and strategies that can support future contaminant monitoring and risk assessment for similar topics.

1.2. Contribution to Occurrence Data Gaps

A second key achievement of this thesis was the generation of new occurrence data for contaminants in food categories that were either insufficiently documented or previously unexplored in Belgium, thereby addressing important data gaps required for risk assessment.

1.2.1. Pesticides in Tea

The analysis of tea samples revealed frequent pesticide contamination, including residues exceeding EU maximum residue limits and the presence of several banned substances. However, these findings must be interpreted cautiously, as compounds such as anthraquinone and biphenyl (often detected) may originate from environmental contamination or drying processes rather than illegal pesticide use. In addition, the complexity of composite tea products—often containing flavourings, herbs, fruits, or essential oils—limits the attribution of contamination to tea only.

These results highlight the difficulty of applying regulatory frameworks to substances located at the interface between plant protection products, environmental contaminants, and processing-related compounds, emphasizing the need for a more global approach to food contamination assessment.

1.2.2. Acrylamide in less-studied foods

This thesis generated 223 acrylamide occurrence data points from 210 food samples belonging to food categories targeted by Recommendation (EU) 2019/1888, including vegetable crisps, potato dough-based products other than fries, and black olives.

Vegetable crisps showed highly variable acrylamide concentrations, with several samples exceeding benchmark levels established for potato crisps, while comparable products exhibited substantially lower concentrations. These findings support the inclusion of vegetable crisps within mitigation strategies similar to those applied to potato crisps, and it is therefore recommended that these products be considered within future EU regulation. This recommendation is particularly relevant given the increasing market availability of such

products and their growing consumption, as they are often perceived as a healthier alternative to conventional potato crisps.

A significant difference was observed between Californian-style and Greek-style black olives, likely linked to the sterilization step used during processing. These results demonstrate that acrylamide formation is not restricted to starchy foods and underline the relevance of including oxidized black olives in future EU regulations, together with the implementation of adapted mitigation measures targeting sterilization processes.

The occurrence data generated in this work were transmitted to EFSA along with datasets from other Member States, contributing to the re-evaluation of acrylamide occurrence in less-studied foods. Recent regulatory developments indicate that upcoming EU legislation extending Regulation (EU) 2017/2158 will include additional food categories such as vegetable-based crisps, sterilized black olives, and potato-based products other than fries. Although these developments result from collective European efforts, they also reflect the contribution of the present work at the Belgian level to the evolution of EU regulation.

1.2.3. PAHs in Spices and Herbs

The analysis of 120 spice and herb samples provided the first Belgian occurrence dataset for PAHs in this category. Although PAHs were detected in most samples, only a limited number exceeded EU maximum levels. Elevated contamination in smoked paprika highlighted the role of specific processing steps such as smoking.

Overall, PAH contamination appeared non-systematic and generally low, confirming that, in this food category, PAHs do not currently pose a significant public health concern under realistic exposure scenarios.

Collectively, the occurrence data generated in this thesis fulfilled the first objective by addressing critical data gaps required for exposure assessment and risk evaluation. These datasets have provided a base for more accurate and up-to-date risk assessments at both national and European levels.

1.3. Integration of Consumer Practices into Risk Assessment

An innovative and key objective of this thesis was to integrate consumer behaviour into food safety research in order to assess its impact on exposure to contaminants.

For acrylamide, controlled laboratory experiments showed that deep-frying could produce acrylamide levels 5 to 25 times higher than oven cooking when strictly applying the cooking instructions provided on product labels. However, results from the pilot consumer study indicated lower acrylamide levels in foods prepared by participants, who relied primarily on sensory cues such as colour and texture rather than strictly following time–temperature instructions. These findings suggest that visual guidance (e.g., promoting a golden-brown colour) may represent a more effective risk mitigation strategy than standardized cooking instructions alone.

In contrast, for pesticides in tea, consumer-controlled parameters such as infusion time, water temperature, and water hardness did not significantly influence transfer factors. Instead, tea type and the physicochemical properties of the compounds were the primary determinants. These findings indicate that exposure through tea infusions is mainly product-dependent rather than consumer-dependent and reinforce the relevance of relying

on standardized TFs databases.

Overall, the second objective of this thesis has been achieved, as it was possible to simulate consumer behaviour through transfer factor studies and to directly involve consumers in the case of acrylamide. These findings demonstrate the value of integrating consumer behaviour into food safety research. Consumer practices may play a variable, but sometimes critical, role in exposure assessment and should therefore be systematically considered whenever relevant to improve exposure estimation and risk evaluation. When specific practices contribute to contaminant formation, targeted consumer communication strategies are recommended as an effective risk mitigation measure.

1.4. Updated Risk Assessment for the Belgian Population

Finally, the last objective of this thesis was to evaluate the risk associated with the three selected contaminant groups for the Belgian population. Using the newly generated occurrence data together with refined exposure scenarios, an updated risk assessment was conducted.

Each contaminant required a specific risk assessment approach, reflecting differences in toxicological profiles:

- MOE-based assessment for acrylamide and PAHs
- ADI- and ARfD-based assessment for pesticides

This final objective was achieved, as it was possible to produce an up-to-date risk assessment for all three contaminant groups with realistic exposure scenarios. PAHs and pesticides did not pose a significant health concern. In contrast, acrylamide remains of concern due to consistently low MOE values for the neoplastic effect. Although very substantial reductions in intake would be required to reach the critical reference value, the additional sources of acrylamide identified in this work indicate that exposure can still be reduced in accordance with the ALARA principle. Based on these findings, continued mitigation efforts are recommended to further reduce acrylamide exposure.

In addition, for pesticides, a practical tool was developed to support risk assessment in cases of MRL exceedance during monitoring activities: a database compiling TFs for numerous tea–pesticide combinations. The predictive models developed in this thesis were incorporated to provide estimates when experimental TF data are unavailable. However, as demonstrated in this work, the recommendation is to prioritise experimentally determined TFs values, as predictive models may significantly diverge from observed data.

This tool is already being used by the Belgian food safety authority, highlighting the practical relevance of this contribution to improved risk assessment.

Together, the findings, tools, and strategies developed in this thesis contribute to advancing food safety assessment toward more realistic exposure and risk evaluation that can evolve alongside changes in food consumption and consumer behaviour.

2 LIMITATIONS AND PERSPECTIVES

2.1. Toward a cumulative food safety assessment

This thesis highlights a key limitation of current food safety assessment frameworks, which continue to evaluate chemical contaminants individually, even though real-world exposure involves the simultaneous presence of multiple substances within a single food item. In this study, for instance, up to fifteen pesticide residues were detected in a single tea sample, underscoring the complexity of realistic dietary exposure.

The frequent co-occurrence of multiple pesticide residues observed in tea illustrates the potential for cumulative exposure to several substances simultaneously. Consumers may therefore be exposed to mixtures of pesticides through contaminated products or composite foods. Such combined exposure, often referred to as the “cocktail effect”, raises concerns because mixture toxicity may differ from evaluations based on individual compounds, particularly when several substances share similar toxicological effects or mechanisms of action. These observations underline a key limitation of single-compound risk assessment approaches.

The study of this cocktail effect on consumer health should be the next step on risk assessment. This kind of study is very challenging because the combinations possible between different pesticides present are numerous and interactions between them are not yet totally known and toxicological data on this combined effect are still lacking (EFSA 2013; Santonja 2022). Several studies have already been initiated to address this ”cocktail effect”. For instance, in 2020, EFSA established Cumulative Assessment Groups (CAGs) for chronic acetylcholinesterase inhibition including 47 active substances (11 N-methyl carbamate and 36 organophosphorus pesticides) (table 7.1) (EFSA 2021).

Table 7.1: Active substances included in the EFSA cumulative assessment group (CAG) for chronic acetylcholinesterase inhibition (2021)

Pesticide	Class	Pesticide	Class
acephate	Organophosphate	malathion	Organophosphate
aldicarb	Carbamate	methamidophos	Organophosphate
azinphos-ethyl	Organophosphate	methidathion	Organophosphate
azinphos-methyl	Organophosphate	methiocarb	Carbamate
benfuracarb	Carbamate	methomyl	Carbamate
cadusafos	Organophosphate	monocrotophos	Organophosphate
carbaryl	Carbamate	omethoate	Organophosphate
carbofuran	Carbamate	oxamyl	Carbamate
carbosulfan	Carbamate	oxydemeton-methyl	Organophosphate
chlorfenvinphos	Organophosphate	parathion	Organophosphate
chlorpyrifos	Organophosphate	parathion-methyl	Organophosphate
chlorpyrifos-methyl	Organophosphate	phenthoate	Organophosphate

Table continued on next page

Pesticide	Class	Pesticide	Class
diazinon	Organophosphate	phosalone	Organophosphate
dichlorvos	Organophosphate	phosmet	Organophosphate
dimethoate	Organophosphate	phoxim	Organophosphate
ethephon	Organophosphonate	pirimicarb	Carbamate
ethion	Organophosphate	pirimiphos-methyl	Organophosphate
ethoprophos	Organophosphate	profenofos	Organophosphate
fenamiphos	Organophosphate	pyrazophos	Organophosphate
fenitrothion	Organophosphate	thiodicarb	Carbamate
fenthion	Organophosphate	toclofos-methyl	Organophosphate
fonofos	Organophosphate	triazophos	Organophosphate
formetanate	Carbamate	trichlorfon	Organophosphate
fosthiazate	Organophosphate		

This work was followed in 2022 by the development of a CAG focusing on craniofacial alterations including 43 active substances of various pesticides classes (table 7.2) (EFSA 2022a).

Table 7.2: Active substances included in the EFSA cumulative assessment group (CAG) for craniofacial alterations (2022)

Pesticide	Class	Pesticide	Class
2,4-D	Herbicide (acidic pesticide)	flusilazole	Fungicide (triazole)
abamectin	Insecticide / Acaricide (avermectin)	flutriafol	Fungicide (triazole)
acephate	Insecticide (organophosphate)	folpet	Fungicide (phthalimide)
acetamiprid	Insecticide (neonicotinoid)	haloxyfop-P	Herbicide (aryloxyphenoxypropionate)
acrinathrin	Insecticide (pyrethroid)	mancozeb	Fungicide (dithiocarbamate)
azadirachtin	Insecticide (botanical)	maneb	Fungicide (dithiocarbamate)
benomyl	Fungicide (benzimidazole)	metconazole	Fungicide (triazole)
bitertanol	Fungicide (triazole)	myclobutanil	Fungicide (triazole)
bromuconazole	Fungicide (triazole)	paclobutrazol	Plant growth regulator (triazole)
carbendazim	Fungicide (benzimidazole)	penconazole	Fungicide (triazole)
chlorpyrifos	Insecticide (organophosphate)	propargite	Acaricide (organosulfur)
cymoxanil	Fungicide (acetamid)	propiconazole	Fungicide (triazole)
cyproconazole	Fungicide (triazole)	propineb	Fungicide (dithiocarbamate)
deltamethrin	Insecticide (pyrethroid)	prosulfocarb	Herbicide (thiocarbamate)
dieldrin	Insecticide (organochlorine)	prothioconazole	Fungicide (triazole)

Table continued on next page

Pesticide	Class	Pesticide	Class
emamectin	Insecticide (avermectin)	spirotetramat	Insecticide (tetramic acid derivative)
epoxiconazole	Fungicide (triazole)	spiroxamine	Fungicide (amine)
ethylene oxide	Fumigant / Sterilant (epoxide)	tebuconazole	Fungicide (triazole)
fenpropidin	Fungicide (morpholine)	tebufenpyrad	Insecticide / Acaricide
fenpropimorph	Fungicide (morpholine)	thiabendazole	Fungicide (benzimidazole)
fenpyrazamine	Fungicide (pyrazole)	thiacloprid	Insecticide (neonicotinoid)
fluazifop-P	Herbicide (aryloxyphenoxypropionate)		

These studies illustrate that while much work remains to be done, regulatory authorities have acknowledged the issue and are actively developing methodologies to assess cumulative toxicological effects.

2.2. Toward multi-contaminant assessment of processing contaminants

Similar limitations apply to processing contaminants. Although no health risk was identified for PAHs in the investigated food categories, recent studies indicate that PAH derivatives such as oxy-PAHs and nitro-PAHs may also occur in food. Initially neglected as they are considered environmental pollutants (Bandowe and Meusel 2017; Sun et al. 2019), these compounds are now known to form during cooking processes such as roasting or barbecuing (Zastrow et al. 2021; Sonego et al. 2022). Concern arises as some derivatives may exhibit higher toxicity and occur at concentrations comparable to or exceeding those of parent PAHs (Schlemitz and Pfannhauser 1997; Deng and Chan 2017; Dos Santos et al. 2019; Sonego et al. 2022). These findings suggest that the current system that focuses solely on monitoring four PAHs may not be sufficient. Additionally, the traditional classification and separation of environmental and processing contaminants may no longer be relevant, as the distinction between the two categories becomes increasingly blurred.

Likewise, acrylamide is not the only contaminant formed during Maillard reactions. Compounds such as furan and alkyl furans may also contribute to food-related carcinogenic risks. Although their toxicological profiles differ, both acrylamide and furan undergo metabolic activation involving CYP2E1, leading to the formation of reactive intermediates capable of interacting with cellular macromolecules. Acrylamide is converted into glycidamide, whereas furan is metabolized to cis-2-butene-1,4-dial. These reactive metabolites can form DNA adducts and have been associated with genotoxic effects that may contribute to carcinogenesis (Burka et al. 1991; Chen et al. 1995; Kraus et al. 2013). The potential co-occurrence of acrylamide, furan and alkyl furans in the same food products (e.g., coffee and fried foods) suggests that cumulative risk assessment approaches may be relevant rather than evaluating each contaminant in isolation. In coffee, for example, studies such as Alsafara (2023) have shown that mitigation strategies aimed at reducing acrylamide concentrations may increase furan and alkyl furan levels, and vice versa. These observations further underline the importance of conducting integrated risk assessments, which consider multiple contaminants

simultaneously, to better reflect real-world exposure scenarios.

2.3. Future data and methodological developments

While the objectives of this thesis were achieved, future developments may further refine exposure assessment. The upcoming Belgian Food Consumption Survey (FCS 2025), which includes a broader range of food categories than the 2014 survey, will enable a more accurate re-evaluation of acrylamide exposure using the occurrence datasets generated in this work. Furthermore, it will improve the identification of major dietary contributors to exposure, which may evolve over time alongside changes in dietary habits.

For instance, the increasing adoption of plant-based diets has led to the development of new food products and cereal-based meat alternatives, some of which may generate higher acrylamide levels than traditional meat or fish products. This trend is also reflected in the growing consumption of foods based on legumes and pulses, which may contribute non-negligibly to dietary acrylamide exposure (Portman et al. 2021).

The analytical tools, occurrence datasets, and TF databases developed during this thesis provide a foundation for future updates of national and European risk assessments, particularly as new consumption data become available.

2.4. Future perspectives for dietary risk assessment

The studies presented in this thesis adopted a targeted approach by focusing on specific contaminants and food categories. Such approaches remain essential as they provide detailed information on contamination sources, occurrence levels and factors influencing contaminant formation or transfer. For example, targeted investigations allow the identification of major contributors to dietary exposure, improve the understanding of contaminant formation mechanisms such as acrylamide generation during food processing, and generate the scientific evidence required to establish mitigation measures and support the application of the ALARA principle. These studies also provide the occurrence data necessary for risk assessment activities and regulatory monitoring programmes.

In parallel, Total Diet Studies (TDS) offer a complementary perspective by estimating dietary exposure at the population level through the analysis of foods as consumed. Compared with targeted monitoring programmes, TDS are generally considered more representative of actual dietary exposure patterns and may provide a more accurate estimate of chronic exposure (WHO, FAO, EFSA 2011). Furthermore, TDS allow the simultaneous assessment of multiple contaminants within a single study, making them particularly attractive from a resource, time and cost-efficiency perspective. Such approaches may therefore play an important role in future risk assessment frameworks, especially in the context of cumulative exposure assessment as previously discussed in this chapter.

However, both approaches address different scientific questions and should not be considered interchangeable. While targeted occurrence studies help identify contamination sources and support risk management measures, TDS primarily aim to estimate overall population exposure. Consequently, both approaches remain necessary and highly complementary for food safety assessment.

An additional step towards a more refined assessment of exposure is the use of human biomonitoring data. In contrast to occurrence studies and TDS, which estimate external

exposure based on food contamination and consumption data, biomonitoring provides information on internal exposure by measuring contaminants, metabolites or biomarkers directly in biological matrices (i.e.blood, hair, mother milk, urine) (DeWoskin et al. 2013; Watzek et al., 2012). Biomonitoring, therefore, offers an opportunity to evaluate the fraction of contaminants that is effectively absorbed by the body and may serve as a tool to support multi-sources exposure assessment.

Nevertheless, the interpretation of biomonitoring data remains challenging. For several contaminants, biomarker levels may be influenced by exposure sources other than food. For example, PAH biomarkers may reflect exposure originating from environmental pollution, smoking or occupational activities in addition to dietary intake (Strickland and Kang 1999; Srogi 2007). Similarly, smoking constitutes a major source of acrylamide exposure and may complicate the interpretation of biomonitoring results when attempting to relate biomarker levels to food consumption (Zhang et al. 2015). Furthermore, biomarker concentrations may be influenced by interindividual differences in absorption, metabolism and excretion processes (for the case of urine), which are partly determined by genetic variability. Consequently, biomonitoring data cannot generally identify the specific dietary sources responsible for exposure and should therefore be viewed as complementary to dietary exposure assessment or for total exposure estimation.

Another limitation is the complexity of collecting human biological samples. Biomonitoring studies require ethical approval and informed consent from participants, as well as qualified personnel for sample collection and handling. In addition, large study populations are often necessary to account for interindividual variability and to obtain reliable population-level estimates. These constraints make biomonitoring studies more resource-intensive than occurrence-based dietary exposure assessments.

Hence, future food safety risk assessment will likely rely on the integration of these different approaches. Combining occurrence data, Total Diet Studies and human biomonitoring may provide a more comprehensive understanding of both external and internal exposure. Such an European initiative is pointing towards this direction, such as the EU PARC project which aims to improve chemical risk assessment through the use of human biomonitoring, the development of new toxicological tools and a better characterization of human exposure (PARC 2026). In the future, these approaches may help to provide a more realistic assessment of the risks associated with dietary exposure to chemical contaminants.

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Appendix A

Supplementary analytical and statistical data for pesticide residues in tea and herbal tea

A1 Materials and methods for the determination of contaminants in tea and tea brew

A1.1. Solvents, reagents and standard solutions

Acetonitrile and methanol (mass spectrometry (MS) grade), di-isopropyl ether, acetone (HPLC grade) and analytical grade formic acid were purchased from Biosolve (Valkenswaard, the Netherlands). Orthophosphoric acid 85 % was from Merck (Darmstadt, Germany). Water was obtained using a milliQ-Gradient A10 system (Millipore, Billerica, USA). Nitric acid (Suprapur, 67-69 %) was purchased from Carlo-Erba (Val-de-Reuil, France). Hydrofluoric acid (48 % >99.99 %) trace metal basis from Sigma-Aldrich (Overijse, Belgium). Sodium chloride, magnesium sulfate, ammonium acetate and ammonium chloride were from VWR chemicals (Leuven, Belgium). Primary Secondary amine (PSA) were from Agilent Technologies, Santa Clara, CA, USA. Multi-element ICP standard (Astasol Mix 26 elements, 100 mg/l) from Analytika (Prague, Czech Republic). Pesticides analytical standards were from LGC standards (Wesel, Germany).

A1.2. Extraction of contaminants from dry materials and infusion

A1.2.1. Extraction of pesticide residues from dry material

Extraction for liquid chromatography- mass spectrometry (LC-MS/MS) analysis

Analytical procedure for pesticide residue analysis by LC-MS/MS from tea and herbal teas has been based on the methodology described by (Hanot et al. 2015). Briefly, 2.5 g of dry herbal material was initially hydrated with 7.5 mL of milliQ water, prior to the extraction with 20 mL of 20 mM ammonium acetate in methanol/water (95:5; v/v) mixture. Next, the samples were blended with a high speed disperser (Ultra-Turrax®) during 1 min and the extracts were subsequently filtered through a glass made Büchner funnel equipped with a 42.5 mm paper filter (pore size 11 µm). The extracts were then transferred to a 50 mL glass cylinder, the internal standard added to the desired quantity and the total volume was adjusted to 30 mL. An aliquot of 300 µL was transferred into a mini-UniPrep Syringeless vial, diluted with 200 µL of mobile phase A and then transferred into a mini-UniPrep Syringeless 0.20 µm polyvinylidene difluoride (PVDF) membrane filter vial before injection in LC-MS/MS system.

Extraction for gas chromatography-mass spectrometry (GC-MS/MS) analysis

Also here 2.5 g of the ground dry herbal material was initially hydrated with 7.5 mL of milliQ water in a sorval stainless steel chamber of 400 mL. Next, 30 mL of acetone was added and samples were mixed with a rotative knife. The obtained extract was filtered through a glass Büchner funnel equipped with a 42.5 mm paper filter with pore size of 11 µm. Also the rotative knife was rinse with a small volume of acetone and was added onto the filter. The filtered extract was transferred to a 50 mL volumetric flask and the volume was adjusted to 50 mL with acetone. Subsequently, a 30 mL aliquot was transferred in a 100 mL Erlenmeyer containing 5 g of celite and 30 mL of ammonium chloride solution (1 % orthophosphoric acid), where the sample was allowed to set for 30 minutes at room temperature. Then, the extract was

filtered through a glass Büchner funnel equipped with a paper filter. The Erlenmeyer was rinsed with a mixture acetone/water (60:40; v/v), then with 100 mL milliQ water. The filtrate was transferred in a 250 mL glass cylinder that contained 20 mL of di-isopropyl ether and 10 mL of a saturated NaCl solution, and the mixture was agitated for one min. Next, 15 mL of the supernatant was taken and passed through 5 g of anhydrous sodium sulfate for water removal. 500 µL of the filtrate was then transferred into a mini-UniPrep Syringeless 0.20 µm PVDF filter vial before injection on the GC-MS/MS system.

A1.2.2. Extraction of pesticide residues from infusions for LC-MS/MS & GC-MS/MS

An adapted Quechers extraction (Lehotay and Collaborators 2007) was performed on tea brew. Briefly, 20 mL of infusion was placed in a 50 mL Falcon tube, where 20 mL of milli-Q water was added with 1 g of NaCl and 8 g of $MgSO_4$. After centrifugation, the organic phase was split in 2 aliquots of 10 mL in Falcon tubes of 15 mL, then spiked with an internal standard. Dispersive solid phase extraction was performed with 250 mg of PSA and 900 mg of $MgSO_4$. After shaking by hand during 1 min, Falcon tubes were centrifuged, then upper phase dry evaporated with a gentle nitrogen stream. For LC-M/SMS analysis the sample was reconstituted with milli-Q water and with di-isopropyl ether for GC-MS/MS analysis. Then samples were placed in mini-UniPrep Syringeless 0.20 µm PVDF membrane before injection on chromatographic systems.

A1.2.3. LC-MS/MS conditions for pesticide determination in leaves and infusions

Ultra-High-Performance Liquid Chromatography (UHPLC)-MS/MS were performed on an UPLC^{TM} system (Waters, Milford, MA) coupled to a Quattro Premier^{TM} mass spectrometer (Waters, Milford, MA) following the protocol of Hanot et al. (2015). Five µL of sample was injected onto a reverse-phase ACQUITY™ UPLC BEH C18 column (1.7 µm, 2.1×100 mm) (Waters, Milford, MA). The mobile phases were composed of water/methanol (90/10 v/v) (mobile phase A) and methanol/water (90/10 v/v) (mobile phase B) both containing 5 mM ammonium acetate. The chromatographic separation was performed at a flow rate of 0.45 mL min^{-1} and with an elution gradient starting with 99.9 % of mobile phase A and linearly evolving to 0.1 % mobile phase A in 10 min, which was maintained for 2 additional minutes prior to re-equilibration during 3 min with 99.9 % of mobile phase A. Three UHPLC runs were necessary to acquire two transitions for each of 200 pesticides. Selected quantities of 200 LC-amenable pesticide residues were added prior to the filtration of the sample preceding the injection. The mass spectrometer source and the desolvation temperatures were set at 130 °C and 450 °C, respectively. The nitrogen gas flow rates were 50 L h^{-1} for the cone and 800 L h^{-1} for desolvation. Argon was used as collision gas with a pressure of 3.5 × 10−3 mbar. Data acquisition was performed in MRM mode. Interpretation of results was performed with the MassLynx software (Waters, Milford, MA) and the obtained relative areas of the analyte and internal standard were used for the quantification.

A1.2.4. GC-MS/MS conditions for pesticides determination in leaves and infusions

GC–MS analyses were performed on an Agilent 7890B GC system (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 7000C GC/MS Triple Quad mass spectrometer (Agilent Technologies) equipped with an electronic impact source. The chromatographic

column used was the Agilent GC column HP-5ms (30 m × 0.25 mm × 0.25 µm) and the liner inlet was a single taper, with wool and 4 mm internal diameter (Agilent Technologies). Five microliters of sample were injected on a PTV injector with the following program: initial condition 40 °C (0.02 min), 200 °C min^{-1} until 300 °C. Helium (≥ 99.9999 %) was used as carrier gas at a flow rate of 1.2 mL min^{-1}. Initial condition for oven was fixed at 70 °C (0.2 min), then 33 °C min^{-1}, 180 °C (0.1 min), 7 °C min^{-1}, 300 °C (4.5 min). Data acquisition was performed in MRM mode. Interpretation of results was performed with the MassHunter software from Agilent (Agilent Technologies) and relative areas of the analyte and internal standard were used for the quantification.

A1.2.5. Chromatograms of pesticides in tea brew at the LOQ

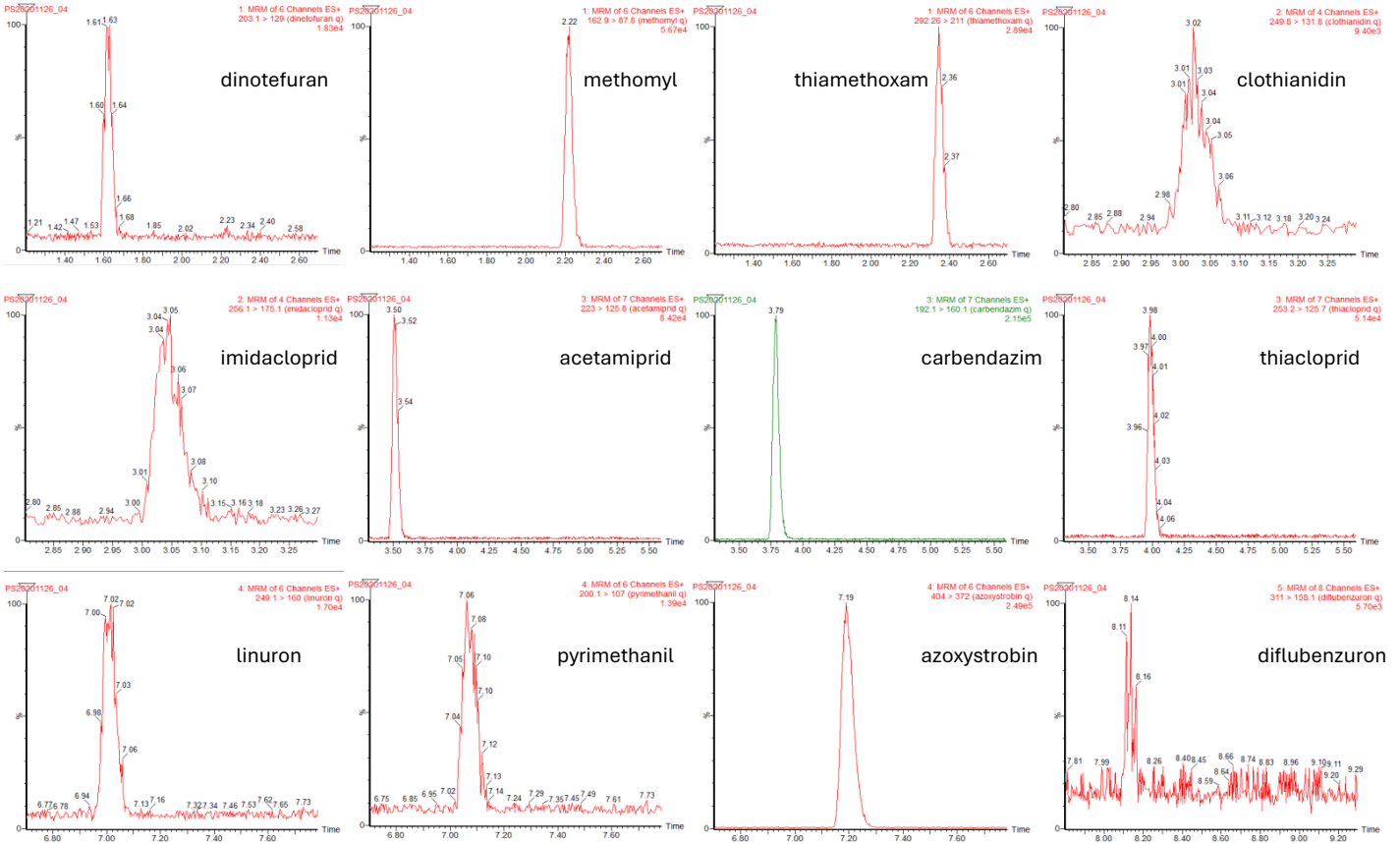

Figure A1: LC-amenable pesticide chromatograms in black tea brew at the LOQ (1 μg L^{-1}) (I).

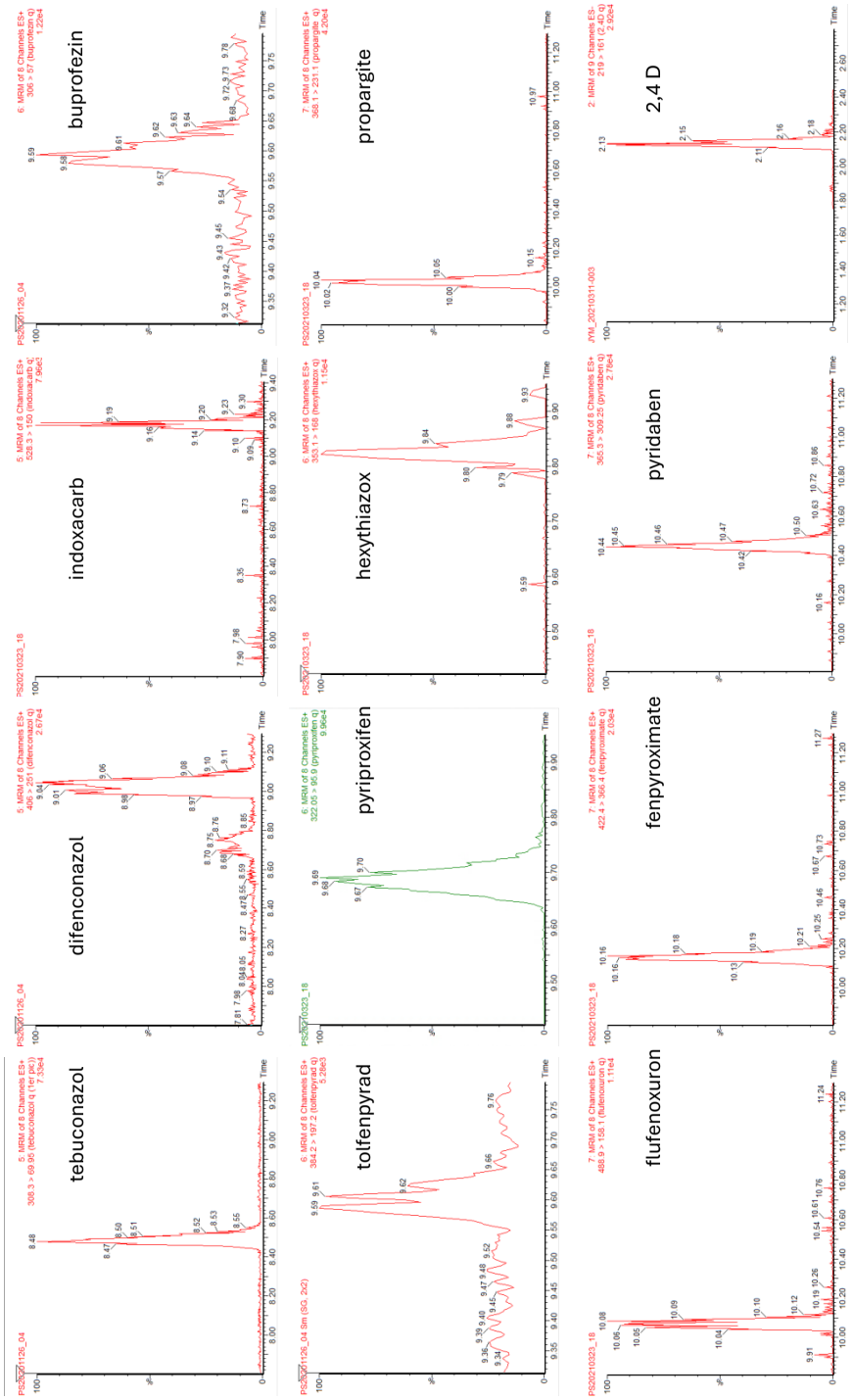

Figure A2: LC-amenable pesticide chromatograms in black tea brew at the LOQ (1 μg L^{-1}) (II).

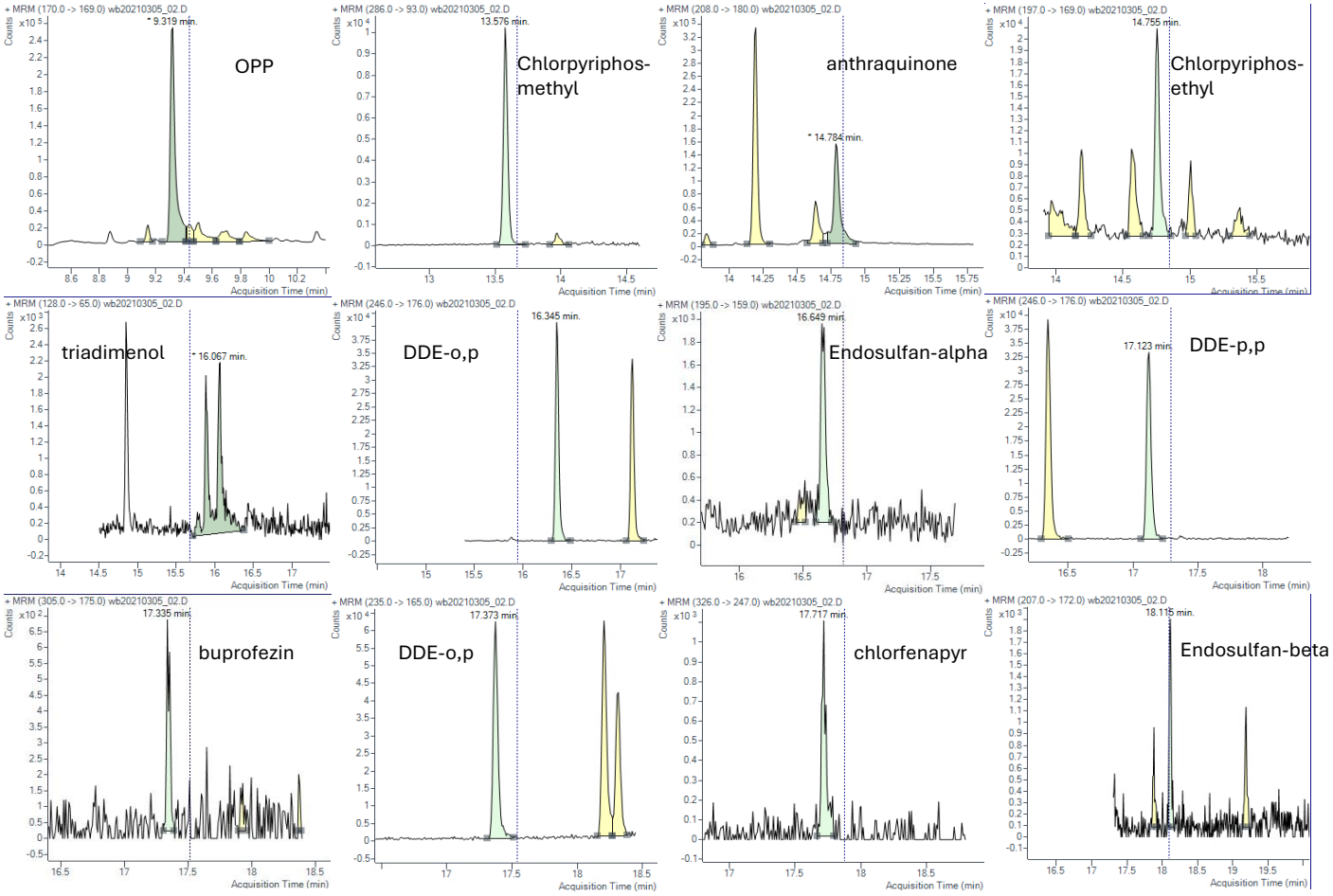

Figure A3: GC-amenable pesticide chromatograms in black tea brew at the LOQ (1 μg L^{-1}) (I).

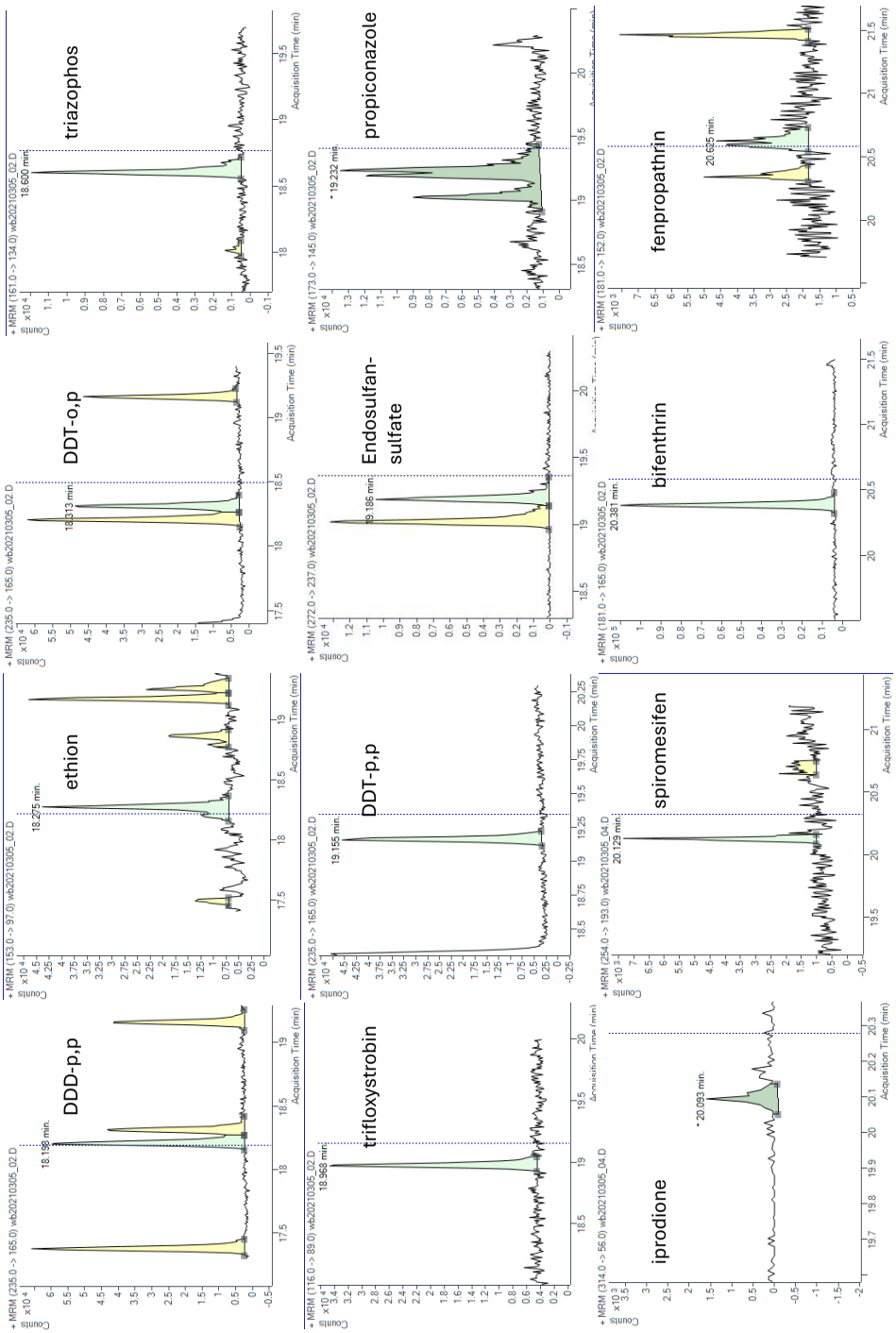

Figure A4: GC-amenable pesticide chromatograms in black tea brew at the LOQ (1 μg L^{-1}) (II).

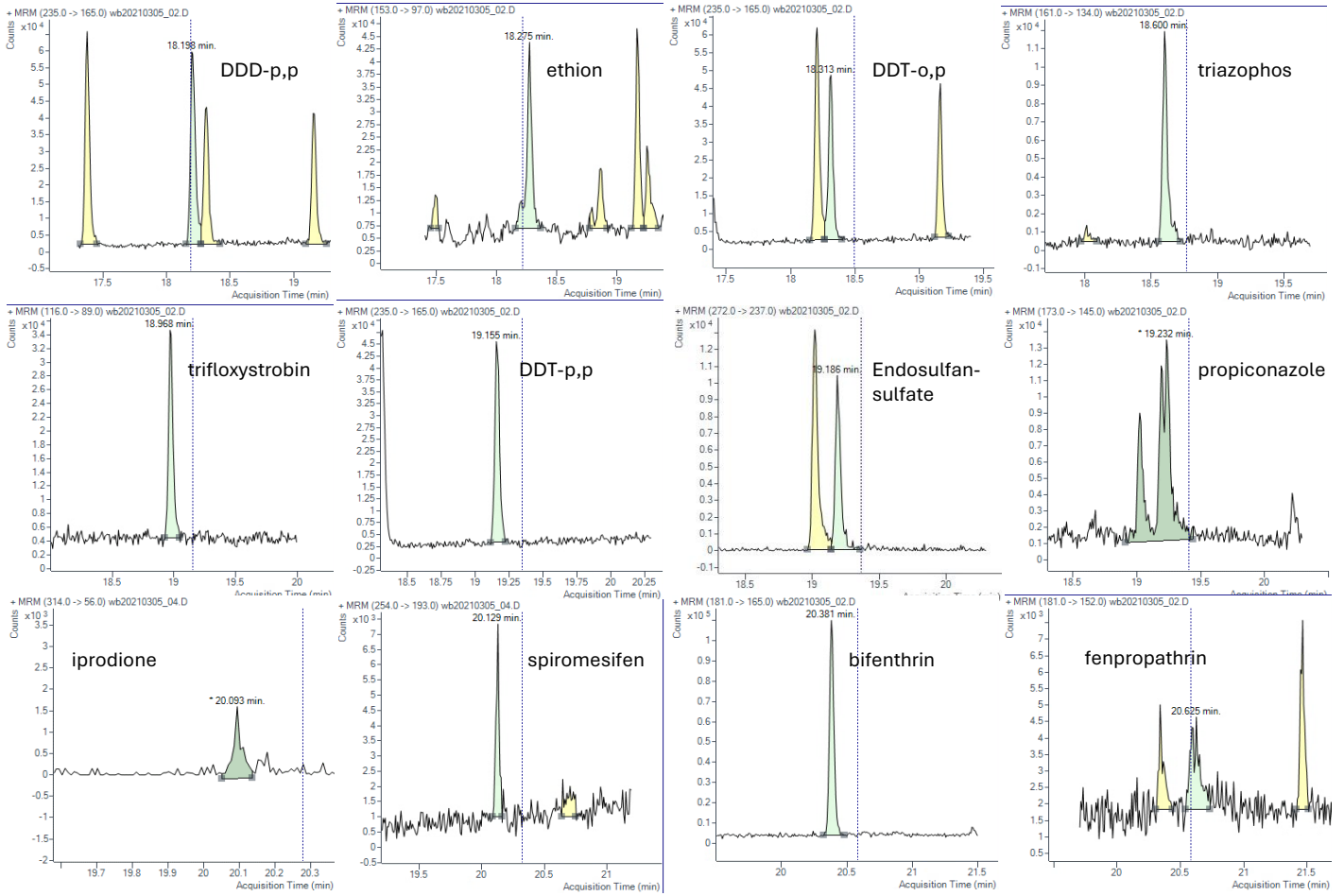

Figure A5: GC-amenable pesticide chromatograms in black tea brew at the LOQ (1 μg L^{-1}) (III).

A1.2.6. Total element concentrations in tea leaves and tea infusion

Total element concentration in tea leaves was determined by digestion (DigiPREP MS Digestion System, SCP Science). Briefly, 0.25 g of the finely ground tea samples was placed in 50 mL polypropylene DigiTUBE (SCP Science) to which 5 mL of concentrated nitric acid (HNO_3, 67-69 %, ultrapure quality) and 1 mL of diluted hydrogen fluoride (HF, 5 % ultrapure quality) were added. Samples were digested at 95 °C during 4 h. The digested samples were diluted with bi-distilled water and the total concentration of As, Cd, cobalt (Co), copper (Cu), Fe, Pb, Mn, nickel (Ni) and Zn, was determined by inductively coupled plasma mass spectroscopy (ICP-MS/MS, Agilent 8800). Blank samples in duplicates and two certified reference materials (CRM) NIST 1515 apple leaves and NIST 1573a tomato leaves were included in each digestion batch and treated in the same way as the tea samples. Recoveries of the CRM for the different elements ranged 74¬108 % for NIST 1515 and 88¬97 % for NIST 1573a. Tea infusions were prepared on selected samples. To select the samples, the total measured elemental concentrations in the leaves (mg kg^{-1}) was recalculated to concentrations in the tea infusion (mg L^{-1}) assuming that all elements were extracted by the water (i.e. dilution factor 50). Based on this theoretical calculation 17 tea samples out of 53 would present element concentration in the infusion of concern and thus were brewed for analysis. Brewing was done following ISO 3103 (2019) as described before. Elemental composition of tea infusions was determined by ICP-MS/MS (Agilent 8800) after sample acidification with concentrated HNO_3.

A1.2.7. Screening for pharmaceutical drugs

All tea and herbal samples were screened for the presence of synthetic drugs or medicines by both GC-MS and LC-MS2, according to the methodology described by Vanhee et al. (2018). Briefly, 30 mg of the tea mixture was solubilized in 10 mL of methanol, sonicated for 15 minutes and the solution was filtered through a 0.2 µm polytetrafluoroethylene (PTFE) filter prior to analysis by full scan GC-MS and LC-MS^n.

The GC-MS analyses were performed in an Agilent 7890A gas chromatograph coupled to an Agilent 5975 mass selective detector. The Column VF-5ms + 10 m EZ-guard was purchased from Agilent technologies (Santa Clara, CA, USA). Full automation was achieved using Agilent MSD ChemStation data acquisition and data handling software. Injections were made in the split mode (3.3:1) at an injection port temperature of 280 °C. The column oven temperature was initially set to 80 °C for 2 min and then raised at 15 °C min^{-1} to 280 °C and held for 17 min, followed by a raise of 10 °C min^{-1} to 310 °C and held for 20 min. The total run time was 55 min. The solvents were separated in an Agilent column (30 m x 0.25 mm x 0.25 µm film thickness). High-purity helium was used as the carrier gas with flow rate of 1 mL min^{-1}. The MS was operated in electron ionisation with electron energy of 70 eV. Data were acquired in full scan mode of m/z 25 to 600.

The LC-MS^n analysis were performed on a Dionex UltiMate 3000 Rapid Separation LC (RSLC) system (Thermo Scientific, Sunnyvale, CA, USA) coupled to an amaZon^{TM} speed ETD mass spectrometer (Bruker Daltonics, Bremen, Germany). The instrument system was calibrated using the manufacturer's calibration mixture, and the mass accuracy was determined to be < 0.1 Da during the period of analysis. A sample volume of 1 µL was injected onto the system. The chromatographic separation was performed at 45 °C on an Acquity™ UPLC BEH C18 Column (150 × 2.1 mm, 1.7 µm particle size) (Waters, Milford, MA, USA) with a mobile

phase consisting of 0.1 % formic acid in water (A) and 0.1 % formic acid in acetonitrile (B). A general LC method, suitable for rapid screening of suspected samples was used. A linear gradient from 1 % B to 99 % B was accomplished in 9 min, followed by an isocratic elution for 2 min and 2 min at 1 % B. The flow rate was 0.5 mL min^{-1}. The mass spectrometer was operated in alternating positive electrospray ionization (ESI+) and negative electrospray ionisation (ESI-) mode, with respective spray voltage of 4.5 kV (ESI+) or 3.5 kV (ESI-) and end plate voltage 500 V. MS spectra were obtained within a mass range of 100–1000 m/z and the smart parameter setting (SPS) was set to 475 m/z. For the MS2 precursor selection, the most intense ion was isolated (including singly charged ions) above the absolute intensity of 2.500 and 5 % relative intensity threshold. The ion charge control (ICC) was set to 200 000 with a maximum accumulation time of 50 ms (ESI+) or the ICC was set to 100 000 with a maximum accumulation time of 200 ms (ESI-). CID (Collision Induced Dissociation) was performed using helium as collision gas.

The LC and MS data were analysed by Compass Data Analysis 4.2 (Bruker Daltonics, Bremen, Germany) and the LC-MS/MS spectra were compared to different libraries, including the home-made library, enclosing a total of about 5000 MS and MS2 spectra. A 0.30 Da precursor tolerance for MS spectra and MS2 spectra were allowed.

A2 Contaminants content in tea and tea brew

Table A1: Pesticides content in tea and herbal tea samples (μg kg^{-1}) and associated maximal residue limits (MRL) and EU status in 2017 - Part 1 (T-1-01 to T-1-22)

Pesticides	EU status	MRL (μg kg^{-1})	T-1 01	T-1 02	T-1 03	T-1 04	T-1 05	T-1 06	T-1 07	T-1 09	T-1 10	T-1 11	T-1 12	T-1 13	T-1 14	T-1 15	T-1 16	T-1 17	T-1 18	T-1 19	T-1 20	T-1 21	T-1 22
Acetamiprid		50								10.8			6.7	25.6			59.1						16.1
Anthraquinone	Forbidden	20		14.4		16.9	81.1			81.4					17.6		241	11.2		14.3	27.2		16.0
Azoxystrobin		50																					
Benalaxyl		100																					
Bentazone		100																					
Bifenthrin		30000		51.3				27.9		86.1			5.6	89.9			308			13.3	7.1		60.2
Biphenyl	Forbidden	50					23.4								14.1		218	62.2					
Boscalid		10																					
Buprofezin		50												44.8		26.8							10.9
Carbendazim	Forbidden	100											11.3			15.4							15.9
Chlorfenapyr		50000		25.6						26.2		826		11.1	12.5								21.6
Chlorothalonil		50								52.9		81.5								222			18.7
Chlorpyriphos		2000												20.3									

Table continued on next page

Table A1 - Part 1 (continued)

Pesticides	EU status	MRL (μg kg^{-1})	T-101	T-102	T-103	T-104	T-105	T-106	T-107	T-109	T-110	T-111	T-112	T-113	T-114	T-115	T-116	T-117	T-118	T-119	T-120	T-121	T-122
-ethyl																							
Clomazone		50																					
Cyflufenamid		50																					
Cyhalothrin-L		10		14.8						22.3			13.0	86.1									26.1
Cypermethrin		500															15.5		57.3		436		
DDD-p,p	Forbidden	200																					
Deltamethrin		5000																					
Diclofop-methyl		50												5.6									
Dimoxystrobin		50													34.8								
Diphenylamine	Forbidden	50																					
Disulfoton	Forbidden	50												10.6									
Disulfoton -sulfoxide	Forbidden	50																					
Endosulfan-alpha		30000 (sum)												44.4									
Endosulfan-beta		30000 (sum)												56.1									
Endosulfan-sulfate		30000 (sum)												30.8			8.5						

Table continued on next page

Table A1 - Part 1 (continued)

Pesticides	EU status	MRL (μg kg^{-1})	T-101	T-102	T-103	T-104	T-105	T-106	T-107	T-109	T-110	T-111	T-112	T-113	T-114	T-115	T-116	T-117	T-118	T-119	T-120	T-121	T-122
Fenamiphos		50																					
Fenpyroximate		50																					
Flufenoxuron	Forbidden	15000		55.7																			
Fluopyram		50																					
Imidacloprid		50								7.3			9.9	21.5		17.8	19.6						
Iprodione	Forbidden	50																					
Mepronil	Forbidden	50						15.5			13.6												
Methomyl		50												15.1			11.8						
Methoxychlor		100											8.1										
OPP		100	18.9		9.0			17.8	15.8		14.4						44.1	24.1					
Pentachloroaniline		100																					
Pirimiphos -methyl		50																					
Procymidone	Forbidden	50																					
Propamocarb		50											15.3										
Propargite	Forbidden	50		55.6																			
Propetamphos	Forbidden	10																					
Propoxur	Forbidden	100																					

Table continued on next page

Table A1 - Part 1 (continued)

Pesticides	EU status	MRL (μg kg^{-1})	T-101	T-102	T-103	T-104	T-105	T-106	T-107	T-109	T-110	T-111	T-112	T-113	T-114	T-115	T-116	T-117	T-118	T-119	T-120	T-121	T-122
Propyzamide		50																					
Pyraclofos	Forbidden	10																					
Pyraclostrobine		100																					
Pyridaben		50																					
Quinalphos	Forbidden	50	36.8																				
Spiroxamine		50																					
Tebufenozid		100																					
Tebufenpyrad		100																			437	4971	
Tecnazene	Forbidden	50																					
Thiacloprid		10000		49.8				36.5		61.7													11.1
Thiamethoxam		20000						57.2		62.4			8.2				21.5						11.5
Tolfenpyrad		10												130	10.8								7.5
Triadimenol		50								43.3													15.7
Zoxamide		50								120													

Table A2: Pesticides content in tea and herbal tea samples (μg kg^{-1}) and associated maximal residue limits (MRL) and EU status in 2017 - Part 2 (T-1-23 to T-2-28)

Pesticides	EU status	MRL (μg kg^{-1})	T-1 23	T-1 24	T-1 25	T-1 26	T-1 27	T-1 28	T-1 29	T-1 30	T-1 31	T-1 32	T-1 33	T-1 34	T-1 35	T-1 36	T-1 37	T-1 38	T-1 39	T-2 25	T-2 26	T-2 27	T-2 28
Acetamiprid		50	33.2				6.2		9.4	9.6								7.1	20.0			10.1	
Anthraquinone	Forbidden	20			21.7	43.6	110				21.9								51.1				
Azoxystrobin		50			5.8																		
Benalaxyl		100																					
Bentazone		100													56.3								
Bifenthrin		30000		338	79.6	137	69.7		46.7	362	230											84.3	
Biphenyl	Forbidden	50																					14.2
Boscalid		10																16.5	5.6				
Buprofezin		50	14.4				7.2			8.7			72.2					14.2	26.8				
Carbendazim	Forbidden	100	15.8				9.2						12.4					22.4	8.6			12.6	10.6
Chlorfenapyr		50000			34.5				50.2		33.7								129				
Chlorothalonil		50					11.1							11.1		39.8	11.1						
Chlorpyriphos -ethyl		2000	23.5						6.5	14.7			16.4					18.3	12.6				48.9
Clomazone		50															10.0						
Cyflufenamid		50																					
Cyhalothrin-L		10	13.2	10.4	27.5	135	12.2		160	445								22.9	19.1			11.0	

Table continued on next page

Table A2 - Part 2 (continued)

Pesticides	EU status	MRL (μg kg^{-1})	T-123	T-124	T-125	T-126	T-127	T-128	T-129	T-130	T-131	T-132	T-133	T-134	T-135	T-136	T-137	T-138	T-139	T-225	T-226	T-227	T-228
Cypermethrin		500	127															142					
DDD-p,p	Forbidden	200						10.0															
Deltamethrin		5000		35.8														34.4					
Dimoxystrobin		50													14.3								
Diphenylamine	Forbidden	50										7.9											
Disulfoton	Forbidden	50																				15.0	
Disulfoton -sulfoxide	Forbidden	50																9.7					
Endosulfan-beta		30000 (sum)			8.4																		
Endosulfan-sulfate		30000 (sum)			6.3																		
Fenamiphos		50																		12.0			
Fenpyroximate		50																					
Flufenoxuron	Forbidden	15000																					
Fluopyram		50																7.5					
Imidacloprid		50	94.1		8.9		31.5			7.9			14.8						9.4				
Iprodione	Forbidden	50																14.2					

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Table A2 - Part 2 (continued)

Pesticides	EU status	MRL (μg kg^{-1})	T-123	T-124	T-125	T-126	T-127	T-128	T-129	T-130	T-131	T-132	T-133	T-134	T-135	T-136	T-137	T-138	T-139	T-225	T-226	T-227	T-228
Mepronil	Forbidden	50							17.3														
OPP		100	18.8				663					19.2											
Pirimiphos -methyl		50															18.9						
Procymidone	Forbidden	50					5.1																
Propamocarb		50																					
Propargite	Forbidden	50							154										39.6				
Propetamphos	Forbidden	10	12.0																				
Propoxur	Forbidden	100															13.2						
Propyzamide		50																21.6					
Pyraclofos	Forbidden	10						38.7															
Pyraclostrobine		100																14.8					
Pyridaben		50									20.2												
Quinalphos	Forbidden	50																					
Spiroxamine		50																					
Tebufenozid		100																	13.6				
Tebufenpyrad		100																					
Tecnazene	Forbidden	50																	15.2				

Table continued on next page

Table A2 - Part 2 (continued)

Pesticides	EU status	MRL (μg kg^{-1})	T-1 23	T-1 24	T-1 25	T-1 26	T-1 27	T-1 28	T-1 29	T-1 30	T-1 31	T-1 32	T-1 33	T-1 34	T-1 35	T-1 36	T-1 37	T-1 38	T-1 39	T-2 25	T-2 26	T-2 27	T-2 28
Thiacloprid		10000		10.1	16.4														73.0				
Thiamethoxam		20000		8.2	16.9				70.7	161												20.8	
Tolfenpyrad		10									106												
Triadimenol		50			13.3																		
Zoxamide		50																					

Table A3: Pesticides content in tea and herbal tea samples (μg kg^{-1}) and associated maximal residue limits (MRL) and EU status in 2017 - Part 3 (T-2-30 to T-2-168)

Pesticides	EU status	MRL (μg kg^{-1})	T-2 30	T-2 58	T-2 59	T-2 61	T-2 64	T-2 93	T-2 98	T-2 99	T-2 160	T-2 162	T-2 164	T-2 167	T-2 168
Acetamiprid		50													
Anthraquinone	Forbidden	20						6.2							
Azoxystrobin		50													
Benalaxyl		100		105											
Bentazone		100													
Bifenthrin		30000		600							8.6				

Table continued on next page

Table A3 - Part 3 (continued)

Pesticides	EU status	MRL (μg kg^{-1})	T-2 30	T-2 58	T-2 59	T-2 61	T-2 64	T-2 93	T-2 98	T-2 99	T-2 160	T-2 162	T-2 164	T-2 167	T-2 168
Biphenyl	Forbidden	50		93.0	14.1				27.2				11.6		
Boscalid		10													7.4
Buprofezin		50													
Carbendazim	Forbidden	100									15.8				
Chlorfenapyr		50000													
Chlorothalonil		50		40.6					39.1				11.2		116
Chlorpyriphos-ethyl		2000													
Clomazone		50													
Cyflufenamid		50		11.4											
Cyhalothrin-L		10													
Cypermethrin		500													
DDD-p,p	Forbidden	200													
Deltamethrin		5000													
Diclofop-methyl		50													
Dimoxystrobin		50													
Diphenylamine	Forbidden	50					6.3								
Disulfoton	Forbidden	50								24.0					
Disulfoton-sulfoxide	Forbidden	50													

Table continued on next page

Table A3 - Part 3 (continued)

Pesticides	EU status	MRL (μg kg^{-1})	T-2 30	T-2 58	T-2 59	T-2 61	T-2 64	T-2 93	T-2 98	T-2 99	T-2 160	T-2 162	T-2 164	T-2 167	T-2 168
Endosulfan-Alpha		30000 (sum)													
Endosulfan-beta		30000 (sum)													
Endosulfan-sulfate		30000 (sum)													
Fenamiphos		50													
Fenpyroximate		50									8.8				25.1
Flufenoxuron	Forbidden	15000													
Fluopyram		50													
Imidacloprid		50			235										
Iprodione	Forbidden	50													
Mepronil	Forbidden	50		228				16.1			14.3				
Methomyl		50													
Methoxychlor		100													
OPP		100						14.5	10.5			14.8			
Pentachloroaniline		100							7.1						
Pirimiphos-methyl		50											12.8		
Procymidone	Forbidden	50													
Propamocarb		50													

Table continued on next page

Table A3 - Part 3 (continued)

Pesticides	EU status	MRL (μg kg^{-1})	T-2 30	T-2 58	T-2 59	T-2 61	T-2 64	T-2 93	T-2 98	T-2 99	T-2 160	T-2 162	T-2 164	T-2 167	T-2 168
Propargite	Forbidden	50									23.3				
Propetamphos	Forbidden	10													
Propoxur	Forbidden	100													
Propyzamide		50								7.8					
Pyraclofos	Forbidden	10													
Pyraclostrobine		100													
Pyridaben		50													
Quinalphos	Forbidden	50													
Spiroxamine		50													11.1
Tebufenozid		100													
Tebufenpyrad		100													
Tecnazene	Forbidden	50													
Thiacloprid		10000													
Thiamethoxam		20000			30.4										
Tolfenpyrad		10													
Triadimenol		50													
Zoxamide		50													

Table A4: Number of pesticides exceedances and corresponding chemical class and pesticides usage (category)

Pesticides	# exceedances	Chemical class	Category
Cyhalothrin-L	9	pyrethoid	insecticide
Anthraquinone	6	PAH	bird repellant and naturally occurring in plants
Chlorothalonil	3	OCP	fungicide
Cypermethrin	3	Pyrethoid	insecticide
Biphenyl	2	PAH	fungicide and naturally occurring in coal tar, crude oil, and natural gas
Imidacloprid	2	neonicotinoid	insecticide
Tebufenpyrad	2	pyrazole	insecticide and acaricide
Tolfenpyrad	2	pyrazole	insecticide and acaricide
Benalaxyl	1	phenylamide	fungicide
Mepronil	1	benzamides	fungicide
Orthophenylphenol	1	OPP	insecticide
Propargite	1	sulphite ester	insecticide
Pyraclofos	1	OCP	insecticide
Zoxamide	1	benzamide	fungicide

Table A5: Trace elements content in dry herbal material samples (mg kg^{-1})

Samples	As	Cd	Pb	Co	Ni	Cu	Fe	Mn	Zn
T-1-01	0.018	0.024	0.177	0.373	5.97	10.25	254	948	18.2
T-1-02	0.091	0.047	1.033	0.317	4.40	10.81	314	1142	21.7
T-1-03	0.404	0.086	1.840	0.320	4.64	13.84	154	1666	27.9
T-1-04	0.185	0.054	1.651	0.367	2.99	14.71	237	1137	26.7
T-1-05	0.134	0.032	0.509	0.203	7.48	14.88	231	528	29.5
T-1-06	0.042	0.028	0.521	0.323	4.99	13.59	298	959	24.3
T-1-07	0.014	0.015	0.785	0.095	4.07	24.00	76	234	29.9
T-1-09	0.090	0.102	0.989	0.351	6.01	10.49	209	1048	22.2

Table continued on next page

Table A5 (continued)

Samples	As	Cd	Pb	Co	Ni	Cu	Fe	Mn	Zn
T-1-10	0.004	0.013	0.161	0.323	4.97	17.74	40	583	28.2
T-1-11	0.003	0.005	0.047	0.080	4.73	12.09	59	493	22.6
T-1-12	0.029	0.037	0.246	0.281	6.19	11.34	77	822	38.5
T-1-13	0.037	0.024	0.355	0.250	4.42	11.29	59	736	35.9
T-1-14	0.151	0.062	0.281	0.187	3.89	13.97	134	531	25.9
T-1-15	0.009	0.031	0.063	0.425	4.19	11.06	42	871	21.8
T-1-16	0.089	0.058	0.620	0.235	5.71	16.06	96	740	27.5
T-1-17	0.004	0.006	0.076	0.474	3.14	11.11	82	778	21.9
T-1-18	0.046	0.030	0.093	0.106	0.57	2.07	87	49	8.9
T-1-19	3.902	0.301	0.910	0.221	2.10	6.75	98	722	47.7
T-1-20	0.171	0.132	1.281	0.252	3.87	13.47	271	1151	25.5
T-1-21	0.104	0.235	0.920	0.440	2.38	11.02	348	415	38.3
T-1-22	0.181	0.073	1.564	0.378	5.92	11.96	312	1230	26.0
T-1-23	0.075	0.027	0.524	0.413	6.64	15.16	109	959	24.4
T-1-24	0.038	0.026	0.356	0.220	2.19	12.00	168	670	19.0
T-1-25	0.089	0.083	1.048	0.305	5.53	11.16	207	1234	21.4
T-1-26	0.129	0.112	1.384	0.430	4.37	8.48	150	1852	19.5
T-1-27	0.667	0.068	2.663	0.411	7.19	22.44	1186	1011	43.0
T-1-28	0.222	0.011	0.479	0.108	0.89	6.35	239	45	14.2
T-1-29	0.099	0.084	1.452	0.288	4.66	13.68	170	1154	23.5
T-1-30	0.059	0.041	0.515	0.192	4.28	11.49	89	875	21.0
T-1-31	0.035	0.032	0.886	0.146	1.58	6.59	104	1254	15.2
T-1-32	0.065	0.048	0.969	0.290	3.07	10.64	110	1206	19.1
T-1-33	0.148	0.078	38.926	0.494	13.84	14.69	240	693	46.6
T-1-34	0.098	0.047	0.820	0.182	2.18	8.52	190	468	13.6
T-1-35	0.147	0.154	0.809	0.206	2.69	7.47	372	289	25.3
T-1-36	0.222	0.095	0.646	0.192	1.71	6.29	435	102	18.7
T-1-37	0.107	0.343	0.569	0.236	2.93	6.96	352	135	32.1
T-1-38	0.106	0.059	0.937	0.263	3.63	11.08	147	902	21.4
T-1-39	0.058	0.147	0.638	0.262	3.57	10.76	132	1231	24.0
T-2-25	0.137	0.057	2.299	0.260	7.83	26.28	406	96	25.8

Table continued on next page

Table A5 (continued)

Samples	As	Cd	Pb	Co	Ni	Cu	Fe	Mn	Zn
T-2-26	0.293	0.087	1.122	1.701	7.68	21.59	3153	117	46.6
T-2-27	0.209	0.059	1.480	0.709	4.46	14.07	1343	611	31.4
T-2-28	0.132	0.085	0.570	0.739	5.72	11.95	1269	97	32.1
T-2-30	0.154	0.047	0.477	0.469	8.28	5.88	513	208	20.9
T-2-59	0.094	0.079	0.285	0.142	1.29	9.97	158	53	34.1
T-2-61	0.202	0.096	0.334	0.240	4.47	9.00	412	37	27.6
T-2-93	0.054	0.033	1.060	0.531	8.30	15.48	358	1359	24.2
T-2-98	0.163	0.036	0.628	0.561	2.37	8.53	1076	120	19.2
T-2-99	0.031	0.014	0.529	0.422	9.39	12.30	362	1288	23.1
T-2-160	0.098	0.077	1.123	0.261	5.32	10.46	267	712	26.2
T-2-162	0.023	0.029	0.148	0.192	5.35	11.25	171	1384	21.2
T-2-164	6.425	0.145	0.544	0.294	3.10	5.61	372	232	36.8
T-2-167	0.083	0.033	0.207	0.182	1.43	10.65	278	42	25.6
T-2-168	0.152	0.116	0.429	0.143	2.08	9.88	295	37	26.0

Table A6: Trace elements content in tea infusions (μg L^{-1})

Samples	As	Cd	Pb	Co	Ni	Cu	Fe	Mn	Zn
T-1-002	0.4863	0.0720	0.8065	2.5689	58.5186	41.5	53.3	3626	100.1
T-1-003	2.4967	0.0805	1.9495	1.6195	48.1873	62.6	26.0	2736	83.4
T-1-004	1.2704	0.0668	2.2493	2.1700	36.7670	53.5	36.1	2588	91.2
T-1-019	38.0127	0.3149	0.6031	1.3390	26.0069	23.8	15.2	1973	142.1
T-1-020	0.7577	0.2815	1.2865	2.2139	57.5209	110.3	44.9	4504	121.2
T-1-022	0.9840	0.1260	1.4651	3.5765	90.7550	83.7	67.9	4793	156.3
T-1-025	0.6437	0.1404	1.2012	3.4810	95.0567	74.8	67.7	4535	149.2
T-1-026	0.7870	0.1362	1.0939	2.9516	53.1145	33.6	68.4	4003	82.7
T-1-027	1.0885	0.0713	1.6168	0.8920	37.2833	6.7	152.8	2266	115.3
T-1-029	0.8011	0.1990	1.9579	2.7638	83.2525	76.7	105.8	4412	167.9
T-1-033	0.7061	0.1196	6.1972	4.7594	181.0389	64.6	27.7	3566	368.7
T-1-037	0.0517	0.1183	0.2287	0.2247	5.9674	9.0	23.0	117	24.3

Table continued on next page

Table A6 (continued)

Samples	As	Cd	Pb	Co	Ni	Cu	Fe	Mn	Zn
T-2-026	0.2575	0.0904	0.3363	1.7389	11.0641	42.4	55.3	165	61.8
T-2-093	0.2956	0.0494	0.3835	2.7528	82.0758	72.7	25.1	4929	121.5
T-2-099	0.2887	0.0855	0.7593	1.6623	52.2900	59.4	36.5	1754	109.3
T-2-027	0.3713	0.0720	1.0551	1.9929	41.0515	63.8	30.1	1462	93.9
T-2-164	49.5507	0.3304	0.7615	1.1493	15.5238	25.7	35.0	1424	201.9

A3 Materials and methods for the determination of pesticides TFs in tea and herbal tea

A3.1. Solvents, reagents and standard solutions

Acetonitrile and methanol (mass spectrometry (MS) grade), di-isopropyl ether and analytical grade formic acid were purchased from Biosolve (Valkenswaard, The Netherlands). Orthophosphoric acid 85 %, sulfuric acid and sodium hydroxide were from Merck (Darmstadt, Germany). Water was obtained using a milliQ-Gradient A10 system (Millipore, Billerica, USA). Sodium chloride (NaCl), magnesium sulfate ($MgSO_4$, ammonium acetate and ammonium chloride were from VWR chemicals (Leuven, Belgium). Primary secondary amine (PSA), graphite carbon black (GCB), and C18 dispersive sorbents were from Agilent Technologies (Santa Clara, CA, USA). Pesticide analytical standards were from LGC standards (Wesel, Germany).

A3.2. Extraction of the pesticides from tea leaves and the brew

A3.2.1. Analysis of pesticides in tea leaves

Pesticides in dry (herbal) tea leaves have been extracted based on an adapted QuEChERS method initially designed by Lehotay et al. (2007). Two g of dry leaves were weighed in a 50 mL Falcon tube. Then 10 mL of milliQ water and 10 mL of ACN were added, followed by 1 g of NaCl and 4 g of $MgSO_4$. Samples were then vortexed and centrifuged for 5 min at 3900 rpm. The supernatant was fortified with the internal standard (100 µL oxfendazole at 1 µg mL^{-1} for LC-MS/MS and 100 µL BaP D12 at 1 µg mL^{-1} for GC-MS/MS). Five mL of supernatant was then purified in a Falcon tube containing dispersive solid phase extraction (d-SPE) sorbents composed of 250 mg of PSA ad 250 mg of C18. Sample extracts were vortexed and centrifuged for 5 min at 3900 rpm. Then, the supernatant was gently concentrated to 500 µL under a nitrogen stream. Finally, the volume was adjusted to 2 mL of water (LC-MS/MS analysis) or 2 mL of di-isopropyl ether (GC-MS/MS analysis) before being placed in auto-filtrating polypropylene vials.

A3.2.2. Analysis of pesticides in the brew

The protocol for pesticide extraction in the brew is an adapted QuEChERS extraction (Lehotay et al. 2007)) and has been described elsewhere (Szternfeld et al. 2022). Briefly, 20 mL of infusion was placed in a 50 mL Falcon tube, where 20 mL of ACN was added with 1 g of NaCl and 8 g of $MgSO^4$. After centrifugation, the organic phase was split into two aliquots of 10 mL in Falcon tubes of 15 mL, then spiked with an internal standard. Dispersive solid phase extraction was performed with 250 mg of PSA and 900 mg of $MgSO_4$. After shaking by hand for 1 min, the solution was centrifuged, and the upper phase was evaporated to dryness with a gentle nitrogen stream. For LC-M/SMS analysis, the sample was reconstituted with 500 µL milli-Q water, while 500 µL di-isopropyl ether was used for GC-MS/MS analysis. Finally, the samples were placed in an auto-filtrating vial before analysis.

A3.3. Analysis of the pesticides

A3.3.1. LC-MS/MS analysis

The LC-MS/MS conditions have been described elsewhere (Szternfeld et al. 2022). Ultra-High-Performance Liquid Chromatography (UHPLC)-MS/MS were performed on an UPLC^{TM} system (Waters, Milford, MA) coupled to a Quattro Premier^{TM} mass spectrometer (Waters, Milford, MA) following the protocol of (Hanot et al. 2015). Five microliters of the obtained extract were injected on a reverse-phase ACQUITY™ UPLC BEH C18 column (1.7 µm, 2.1×100 mm) (Waters, Milford, MA). The mobile phases were composed of water/methanol (90/10 v/v) (mobile phase A) and methanol/water (90/10 v/v) (mobile phase B), both containing five mM ammonium acetate. The chromatographic separation was performed at a flow rate of 0.45 mL min^{-1} and with an elution gradient starting with 99.9 % of mobile phase A, linearly evolving to 0.1 % mobile phase A in 10 min, which was maintained for two additional minutes prior to re-equilibration during 3 min with 99.9 % of mobile phase A.

The mass spectrometer source and desolvation temperatures were set at 130 °C and 450 °C, respectively. The nitrogen gas flow rates were 50 L h^{-1} for the cone and 800 L h^{-1} for desolvation. Argon was used as collision gas with a pressure of 3.5 × 10−3 mbar. Data acquisition was performed in MRM mode. Retention time (Rt), precursor ions, product ions are available in Table A1. Interpretation of the results was performed with the MassLynx software (Waters, Milford, MA), and the obtained relative areas of the analytes and the internal standard were used for the quantification. Calibration curves ranged from 1.25 ng mL^{-1} to 100 ng mL in linear mode with a weight factor of 1/x.

A3.3.2. GC-MS/MS analysis

GC–MS/MS conditions have been described elsewhere (Szternfeld et al. 2022). Analyses were performed on an Agilent 7890B GC system (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 7000C GC/MS Triple Quad mass spectrometer (Agilent Technologies) equipped with an electronic impact source. The chromatographic column used was the Agilent GC column HP-5ms (30 m × 0.25 mm × 0.25 µm), and the liner inlet was a single taper, with wool and 4 mm internal diameter (Agilent Technologies). Five µL of sample were injected on a PTV injector with the following program: initial condition 40 °C (0.02 min), 200 °C min^{-1} until 300 °C. Helium (≥ 99.9999 %) was used as carrier gas at a flow rate of 1.2 mL min^{-1}.

Initial conditions for the oven were fixed at 70 °C (0.2 min), then 33 °C min^{-1}, 180 °C

(0.1 min), 7 °C min^{-1}, 300 °C (4.5 min). Data acquisition was performed in MRM mode. Rt, precursor ions, and product ions are available in Table A1. Interpretation of results was performed with the MassHunter software from Agilent (Agilent Technologies), and relative areas of the analyte and internal standard were used for the quantification. Calibration curves ranged from 0.75 ng mL^{-1} to 37.5 ng mL^{-1} in linear mode with a weighting factor of 1/x.

A3.4. Quality control and quality insurance

In order to ensure the accuracy and reliability of the results, a quality control plan has been established. Acceptability criteria were based on the SANTE document dedicated to validating and quality control for analysing pesticides in food (European Commission 2021).

The sensitivity and stability of the system were verified using a solution at the limit of quantification (LOQ) level at the beginning and end of each run. A signal-to-noise ratio > 6 for the quantification and the confirmation transitions for all pesticides ensures no sensitivity loss. All concentrations of the calibration levels were back-calculated using the regression equation of the calibration curve. A deviation lower than |±20 %| illustrated an accurate pesticide quantification. The absence of pesticide contamination (<limit of detection, LOD) and/or carry-over in the chromatographic systems were assessed by the analysis of a procedural blank for each analytical batch. Furthermore, all experiments were performed using matrix-matched calibration curves.

A4 Statistical data for the determination of parameters influencing pesticides TFs

Table A7: Analytical technique, limit of quantification (LOQ) and physico-chemical parameters of the pesticides

Pesticides	Technique	Transition (m/z)	CE (eV)	Confirm. transition (m/z)	CE (eV)	RT (min)	LOQ – dry (mg kg^{-1})	LOQ – brew (mg kg^{-1})	Log(P)	Log(S)	Log(H)	Log(VP)
Acetamiprid	LC-MS/MS	223.0 → 125.8	26	223.0 → 89.9	35	3.5	0.005	0.001	0.80	3.62	-7.28	-3.00
Anthraquinone	GC-MS/MS	208.0 → 180.0	10	208.0 → 151.0	30	13.6	0.005	0.001	3.66	-1.08	-1.91	-2.30
Azoxystrobin	LC-MS/MS	404.0 → 372.0	23	404.0 → 329.2	30	7.2	0.005	0.001	2.50	0.78	-8.14	-6.96
Bifenthrin	GC-MS/MS	181.0 → 165.0	35	181.0 → 166.0	20	19.3	0.005	0.001	7.30	-4.00	-0.99	-2.75
Boscalid	GC-MS/MS	140.0 → 112.0	10	140.0 → 76.0	30	23.2	0.005	0.001	3.00	0.66	-4.29	-3.14
Buprofezin	LC-MS/MS	306.0 → 57.0	22	306.0 → 115.8	16	9.5	0.005	0.001	4.93	-0.41	-1.55	-1.38
Carbendazim	LC-MS/MS	192.1 → 160.1	25	192.1 → 132.1	30	3.8	0.005	0.001	1.51	0.90	-2.44	-1.05
Chlorantraniliprole	LC-MS/MS	484.4 → 453.1	20	484.4 → 286.1	14	6.8	0.005	0.001	2.76	-0.02	-8.49	-7.68
Chlorfenapyr	GC-MS/MS	326.0 → 247.0	20	247.0 → 227.0	20	16.7	0.005	0.001	4.83	-0.85	-3.24	-2.01
Chlorpyriphos-ethyl	GC-MS/MS	314.0 → 258.0	15	316.0 → 260.0	15	13.7	0.005	0.001	4.70	0.15	-0.17	0.43
Chlorpyriphos-methyl	GC-MS/MS	286.0 → 93.0	25	286.0 → 208.0	20	12.5	0.005	0.001	4.28	0.41	-0.43	0.48

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Table A7 (continued)

Pesticides	Technique	Transition (m/z)	CE (eV)	Confirm. transition (m/z)	CE (eV)	RT (min)	LOQ – dry (mg kg^{-1})	LOQ – brew (mg kg^{-1})	Log(P)	Log(S)	Log(H)	Log(VP)
Clothianidin	LC-MS/MS	249.8 → 131.8	21	249.8 → 168.8	12	3.1	0.005	0.001	0.70	2.48	-10.5	-7.42
Cyhalothrin-L	GC-MS/MS	163.0 → 127.0	15	163.0 → 91.0	5	22.9	0.005	0.001	7.00	-2.30	-1.70	-3.70
Cyfluthrin	GC-MS/MS	181.0 → 152.0	25	197.0 → 141.0	20	20.7	0.005	0.001	5.95	-2.64	-1.28	-3.57
Cypermethrin	GC-MS/MS	163.0 → 127.0	15	163.0 → 91.0	5	23.2	0.005	0.001	6.60	-2.40	-1.70	-3.70
o,p-DDD	GC-MS/MS	235.0 → 165.0	35	235.0 → 199.0	20	16.3	0.005	0.001	6.02	0.09	-0.17	-0.74
p,p'-DDD	GC-MS/MS	235.0 → 165.0	30	237.0 → 165.0	30	17.1	0.005	0.001	6.02	0.09	-0.17	-0.74
o,p-DDE	GC-MS/MS	248.0 → 176.0	40	318.0 → 248.0	25	15.3	0.005	0.001	6.51	0.07	0.62	-0.10
p,p'-DDE	GC-MS/MS	248.0 → 176.0	40	318.0 → 248.0	25	16.0	0.005	0.001	6.51	0.07	0.62	-0.10
o,p-DDT	GC-MS/MS	235.0 → 165.0	30	237.0 → 165.0	30	17.2	0.005	0.001	6.91	0.03	-2.07	-1.60
p,p'-DDT	GC-MS/MS	235.0 → 165.0	30	237.0 → 165.0	30	18.1	0.005	0.001	6.91	0.03	-2.07	-1.60
Deltamethrin	GC-MS/MS	181.0 → 152.0	22	253.0 → 93.0	15	25.6	0.005	0.001	4.60	-3.70	-1.50	-4.91
Difenoconazole	LC-MS/MS	406.0 → 251.0	37	408.0 → 253.0	25	8.9	0.005	0.001	4.40	1.18	-6.05	-4.48
Diflubenzuron	LC-MS/MS	311.0 → 158.1	35	311.0 → 140.9	30	8.1	0.005	0.001	4.00	-1.10	-3.33	-3.92

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Table A7 (continued)

Pesticides	Technique	Transition (m/z)	CE (eV)	Confirm. transition (m/z)	CE (eV)	RT (min)	LOQ – dry (mg kg^{-1})	LOQ – brew (mg kg^{-1})	Log(P)	Log(S)	Log(H)	Log(VP)
Dinotefuran	LC-MS/MS	203.1 → 129.0	15	203.1 → 156.9	7	1.7	0.005	0.001	-0.55	4.60	-8.06	-2.77
Endosulfan α	GC-MS/MS	195.0 → 159.0	5	195.0 → 125.0	25	15.5	0.005	0.001	4.74	0.32	-0.17	-0.08
Endosulfan β	GC-MS/MS	195.0 → 159.0	10	195.0 → 125.0	30	17.0	0.005	0.001	4.79	0.33	-1.15	-0.08
Endosulfan sulfate	GC-MS/MS	272.0 → 237.0	20	274.0 → 239.0	15	18.1	0.005	0.001	3.70	0.22	-2.92	-5.88
Epoxiconazole	LC-MS/MS	330.3 → 102.9	26	330.3 → 100.9	46	7.93	0.005	0.001	3.33	0.82	-3.33	-5.00
Ethion	GC-MS/MS	231.0 → 175.0	10	153.0 → 97.0	15	17.2	0.005	0.001	4.40	0.30	-1.41	-1.70
Fenazaquin	GC-MS/MS	145.0 → 117.0	5	160.0 → 145.0	20	19.7	0.005	0.001	5.51	-0.99	-2.32	-2.47
Fenpropathrin	GC-MS/MS	265.0 → 210.0	5	265.0 → 89.0	40	19.5	0.005	0.001	6.00	-1.85	/	-0.14
Fenpyroximate	LC-MS/MS	422.4 → 366.4	28	422.4 → 231.4	24	10.0	0.005	0.001	5.01	-1.64	-0.87	-2.13
Fenvalerate (sum)	GC-MS/MS	167.0 → 125.0	5	181.0 → 152.0	20	24.4	0.005	0.001	5.01	-1.62	-2.46	-3.70
Flufenoxuron	LC-MS/MS	488.9 → 158.1	25	488.9 → 141.0	50	9.9	0.005	0.001	4.24	-2.82	-5.13	-8.19
Flutolanil	LC-MS/MS	324.3 → 262.2	28	324.3 → 242.2	25	7.5	0.005	0.001	3.17	-3.39	-4.78	0.90
Fosthiazate	LC-MS/MS	284.3 → 103.7	21	284.3 → 227.9	10	6.0	0.005	0.001	1.68	3.99	-4.76	-0.25

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Table A7 (continued)

Pesticides	Technique	Transition (m/z)	CE (eV)	Confirm. transition (m/z)	CE (eV)	RT (min)	LOQ – dry (mg kg^{-1})	LOQ – brew (mg kg^{-1})	Log(P)	Log(S)	Log(H)	Log(VP)
Hexythiazox	LC-MS/MS	353.1 → 168.0	27	353.1 → 228.0	25	9.7	0.005	0.001	2.53	-0.30	-2.62	-2.47
Imazalil	LC-MS/MS	297.1 → 159.0	30	297.1 → 69.1	20	8.4	0.005	0.001	3.82	2.35	-3.49	-0.80
Imidacloprid	LC-MS/MS	256.1 → 175.1	22	256.1 → 209.2	16	3.1	0.005	0.001	0.57	2.79	-9.77	-6.40
Indoxacarb	LC-MS/MS	528.3 → 150.0	28	528.3 → 218.2	20	9.0	0.005	0.001	4.65	-0.70	-4.22	-4.60
Iprodione	GC-MS/MS	314.0 → 56.0	20	314.0 → 245.0	10	19.0	0.005	0.001	3.00	1.11	-5.15	-3.30
Linuron	LC-MS/MS	249.1 → 160.0	28	249.1 → 182.1	15	7.0	0.005	0.001	3.08	1.80	-3.70	-1.29
Metalaxyl	GC-MS/MS	280.1 → 220.2	13	280.1 → 192.2	17	6.52	0.005	0.001	1.75	3.92	-4.80	-0.12
Methomyl	LC-MS/MS	162.9 → 87.8	15	162.9 → 105.9	10	2.2	0.005	0.001	0.09	4.76	-5.68	-0.14
Ortho-phenyl-phenol	GC-MS/MS	170.0 → 169.0	15	170.0 → 141.0	30	8.5	0.005	0.001	3.34	2.85	-0.85	1.85
Permethrin (sum)	GC-MS/MS	183.0 → 168.0	10	183.0 → 155.0	10	21.9	0.005	0.001	6.10	-0.99	-2.37	-2.72
Propargite	LC-MS/MS	368.1 → 231.1	16	368.1 → 174.9	16	9.9	0.005	0.001	5.70	-0.67	-1.19	-1.40
Propiconazole	GC-MS/MS	259.0 → 173.0	20	259.0 → 145.0	40	18.1	0.005	0.001	3.89	2.00	-4.04	-1.57
Pyridaben	LC-MS/MS	365.3 → 147.0	19	365.3 → 309.3	13	10.3	0.005	0.001	6.37	-1.92	-0.52	-0.52
Pyrimethanil	LC-MS/MS	200.1 → 107.0	42	200.1 → 82.0	25	7.1	0.005	0.001	2.96	2.08	-2.44	0.34
Pyriproxyfen	LC-MS/MS	322.1 → 95.9	18	322.1 → 184.9	24	9.6	0.005	0.001	5.37	-0.17	-4.19	-1.89

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Table A7 (continued)

Pesticides	Technique	Transition (m/z)	CE (eV)	Confirm. transition (m/z)	CE (eV)	RT (min)	LOQ – dry (mg kg^{-1})	LOQ – brew (mg kg^{-1})	Log(P)	Log(S)	Log(H)	Log(VP)
Spiromesifen	GC-MS/MS	254.0 → 193.0	20	254.0 → 208.0	10	19.0	0.005	0.001	4.55	-0.89	-1.70	-2.15
Tebuconazole	LC-MS/MS	308.3 → 69.9	30	310.3 → 69.95	20	8.4	0.005	0.001	3.72	1.56	-5.00	-2.77
Thiabendazole	LC-MS/MS	202.0 → 175.1	40	202.0 → 131.0	32	4.5	0.005	0.001	2.39	1.48	-5.43	-3.28
Thiacloprid	LC-MS/MS	253.2 → 125.7	29	253.2 → 186.2	14	4.0	0.005	0.001	0.74	2.27	-9.39	-6.52
Thiamethoxam	LC-MS/MS	292.3 → 211.0	19	292.3 → 180.9	24	2.4	0.005	0.001	-0.13	3.61	-9.33	-5.18
Tolfenpyrad	LC-MS/MS	384.2 → 197.2	38	384.2 → 145.1	28	9.5	0.005	0.001	5.61	-1.06	-2.66	-3.30
Triadimenol	GC-MS/MS	128.0 → 65.0	10	168.0 → 70.0	25	15.0	0.005	0.001	3.09	1.75	-5.52	-3.22
Triazophos	GC-MS/MS	161.0 → 134.0	5	257.0 → 162.0	5	17.5	0.005	0.001	3.52	1.59	-2.50	-0.41
Trifloxystrobin	GC-MS/MS	222.0 → 130.0	20	172.0 → 145.0	15	17.9	0.005	0.001	4.50	-0.21	-2.64	-2.47

P: partition coefficient between octanol and water; S: solubility; H: Henry; VP: vapour pressure

Table A8: Minimal, maximal and mean transfer factors over three replicates for pesticides in black tea for different brewing times. Associated Mann-Whitney tests

Pesticides	Transfer factor (%) in black tea								Mann-Whitney test		
	2 min		3 min		5 min		10 min		2 vs 3	2 vs 5	2 vs 10
	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	p	p	p
Acetamiprid	60–78	67 ± 10	53–76	65 ± 14	62–83	73 ± 13	78–94	87 ± 9.4	0.500	0.331	0.040
Anthraquinone	7.3–19	13 ± 6.7	16–21	18 ± 2.8	19–20	20 ± 0.8	16–24	20 ± 4.6	0.191	0.040	0.191
Azoxystrobin	40–53	44 ± 7.9	31–50	42 ± 11	40–59	49 ± 11	47–59	51 ± 7.0	0.500	0.191	0.191
Bifenthrin	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	1.000	1.000	1.000
Boscalid	13–21	16 ± 4.3	19–24	21 ± 2.7	22–23	22 ± 0.8	19–24	22 ± 3.0	0.191	0.404	0.952
Buprofezin	5.2–6.5	6.0 ± 0.8	5.3–9.3	7.0 ± 2.4	4.8–18	9.7 ± 7.8	1.3–3.7	2.4 ± 1.4	0.253	0.588	0.040
Carbendazim	55–73	62 ± 11	45–67	58 ± 13	59–79	68 ± 12	71–88	76 ± 10	0.500	0.191	0.191
Chlorfenapyr	0.0–0.5	0.2 ± 0.3	1.2–5.4	2.6 ± 2.1	1.5–5.5	3.4 ± 2.4	0.0–6.3	2.8 ± 3.7	0.038	0.038	0.177
Chlorpyriphos-ethyl	0.0–0.8	0.3 ± 0.5	0.7–2.0	1.2 ± 0.8	0.9–2.3	1.6 ± 0.8	0.4–4.7	2.0 ± 2.5	0.038	0.038	0.131
Chlorpyriphos-methyl	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–1.5	0.5 ± 0.9	0.0–7.0	2.3 ± 4.1	1.000	0.098	0.253
Clothianidin	58–76	70 ± 11	51–69	62 ± 10	62–88	76 ± 15	82–98	92 ± 9.6	0.191	0.191	0.404
Cyfluthrin	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–6.6	2.2 ± 3.9	1.000	1.000	0.253
Cyhalothrin-L	0.3–1.0	0.6 ± 0.4	0.6–1.1	0.8 ± 0.3	0.6–1.4	1.1 ± 0.5	0.6–1.0	0.8 ± 0.2	0.191	0.095	0.253
Cypermethrin	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–5.9	2.0 ± 3.5	1.000	1.000	0.253
DDD-o,p	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	1.000	1.000	1.000
DDD-p,p	0.0–0.0	0.0 ± 0.0	0.0–0.7	0.2 ± 0.4	0.0–0.4	0.1 ± 0.2	0.0–1.1	0.4 ± 0.6	0.253	0.253	0.253
DDE-o,p	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	1.000	1.000	1.000
DDE-p,p	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	1.000	1.000	1.000
DDT-o,p	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	1.000	1.000	1.000
DDT-p,p	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	1.000	1.000	1.000

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Table A8 (continued)

Pesticides	Transfer factor (%) in black tea								Mann-Whitney test		
	2 min		3 min		5 min		10 min		2 vs 3	2 vs 5	2 vs 10
	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	p	p	p
Deltamethrin	1.0–1.3	1.1 ± 0.2	1.2–2.8	2.1 ± 0.9	2.3–2.8	2.5 ± 0.3	1.2–3.1	2.1 ± 1.1	0.092	0.038	0.092
Difenoconazole	8.4–16	12 ± 4.3	11–17	14 ± 3.7	14–25	18 ± 6.4	7.0–12	8.8 ± 2.7	0.331	0.095	0.095
Diflubenzuron	25–40	33 ± 9.1	23–48	35 ± 15	32–53	42 ± 12	19–32	24 ± 8.0	0.500	0.191	0.095
Dinetofuran	77–95	83 ± 11	72–86	77 ± 8.7	71–97	88 ± 16	93–115	102 ± 13	0.191	0.500	0.952
Endosulfan-alpha	0.0–1.9	0.7 ± 1.1	0.6–2.8	1.5 ± 1.3	1.3–2.6	2.0 ± 0.8	0.0–3.6	1.5 ± 2.1	0.191	0.095	0.412
Endosulfan-beta	0.0–3.3	1.6 ± 1.9	3.1–7.3	4.8 ± 2.5	2.5–5.7	4.5 ± 1.9	0.0–8.9	4.1 ± 5.3	0.095	0.952	0.253
Endosulfan-sulfate	2.2–10	6.1 ± 4.3	8.5–16	11 ± 4.1	10–14	12 ± 2.3	3.7–15	8.8 ± 6.4	0.134	0.404	0.331
Esfenvalerate	0.0–1.0	0.4 ± 0.6	0.0–1.1	0.7 ± 0.4	0.6–1.1	0.9 ± 0.3	0.6–1.3	1.1 ± 0.4	0.191	0.092	0.092
Ethion	0.4–4.3	1.8 ± 2.3	4.5–5.9	5.0 ± 0.8	7.4–12	10 ± 2.8	2.2–17	10 ± 8.9	0.040	0.040	0.095
Fenazaquin	0.0–0.8	0.3 ± 0.5	0.5–2.7	1.4 ± 1.3	1.0–2.5	1.8 ± 0.9	0.0–3.8	2.0 ± 2.2	0.092	0.038	0.177
Fenpropathrin	1.9–3.1	2.4 ± 0.7	4.4–6.8	5.3 ± 1.4	5.8–14	8.8 ± 4.8	5.1–9.2	6.6 ± 2.4	0.040	0.040	0.040
Fenpyroximate	1.0–2.1	1.6 ± 0.6	0.0–0.7	0.5 ± 0.4	0.2–7.6	2.7 ± 4.4	0.0–0.0	0.0 ± 0.0	0.038	0.081	0.032
Fenvalerate	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	1.000	1.000	1.000
Hexythiazox	1.1–3.2	2.4 ± 1.2	1.5–5.7	3.0 ± 2.5	1.9–14	6.1 ± 7.0	0.0–0.0	0.0 ± 0.0	0.500	0.500	0.032
Imidacloprid	65–81	71 ± 10	52–79	68 ± 16	64–88	76 ± 14	67–105	84 ± 22	0.500	0.500	0.331
Indoxacarb	6.9–14	10 ± 4.3	0.0–5.2	3.1 ± 3.1	3.2–23	10 ± 11	0.0–0.0	0.0 ± 0.0	0.040	0.081	0.032
Iprodione	6.4–17	10 ± 6.1	16–18	17 ± 1.2	17–18	17 ± 0.6	15–17	16 ± 0.8	0.095	0.095	0.331
Linuron	36–52	43 ± 10	32–48	40 ± 9.4	43–59	50 ± 9.4	45–62	53 ± 10	0.412	0.191	0.191
Methomyl	80–93	85 ± 8.0	56–87	74 ± 18	73–104	92 ± 19	89–104	96 ± 8.7	0.331	0.331	0.952
Orthophenyl phenol	16–36	27 ± 12	33–36	34 ± 2.1	25–28	26 ± 1.7	24–31	28 ± 4.7	0.331	0.331	0.500

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Table A8 (continued)

Pesticides	Transfer factor (%) in black tea								Mann-Whitney test		
	2 min		3 min		5 min		10 min		2 vs 3	2 vs 5	2 vs 10
	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	p	p	p
Permethrin	1.5–3.5	2.6 ± 1.2	2.1–2.9	2.6 ± 0.5	2.6–4.7	3.3 ± 1.2	2.3–2.7	2.4 ± 0.2	0.588	0.500	0.329
Propargite	0.8–3.1	1.9 ± 1.4	0.0–1.5	0.9 ± 0.9	0.0–13	4.5 ± 7.7	0.0–0.0	0.0 ± 0.0	0.191	0.081	0.032
Propiconazole	11–22	15 ± 6.2	19–24	21 ± 3.0	21–24	23 ± 1.8	21–26	23 ± 2.7	0.191	0.095	0.095
Pyridaben	0.0–0.7	0.3 ± 0.4	0.0–0.0	0.0 ± 0.0	0.0–5.2	1.7 ± 3.1	0.0–0.0	0.0 ± 0.0	0.098	0.679	0.098
Pyrimethanil	26–44	34 ± 11	24–44	37 ± 12	40–50	44 ± 5.9	39–56	47 ± 10	0.669	0.191	0.095
Pyriproxifen	1.5–3.5	2.6 ± 1.2	0.1–2.7	1.4 ± 1.5	0.8–11	4.4 ± 5.9	0.0–0.0	0.0 ± 0.0	0.184	0.809	0.032
Spiromesifen	1.2–2.4	1.8 ± 0.7	3.0–4.4	3.8 ± 0.8	1.9–4.6	3.4 ± 1.8	2.6–4.2	3.4 ± 0.9	0.040	0.095	0.040
Tebuconazole	22–34	28 ± 7.2	19–34	27 ± 8.6	32–41	36 ± 5.4	29–39	33 ± 6.1	0.500	0.191	0.191
Thiacloprid	50–66	57 ± 9.1	41–65	55 ± 14	54–77	66 ± 13	67–81	72 ± 8.0	0.500	0.191	0.040
Thiamethoxam	93–106	100 ± 7.7	78–114	99 ± 21	91–116	100 ± 15	64–96	74 ± 19	0.669	0.500	0.952
Tolfenpyrad	2.4–4.0	3.2 ± 0.9	2.7–4.4	3.4 ± 1.0	3.2–14	7.0 ± 6.1	0.0–0.0	0.0 ± 0.0	0.500	0.191	0.032
Triadimenol	26–37	30 ± 6.9	33–37	35 ± 2.4	35–37	36 ± 1.6	33–38	35 ± 3.1	0.331	0.331	0.191
Triazophos	16–26	19 ± 6.0	24–29	26 ± 3.0	28–30	29 ± 0.9	27–31	28 ± 2.9	0.134	0.040	0.040
Trifloxystrobin	2.0–6.1	3.9 ± 2.4	7.6–11	8.8 ± 1.8	7.1–10	8.7 ± 1.8	6.3–13	9.2 ± 4.1	0.040	0.040	0.040

Table A9: Minimal, maximal and mean transfer factors over three replicates for pesticides in green tea for different infusion times. Associated Mann-Whitney tests

Pesticides	Transfer factor (%) in green tea								Mann-Whitney test		
	2 min		3 min		5 min		10 min		2 vs 3	2 vs 5	2 vs 10
	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	p	p	p
Acetamiprid	46–61	54 ± 9.1	62–69	64 ± 4.2	61–70	66 ± 5.0	64–75	68 ± 6.5	0.040	0.040	0.040
Anthraquinone	12–16	14 ± 2.2	18–28	23 ± 5.9	16–38	27 ± 13	11–17	14 ± 3.3	0.040	0.061	0.500
Azoxystrobin	36–46	41 ± 5.2	42–53	48 ± 6.4	33–63	48 ± 18	14–36	25 ± 13	0.095	0.331	0.040
Bifenthrin	1.9–2.4	2.1 ± 0.3	6.7–7.7	7.1 ± 0.7	4.3–5.3	4.8 ± 0.5	1.7–2.1	1.9 ± 0.3	0.040	0.040	0.095
Boscalid	23–25	24 ± 0.8	28–35	32 ± 3.8	18–47	32 ± 17	7.9–21	14 ± 8.0	0.040	0.331	0.040
Buprofezin	1.7–4.0	2.8 ± 1.3	1.9–3.2	2.5 ± 0.8	2.1–3.2	2.5 ± 0.6	2.5–3.1	2.9 ± 0.3	0.500	0.500	0.500
Carbendazim	44–59	52 ± 8.8	55–64	60 ± 4.8	65–75	71 ± 5.7	71–83	76 ± 6.9	0.095	0.040	0.040
Chlorfenapyr	4.6–6.9	5.8 ± 1.4	10–10	10 ± 0.4	7.4–25	16 ± 11	5.0–6.3	5.6 ± 0.8	0.040	0.040	0.500
Chlorpyriphos-ethyl	2.9–3.4	3.1 ± 0.3	7.7–8.1	7.9 ± 0.2	4.0–8.8	6.4 ± 2.8	2.3–3.1	2.7 ± 0.4	0.040	0.040	0.095
Chlorpyriphos-methyl	8.5–10	9.0 ± 0.6	13–13	13 ± 0.2	8.9–24	16 ± 8.7	5.7–8.6	7.1 ± 1.7	0.040	0.191	0.095
Clothianidin	50–66	59 ± 9.4	62–68	66 ± 3.5	66–75	71 ± 5.0	68–80	73 ± 7.0	0.134	0.095	0.040
Cyfluthrin	1.0–3.2	2.1 ± 1.3	7.8–9.3	8.6 ± 0.9	6.2–12	9.1 ± 3.4	5.7–8.7	7.2 ± 1.7	0.040	0.040	0.040
Cyhalothrin-L	2.0–2.9	2.4 ± 0.5	7.9–10	8.9 ± 1.2	5.6–6.1	5.9 ± 0.3	2.3–2.6	2.4 ± 0.2	0.040	0.040	0.331
Cypermethrin	1.0–3.1	2.0 ± 1.2	6.9–9.1	8.0 ± 1.3	5.1–12	8.8 ± 4.3	2.2–5.5	3.8 ± 1.9	0.040	0.040	0.095
DDD-o,p	2.4–2.7	2.6 ± 0.2	6.9–7.3	7.1 ± 0.2	4.0–6.4	5.2 ± 1.4	2.1–2.3	2.2 ± 0.1	0.040	0.040	0.040
DDD-p,p	2.3–2.6	2.4 ± 0.2	5.7–7.0	6.3 ± 0.8	4.2–6.4	5.3 ± 1.3	2.1–2.1	2.1 ± 0.0	0.040	0.040	0.040
DDE-o,p	1.8–1.9	1.9 ± 0.0	5.9–6.6	6.3 ± 0.4	3.5–5.0	4.2 ± 0.9	1.4–1.6	1.5 ± 0.1	0.040	0.040	0.331
DDE-p,p	1.9–2.1	2.0 ± 0.1	5.7–6.4	6.1 ± 0.4	3.6–4.5	4.0 ± 0.6	1.5–2.0	1.8 ± 0.3	0.040	0.040	0.134
DDT-o,p	2.4–2.4	2.4 ± 0.0	6.8–7.4	7.1 ± 0.4	4.2–5.1	4.7 ± 0.5	1.8–2.4	2.1 ± 0.3	0.032	0.032	0.032
DDT-p,p	3.0–3.0	3.0 ± 0.0	7.3–7.9	7.6 ± 0.4	5.0–6.4	5.7 ± 0.8	2.2–3.0	2.6 ± 0.5	0.032	0.032	0.098

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Table A9 (continued)

Pesticides	Transfer factor (%) in green tea								Mann-Whitney test		
	2 min		3 min		5 min		10 min		2 vs 3	2 vs 5	2 vs 10
	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	p	p	p
Deltamethrin	2.8–3.2	3.0 ± 0.3	9.3–13	11 ± 2.1	2.1–6.2	4.1 ± 2.4	1.8–1.9	1.8 ± 0.0	0.040	0.331	0.040
Difenoconazole	4.8–14	9.3 ± 5.3	2.4–14	8.2 ± 6.7	4.5–7.9	6.3 ± 2.0	2.9–12	7.0 ± 6.5	0.412	0.191	0.331
Diflubenzuron	5.7–16	10 ± 6.1	3.1–16	11 ± 7.5	4.1–5.9	4.9 ± 1.0	0.0–10	6.2 ± 5.9	0.669	0.095	0.500
Dinetofuran	63–84	76 ± 12	82–87	84 ± 2.9	78–88	84 ± 6	88–100	92 ± 7.4	0.191	0.191	0.038
Endosulfan-alpha	1.1–1.9	1.5 ± 0.5	7.3–10	8.4 ± 1.4	2.7–11	6.9 ± 4.9	1.1–3.7	2.4 ± 1.5	0.040	0.040	0.253
Endosulfan-beta	5.8–8.0	6.9 ± 1.3	14–16	15 ± 1.2	8.4–18	13 ± 5.5	5.3–5.7	5.5 ± 0.2	0.040	0.061	0.040
Endosulfan-sulfate	7.4–10	8.6 ± 1.4	14–15	14 ± 0.3	1.4–25	13 ± 14	4.7–7.4	6.0 ± 1.6	0.040	0.331	0.061
Esfenvalerate	3.3–3.5	3.2 ± 0.3	7.4–8.5	8.0 ± 0.7	5.7–7.0	6.4 ± 0.8	2.3–3.1	2.7 ± 0.4	0.040	0.040	0.061
Ethion	0.0–0.0	0.0 ± 0.0	4.3–15	10 ± 6.3	4.1–7.9	6.0 ± 2.2	3.5–11	7.2 ± 4.4	0.032	0.032	0.032
Fenazaquin	2.2–3.0	2.6 ± 0.5	7.5–7.6	7.6 ± 0.0	4.1–8.2	6.1 ± 2.5	2.9–3.0	3.0 ± 0.1	0.040	0.040	0.098
Fenpropathrin	0.0–0.7	0.3 ± 0.4	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.098	0.098	0.098
Fenpyroximate	0.8–1.5	1.0 ± 0.4	0.8–1.7	1.2 ± 0.6	0.0–1.5	1.0 ± 0.9	0.7–1.1	0.8 ± 0.2	0.321	0.681	0.321
Fenvalerate	1.4–2.1	1.8 ± 0.4	6.1–7.6	6.9 ± 0.9	4.5–4.8	4.7 ± 0.2	1.6–1.8	1.7 ± 0.1	0.040	0.040	0.253
Flufenoxuron	1.2–6.9	4.8 ± 3.4	2.1–2.8	2.4 ± 0.4	3.1–3.6	3.2 ± 0.3	1.5–17	7.1 ± 9.2	0.300	0.330	0.500
Hexythiazox	0.9–1.7	1.4 ± 0.5	1.1–2.1	1.6 ± 0.6	0.9–1.5	1.2 ± 0.3	0.9–5.3	2.4 ± 2.6	0.500	0.253	0.588
Imidacloprid	45–61	55 ± 10	61–66	64 ± 3.1	69–82	76 ± 7.6	73–93	81 ± 12	0.095	0.040	0.040
Indoxacarb	1.9–5.2	3.1 ± 2.0	1.2–6.7	3.3 ± 3.2	1.2–3.1	2.2 ± 1.1	1.3–5.1	2.8 ± 2.2	0.669	0.500	0.253
Iprodione	17–22	19 ± 3.4	27–34	30 ± 4.1	19–44	31 ± 15	11–27	19 ± 9.4	0.040	0.191	0.500
Linuron	22–41	30 ± 11	30–32	31 ± 1.3	32–41	37 ± 5.1	33–46	39 ± 7.6	0.331	0.253	0.191
Methomyl	58–76	68 ± 10	73–78	76 ± 2.9	68–79	73 ± 6.5	72–88	81 ± 10	0.095	0.331	0.095

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Table A9 (continued)

Pesticides	Transfer factor (%) in green tea								Mann-Whitney test		
	2 min		3 min		5 min		10 min		2 vs 3	2 vs 5	2 vs 10
	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	p	p	p
Orthophenyl phenol	22–46	34 ± 14	53–65	59 ± 7.2	34–69	52 ± 21	26–43	34 ± 10	0.040	0.191	0.500
Permethrin	0.1–2.0	1.1 ± 1.1	4.5–7.4	5.9 ± 1.7	2.6–12	7.2 ± 5.4	0.9–1.4	1.2 ± 0.3	0.040	0.040	0.500
Propargite	1.9–3.1	2.3 ± 0.7	1.4–1.6	1.5 ± 0.1	1.3–1.6	1.5 ± 0.2	1.4–1.5	1.4 ± 0.1	0.038	0.040	0.038
Propiconazole	17–21	19 ± 2.5	28–36	32 ± 4.7	19–47	33 ± 17	16–29	22 ± 7.5	0.040	0.191	0.331
Pyridaben	1.3–1.5	1.4 ± 0.1	1.3–1.7	1.5 ± 0.2	0.8–1.6	1.3 ± 0.5	1.2–1.3	1.2 ± 0.1	0.240	0.816	0.079
Pyrimethanil	18–42	28 ± 14	24–25	24 ± 0.7	29–35	32 ± 3.3	28–41	36 ± 7.7	0.809	0.331	0.331
Pyriproxifen	0.5–0.9	0.8 ± 0.2	0.7–0.9	0.8 ± 0.2	0.5–0.8	0.7 ± 0.2	0.5–0.5	0.5 ± 0.0	0.500	0.327	0.098
Spiromesifen	0.0–2.4	1.2 ± 1.4	7.7–9.2	8.4 ± 0.9	8.5–14	11 ± 3.3	2.8–5.2	4.0 ± 1.4	0.040	0.040	0.040
Tebuconazole	11–27	20 ± 9.5	5.1–23	16 ± 11	12–20	17 ± 4.4	10–26	20 ± 9.5	0.331	0.331	0.669
Thiacloprid	36–48	42 ± 6.6	44–47	45 ± 1.6	44–49	47 ± 3.3	43–52	46 ± 5.0	0.331	0.134	0.191
Thiamethoxam	56–72	62 ± 10	45–58	50 ± 7.2	48–52	50 ± 2.4	38–44	42 ± 3.5	0.095	0.040	0.040
Tolfenpyrad	0.8–2.3	1.3 ± 0.9	0.8–2.8	1.8 ± 1.2	0.5–1.9	1.2 ± 0.8	0.5–19	6.6 ± 11	0.412	0.500	0.809
Triadimenol	27–43	35 ± 9.3	46–60	53 ± 8.0	36–72	54 ± 21	25–50	38 ± 15	0.040	0.095	0.500
Triazophos	19–26	22 ± 4.0	31–39	35 ± 4.6	21–53	37 ± 19	12–27	19 ± 8.8	0.040	0.191	0.500
Trifloxystrobin	5.1–6.8	5.9 ± 1.0	12–12	12 ± 0.5	6.5–18	12 ± 6.8	5.5–6.0	5.7 ± 0.3	0.040	0.095	0.412

Table A10: Minimal, maximal and mean transfer pesticides factors in black tea and green tea. Associated Mann-Whitney test

Pesticides	Transfer factor (%) in black tea		Transfer factor (%) in green tea		Mann-Whitney test
	min–max	mean ± SD	min–max	mean ± SD	p-value
Acetamiprid	53–94	73 ± 12	45–75	63 ± 7.5	0.023
Anthraquinone	7.3–24	18 ± 4.3	11–38	19 ± 9.0	0.768
Azoxystrobin	23–36	32 ± 4.0	14–63	40 ± 14	0.018
Bifenthrin	0.0–0.0	0.0 ± 0.0	1.7–7.7	4.0 ± 2.3	0.013
Boscalid	13–24	20 ± 3.7	7.9–46	26 ± 11	$< 0.001^*$
Buprofezin	1.3–18	6.3 ± 4.3	1.7–4.0	2.7 ± 0.7	0.088
Carbendazim	45–88	66 ± 12	44–83	65 ± 11	0.002^*
Chlorfenapyr	0.0–6.3	2.3 ± 2.3	4.6–25	9.4 ± 6.7	0.026
Chlorpyriphos-ethyl	0.0–4.7	1.3 ± 1.3	2.3–8.8	5.0 ± 2.7	0.443
Chlorpyriphos-methyl	0.0–7.0	0.7 ± 2.0	5.7–24	11 ± 5.5	0.001^*
Clothianidin	51–98	75 ± 15	50–80	67 ± 7.3	0.001^*
Cyfluthrin	0.0–6.6	0.5 ± 1.9	1.0–12	6.7 ± 3.5	$< 0.001^*$
Cyhalothrin-L	0.3–1.4	0.8 ± 0.3	2.0–10	4.9 ± 2.9	0.063
Cypermethrin	0.0–5.9	0.5 ± 1.7	1.0–13	5.7 ± 3.8	$< 0.001^*$
DDD-o,p	0.0–0.0	0.0 ± 0.0	2.1–7.3	4.3 ± 2.3	$< 0.001^*$
DDD-p,p	0.0–1.0	0.2 ± 0.4	2.1–7.0	4.0 ± 2.1	$< 0.001^*$
DDE-o,p	0.0–0.0	0.0 ± 0.0	1.4–6.6	3.5 ± 2.1	$< 0.001^*$
DDE-p,p	0.0–0.0	0.0 ± 0.0	1.5–6.4	3.5 ± 1.9	$< 0.001^*$
DDT-o,p	0.0–0.0	0.0 ± 0.0	1.8–7.4	4.1 ± 2.2	$< 0.001^*$
DDT-p,p	0.0–0.0	0.0 ± 0.0	2.2–7.9	4.7 ± 2.2	$< 0.001^*$
Deltamethrin	1.0–3.1	2.0 ± 0.8	1.8–13	5.0 ± 4.1	$< 0.001^*$
Difenoconazole	7.1–25	13 ± 4.9	2.4–14	7.7 ± 4.0	$< 0.001^*$
Diflubenzuron	19–53	34 ± 11	0.0–16	8.0 ± 5.1	0.027
Dinetofuran	71–115	87 ± 14	63–100	84 ± 8.5	0.006^*
Endosulfan-alpha	0.0–3.6	1.4 ± 1.2	1.1–11	4.8 ± 3.9	$< 0.001^*$
Endosulfan-beta	0.0–8.9	3.8 ± 2.7	5.3–18	10 ± 5.0	0.354
Endosulfan-sulfate	2.2–15	10 ± 4.2	1.4–25	10 ± 7.2	0.018
Esfenvalerate	0.0–1.3	0.8 ± 0.4	2.3–8.5	5.1 ± 2.4	0.002^*
Ethion	0.4–17	6.8 ± 5.1	0.0–15	5.7 ± 5.2	0.515
Fenazaquin	0.0–3.8	1.4 ± 1.2	2.2–8.2	4.8 ± 2.5	$< 0.001^*$
Fenpropathrin	1.9–14	5.8 ± 3.3	0.0–0.7	0.1 ± 0.2	0.220
Fenpyroximate	0.0–7.6	1.2 ± 2.1	0.0–1.7	1.0 ± 0.5	0.001^*
Fenvalerate	0.0–0.0	0.0 ± 0.0	1.4–7.6	3.8 ± 2.3	$< 0.001^*$
Hexythiazox	0.0–14	2.9 ± 3.8	0.9–5.3	1.7 ± 1.2	0.919

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Table A10 (continued)

Pesticides	**Transfer factor (%) in black tea**		**Transfer factor (%) in green tea**		**Mann-Whitney test**
	min–max	**mean ± SD**	**min–max**	**mean ± SD**	p**-value**
Imidacloprid	52–105	75 ± 14	45–93	69 ± 13	$< 0.001^*$
Indoxacarb	0.0–23	5.7 ± 6.8	1.2–6.7	2.9 ± 1.8	0.185
Iprodione	6.4–18	15 ± 4.3	11–44	25 ± 10	0.156
Linuron	32–62	46 ± 9.0	22–46	34 ± 6.8	0.226
Methomyl	56–104	87 ± 14	58–88	74 ± 7.5	0.008^*
Orthophenyl phenol	16–36	29 ± 5.8	22–69	45 ± 17	0.002^*
Permethrin	1.5–4.7	2.7 ± 0.8	0.1–12	3.8 ± 3.9	0.004^*
Propargite	0.0–13	1.8 ± 3.7	1.3–3.1	1.7 ± 0.5	0.021
Propiconazole	11–26	20 ± 4.3	16–47	27 ± 11	0.577
Pyridaben	0.0–5.2	0.5 ± 1.5	0.8–1.7	1.4 ± 0.2	0.976
Pyrimethanil	24–56	40 ± 9.3	18–42	30 ± 7.7	0.220
Pyriproxifen	0.0–11	2.1 ± 3.1	0.5–0.9	0.7 ± 0.2	$< 0.001^*$
Spiromesifen	1.2–4.6	3.1 ± 1.1	0.0–14	6.2 ± 4.5	0.004^*
Tebuconazole	19–41	31 ± 6.3	5.1–27	18 ± 7.1	0.122
Thiacloprid	41–81	62 ± 11	36–52	45 ± 3.9	0.061
Thiamethoxam	64–116	94 ± 17	38–72	51 ± 8.9	$< 0.001^*$
Tolfenpyrad	0.0–14	3.4 ± 3.6	0.5–19	2.7 ± 5.1	$< 0.001^*$
Triadimenol	26–38	34 ± 3.8	25–71	45 ± 16	$< 0.001^*$
Triazophos	16–31	26 ± 4.9	12–53	28 ± 13	0.078
Trifloxystrobin	2.0–13	7.7 ± 3.0	5.1–18	8.9 ± 4.5	0.057

* indicates a significant statistical difference (p-value < 0.01)

Table A11: Minimal, maximal and mean pesticides transfer factors for pesticides in unflavoured and flavoured green tea and associated Mann-Whitney tests

Pesticides	Transfer factor (%) in natural green tea and flavoured green teas								Mann-Whitney test		
	Green unflavoured		Green – orange		Green – lemon		Green – mint		Unflav. vs orange	Unflav. vs lemon	Unflav. vs mint
	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	p-value	p-value	p-value
Acetamiprid	45–75	63 ± 7.5	37–50	43 ± 7.7	40–54	45 ± 8.2	57–66	61 ± 5.1	0.009*	0.009*	0.214
Anthraquinone	11–38	19 ± 9.0	17–27	21 ± 5.6	31–40	35 ± 5.6	27–47	36 ± 12	0.179	0.026	0.041
Azoxystrobin	14–63	40 ± 14	32–42	38 ± 5.6	45–54	50 ± 4.8	37–60	50 ± 14	0.305	0.131	0.179
Bifenthrin	1.7–7.7	4.0 ± 2.3	1.5–2.0	1.8 ± 0.3	3.1–4.5	3.8 ± 0.8	1.5–4.7	2.7 ± 1.9	0.062	0.620	0.207
Boscalid	7.9–46	26 ± 11	17–26	22 ± 5.3	24–30	28 ± 3.7	25–43	35 ± 11	0.305	0.237	0.092
Buprofezin	1.7–4.0	2.7 ± 0.7	2.5–6.4	4.1 ± 2.3	5.6–6.0	5.8 ± 0.2	11–14	12 ± 1.7	0.459	0.131	0.041
Carbendazim	44–83	65 ± 11	33–45	39 ± 7.1	38–55	44 ± 10	51–59	54 ± 4.7	0.009*	0.013	0.048
Chlorfenapyr	4.6–25	9.4 ± 6.7	2.3–4.2	3.1 ± 1.1	3.4–4.0	3.7 ± 0.4	5.7–9.2	7.7 ± 2.1	0.009*	0.009*	0.541
Chlorpyriphos-ethyl	2.3–8.8	5.0 ± 2.7	0.4–1.9	1.4 ± 0.9	4.0–4.1	4.1 ± 4.0	3.0–6.4	5.2 ± 2.0	0.009*	0.697	0.500
Chlorpyriphos-methyl	5.7–24	11 ± 5.5	0.0–0.0	0.0 ± 0.0	14–18	17 ± 2.7	0.0–17	7.4 ± 10	0.009*	0.041	0.179
Clothianidin	50–80	67 ± 7.3	39–53	46 ± 8.3	41–53	46 ± 6.9	56–64	59 ± 4.8	0.009*	0.009*	0.035
Cyfluthrin	1.0–12	6.7 ± 3.5	0.0–0.0	0.0 ± 0.0	4.9–6.8	5.7 ± 1.1	0.0–0.0	0.0 ± 0.0	0.009*	0.237	0.009*
Cyhalothrin-L	2.0–10	4.9 ± 2.9	1.6–1.8	1.7 ± 0.1	2.9–3.9	3.4 ± 0.6	2.3–3.0	2.7 ± 0.4	0.009*	0.419	0.178
Cypermethrin	1.0–13	5.7 ± 3.8	2.7–2.8	2.7 ± 0.0	9.1–10	10 ± 0.8	3.1–8.8	6.4 ± 3.4	0.128	0.041	0.341
DDD-o,p	2.1–7.3	4.3 ± 2.3	1.4–1.7	1.5 ± 0.2	2.6–3.4	3.0 ± 0.5	2.9–4.3	3.7 ± 0.8	0.009*	0.459	0.659
DDD-p,p	2.1–7.0	4.0 ± 2.1	1.8–2.0	1.9 ± 0.1	2.6–3.3	3.0 ± 0.4	2.9–4.4	3.8 ± 0.9	0.012	0.500	0.695
DDE-o,p	1.4–6.6	3.5 ± 2.1	1.6–1.7	1.7 ± 0.1	2.5–3.4	3.0 ± 0.5	2.1–4.1	2.9 ± 1.2	0.041	0.541	0.620
DDE-p,p	1.5–6.4	3.5 ± 1.9	1.5–1.8	1.6 ± 0.2	2.9–3.9	3.4 ± 0.6	1.9–3.9	2.7 ± 1.2	0.033	0.541	0.341
DDT-o,p	1.8–7.4	4.1 ± 2.2	0.7–0.8	0.7 ± 0.1	2.7–3.5	3.0 ± 0.5	0.9–3.0	1.8 ± 1.2	0.009*	0.541	0.062
DDT-p,p	2.2–7.9	4.7 ± 2.2	1.3–1.4	1.3 ± 0.1	2.5–3.5	3.0 ± 0.6	1.5–3.6	2.6 ± 1.2	0.009*	0.202	0.090

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Table A11 (continued)

Pesticides	Transfer factor (%) in natural green tea and flavoured green teas								Mann-Whitney test		
	Green unflavoured		Green – orange		Green – lemon		Green – mint		Unflav. vs orange	Unflav. vs lemon	Unflav. vs mint
	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	p-value	p-value	p-value
Deltamethrin	1.8–13	5.0 ± 4.1	2.1–3.3	2.8 ± 0.7	4.1–6.1	5.0 ± 1.2	2.9–3.5	3.1 ± 0.4	0.500	0.821	0.620
Difenoconazol	2.4–14	7.7 ± 4.0	1.0–9.0	5.7 ± 4.7	4.6–7.1	5.5 ± 1.5	3.2–11	6.0 ± 4.8	0.307	0.235	0.258
Diflubenzuron	0.0–16	8.0 ± 5.1	5.0–16	11 ± 6.5	8.5–16	12 ± 4.6	8.5–19	12 ± 6.0	0.214	0.085	0.110
Dinetofuran	63–100	84 ± 8.5	50–68	60 ± 11	52–65	58 ± 7.8	77–81	79 ± 2.4	0.009*	0.009*	0.048
Endosulfan-alpha	1.1–11	4.8 ± 3.9	1.6–3.5	2.5 ± 1.1	4.7–6.0	5.3 ± 0.8	6.3–8.4	7.6 ± 1.2	0.305	0.305	0.179
Endosulfan-beta	5.3–18	10 ± 5.0	1.8–4.0	2.8 ± 1.3	4.6–7.0	5.7 ± 1.4	9.1–11	10 ± 0.8	0.009*	0.062	0.763
Endosulfan-sulfate	1.4–25	10 ± 7.2	0.0–0.0	0.0 ± 0.0	6.0–10	8.1 ± 2.2	0.0–11	6.7 ± 6.5	0.009*	0.459	0.305
Esfenvalerate	2.3–8.5	5.1 ± 2.4	1.3–1.7	1.5 ± 0.2	3.9–4.5	4.1 ± 0.4	1.7–3.6	2.5 ± 1.1	0.009*	0.419	0.041
Ethion	0.0–15	5.7 ± 5.2	2.7–3.4	3.0 ± 0.4	3.6–6.1	5.1 ± 1.5	6.2–13	10 ± 4.0	0.130	0.621	0.092
Fenazaquin	2.2–8.2	4.8 ± 2.5	3.0–3.7	3.3 ± 0.4	3.8–4.6	4.2 ± 0.5	5.1–6.4	5.9 ± 0.8	0.417	0.697	0.303
Fenpropathrin	0.0–0.7	0.1 ± 0.2	0.0–3.4	1.1 ± 2.0	3.8–8.4	6.0 ± 2.7	0.0–8.7	4.5 ± 5.1	0.225	0.003*	0.035
Fenpyroximate	0.0–1.7	1.0 ± 0.5	2.0–3.0	2.7 ± 0.6	2.5–2.6	2.5 ± 0.1	2.0–3.2	2.4 ± 0.7	0.005*	0.005*	0.005*
Fenvalerate	1.4–7.6	3.8 ± 2.3	2.2–2.7	2.5 ± 0.3	4.1–5.0	4.4 ± 0.5	2.7–4.9	3.6 ± 1.3	0.541	0.419	0.621
Flufenoxuron	1.2–17	4.4 ± 4.4	2.0–3.0	2.3 ± 0.6	2.0–2.5	2.3 ± 0.3	1.6–3.0	2.2 ± 0.8	0.139	0.110	0.139
Hexythiazox	0.9–5.3	1.7 ± 1.2	0.0–4.0	2.0 ± 2.4	1.5–1.7	1.6 ± 0.1	0.7–3.1	1.5 ± 1.4	0.358	0.893	0.173
Imidacloprid	45–93	69 ± 13	38–53	46 ± 8.9	42–60	48 ± 10	59–74	65 ± 8.8	0.013	0.013	0.307
Indoxacarb	1.2–6.7	2.9 ± 1.8	1.0–8.0	5.0 ± 4.1	4.2–4.8	4.4 ± 0.4	2.5–9.5	5.1 ± 4.1	0.258	0.110	0.064
Iprodione	11–44	25 ± 10	14–23	19 ± 5.6	20–25	23 ± 2.7	26–38	32 ± 7.1	0.237	0.459	0.179
Linuron	22–46	34 ± 6.8	13–25	19 ± 7.1	16–30	22 ± 8.4	18–28	22 ± 6.1	0.009*	0.018	0.009*
Methomyl	58–88	74 ± 7.5	42–55	49 ± 7.7	42–57	49 ± 9.0	65–69	67 ± 2.5	0.006*	0.006*	0.026
OPP	22–69	45 ± 17	37–42	39 ± 3.0	43–71	62 ± 17	62–108	84 ± 27	0.305	0.076	0.026

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Table A11 (continued)

Pesticides	Transfer factor (%) in natural green tea and flavoured green teas								Mann-Whitney test		
	Green unflavoured		Green – orange		Green – lemon		Green – mint		Unflav. vs orange	Unflav. vs lemon	Unflav. vs mint
	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	p-value	p-value	p-value
Permethrin	0.1–12	3.8 ± 3.9	0.0–0.0	0.0 ± 0.0	2.2–3.8	3.2 ± 0.9	0.0–11	3.6 ± 6.4	0.009*	0.695	0.179
Propargite	1.3–3.1	1.7 ± 0.5	0.0–2.0	1.3 ± 1.2	1.0–1.4	1.2 ± 0.2	0.5–2.6	1.3 ± 1.2	0.696	0.016	0.171
Propiconazole	16–47	27 ± 11	18–26	22 ± 5.1	24–33	29 ± 5.6	32–46	39 ± 7.9	0.380	0.237	0.063
Pyridaben	0.8–1.7	1.4 ± 0.2	1.0–2.0	1.7 ± 0.6	1.4–1.9	1.7 ± 0.3	1.0–2.0	1.4 ± 0.6	0.171	0.034	0.744
Pyrimethanil	18–42	30 ± 7.7	10–20	15 ± 5.9	13–26	18 ± 7.1	16–25	19 ± 5.6	0.009*	0.036	0.030
Pyriproxifen	0.5–0.9	0.7 ± 0.2	0.0–3.0	1.7 ± 1.8	1.0–1.3	1.2 ± 0.2	0.2–2.3	0.9 ± 1.2	0.208	0.005*	0.832
Spiromesifen	0.0–14	6.2 ± 4.5	0.0–0.0	0.0 ± 0.0	2.4–4.0	3.0 ± 0.9	0.0–0.0	0.0 ± 0.0	0.018	0.153	0.018
Tebuconazole	5.1–27	18 ± 7.1	9.0–21	16 ± 7.1	12–23	17 ± 6.5	13–23	17 ± 6.1	0.214	0.307	0.386
Thiaclorpid	36–52	45 ± 3.9	27–39	33 ± 7.1	32–46	38 ± 8.0	42–51	46 ± 5.0	0.009*	0.048	0.471
Thiamethoxam	38–72	51 ± 8.9	37–44	40 ± 4.1	33–40	38 ± 4.1	59–62	61 ± 1.6	0.018	0.013	0.018
Tolfenpyrad	0.5–19	2.7 ± 5.1	0.0–4.0	2.0 ± 2.4	1.9–2.4	2.1 ± 0.3	0.8–4.0	2.0 ± 1.9	0.641	0.926	0.694
Triadimenol	25–71	45 ± 16	35–44	41 ± 5.1	45–58	53 ± 7.3	53–63	59 ± 6.1	0.380	0.237	0.063
Triazophos	12–53	28 ± 13	13–21	18 ± 4.9	28–36	33 ± 4.6	20–38	31 ± 10	0.131	0.179	0.380
Trifloxystrobin	5.1–18	8.9 ± 4.5	6.2–14	9.4 ± 4.5	7.2–9.2	8.4 ± 1.2	14–15	14 ± 0.6	0.236	0.764	0.041

* indicates a significant statistical difference (p-value < 0.01)

Table A12: Minimal, maximal and mean pesticides transfer factors for pesticides in herbal teas. Associated Mann-Whitney tests

Pesticides	Transfer factor (%) in different herbal teas						Mann–Whitney test		
	Chamomile		Linden		Mint		Cham. vs lind.	Cham. vs mint	Lind. vs mint
	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	p	p	p
Acetamiprid	64–87	78 ± 14	73–75	74 ± 1.22	100–108	105 ± 4.6	0.386	0.040	0.075
Anthraquinone	35–43	40 ± 5.2	48–55	51 ± 4.0	45–67	57 ± 13	0.075	0.040	0.075
Azoxystrobin	44–52	49 ± 4.2	42–49	45 ± 3.6	54–65	58 ± 6.2	0.193	0.040	0.075
Bifenthrin	0.0–8.3	4.1 ± 4.9	5.0–5.6	5.3 ± 0.4	4.5–8.1	6.3 ± 2.1	0.386	0.331	0.386
Boscalid	8.1–18	14 ± 6.1	15–22	18 ± 3.8	14–25	20 ± 6.4	0.386	0.191	0.500
Buprofezin	3.6–5.2	4.4 ± 0.9	2.8–3.9	3.4 ± 0.6	3.6–11	7.0 ± 4.1	0.193	0.253	0.193
Carbendazim	63–86	74 ± 13	70–70	70 ± 0.2	96–105	99 ± 5.2	0.386	0.040	0.075
Chlorfenapyr	0.0–13	4.3 ± 7.6	0.0–5.3	2.7 ± 3.1	0.0–6.1	3.7 ± 3.6	0.627	0.679	0.500
Chlorpyriphos-ethyl	0.0–6.2	3.8 ± 3.7	0.0–0.0	0.0 ± 0.0	0.0–8.1	4.5 ± 4.8	0.167	0.412	0.167
Chlorpyriphos-methyl	3.0–6.9	4.5 ± 2.3	3.3–7.0	5.2 ± 2.2	4.6–8.7	6.6 ± 2.4	0.386	0.191	0.386
Clothianidin	63–84	77 ± 12	85–92	88 ± 4.0	104–110	108 ± 3.7	0.075	0.040	0.075
Cyfluthrin	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	1.000	1.000	1.000
Cyhalothrin-L	0.0–12	3.9 ± 6.9	2.7–5.1	3.9 ± 1.4	2.8–6.2	5.1 ± 2.0	0.384	0.327	0.187
Cypermethrin	0.0–0.0	0.0 ± 0.0	0.0–3.5	1.8 ± 2.1	0.0–2.2	1.1 ± 1.3	0.207	0.098	0.500
DDD-o,p	3.0–7.1	4.7 ± 2.4	3.9–4.6	4.3 ± 0.4	3.4–6.5	5.0 ± 1.8	0.723	0.500	0.386
DDD-p,p	2.8–8.5	5.1 ± 3.4	4.6–4.9	4.8 ± 0.2	3.9–6.9	5.5 ± 1.8	0.807	0.412	0.386
DDE-o,p	1.8–2.8	2.4 ± 0.6	2.1–3.7	2.9 ± 0.9	2.1–5.7	3.5 ± 2.1	0.386	0.253	0.500
DDE-p,p	2.7–5.4	4.2 ± 1.6	3.2–4.4	3.8 ± 0.7	3.1–7.0	4.7 ± 2.3	0.386	0.500	0.614
DDT-o,p	3.1–7.2	4.9 ± 2.4	4.0–4.8	4.4 ± 0.5	3.7–7.8	5.6 ± 2.4	0.614	0.331	0.386
DDT-p,p	0.0–6.7	2.2 ± 4.0	0.0–5.4	2.7 ± 3.2	5.1–8.7	6.7 ± 2.1	0.627	0.188	0.932
Deltamethrin	0.0–10	4.3 ± 5.6	9.0–11	10 ± 0.9	6.9–7.9	7.3 ± 0.6	0.193	0.331	0.075
Difenconazole	13–17	15 ± 2.5	9.3–13	11 ± 2.2	15–28	20 ± 8.0	0.193	0.095	0.075

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Table A12 (continued)

Pesticides	Transfer factor (%) in different herbal teas						Mann-Whitney test		
	Chamomile		Linden		Mint		Cham. vs lind.	Cham. vs mint	Lind. vs mint
	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	p	p	p
Diflubenzuron	24–29	27 ± 3.0	23–28	25 ± 2.7	31–43	35 ± 7.0	0.386	0.040	0.075
Dinetofuran	63–96	85 ± 20	79–83	81 ± 2.5	110–128	120 ± 11	0.386	0.040	0.075
Endosulfan-alpha	0.0–5.3	1.8 ± 3.1	0.0–0.0	0.0 ± 0.0	6.6–15	10 ± 5.2	0.342	0.038	0.069
Endosulfan-beta	0.0–7.8	2.6 ± 4.6	0.0–5.6	2.8 ± 3.3	0.0–8.2	5.1 ± 4.8	0.627	0.321	0.277
Endosulfan-sulfate	4.7–13	8.9 ± 4.8	5.0–11	7.9 ± 3.4	7.2–10	8.8 ± 1.5	0.614	0.588	0.614
Esfenvalerate	0.0–12	5.1 ± 6.8	5.3–7.0	6.2 ± 1.0	5.3–8.0	7.0 ± 1.62	0.386	0.331	0.277
Ethion	0.0–6.0	2.0 ± 3.5	0.0–0.0	0.0 ± 0.0	0.0–8.4	5.2 ± 5.0	0.342	0.177	0.167
Fenazaquin	0.0–7.8	2.6 ± 4.6	0.0–7.1	3.6 ± 4.2	0.0–9.1	5.4 ± 5.4	0.627	0.321	0.381
Fenpropathrin	0.0–0.0	0.0 ± 0.0	0.0–27	13 ± 16	0.0–20	6.8 ± 18	0.207	0.253	0.373
Fenpyroximate	1.9–2.8	2.4 ± 0.5	1.5–1.6	1.6 ± 0.1	1.6–11	6.7 ± 5.7	0.075	0.331	0.118
Fenvalerate	6.0–13	9.2 ± 4.1	6.2–10	7.9 ± 2.0	5.3–10	8.0 ± 2.7	0.614	0.500	0.614
Flufenoxuron	1.5–2.5	2.0 ± 0.6	0.9–1.1	1.0 ± 0.1	1.2–15	7.7 ± 7.9	0.075	0.331	0.075
Hexythiazox	2.1–3.2	2.7 ± 0.6	2.0–2.1	2.1 ± 0.1	2.0–9.2	6.2 ± 4.3	0.118	0.331	0.277
Imidacloprid	56–84	73 ± 16	93–93	93 ± 0.2	101–132	121 ± 18	0.075	0.040	0.075
Indoxacarb	7.9–9.6	8.9 ± 1.0	5.6–8.0	6.8 ± 1.4	8.9–22	15 ± 7.4	0.193	0.191	0.075
Iprodione	15–17	16 ± 1.4	0.0–13	6.7 ± 7.9	0.0–31	10 ± 19	0.075	0.329	0.627
Linuron	43–45	44 ± 0.9	45–50	48 ± 2.8	51–62	56 ± 7.0	0.069	0.038	0.075
Methomyl	62–93	81 ± 18	69–77	73 ± 4.3	95–115	106 ± 12	0.386	0.040	0.075
OPP	4.3–27	16 ± 14	9.2–36	22 ± 16	15–23	20 ± 4.8	0.386	0.500	0.614
Permethrin	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–23	7.6 ± 13	1.000	0.253	0.342
Propargite	1.7–2.5	2.2 ± 0.5	1.2–1.7	1.5 ± 0.3	1.5–9.3	5.6 ± 4.6	0.118	0.331	0.193
Propiconazole	0.0–24	14 ± 14	11–28	20 ± 10	16–25	20 ± 5.8	0.386	0.331	0.614

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Table A12 (continued)

Pesticides	Transfer factor (%) in different herbal teas						Mann-Whitney test		
	Chamomile		Linden		Mint		Cham. vs lind.	Cham. vs mint	Lind. vs mint
	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	p	p	p
Pyridaben	1.1–1.7	1.4 ± 0.4	0.9–1.2	1.1 ± 0.2	0.9–11	5.9 ± 5.8	0.193	0.331	0.277
Pyrimethanil	32–39	36 ± 4.2	29–31	30 ± 1.3	38–44	41 ± 3.5	0.075	0.191	0.075
Pyriproxifen	1.7–2.9	2.3 ± 0.7	1.6–1.9	1.8 ± 0.2	1.7–8.1	5.7 ± 3.8	0.193	0.253	0.193
Spiromesifen	0.0–0.0	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–12	8.1 ± 7.2	1.000	0.098	0.167
Tebuconazole	30–36	34 ± 3.1	26–34	30 ± 4.4	34–45	38 ± 6.6	0.187	0.188	0.118
Thiacloprid	64–76	71 ± 7.6	64–70	67 ± 3.5	90–91	90 ± 0.8	0.386	0.040	0.075
Thiamethoxam	70–96	85 ± 16	83–86	85 ± 1.8	36–150	103 ± 67	0.386	0.331	0.386
Tolfenpyrad	2.7–3.9	3.4 ± 0.7	2.5–2.8	2.7 ± 0.2	2.9–12	7.6 ± 5.1	0.193	0.191	0.075
Triadimenol	4.8–37	23 ± 19	18–38	28 ± 12	22–33	28 ± 6.3	0.386	0.500	0.614
Triazophos	6.4–13	10 ± 3.8	7.8–14	11 ± 3.6	13–29	20 ± 9.2	0.386	0.040	0.193
Trifloxystrobin	0.0–15	8.5 ± 8.9	12–12	12 ± 0.1	8.8–15	13 ± 3.4	0.386	0.500	0.386

* indicates a significant statistical difference (p-value < 0.01)

Table A13: Minimal, maximal and mean pesticides transfer factors for pesticides in black tea, green tea and herbal tea. Associated Mann-Whitney tests

Pesticides	Transfer factor in black tea (%)		Transfer factor in green tea (%)		Transfer factor in herbal tea (%)		Mann-Whitney test	
	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	Black vs herbal	Green vs herbal
							p-value	p-value
Acetamiprid	53–94	73 ± 12	45–75	63 ± 7.5	64–108	87 ± 17	0.049	0.001^*
Anthraquinone	7.3–24	18 ± 4.3	11–38	19 ± 9.0	35–67	49 ± 10	$< 0.001^*$	$< 0.001^*$
Azoxystrobin	23–36	32 ± 4.0	14–63	40 ± 14	42–65	51 ± 6.9	0.101	0.013
Bifenthrin	0.0–0.0	0.0 ± 0.0	1.7–7.7	4.0 ± 2.3	0.0–8.3	5.2 ± 2.6	$< 0.001^*$	0.116
Boscalid	13–24	20 ± 3.7	7.9–46	26 ± 11	8.1–25	18 ± 5.3	0.208	0.035
Buprofezin	1.3–18	6.3 ± 4.3	1.7–4.0	2.7 ± 0.7	2.8–11	5.1 ± 2.5	0.177	0.665
Carbendazim	45–88	66 ± 12	44–83	65 ± 11	63–105	83 ± 15	0.017	0.015
Chlorfenapyr	0.0–6.3	2.3 ± 2.3	4.6–25	9.4 ± 6.7	0.0–13	3.7 ± 4.6	0.453	0.014
Chlorpyriphos-ethyl	0.0–4.7	1.3 ± 1.3	2.3–8.8	5.0 ± 2.7	0.0–8.1	3.1 ± 3.4	0.331	0.115
Chlorpyriphos-methyl	0.0–7.0	0.7 ± 2.0	5.7–24	11 ± 5.5	3.0–8.7	5.5 ± 2.1	$< 0.001^*$	0.001^*
Clothianidin	51–98	75 ± 15	50–80	67 ± 7.3	63–110	92 ± 16	0.014	0.001^*
Cyfluthrin	0.0–6.6	0.5 ± 1.9	1.0–12	6.7 ± 3.5	0.0–0.0	0.0 ± 0.0	0.133	$< 0.001^*$
Cyhalothrin-L	0.3–1.4	0.8 ± 0.3	2.0–10	4.9 ± 2.9	0.0–12	4.3 ± 3.9	0.019	0.393
Cypermethrin	0.0–5.9	0.5 ± 1.7	1.0–13	5.7 ± 3.8	0.0–3.5	0.9 ± 1.3	0.168	0.001^*
DDD-o,p	0.0–0.0	0.0 ± 0.0	2.1–7.3	4.3 ± 2.3	3.0–7.1	4.7 ± 1.5	$< 0.001^*$	0.198
DDD-p,p	0.0–1.0	0.2 ± 0.4	2.1–7.0	4.0 ± 2.1	2.8–8.5	5.2 ± 1.8	$< 0.001^*$	0.132
DDE-o,p	0.0–0.0	0.0 ± 0.0	1.4–6.6	3.5 ± 2.1	1.8–5.7	3.0 ± 1.3	$< 0.001^*$	0.500

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Table A13 (continued)

Pesticides	Transfer factor in black tea (%)		Transfer factor in green tea (%)		Transfer factor in herbal tea (%)		Mann-Whitney test	
	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	Black vs herbal	Green vs herbal
							p-value	p-value
DDE-p,p	0.0–0.0	0.0 ± 0.0	1.5–6.4	3.5 ± 1.9	2.7–7.0	4.3 ± 1.4	$< 0.001^*$	0.140
DDT-o,p	0.0–0.0	0.0 ± 0.0	1.8–7.4	4.1 ± 2.2	3.1–7.8	5.0 ± 1.7	$< 0.001^*$	0.082
DDT-p,p	0.0–0.0	0.0 ± 0.0	2.2–7.9	4.7 ± 2.2	0.0–8.7	4.0 ± 3.5	0.004^*	0.364
Deltamethrin	1.0–3.1	2.0 ± 0.8	1.8–13	5.0 ± 4.1	0.0–11	6.8 ± 3.5	0.003^*	0.132
Difenoconazole	7.1–25	13 ± 4.9	2.4–14	7.7 ± 4.0	9.3–28	16 ± 5.6	0.149	0.001^*
Diflubenzuron	19–53	34 ± 11	0.0–16	8.0 ± 5.1	23–43	30 ± 6.1	0.393	$< 0.001^*$
Dinetofuran	71–115	87 ± 14	63–100	84 ± 8.5	63–128	97 ± 22	0.149	0.116
Endosulfan-alpha	0.0–3.6	1.4 ± 1.2	1.1–11	4.8 ± 3.9	0.0–15	4.3 ± 5.5	0.422	0.148
Endosulfan-beta	0.0–8.9	3.8 ± 2.7	5.3–18	10 ± 5.0	0.0–8.2	3.6 ± 3.9	0.319	0.012
Endosulfan-sulfate	2.2–15	10 ± 4.2	1.4–25	10 ± 7.2	4.7–13	8.6 ± 2.8	0.393	0.350
Esfenvalerate	0.0–1.3	0.8 ± 0.4	2.3–8.5	5.1 ± 2.4	0.0–12	6.1 ± 3.4	0.002^*	0.243
Ethion	0.4–17	6.8 ± 5.1	0.0–15	5.7 ± 5.2	0.0–8.4	2.7 ± 3.8	0.023	0.076
Fenazaquin	0.0–3.8	1.4 ± 1.2	2.2–8.2	4.8 ± 2.5	0.0–9.1	3.9 ± 4.2	0.332	0.230
Fenpropathrin	1.9–14	5.8 ± 3.3	0.0–0.7	0.1 ± 0.2	0.0–27	5.9 ± 11	0.033	0.272
Fenpyroximate	0.0–7.6	1.2 ± 2.1	0.0–1.7	1.0 ± 0.5	1.5–11	3.8 ± 3.6	0.010	$< 0.001^*$
Fenvalerate	0.0–0.0	0.0 ± 0.0	1.4–7.6	3.8 ± 2.3	5.3–13	8.4 ± 2.5	$< 0.001^*$	0.001^*
Hexythiazox	0.0–14	2.9 ± 3.8	0.9–5.3	1.7 ± 1.2	2.0–9.2	3.8 ± 2.8	0.165	0.001^*
Imidacloprid	52–105	75 ± 14	45–93	69 ± 13	56–132	96 ± 25	0.014	0.006^*

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Table A13 (continued)

Pesticides	Transfer factor in black tea (%)		Transfer factor in green tea (%)		Transfer factor in herbal tea (%)		Mann-Whitney test	
	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD	Black vs herbal	Green vs herbal
							p-value	p-value
Indoxacarb	0.0–23	5.7 ± 6.8	1.2–6.7	2.9 ± 1.8	5.6–22	11 ± 5.0	0.056	$< 0.001^*$
Iprodione	6.4–18	15 ± 4.3	11–44	25 ± 10	0.0–31	12 ± 11	0.100	0.006^*
Linuron	32–62	46 ± 9.0	22–46	34 ± 6.8	43–62	49 ± 6.6	0.232	$< 0.001^*$
Methomyl	56–104	87 ± 14	58–88	74 ± 7.5	62–115	89 ± 18	0.336	0.053
OPP	16–36	29 ± 5.8	22–69	45 ± 17	4.3–36	19 ± 10	0.024	0.001^*
Permethrin	1.5–4.7	2.7 ± 0.8	0.1–12	3.8 ± 3.9	0.0–23	2.9 ± 8.1	0.002^*	0.003
Propargite	0.0–13	1.8 ± 3.7	1.3–3.1	1.7 ± 0.5	1.2–9.3	3.3 ± 2.9	0.033	0.048
Propiconazole	11–26	20 ± 4.3	16–47	27 ± 11	0.0–28	18 ± 9.1	0.454	0.041
Pyridaben	0.0–5.2	0.5 ± 1.5	0.8–1.7	1.4 ± 0.2	0.9–11	3.0 ± 3.6	0.001^*	0.500
Pyrimethanil	24–56	40 ± 9.3	18–42	30 ± 7.7	29–44	36 ± 5.4	0.076	0.035
Pyriproxifen	0.0–11	2.1 ± 3.1	0.5–0.9	0.7 ± 0.2	1.6–8.1	3.4 ± 2.7	0.186	$< 0.001^*$
Spiromesifen	1.2–4.6	3.1 ± 1.1	0.0–14	6.2 ± 4.5	0.0–12	3.0 ± 5.6	0.031	0.038
Tebuconazole	19–41	31 ± 6.3	5.1–27	18 ± 7.1	26–45	35 ± 5.3	0.076	$< 0.001^*$
Thiacloprid	41–81	62 ± 11	36–52	45 ± 3.9	64–91	77 ± 12	0.017	$< 0.001^*$
Thiamethoxam	64–116	94 ± 17	38–72	51 ± 8.9	36–150	92 ± 34	0.531	0.004^*
Tolfenpyrad	0.0–14	3.4 ± 3.6	0.5–19	2.7 ± 5.1	2.5–12	4.8 ± 3.4	0.454	0.002^*
Triadimenol	26–38	34 ± 3.8	25–71	45 ± 16	4.8–38	26 ± 11	0.131	0.005^*
Triazophos	16–31	26 ± 4.9	12–53	28 ± 13	6.4–29	14 ± 7.0	0.005^*	0.002^*

Table continued on next page

Table A13 (continued)

Pesticides	Transfer factor in black tea (%)		Transfer factor in green tea (%)		Transfer factor in herbal tea (%)		Mann-Whitney test	
	min–max	mean $\pm$ SD	min–max	mean $\pm$ SD	min–max	mean $\pm$ SD	Black vs herbal	Green vs herbal
							p-value	p-value
Trifloxystrobin	2.0–13	7.7 $\pm$ 3.0	5.1–18	8.9 $\pm$ 4.5	0.0–15	11 $\pm$ 4.9	0.057	0.140

* indicates a significant statistical difference (p-value < 0.01)

Table A14: Minimal, maximal and mean pesticides transfer factors in black tea infused with tap water at 100 °C and 80 °C. Associated Mann-Whitney test

Pesticides	Transfer factor in black tea (100 °C) (%)		Transfer factor in black tea (80 °C) (%)		Mann-Whitney
	min–max	mean ± SD	min–max	mean ± SD	p-value
Acetamiprid	53–76	65 ± 14	51–57	54 ± 3.3	0.040
Azoxystrobin	31–50	42 ± 11	29–32	30 ± 1.9	0.040
Buprofezin	5.3–9.3	7.1 ± 2.4	2.4–3.7	3.0 ± 0.8	0.191
Carbendazim	45–67	58 ± 13	46–53	50 ± 3.8	0.040
Clothianidin	51–69	62 ± 11	54–60	57 ± 3.5	0.331
Difenoconazole	11–17	14 ± 3.7	6.9–8.0	7.5 ± 0.6	0.331
Diflubenzuron	23–48	35 ± 15	11–15	13 ± 2.2	0.191
Dinetofuran	72–86	77 ± 8.7	57–59	58 ± 0.8	0.331
Fenpyroximate	0.0–0.7	0.5 ± 0.4	1.9–2.1	2.0 ± 0.2	0.095
Hexythiazox	1.5–5.7	3.0 ± 2.5	0.8–0.8	0.8 ± 0.0	0.040
Imidacloprid	52–79	68 ± 16	48–50	49 ± 1.6	0.040
Indoxacarb	0.0–5.2	3.1 ± 3.1	4.3–5.9	5.0 ± 0.9	0.191
Linuron	32–48	40 ± 24	24–28	26 ± 2.7	0.040
Methomyl	56–87	74 ± 24	50–61	56 ± 6.8	0.040
Propargite	0.0–1.5	0.9 ± 0.9	1.6–1.9	1.7 ± 0.2	0.095
Pyridaben	0.0–0.0	0.0 ± 0.0	2.1–2.4	2.2 ± 0.2	0.040
Pyrimethanil	24–44	37 ± 12	17–21	19 ± 14	0.032
Pyriproxifen	0.1–2.7	1.4 ± 1.5	1.6–1.9	1.7 ± 0.2	0.329
Tebuconazole	19–34	27 ± 8.6	15–19	18 ± 2.4	0.040
Thiacloprid	41–65	55 ± 14	42–46	44 ± 2.2	0.036
Thiamethoxam	78–114	99 ± 21	53–65	59 ± 6.9	0.038
Tolfenpyrad	2.7–4.4	3.4 ± 1.0	1.1–1.9	1.5 ± 0.5	0.030

Table A15: Minimal, maximal and mean pesticides transfer factors in green tea infused with tap water at 100 °C and 80 °C. Associated Mann-Whitney test

Pesticides	**Transfer factor in green tea (100 °C) (%)**		**Transfer factor in green tea (80 °C) (%)**		**Mann-Whitney**
	min–max	**mean ± SD**	**min–max**	**mean ± SD**	p**-value**
Acetamiprid	45–61	54 ± 9.1	57–79	67 ± 13	0.095
Azoxystrobin	21–76	46 ± 32	31–78	37 ± 6.5	0.500
Buprofezin	1.7–4.0	2.8 ± 1.3	2.1–4.0	3.0 ± 1.1	0.253
Carbendazim	44–59	52 ± 8.8	58–79	67 ± 11	0.095
Clothianidin	50–66	59 ± 9.4	59–85	69 ± 15	0.191
Difenoconazole	4.8–14	9.3 ± 5.3	8.0–15	12 ± 4.1	0.669
Diflubenzuron	5.7–16	9.8 ± 6.1	11–22	18 ± 6.5	0.191
Dinetofuran	63–84	76 ± 12	59–90	72 ± 18	0.331
Fenpyroximate	0.8–1.5	1.0 ± 0.4	1.3–3.2	2.1 ± 1.1	0.331
Flufenoxuron	1.2–6.9	4.8 ± 3.4	1.6–5.1	3.0 ± 2.0	0.095
Hexythiazox	0.9–1.7	1.4 ± 0.5	1.6–2.9	2.1 ± 0.8	0.588
Imidacloprid	45–61	55 ± 9.7	49–74	60 ± 15	0.191
Indoxacarb	1.9–5.2	3.1 ± 2.0	5.1–8.5	7.2 ± 2.0	0.191
Linuron	22–41	30 ± 11	31–42	36 ± 5.5	0.331
Methomyl	58–76	68 ± 10	28–90	45 ± 16	0.331
Propargite	1.9–3.1	2.3 ± 0.7	0.8–1.9	1.3 ± 0.6	0.040
Pyridaben	1.3–1.5	1.4 ± 0.1	1.1–2.4	1.7 ± 0.8	0.500
Pyrimethanil	18–42	28 ± 15	25–40	33 ± 8.2	0.331
Pyriproxifen	0.5–0.9	0.8 ± 0.2	1.1–2.4	1.8 ± 0.8	0.040
Tebuconazole	11–27	20 ± 9.5	19–28	25 ± 5.0	0.191
Thiacloprid	36–48	42 ± 6.6	49–85	58 ± 9.1	0.040
Thiamethoxam	56–72	62 ± 9.5	41–68	52 ± 16	0.669
Tolfenpyrad	0.8–2.3	1.3 ± 0.9	1.6–3.2	2.4 ± 0.9	0.191

Table A16: Comparison of pesticides transfer factors in black tea infused with milli-Q water and tap water. Associated Mann-Whitney test

Pesticides	**Transfer factor (black tea) - milli-Q water (%)**		**Transfer factor (black tea) - tap water (%)**		**Mann-Whitney**
	min–max	**mean ± SD**	**min–max**	**mean ± SD**	p**-value**
Acetamiprid	58–80	70 ± 13	53–76	65 ± 14	0.331
Azoxystrobin	34–60	47 ± 15	31–50	42 ± 11	0.331
Buprofezin	5.9–13	10 ± 4.1	5.3–9.3	7.1 ± 2.4	0.191
Carbendazim	51–73	63 ± 13	45–67	58 ± 13	0.331
Clothianidin	66–80	75 ± 8.0	51–69	62 ± 10	0.191
Difenoconazole	15–31	21 ± 9.1	11–17	14 ± 3.7	0.095
Diflubenzuron	26–34	30 ± 4.7	23–48	35 ± 15	0.331
Dinetofuran	69–85	76 ± 10	72–86	77 ± 8.7	0.500
Fenpyroximate	2.0–12	7.0 ± 5.7	0.0–0.7	0.5 ± 0.4	0.038
Hexythiazox	2.0–16	10 ± 8.3	1.5–5.7	3.0 ± 2.5	0.095
Imidacloprid	58–77	70 ± 11	52–79	68 ± 16	0.500
Indoxacarb	7.7–21	13 ± 8.0	0.0–5.2	3.1 ± 3.1	0.040
Linuron	33–59	47 ± 16	32–48	40 ± 9.4	0.191
Methomyl	61–78	67 ± 10	56–87	74 ± 18	0.331
Propargite	1.7–14	7.8 ± 7.2	0.0–1.5	0.9 ± 0.9	0.040
Pyridaben	1.6–17	8.5 ± 8.8	0.0–0.0	0.0 ± 0.0	0.032
Pyrimethanil	30–50	40 ± 12	24–44	37 ± 12	0.500
Pyriproxifen	2.3–25	13 ± 13	0.1–2.7	1.4 ± 1.5	0.095
Tebuconazole	26–47	36 ± 13	19–34	27 ± 8.6	0.191
Thiacloprid	54–80	66 ± 16	41–65	55 ± 14	0.191
Thiamethoxam	58–82	70 ± 14	78–114	99 ± 21	0.095
Tolfenpyrad	2.3–12	8.0 ± 5.7	2.7–4.4	3.4 ± 1.0	0.331

Table A17: Comparison of pesticides transfer factors in green tea infused with milli-Q water and tap water and associated Mann-Whitney test

Pesticides	Transfer factor (green tea) - milli-Q water (%)		Transfer factor (green tea) - tap water (%)		Mann-Whitney
	min–max	mean $\pm$ SD	min–max	mean $\pm$ SD	p-value
Acetamiprid	49–54	51 $\pm$ 2.9	46–61	54 $\pm$ 9.1	0.331
Azoxystrobin	25–31	28 $\pm$ 3.3	21–76	46 $\pm$ 32	0.331
Buprofezin	1.3–2.7	2.0 $\pm$ 0.8	1.7–4.0	2.8 $\pm$ 1.3	0.191
Carbendazim	49–55	51 $\pm$ 3.5	44–59	52 $\pm$ 8.8	0.500
Clothianidin	52–55	54 $\pm$ 1.8	50–66	59 $\pm$ 9.4	0.331
Difenoconazole	5.3–11	7.5 $\pm$ 3.2	4.8–14	9.3 $\pm$ 5.3	0.500
Diflubenzuron	12–19	14 $\pm$ 3.7	5.7–16	10 $\pm$ 6.1	0.188
Dinetofuran	67–71	69 $\pm$ 2.0	63–84	76 $\pm$ 12	0.331
Fenpyroximate	0.8–2.1	1.5 $\pm$ 0.8	0.8–1.5	1.0 $\pm$ 0.4	0.177
Flufenoxuron	2.1–2.7	2.4 $\pm$ 0.3	1.2–6.9	4.8 $\pm$ 3.4	0.331
Hexythiazox	0.8–1.9	1.2 $\pm$ 0.6	0.9–1.7	1.4 $\pm$ 0.5	0.412
Imidacloprid	46–52	49 $\pm$ 3.8	45–61	55 $\pm$ 10	0.331
Indoxacarb	5.2–5.3	5.2 $\pm$ 0.1	1.9–5.2	3.1 $\pm$ 2.0	0.082
Linuron	22–28	26 $\pm$ 3.4	22–41	30 $\pm$ 11	0.191
Methomyl	59–72	64 $\pm$ 8.0	58–76	68 $\pm$ 10	0.500
Propargite	0.8–1.6	1.1 $\pm$ 0.5	1.9–3.1	2.3 $\pm$ 0.7	0.040
Pyridaben	0.7–1.1	0.9 $\pm$ 0.2	1.3–1.5	1.4 $\pm$ 0.1	0.038
Pyrimethanil	17–22	20 $\pm$ 2.4	18–42	28 $\pm$ 14	0.191
Pyriproxifen	0.9–1.3	1.1 $\pm$ 0.2	0.5–0.9	0.8 $\pm$ 0.2	0.082
Tebuconazole	17–24	20 $\pm$ 3.9	11–27	20 $\pm$ 10	0.500
Thiacloprid	35–40	39 $\pm$ 2.9	36–48	42 $\pm$ 6.6	0.191
Thiamethoxam	34–42	37 $\pm$ 4.6	56–72	62 $\pm$ 10	0.040
Tolfenpyrad	1.2–2.0	1.6 $\pm$ 0.5	0.8–2.3	1.3 $\pm$ 0.9	0.331

A5 Validation data for the modelling of pesticides transfer

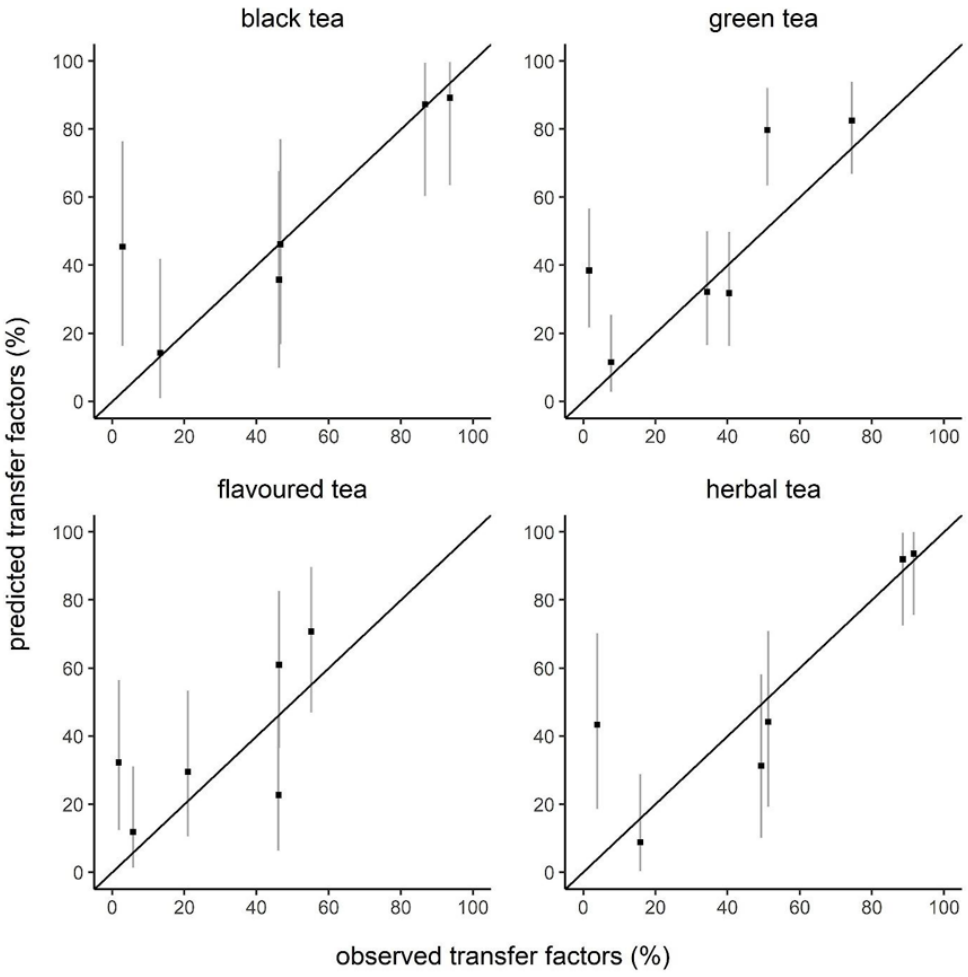

Figure A6: Predicted TF vs observed TF for black tea (a), green tea (b), flavoured tea (c), and herbal tea (d) for pesticides used for the model validations

Table A18: Mean squared errors (MSE) for selected pesticides for the four model validations (black tea, green tea, flavoured green tea and herbal tea) and associated crossed validation means

	Black tea	**Green tea**	**Flavoured green tea**	**Herbal tea**
MSE Methomyl	0.249	74.0	552	50.3
MSE Thiamethoxam	0.800	14.5	36.0	49.9
MSE Linuron	1810	1350	928	1560
MSE Difenoconazole	111	4.90	71.0	325
MSE Azoxystrobin	0.216	63.8	242	11.6
MSE Hexythiazox	20.0	825	215	3.89
Model mean MSE	**324**	**389**	**341**	**333**
Crossed validation mean MSE $\pm$ SD	**317 $\pm$ 146**	**148 $\pm$ 101**	**196 $\pm$ 108**	**268 $\pm$ 100**

Appendix B

Supplementary data related to acrylamide analysis

B1 Occurence data of acrylamide in foodstuffs

Table B1: Acrylamide content of samples analyzed as ready to eat or following a cooking process

Sample ID	Category	Sub-category	Relevant information on composition	Organic food	Add. cooking step	Acrylamide content – as ready to eat (μg kg^{-1})	Acrylamide content – deep fryer cooked (μg kg^{-1})	Acrylamide content – oven cooked (μg kg^{-1})	Acrylamide content – microwave cooked (μg kg^{-1})
AOF-166	Black olives		'Californian-style'	no	no	431			
AOF-167	Black olives		'Greek-style'	no	no	102			
AOF-168	Black olives		'Californian-style'	no	no	575			
AOF-169	Black olives		'Greek-style'	yes	no	31.9			
AOF-170	Black olives		'Greek-style'	yes	no	56.8			
AOF-031	Bread	Buns		no	no			<LOQ	
AOF-032	Bread	Buns		no	no			<LOQ	
AOF-033	Bread	Buns		no	no			22.9	
AOF-034	Bread	Milk bread		no	no	<LOD			
AOF-035	Bread	Milk bread		no	no	<LOD			
AOF-036	Bread	Pita bread		no	no			<LOD	
AOF-037	Bread	Pita bread		no	no			<LOD	
AOF-038	Bread	Pita bread		no	no			<LOD	
AOF-039	Bread	Pita bread		no	no			<LOD	
AOF-040	Bread	Tortilla		no	no	<LOD			
AOF-041	Bread	Tortilla		no	no			<LOD	
AOF-042	Bread	Tortilla		no	no			<LOD	
AOF-043	Bread	Tortilla		no	no	<LOD			
AOF-063	Bread	Special bread	With hazelnuts and grapes	no	no	<LOD			
AOF-064	Bread	Special bread	With grapes	no	no	<LOD			
AOF-065	Bread	Special bread	Rye bread	yes	no	20.2			
AOF-066	Bread	Special bread	Rye and oat bread	yes	no	<LOD			
AOF-067	Bread	Special bread	With sesam and grapes	yes	no	<LOQ			
AOF-068	Bread	Special bread	Rye bread	yes	no	76.2			

Table continued on next page

Table B1 (continued)

AOF-069	Bread	Special bread	With black olives	yes	no	98
AOF-070	Bread	Special bread	With grapes	no	no	<LOQ
AOF-181	Bread	Special bread	Chia-spelt bread	no	no	47.2
AOF-182	Bread	Special bread	Multigrain bread	no	no	<LOD
AOF-183	Bread	Special bread	Whole bread with chia seeds	yes	no	<LOD
AOF-184	Bread	Special bread	Spelt bread with chia and flax seeds	yes	no	<LOD
AOF-185	Bread	Special bread	Spelt bread with chia seeds	yes	no	<LOD
AOF-186	Bread	Special bread	Whole bread with chia seeds	yes	no	<LOD
AOF-193	Bread	Rusk	With chia seeds	no	no	211
AOF-194	Bread	Rusk	Chia and oats	yes	no	<LOD
AOF-200	Bread	Tortilla	With chia seeds	yes	no	<LOD
AOF-161	Cocoa powder for beverage		100 % cocoa	no	no	141
AOF-162	Cocoa powder for beverage		32 % cocoa	no	no	<LOQ
AOF-163	Cocoa powder for beverage		13 % cocoa	no	no	55.8
AOF-164	Cocoa powder for beverage		21,5 % cocoa	no	no	27.5
AOF-165	Cocoa powder for beverage		100 % cocoa	yes	no	55.2
AOF-171	Coffee substitutes		Lupin	yes	no	28.7
AOF-172	Coffee substitutes		Roasted dandelion roots	yes	no	184
AOF-173	Coffee substitutes		Fruits / rice / matcha	yes	no	<LOD
AOF-174	Coffee substitutes		Carob nuts / chicory	yes	no	1057

Table continued on next page

Table B1 (continued)

AOF-175	Coffee substitutes		Carob nuts / chicory and dandelion roots	yes	no	642
Add13	Coffee substitutes		Jerusalem artichoke	no	no	4389
Add14	Coffee substitutes		Acorn	no	no	207
AOF-081	Cereals and snacks	Crackers and snacks	Rice cracker	yes	no	124
AOF-082	Cereals and snacks	Crackers and snacks	Rice cracker	yes	no	259
AOF-083	Cereals and snacks	Crackers and snacks	Rice cracker	yes	no	192
AOF-084	Cereals and snacks	Crackers and snacks	Rice cracker	yes	no	149
AOF-085	Cereals and snacks	Crackers and snacks	Rice cracker	no	no	165
AOF-086	Cereals and snacks	Crackers and snacks	Rice cracker	yes	no	41
AOF-087	Cereals and snacks	Crackers and snacks	Rice cracker	yes	no	199
AOF-088	Cereals and snacks	Crackers and snacks	Rice cracker	no	no	135
AOF-089	Cereals and snacks	Crackers and snacks	Rice cracker	yes	no	43
AOF-090	Cereals and snacks	Crackers and snacks	Rice cracker	yes	no	<LOQ
AOF-091	Cereals and snacks	Crackers and snacks	Maize cracker	yes	no	53.4
AOF-092	Cereals and snacks	Crackers and snacks	Maize cracker	yes	no	114
AOF-093	Cereals and snacks	Crackers and snacks	Maize cracker	yes	no	118
AOF-094	Cereals and snacks	Crackers and snacks	Maize cracker	no	no	49.7
AOF-095	Cereals and snacks	Crackers and snacks	Maize cracker	yes	no	169

Table continued on next page

Table B1 (continued)

AOF-096	Cereals and snacks	Crackers and snacks	Maize cracker	no	no	110
AOF-097	Cereals and snacks	Crackers and snacks	Maize cracker	yes	no	106
AOF-098	Cereals and snacks	Crackers and snacks	Maize cracker	yes	no	<LOD
AOF-099	Cereals and snacks	Crackers and snacks	Maize cracker	yes	no	62.1
AOF-100	Cereals and snacks	Crackers and snacks	Maize cracker	yes	no	59
AOF-101	Cereals and snacks	Crackers and snacks	Maize snacks	no	no	55.1
AOF-102	Cereals and snacks	Crackers and snacks	Maize snacks	no	no	300
AOF-103	Cereals and snacks	Crackers and snacks	Maize snacks	no	no	68
AOF-104	Cereals and snacks	Crackers and snacks	Maize snacks	no	no	295
AOF-105	Cereals and snacks	Crackers and snacks	Maize snacks	no	no	<LOD
AOF-106	Cereals and snacks	Crackers and snacks	Maize/wheat snack	no	no	317
AOF-107	Cereals and snacks	Crackers and snacks	Wheat snack	no	no	207
AOF-108	Cereals and snacks	Crackers and snacks	Wheat snack	no	no	94.8
AOF-109	Cereals and snacks	Crackers and snacks	Maize snacks	no	no	132
AOF-110	Cereals and snacks	Crackers and snacks	Maize snacks	no	no	337
AOF-111	Cereals and snacks	Crackers and snacks	Maize snacks	no	no	43.5
AOF-112	Cereals and snacks	Crackers and snacks	Maize snacks	no	no	49
AOF-113	Cereals and snacks	Crackers and snacks	Maize snacks	no	no	68.2

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Table B1 (continued)

AOF-114	Cereals and snacks	Crackers and snacks	Maize snacks	yes	no	46.7
AOF-115	Cereals and snacks	Crackers and snacks	Maize snacks	no	no	<LOQ
AOF-116	Cereals and snacks	Crackers and snacks	Wheat snack	no	no	63.2
AOF-117	Cereals and snacks	Crackers and snacks	Maize/rice snack	no	no	112
AOF-118	Cereals and snacks	Crackers and snacks	Maize/rice snack	no	no	45
AOF-119	Cereals and snacks	Crackers and snacks	Manioc snack	no	no	<LOD
AOF-120	Cereals and snacks	Crackers and snacks	Manioc snack	no	no	<LOD
AOF-121	Cereals and snacks	Crackers and snacks	Honey roasted muesli	no	no	353
AOF-122	Cereals and snacks	Crackers and snacks	Honey roasted muesli	no	no	53.2
AOF-123	Cereals and snacks	Crackers and snacks	Honey roasted muesli	yes	no	<LOQ
AOF-124	Cereals and snacks	Crackers and snacks	Honey roasted muesli	yes	no	<LOQ
AOF-125	Cereals and snacks	Crackers and snacks	Honey roasted muesli	yes	no	34.2
AOF-126	Cereals and snacks	Crackers and snacks	Honey roasted muesli	no	no	56.5
AOF-127	Cereals and snacks	Crackers and snacks	Honey roasted muesli	no	no	42.3
AOF-128	Cereals and snacks	Crackers and snacks	Honey roasted muesli	no	no	30.9
AOF-129	Cereals and snacks	Crackers and snacks	Honey roasted muesli	no	no	<LOQ
AOF-130	Cereals and snacks	Crackers and snacks	Honey roasted muesli	yes	no	<LOD
AOF-187	Cereals and snacks	Crackers and snacks	Cookies (chia seeds 5.3%)	no	no	67.6

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Table B1 (continued)

AOF-188	Cereals and snacks	Crackers and snacks	Cookies (chia seeds 5%)	no	no	<LOD
AOF-189	Cereals and snacks	Crackers and snacks	Cookies (chia seeds 4%)	no	no	<LOD
AOF-190	Cereals and snacks	Crackers and snacks	Cookies (chia seeds 2%)	yes	no	<LOQ
AOF-191	Cereals and snacks	Crackers and snacks	Cookies (chia seeds 8%)	no	no	63
AOF-192	Cereals and snacks	Crackers and snacks	Cookies (chia seeds 4%)	no	no	21.7
AOF-195	Cereals and snacks	Crackers and snacks	Cookies (chia seeds 6%)	yes	no	<LOD
AOF-196	Cereals and snacks	Crackers and snacks	Cookies (chia seeds 5%)	no	no	<LOD
AOF-197	Cereals and snacks	Crackers and snacks	Cookies (chia seeds 10%)	yes	no	134
AOF-198	Cereals and snacks	Crackers and snacks	Cookies (chia seeds 5%)	no	no	<LOD
AOF-199	Cereals and snacks	Crackers and snacks	Cookies (chia seeds 8%)	yes	no	190
Add11	Cereals and snacks	Crackers and snacks	Rice cracker	yes	no	41.5
Add12	Cereals and snacks	Crackers and snacks	Wheat/rice snack	yes	no	98.4
Add15	Cereals and snacks	Crackers and snacks	Honey roasted muesly	no	no	29.8
AOF-155	Dried fruits -fudges -caramel	Dried fruits	Dried grapes	yes	no	<LOD
AOF-156	Dried fruits -fudges -caramel	Dried fruits	Dried grapes	yes	no	<LOD
AOF-157	Dried fruits -fudges -caramel	Dried fruits	Dried plums	yes	no	<LOD

Table continued on next page

Table B1 (continued)

AOF-158	Dried fruits -fudges -caramel	Dried fruits	Dried apricots	no	no	<LOD
AOF-159	Dried fruits -fudges -caramel	Dried fruits	Dried peachs	no	no	<LOD
AOF-160	Dried fruits -fudges -caramel	Dried fruits	Dried figs	yes	no	<LOD
AOF-176	Dried fruits -fudges -caramel	Nougat		no	no	<LOQ
AOF-177	Dried fruits -fudges -caramel	Nougat		no	no	25.4
AOF-178	Dried fruits -fudges -caramel	Fudge		no	no	<LOD
AOF-179	Dried fruits -fudges -caramel	Fudge		no	no	<LOD
AOF-180	Dried fruits -fudges -caramel	Fudge		no	no	59.6
AOF-139	Nuts and oilseeds (roasted)	Nuts (roasted)	Cashew	no	no	<LOD
AOF-140	Nuts and oilseeds (roasted)	Nuts (roasted)	Cashew	no	no	<LOD
AOF-141	Nuts and oilseeds (roasted)	Nuts (roasted)	Pistachios	no	no	<LOD
AOF-142	Nuts and oilseeds (roasted)	Nuts (roasted)	Peanuts	no	no	25.7

Table continued on next page

Table B1 (continued)

AOF-143	Nuts and oilseeds (roasted)	Nuts (roasted)	Mix	no	no	75.6
AOF-144	Nuts and oilseeds (roasted)	Nuts (roasted)	Peanuts	no	no	<LOQ
AOF-145	Nuts and oilseeds (roasted)	Nuts (roasted)	Peanuts	no	no	32.5
AOF-146	Nuts and oilseeds (roasted)	Nuts (roasted)	Peanuts	no	no	25.3
AOF-147	Nuts and oilseeds (roasted)	Nuts (roasted)	Mix	no	no	28.7
AOF-148	Nuts and oilseeds (roasted)	Nuts (roasted)	Macadamia	no	no	<LOD
AOF-149	Nuts and oilseeds (roasted)	Oilseeds (roasted)	Sunflower	no	no	<LOQ
AOF-150	Nuts and oilseeds (roasted)	Oilseeds (roasted)	Sunflower	no	no	<LOQ
AOF-151	Nuts and oilseeds (roasted)	Oilseeds (roasted)	Sesame	no	no	47.1
AOF-152	Nuts and oilseeds (roasted)	Oilseeds (roasted)	Mix of nuts	no	no	<LOD
AOF-153	Nuts and oilseeds (roasted)	Oilseeds (roasted)	Sunflower	no	no	155
AOF-154	Nuts and oilseeds (roasted)	Oilseeds (roasted)	Peanuts	no	no	42.1

Table continued on next page

Table B1 (continued)

AOF-044	Pastries	Viennoiserie	no	yes		32.5
AOF-045	Pastries	Viennoiserie	no	yes		24.9
AOF-046	Pastries	Viennoiserie	no	yes		25.4
AOF-047	Pastries	Viennoiserie	no	no	<LOD	
AOF-048	Pastries	Viennoiserie	no	no	<LOQ	
AOF-049	Pastries	Viennoiserie	no	no	<LOD	
AOF-050	Pastries	Viennoiserie	no	no	<LOD	
AOF-051	Pastries	Viennoiserie	no	no	<LOQ	
AOF-052	Pastries	Viennoiserie	no	no	<LOQ	
AOF-053	Pastries	Viennoiserie	no	no	<LOD	
AOF-054	Pastries	Viennoiserie	no	no	22.2	
AOF-055	Pastries	Viennoiserie	no	no	<LOD	
AOF-056	Pastries	Viennoiserie	no	no	<LOD	
AOF-057	Pastries	Viennoiserie	no	no	20.8	
AOF-058	Pastries	Donut	no	no	<LOD	
AOF-059	Pastries	Donut	no	no	<LOD	
AOF-060	Pastries	Donut	no	no	<LOD	
AOF-061	Pastries	Donut	no	no	<LOD	
AOF-062	Pastries	Donut	no	no	<LOD	
AOF-71	Pastries	Pancake	yes	no	28.2	
AOF-72	Pastries	Pancake	no	no	<LOQ	
AOF-73	Pastries	Pancake	no	no	<LOQ	
AOF-74	Pastries	Pancake	no	no	24.2	
AOF-75	Pastries	Pancake	no	no	<LOD	
AOF-76	Pastries	Beignet	no	no	<LOD	
AOF-77	Pastries	Beignet	no	no	<LOD	
AOF-78	Pastries	Churros	no	no	<LOD	
AOF-79	Pastries	Churros	no	no	<LOQ	
AOF-80	Pastries	Beignet	no	yes	31.3	
Add10	Pastries	Zakouski	no	yes	31.9	
Add8	Pastries	Zakouski	no	yes		23.8
Add9	Pastries	Zakouski	no	yes		<LOD
AOF-001	Potato based products other than fries	Rosti	no	yes		123

Table continued on next page

Table B1 (continued)

AOF-002	Potato based products other than fries	Rosti	no	yes	506	63.6
AOF-003	Potato based products other than fries	Rosti	no	yes	1199	216
AOF-006	Potato based products other than fries	Potato cubes	no	yes	559	
AOF-007	Potato based products other than fries	Duchesse potatoes	no	yes	381	<LOQ
AOF-008	Potato based products other than fries	Duchesse potatoes	no	yes	419	57.7
AOF-009	Potato based products other than fries	Duchesse potatoes	no	yes	755	27.7
AOF-010	Potato based products other than fries	Duchesse potatoes	no	yes	737	<LOQ
AOF-011	Potato based products other than fries	Croquettes	no	yes	133	
AOF-012	Potato based products other than fries	Croquettes	no	yes		370

Table continued on next page

Table B1 (continued)

AOF-013	Potato based products other than fries	Croquettes	no	yes		141
AOF-014	Potato based products other than fries	Croquettes	no	yes	292	
AOF-015	Potato based products other than fries	Croquettes	no	yes	57.3	
AOF-016	Potato based products other than fries	Croquettes	no	yes	142	
AOF-017	Potato based products other than fries	Croquettes	no	yes		377
AOF-018	Potato based products other than fries	Croquettes	no	yes	154	
AOF-019	Potato based products other than fries	Croquettes	no	no	107	
AOF-020	Potato based products other than fries	Croquettes	no	no	218	
AOF-021	Potato based products other than fries	Potato balls	no	yes	107	54.5

Table continued on next page

Table B1 (continued)

AOF-022	Potato based products other than fries	Potato balls	no	yes	1248	81
AOF-023	Potato based products other than fries	Potato balls	no	yes		224
AOF-024	Potato based products other than fries	Potato balls	no	yes	516	<LOQ
AOF-025	Potato based products other than fries	Potato balls	no	yes	172	<LOD
ADD1	Potato based products other than fries	Rosti	no	yes		140
ADD2	Potato based products other than fries	Potato cubes	no	yes	214	
ADD3	Potato based products other than fries	Potato cubes	no	yes	1503	
ADD4	Potato based products other than fries	Duchesse potatoes	no	yes	813	34.7
ADD5	Potato based products other than fries	Sweet potato fries	no	no	717	

Table continued on next page

Table B1 (continued)

ADD6	Potato based products other than fries	Sweet potato fries		no	no		514	
ADD7	Potato based products other than fries	Sweet potato fries		no	yes		981	46.7
Supp 12	Potato based products other than fries	Sweet potato fries		no	yes		876	
Supp 15	Potato based products other than fries	Croquettes		no	yes		1071	199
AOF-131	Vegetables chips		Taro / sweet potatoes / batata / tapioca / parsnip	no	no	238		
AOF-132	Vegetables chips		Beetroot / parsnip / sweet potatoes	no	no	2183		
AOF-133	Vegetables chips		Carrot / parsnip / beetroot / sweet potatoes	no	no	160		
AOF-134	Vegetables chips		Carrot / parsnip / beetroot / sweet potatoes	no	no	156		
AOF-135	Vegetables chips		Sweet potatoes	no	no	642		
AOF-136	Vegetables chips		Carrot / parsnip / beetroot / sweet potatoes	yes	no	1554		
AOF-137	Vegetables chips		Manioc	yes	no	3063		
AOF-138	Vegetables chips		Banana	yes	no	98		

B2 Validation of the acrylamide analytical method

B2.1. Validation protocol

Linearity and matrix effects

Height concentration points from 20 to 4000 µg kg^{-1} were used for calibration curves to investigate the linearity of the detector response. Slopes of matrix-matched calibration curves (Cereal-based babyfood, crisps, fried potato products, black olives, pancakes, pastries, oil seeds and nuts, dried fruits and confectionaries, cocoa powder) were compared with a calibration curve prepared in the neat solvent. According to the SANTE document (SANTE/12682/2019 2020), it can be concluded that there is no significant matrix effect if slopes differ less than ± 20 %. The ratio of the surface areas relative to acrylamide and acrylamide D3 was plotted on a graph as function of the acrylamide contentration.

Detection and quantification limits

Regulation (EU) 2017/2158 fixed a limit of quantification (LOQ) of 20 µg kg^{-1} and ≤ 50 µg kg^{-1} for food with a benchmark (BML) limit < 125 µg kg^{-1} and for food with BML ≥ 125 µg kg^{-1}, respectively. The limit of detection (LOD) is set at one-third of the LOQ. LOD and LOQ were both determined using four matrices (bread, nuts, black olive and nougat) with different physico-chemical properties contaminated at a level close to the expected LOQ, inducing a peak with a signal-to-noise (S/N) ratio of at least 3 for LOD and 10 for LOQ. S/Ns have been calculated using raw chromatograms for both quantifier and qualifier transitions.

Precision and recovery

All types of food included in this study were first grouped based on their nature and composition (e.g. water content, fat content, sugar content) (table B2 and **??**). Blank samples have been fortified at relevant levels based on the benchmark levels from the Regulation (EU) 2017/2158 (European Commission 2017). If no benchmark levels were set for a food category, fortification levels were chosen based on the typical concentration in the concerned food matrix. When no blank samples were available, the food item selected for validation without acrylamide fortification was analyzed on each day of validation. The average value obtained was subtracted from the value corresponding to the fortified sample to calculate the recovery. Methods have been validated according to 2 validation schemes (primary and secondary validations). For primary validations, precision was evaluated with fortified samples at three concentration levels on three different days with at least two different operators. The same approach was used for secondary validation but only at the lowest concentration level. A Cochran test has been used to identify outliers. The relative standard deviations for the repeatability (RSDr) and the within laboratory reproducibility (RSDRW) were calculated for the 3 levels as described by ISO 5725-2 guidelines (ISO 5725 2019).

Measurement uncertainty (MU)

The expanded uncertainty with a coverage factor of 95 % (k=2) was calculated using the following equation: U = |bias| + 2 RSDRW. Both bias and RSDRW data came from the control charts, intially generated from validation data. All matrices were merged on a control chart, except the categories 'coffee and coffee substitutes', and 'cocoa powder for beverages' for which a specific control chart was generated due to the specific protocol used

for the determination of acrylamide content. Depending on the intra-food category variability, different samples were taken to reflect the variability within a food category as much as possible to obtain a realistic measurement uncertainty and reflect the variety in the category.

Trueness

Finally, the trueness was assessed with 4 proficiency tests on potato chips, bread, cereal-based baby food, and coffee from the European Reference Laboratory for processing contaminants (EURL-PC) (formerly called EURL-PAH) or if absent for a category with an internal laboratory blind sample meaning a fortified sample of which the analyst doesn't know the concentration. The good method trueness was illustrated with a z-score inferior to ± |2| for proficiency tests and the match between the fortification level and the result, including the measurement uncertainty for the blind samples.

B2.2. Validation data

The linear model with a 1/x weighting factor was selected as all of the residuals were within ± 20 %. Matrix effects were less than 20 % for the four matrices tested using IL-IS acrylamide D3. Therefore, clean solvent calibration curves were selected (table B2). LOD and LOQ have been set at 7 µg kg^{-1} and 20 µg kg^{-1}, respectively. Seven primary validations and 3 secondary validations have been performed, and recoveries for all concentration levels met the EU criteria (70 – 110 %) and are close to 100 % (from 91.7 to 106 %)(table B3). All RSDr and RSDRW values were less than 15 %, even when 3 different matrices were used during each validation day, and met the EU criteria set at 14.5 % and 22 %, respectively (European Commission 2017). These results demonstrate the method's suitability for food categories with a wide range of characteristics. The practical expanded MU has been set at 15 % except for the categories "coffee, coffee substitutes and chocolate powder for beverages" (20 %). Finally, the method has been successfully tested on 4 proficiency tests (PTs) materials and 6 blind samples. Z-scores < |±1| were obtained for the 4 PTs, and results obtained ± the MU covered the fortified value in the case of blind samples (table 4.2). These extensive validation data demonstrate the added value of having a generic extraction step followed by a specific clean-up adapted for each food category. Finally, this data illustrates that the method is fit for purpose.

Table B2: Slope differences (%) between calibration curves in neat solvent and matrix with acrylamide D3 correction

Food category	Difference between neat solvent and matrix slopes (%)	Matrix effect (Yes/No)
Babyfood cereals	3.1	No
Crisps	0.1	No
Fried potato products	0	No
Black olives	16.7	No

Table continued on next page

Table B2 (continued)

Food category	Difference between neat solvent and matrix slopes (%)	Matrix effect (Yes/No)
Pancakes	-3.4	No
Pastries	-3.3	No
Oil seeds and nuts (roasted)	-3.6	No
Dried fruits and confectionaries	3.3	No
Cocoa powder	-9.3	No
Coffee and substitute coffee	-3.2	No

Table B3: Recovery and precision data for primary (n=9) and secondary (n=3) validations for acrylamide in food

Food	Fortified concentration (μg kg^{-1})	Average recovery (%)	RSDr (%)	RSDrw (%)
Black olives	50	97.3	12.4	13.0
Black olives	100	98.4	4.9	9.8
Black olives	200	103	3.6	4.6
Cereals in babyfood	25	97.6	3.7	6.6
Cereals in babyfood	50	100	7.4	7.4
Cereals in babyfood	200	106	4.7	4.7
Cocoa powder	25	106	2.8	3.6
Cocoa powder	50	102	2.0	2.3
Cocoa powder	100	98.0	2.1	5.3
Coffee and coffee substitute	20	97.0	5.9	6.2
Coffee and coffee substitute	100	94.0	3.7	6.2
Coffee and coffee substitute	200	94.0	2.1	3.1
Crisps	100	102	3.3	4.3
Crisps	250	102	3.5	3.8
Crisps	750	99.0	2.1	3.6
Dried fruits and confectionaries	50	99.7	7.7	7.7
Dried fruits and confectionaries	100	91.7	9.4	10.5
Dried fruits and confectionaries	200	103	2.7	5.1
Fried potato products*	250	102	2.2	2.2
Oil seeds and nuts (roasted)	50	97.8	6.8	10.7
Oil seeds and nuts (roasted)	100	98.0	4.0	4.0
Oil seeds and nuts (roasted)	200	98.8	2.6	5.3

Table continued on next page

Table B3 (continued)

Food	Fortified concentration (μg kg^{-1})	Average recovery (%)	RSDr (%)	RSDrw (%)
Pancakes*	100	99.0	1.4	1.7
Pastries*	100	101	3.0	4.7

*Secondary validation

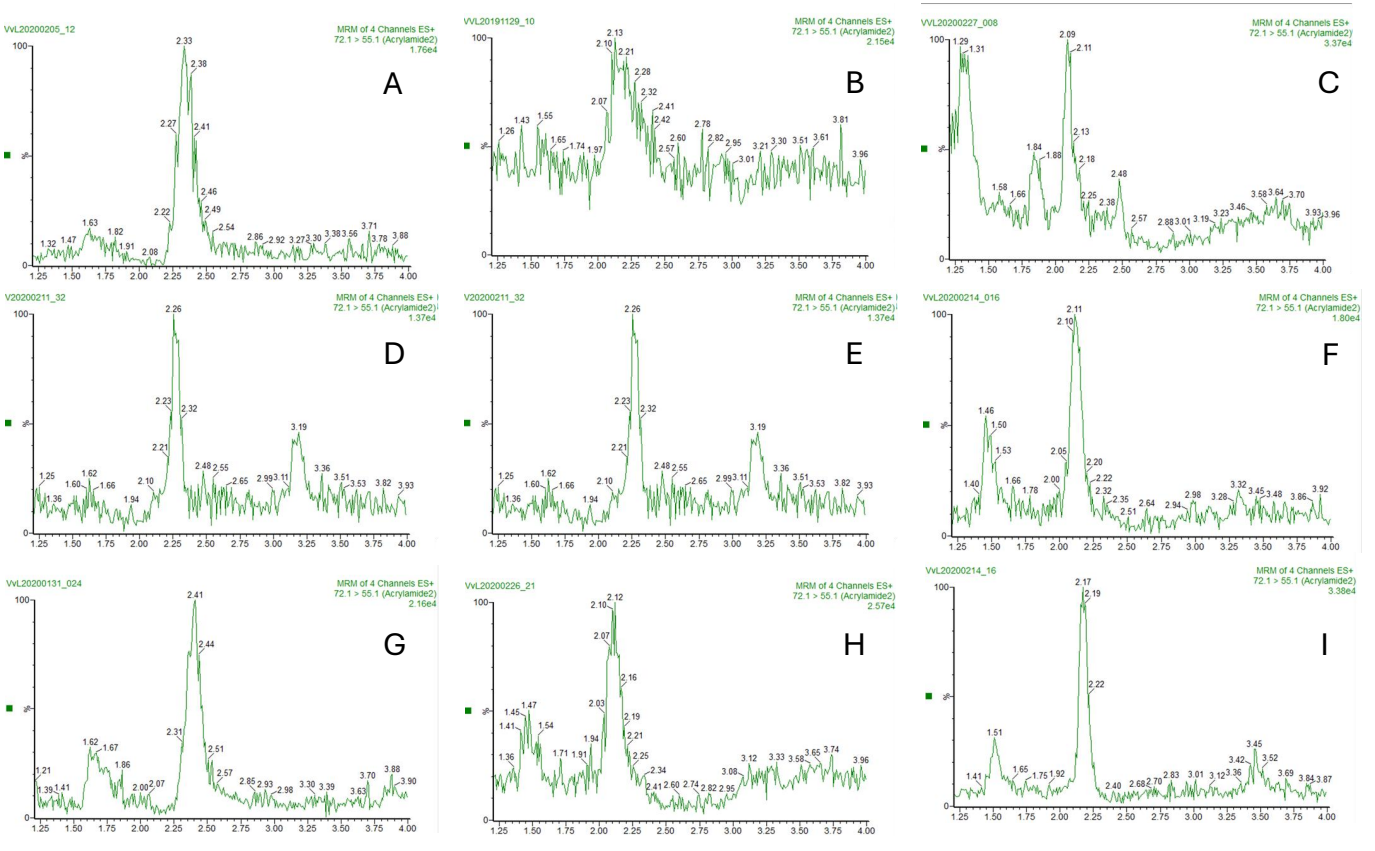


Figure B1: Acrylamide chromatogram at a level close to the LOQ for food categories investigated. Black olives at 31.9 μg kg^{-1} (A), bread buns at 21.2 μg kg^{-1} (B), cocoa powder at 27.5 μg kg^{-1} (C), coffee substitutes at 28.7 μg kg^{-1} (D), cookies at 21.7 μg kg^{-1} (E), nougat at 25.4 μg kg^{-1} (F), pastries at 20.8 μg kg^{-1} (G), duchesse potatoe at 27.7 μg kg^{-1} (H), roasted peanuts at 25.3 μg kg^{-1} (I)

B3 Questionnaire sent to participants for the study of Belgian cooking habits

Questionnaire dans le cadre de l'étude pilote sur les habitudes de cuisson de la population belge.

Merci d'avoir accepté de participer à cette petite étude pilote visant à recueillir certaines informations sur les habitudes de cuisson de la population belge. Un ou plusieurs aliments vous a été donné avec pour instruction de les cuire. Vous devez cuire ces aliments comme vous avez l'habitude de le faire, comme vous aimez les manger et ensuite répondre aux questions ci-dessous (1 questionnaire par échantillon).

Consignes et remarques:

Les sachets d'aliments vous sont offerts, nous demandons seulement de nous renvoyer dans les barquettes en plastiques fournies, les quantités suivantes :

- Croquettes : 5-6 croquettes
- Frites de patates douces : 10-12 frites
- Buns (pain burger) : 1 pain

Quand vous prélevez les aliments pour la barquette, essayez dans la mesure du possible de prendre des croquettes/frites à différents endroits du four (côtés, arrière, avant, milieu). Dans le cas de la friteuse, essayer de mélanger la portion avant de prélever la quantité adéquate. Si vous n'avez pas pour habitude de cuire des frites de patate douce, le mieux est de faire des tests sur des petites portions au préalable afin de juger si la cuisson est à votre convenance. En effet, leur cuisson diffère des frites traditionnelles.

Une fois les aliments cuits, vous pouvez nous les rapporter dans la barquette fournie. Si vous rendez les aliments prélevés, le lendemain, ceux-ci peuvent être stockés au frigo jusqu'au lendemain. Si vous devez les rendre dans un délai plus long, il est préférable de les stocker au congélateur en attendant.

Questions:

- Quel est le numéro qui vous a été attribué ?
- Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
- Quel était le mode de cuisson que vous avez utilisé ?
- Quelle était la température de cuisson ?
- Quelle a été la durée de la cuisson ?
- Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
- Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?

- Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
- Correspondent-elles à vos attentes d'un point de vue organoleptique ?
- Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez pas.
- Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
- A quelle fréquence changez-vous l'huile de votre friture ?
- Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
- Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
- Si vous avez répondu oui ou non, dites-nous pourquoi ?
- Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez.
- Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
- Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détailler et expliquer votre raisonnement/pratique.
- Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
- Comment avez-vous toasté l'aliment ? Était-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

B4 Questionnaires filled by participants during the study on Belgian cooking habits

Candidat 1 – croquettes - friteuse

Quel est le numéro qui vous a été attribué ?
1
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Croquette
Quel était le mode de cuisson que vous avez utilisé ?
Friteuse
Quelle était la température de cuisson ?
175 °C
Quelle a été la durée de la cuisson ?
6 min
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
Bien cuites
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
Bonnes
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Non
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
/
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
/
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
100% Tournesol
A quelle fréquence changez-vous l'huile de votre friture ?
Régulièrement
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Selon la quantité de friture
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
non
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

Candidat 2 – croquettes - four

Quel est le numéro qui vous a été attribué ?
2
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Croquettes
Quel était le mode de cuisson que vous avez utilisé ?
Four
Quelle était la température de cuisson ?
220 °C
Quelle a été la durée de la cuisson ?
17 min
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
Suffisamment dorées
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
La température est souvent correcte, le temps de cuisson souvent trop court
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Non
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
Pas toujours
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
Je n'utilise en général pas le four pour cuire ce genre d'aliments (moins croquant, cuisson plus irrégulière), mais dans ce cas précis, les instructions semblaient assez correctes
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
A quelle fréquence changez-vous l'huile de votre friture ?
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Chaleur tournante 220 °C, milieu du four
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Oui, particulièrement quand je n'ai pas l'habitude de la cuisson d'un aliment ou que le temps de cuisson est court et précis.
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Je retourne les aliments, pas la plaque, car les aliments sont sur la plaque et quand on la retourne, ils tombent. Lorsque l'aliment doit dorer, je les retourne pour qu'il soit doré de tous côtés.
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

Candidat 2 – frites de patate douce - friteuse

Quel est le numéro qui vous a été attribué ?
2
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Frites de patate douce
Quel était le mode de cuisson que vous avez utilisé ?
Friteuse
Quelle était la température de cuisson ?
170 °C
Quelle a été la durée de la cuisson ?
6,5 min
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
Dorées + croquantes
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
La température est souvent correcte, le temps de cuisson souvent trop court.
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Non
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
Pas toujours
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
Le temps de cuisson est régulièrement trop court et n'offre pas le croustillant attendu.
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
Mélange d'huile (palme/colza/tournesol)
A quelle fréquence changez-vous l'huile de votre friture ?
Environ toutes les 10 fritures
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Quand il y a des résidus dans la friteuse/quand ça fait trop longtemps qu'on a utilisé la friteuse, pour que je puisse me souvenir de quand l'huile date.
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
non
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Je ne les lis pas
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

Candidat 3 – frites de patate douce - four

Quel est le numéro qui vous a été attribué ?
3
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Frites de patate douce
Quel était le mode de cuisson que vous avez utilisé ?
Four
Quelle était la température de cuisson ?
220 °C
Quelle a été la durée de la cuisson ?
20 min
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
L'aspect croustillant
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
Consignes claires
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
oui
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
Même mieux qu'attendu
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
Les frites ne donnaient initialement pas spécialement envie une fois cuites car pas l'air très croustillantes. Au goût par contre, pas mal !
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
A quelle fréquence changez-vous l'huile de votre friture ?
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Chaleur tournante
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Oui pour que les frites soient « saisies »
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Je retourne les aliments (car si je retourne la plaque… les frites au sol… c'est pas très bon)
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

Candidat 3 – frites de patate douce - airfryer

Quel est le numéro qui vous a été attribué ?
3
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Frites de patate douce
Quel était le mode de cuisson que vous avez utilisé ?
airfryer
Quelle était la température de cuisson ?
180 °C
Quelle a été la durée de la cuisson ?
12 min
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
L'aspect croustillant
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
Consignes claires
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
oui
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
Même mieux qu'attendu
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
Les frites ne donnaient initialement pas spécialement envie une fois cuites car pas l'air très croustillantes. Au goût par contre, pas mal !
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
A quelle fréquence changez-vous l'huile de votre friture ?
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

Candidat 4 – croquettes - four

Quel est le numéro qui vous a été attribué ?
4
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Croquettes
Quel était le mode de cuisson que vous avez utilisé ?
Four
Quelle était la température de cuisson ?
220 °C
Quelle a été la durée de la cuisson ?
18 min
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
Couleur dorée Goût d'une croquette
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
Elles sont utiles pour avoir une estimation de la durée/T°C de cuisson, mais surtout indicatif
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Oui
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
En règle générale oui
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
Il arrive que l'aliment ne soit parfois pas assez cuit à l'intérieur. Donc je prolonge la cuisson.
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
A quelle fréquence changez-vous l'huile de votre friture ?
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Normal
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Oui, afin d'être certaine d'atteindre la température adéquate
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Oui, les aliments je les retourne si je constate que la cuisson n'est pas uniforme
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

Candidat 5 – croquettes - four

Quel est le numéro qui vous a été attribué ?
5
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Croquettes
Quel était le mode de cuisson que vous avez utilisé ?
Four
Quelle était la température de cuisson ?
220 °C
Quelle a été la durée de la cuisson ?
16 min
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
- Couleur des croquettes - J'ai piqué la pointe d'un couteau dans la croquette pour voir si c'était chaud à l'intérieur
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
Je les respectes, mais je reste vigilante et surveille la cuisson. Lors d'une utilisation ultérieure, j'adapte éventuellement te temps ou la température en fonction de mon expérience.
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Oui, la première fois toujours
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
Oui, la plupart du temps
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
Les croquettes sont ben croquantes
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
A quelle fréquence changez-vous l'huile de votre friture ?
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Normal J'ai respecté les consignes de temps et de chaleur indiquées sur le paquet
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Oui, car les aliments doivent être mis dans le four à la bonne température de cuisson
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Cela dépend des instructions du fabriquant. Dans le cas des croquettes, j'ai retourné les croquettes à mi-cuisson (comme conseillé sur l'emballage). Je l'ai fait au moyen d'une fourchette et de ma main pour aller plus vite et ne pas refroidir le four. Il est nécessaire de retourner les croquettes sinon, elles brunissent trop à la surface supérieur (cuisson pas uniforme).
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

Candidat 6 – croquettes - friteuse

Quel est le numéro qui vous a été attribué ?
6
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Croquette
Quel était le mode de cuisson que vous avez utilisé ?
Friteuse
Quelle était la température de cuisson ?
175 °C
Quelle a été la durée de la cuisson ?
4.5 min
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
La croute devait être dorée.
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
Nous suivons les annotations du mode de cuisson
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
oui
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
oui
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
- Elles (les croquettes) sont croustillantes et fondent en bouche - La vue des croquettes et leur couleur et odeur donne réellement envie de les déguster
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
Huile végétale pour friture (mélange huile de tournesol, colza et maïs)
A quelle fréquence changez-vous l'huile de votre friture ?
On utilise très peu la friteuse. Après 7 utilisation de l'huile, nous la changeons
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
La couleur devient brunâtre
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
oui
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Nous respectons les consignes ca de l'huile de friteuse brulée (ou chauffé trop fort, peut être cancérigène) => nous suivons de préférences, les consignes indiqués.
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

Candidat 7 – croquettes - four

Quel est le numéro qui vous a été attribué ?
7
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Croquettes
Quel était le mode de cuisson que vous avez utilisé ?
Four
Quelle était la température de cuisson ?
220 °C
Quelle a été la durée de la cuisson ?
22 min
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
A la couleur des croquettes
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
Je les lis toujours et j'adapte en fonction de mon four. Souvent, je laisse un peu plus longtemps que le temps indiqué pour la même température.
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Oui, mais en les modifiant à mon goût
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
Non, car je les aime plus cuites, plus croustillantes et je les ai laissées donc plus longtemps.
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
A quelle fréquence changez-vous l'huile de votre friture ?
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Normal 9 grill car nos n'avons pas de chaleur tournante.
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Oui car pour les produits surgelés, il est conseillé de « saisir » plutôt que de les cuire lentement
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Oui si c'est indiqué dans les consignes. Sinon, non
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

Candidat 7 – Buns - four

Quel est le numéro qui vous a été attribué ?
7
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Bun
Quel était le mode de cuisson que vous avez utilisé ?
Four - grill
Quelle était la température de cuisson ?
Grill – pas de température mentionnée
Quelle a été la durée de la cuisson ?
2 min de chaque côté du bun
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
La couleur du bun
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
Dans ce cas, avec les buns, je n'en tiens pas compte. Je le fais au feeling
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Pour certaines cuissons oui, mais pas pour les buns
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
Souvent j'ajoute du temps de cuisson ou la température du fou
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
J'aime quand les ingrédients sont bien cuits
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
A quelle fréquence changez-vous l'huile de votre friture ?
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Grill
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Oui
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Oui. Je retourne à la fois les aliments et la plaque de cuisson à mi-cuisson
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

Candidat 8 – Buns - toaster

Quel est le numéro qui vous a été attribué ?
8
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Bun
Quel était le mode de cuisson que vous avez utilisé ?
toaster
Quelle était la température de cuisson ?
-
Quelle a été la durée de la cuisson ?
2 min
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
La couleur et le croustillant
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
Peu d'instructions au niveau du bun, mais cela reste clair pour des buns que l'on doit juste toaster
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Oui lorsque nous testons le produit pour la première fois, puis nous adaptons
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
D'une manière générale, oui
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
Le bun était bien croquant après avoir été toasté sans avoir été carbonisé
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
A quelle fréquence changez-vous l'huile de votre friture ?
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Notre toaster n'a pas vraiment de mode. Nous pouvons choisir la durée de « toastage » de l'aliment, entre 2 et 5 min. Nous avons mis notre toaster sur 2 et couplé le bun en 2
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?
L'aliment a été placé à l'intérieur du toaster

Candidat 8 – frites de patate douce - four

Quel est le numéro qui vous a été attribué ?
8
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Frite de patate douce
Quel était le mode de cuisson que vous avez utilisé ?
Four
Quelle était la température de cuisson ?
220 °C
Quelle a été la durée de la cuisson ?
10-12 min (moins que les 15-20 prévues)
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
Le fait que certaines commencent à roussir. Nous les avons goûtées
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
Les instructions présentes au dos étaient claires. Néanmoins, seul le logo friteuse, four figurait sur le devant sans durée ni température, ce qui est (-) pour la visibilité/pratique
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Oui lorsque nous testons le produit pour la première fois, puis nous adaptons
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
D'une manière générale, oui
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
Les frites de patate douce avaient un goût très agréable mais le côté croustillant n'était pas présent.
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
A quelle fréquence changez-vous l'huile de votre friture ?
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Nous avons utilisé le mode chaleur tournante dit turbo pizza. Pour cuire sur un gradin des mets nécessitant un brunissage intensif et un fond croustillant. C'est notre mode de cuisson habituel pour n'importe quel met au four.
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Oui pour une cuisson plus rapide
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Nous n'avons pas retourné l'aliment malgré les instructions du fournisseur. Nous n'avons pas eu le temps car en 10 min les petites frites étaient cuites et commençaient à roussir. Après, nous avons donc arrêter la cuisson suite à un test de goût. Néanmoins les plus grosses et longues frites semblaient moins cuites à la vue. Au goût, elles étaient très bonnes bien que un peu molle. La couleur est aussi à notre goût.
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

Candidat 9 – croquettes - friteuse

Quel est le numéro qui vous a été attribué ?
9
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Croquettes
Quel était le mode de cuisson que vous avez utilisé ?
Friteuse
Quelle était la température de cuisson ?
175 °C
Quelle a été la durée de la cuisson ?
8 min
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
Je vérifie si l'huile bout et si les aliments sont assez bruns ou changent de couleur
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
Je pense que ce n'est pas assez long
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Non
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
oui
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
Les croquettes étaient assez cuites, chaudes et croquantes
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
Mélange tournesol et colza
A quelle fréquence changez-vous l'huile de votre friture ?
+/- après l'avoir utilisé 15 fois
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Si l'huile devient brune et si il y a beaucoup de résidu sur le fond
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Non
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Je ne l'ai jamais vérifié ca je ne savais pas qu'il y avait des consignes concernant l'utilisation sur l'emballage.
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

Candidat 9 – frites de patate douce - friteuse

Quel est le numéro qui vous a été attribué ?
9
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Frites de patate douce
Quel était le mode de cuisson que vous avez utilisé ?
Friteuse
Quelle était la température de cuisson ?
170 °C
Quelle a été la durée de la cuisson ?
6.5 min
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
Je vérifie si l'huile bout et si les aliments sont assez bruns ou changent de couleur
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
Je pense que ce n'est pas assez long. Il y avait marqué 175°C pour 3 min. je préfère des frites un peu croquantes donc 3 min ce n'est pas assez.
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Non
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
oui
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
Les frites doivent être cuites, chaudes et croquantes. Sauf qu'elles n'étaient pas aussi croquantes comme j'espérais.
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
Mélange tournesol et colza
A quelle fréquence changez-vous l'huile de votre friture ?
+/- après l'avoir utilisé 15 fois
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Si l'huile devient brune et si il y a beaucoup de résidu sur le fond
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Non
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Je ne l'ai jamais vérifié ca je ne savais pas qu'il y avait des consignes concernant l'utilisation sur l'emballage.
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

Candidat 10 – Croquettes - friteuse

Quel est le numéro qui vous a été attribué ?
10
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Croquettes
Quel était le mode de cuisson que vous avez utilisé ?
Friteuse
Quelle était la température de cuisson ?
180 °C
Quelle a été la durée de la cuisson ?
4 min, croquette un peu décongelées
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
- Le chrono - L'apparence un peu plus foncée à l'extérieur
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
Utile pour les premières cuissons, après on retient
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Oui
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
oui
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
Croustillant à l'extérieur, bien rempli, bon goût, bonne texture
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
Huile végétale 99% (tournesol et tournesol à haute teneur en acide oléique
A quelle fréquence changez-vous l'huile de votre friture ?
~4x/an (~toutes les 10 cuissons)
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
- Couleur + foncée - Beaucoup de dépôts dans le fond
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Oui
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Oui : on fait comme depuis toujours et cela correspond aux consignes. La qualité du produit nous convient
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

Candidat 10 – frites de patate douce - friteuse

Quel est le numéro qui vous a été attribué ?
10
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Frites de patate douce
Quel était le mode de cuisson que vous avez utilisé ?
Friteuse
Quelle était la température de cuisson ?
180 °C
Quelle a été la durée de la cuisson ?
2 min, les frites étaient un peu décongelées
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
- Le chrono - L'apparence un peu plus foncée à l'extérieur
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
Utile pour les premières cuissons, après on retient
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Oui
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
Oui. Croustillant à l'extérieur. Un peu de sel a été rajouté post-cuisson
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
Bon goût
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
Huile végétale 99% (tournesol et tournesol à haute teneur en acide oléique
A quelle fréquence changez-vous l'huile de votre friture ?
~4x/an (~toutes les 10 cuissons)
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
- Couleur + foncée - Beaucoup de dépôts dans le fond
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Oui
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Oui : on fait comme depuis toujours et cela correspond aux consignes. La qualité du produit nous convient
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

Candidat 10 – buns - toaster

Quel est le numéro qui vous a été attribué ?
10
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Buns
Quel était le mode de cuisson que vous avez utilisé ?
Toaster
Quelle était la température de cuisson ?
-
Quelle a été la durée de la cuisson ?
1 min
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
A quelle fréquence changez-vous l'huile de votre friture ?
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Non, sur l'échelle de 1 à 8, nous sommes à 4. J'ai arrêté manuellement pour contrôler la cuisson. J'arrêt quand les pains sont bien chauds et légèrement croustillants/dures, dans ce cas-ci, 1min
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?
A l'intérieur

Candidat 11 – croquettes - four

Quel est le numéro qui vous a été attribué ?
11
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Croquettes
Quel était le mode de cuisson que vous avez utilisé ?
Four
Quelle était la température de cuisson ?
220 °C
Quelle a été la durée de la cuisson ?
16 – 17 min
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
Temps indiqué sur l'emballage écoulé + croquettes dorées
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
Utiles
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Oui
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
Oui
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
Croquettes suffisamment dorées et croquantes à l'extérieur et cuites/chaudes à l'intérieur
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
A quelle fréquence changez-vous l'huile de votre friture ?
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Chaleur tournante
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Oui
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Non (si j'utilise le mode chaleur tournante) puisque celui-ci est censé cuire de façon homogène.
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

Candidat 12 – croquettes - four

Quel est le numéro qui vous a été attribué ?
12
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Croquettes
Quel était le mode de cuisson que vous avez utilisé ?
Four
Quelle était la température de cuisson ?
220 °C
Quelle a été la durée de la cuisson ?
16 min
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
Instructions de préparation
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
C'est une bonne indication de base
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Oui (dans un premier temps)
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
Oui
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
Les croquettes avaient une couleur orange dorée très appétissante et une texture agréable
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
A quelle fréquence changez-vous l'huile de votre friture ?
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Chaleur tournante
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Oui, afin de démarrer à la bonne température et que la durée/température de cuisson correspondent aux indications fournisseurs/producteurs
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
A mi-cuisson, les aliments sont retournés. Après 8 min de cuisson 1.5 min pour tout retourner à la spatule 8 minutes de cuisson
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

Candidat 12 – frites de patate douce - four

Quel est le numéro qui vous a été attribué ?
12
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
frites de patate douce
Quel était le mode de cuisson que vous avez utilisé ?
Four
Quelle était la température de cuisson ?
220 °C
Quelle a été la durée de la cuisson ?
17 min
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
Couleur de l'aliment Texture de l'aliment (mou ou encore ferme)
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
C'est une bonne indication de base
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Oui (dans un premier temps)
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
Oui
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
Les frites de patate douce sentaient très bon à la cuisson. Une couleur un peu orange-rouge qui laissait supposer une couche d'épices comme du paprika. Très surprenante au goût puisque l'apparence épicée laissait place à un goût sucré et doux et non pas épicé.
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
A quelle fréquence changez-vous l'huile de votre friture ?
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Chaleur tournante
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Oui, afin de démarrer à la bonne température et que la durée/température de cuisson correspondent aux indications fournisseurs/producteurs
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
15 min de cuisson + 2 min extra Pas de retournement des frites à mi-cuisson (trop fastidieux)
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

Candidat 13 – croquettes - four

Quel est le numéro qui vous a été attribué ?
13
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Croquettes
Quel était le mode de cuisson que vous avez utilisé ?
Four
Quelle était la température de cuisson ?
190 °C
Quelle a été la durée de la cuisson ?
16 min
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
A la couleur + timer
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
C'est une base à suivre et à adapter si nécessaire
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Oui
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
Oui, après le temps indiqué, les croquettes avaient l'aspect désiré, elles étaient dégelées entièrement sans être trop cuites. Il est important qu'elles ne soient pas trop foncées. Elles étaient croustillantes à l'extérieur et molle à l'intérieur sans être trop sèches.
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
A quelle fréquence changez-vous l'huile de votre friture ?
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Chaleur tournante
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Oui, car telles furent les consignes
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Oui, on retourne la plaque de cuisson car le four ne chauffe pas uniformément malgré la chaleur tournante0 On a aussi retournée les croquettes à mi-cuisson pour qu'elles se dorent des 2 côtés.
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

Candidat 13 – buns - toaster

Quel est le numéro qui vous a été attribué ?
13
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Buns
Quel était le mode de cuisson que vous avez utilisé ?
Toaster
Quelle était la température de cuisson ?
Niveau 2/6
Quelle a été la durée de la cuisson ?
-
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
A l'apparence + fin du timer
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
C'est une base à suivre et à adapter si nécessaire
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Oui
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
Non, les consignes sont très vagues. Pas de temps, ni de température, 2 modes de cuisson possibles. Contrôle constant pour éviter que le pain ne brûle. Nous désirons que le pain soit légèrement grillé/brun d'un seul des 2 côtés.
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
A quelle fréquence changez-vous l'huile de votre friture ?
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Niveau 2/6. Pas de mode spécifique
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?
Sur le dessus, car ils sont trop épais pour aller dedans et qu'on les souhaite grillé d'un seul côté. Ils ont donc grillé sur une ligne.

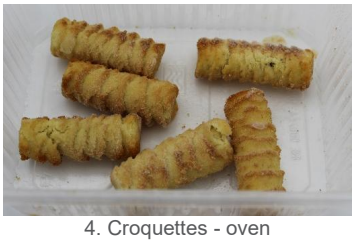
Candidat 14 – croquettes - four

Quel est le numéro qui vous a été attribué ?
14
Quel aliment avez-vous cuit pour cette étude ? (croquette, frite de patate douce, bun)
Croquettes
Quel était le mode de cuisson que vous avez utilisé ?
Four
Quelle était la température de cuisson ?
180 °C
Quelle a été la durée de la cuisson ?
29 min après avoir préchauffé le four à 200 °C
Quels étaient vos critères pour estimer que la cuisson devait être arrêtée ?
Couleur doit être dorée
Sur les emballages des aliments à cuire/frire, il y a souvent des annotations spécifiant le mode de cuisson, la température et la durée de cuisson. Que pensez-vous de ces annotations ?
Elles servent selon moi aux premiers essais. Après une première cuisson, on a compris et on s'adapte pour la fois suivante
Avez-vous pour habitudes de suivre ces consignes ? (oui/non)
Non
Correspondent-elles à vos attentes d'un point de vue organoleptique ?
Non, souvent pas assez cuit ou trop gras quand ce sont les aliments/graisses à ajouter.
Si oui ou non, pourquoi ? Vous pouvez donner un maximum de détails, n'hésitez-pas.
J'aime qu'on m'aiguille/qu'on me donne des exemples de comment procéder, mais je n'aime pas avoir l'impression de suivre des ordres ou exécuter. J'aime apporter ma touche et faire quelque chose de spécial, autre que ce que les instructions standards me préconisent.
Si vous avez utilisé une friteuse pour cuire vos aliments, quelle huile avez-vous utilisée ? (huile de tournesol, colza, mélange, autres ?)
A quelle fréquence changez-vous l'huile de votre friture ?
Quel est votre critère pour juger qu'une huile n'est plus bonne et que vous décidez de la changer ?
Respectez-vous les consignes sur l'emballage de votre bouteille d'huile ? (oui/non)
Si vous avez répondu oui ou non, dites-nous pourquoi ?
Si vous avez utilisé un four, quel mode de cuisson du four avez-vous utilisé ? (normal, chaleur tournante, grill, autres, mix de mode ?) Détaillez
Mode normal, standard. J'utilise le grill si je suis pressée. Je n'utilise jamais la chaleur tournante car cela dessèche les aliments. Je place toujours les aliments sur une grille (jamais sur la plaque) pour que ça cuise plus vite, et je place toujours la grille ans la moitié supérieure du four.
Utilisez-vous un four préchauffé ? (oui/non, pourquoi ?)
Oui, pour repère temporel et pour saisir l'aliment (idem pour cuire de la viande, je la pose sur la poêle déjà chaude).
Retournez-vous les aliments lors de la cuisson ou retournez-vous la plaque de cuisson ? N'hésitez pas à détaillé et expliquer votre raisonnement/pratique.
Je retourne les aliments à mi-temps pour éviter que le dessous colle au plat, et pour que la cuisson soit homogène (même couleur et même texture sur tous les côtés de l'aliment).
Si vous avez utilisé un toaster, utilisez-vous un mode spécial sur votre toaster ? Ces modes ne sont pas toujours standardisés, décrivez-nous dans la mesure du possible le mode que vous avez utilisé.
Comment avez-vous toasté l'aliment ? Etait-il placé à l'intérieur du toaster ? Sur le dessus ? autre ?

B5 Pictures of samples cooked by participants

1.Croquettes - oven

2.Croquettes - oven



4. Croquettes - oven

5. Croquettes - oven

6. Croquettes – deep fryer

7.Croquettes - oven

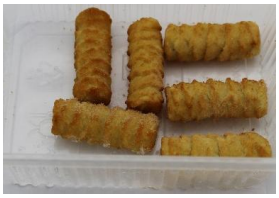
9. Croquettes – deep fryer

10.Croquettes – deep fryer

11. Croquettes - oven

12. Croquettes - oven

13. Croquettes - oven



14. Croquettes - oven

2.Fries – deep fryer

3.Fries - oven

3.Fries - airfryer

8. Fries - oven

9. Fries – deep fryer

10.Fries – deep fryer

11.Fries - oven

12.Fries - oven

Frozen fries -lot:CL4919257


7. Buns - toaster


8. Buns - toaster


10. Buns - toaster


13. Buns - toaster

B6 Food categorization and food grouping for acrylamide exposure

Acrofood_groups	Category_project			Count
apero	Pâtes feuilletées -Apé	Add10	Aperitiefhapje,bladerdeeghapje	1
		Add8	Aperitiefhapje,bladerdeeghapje	1
		Add9	Aperitiefhapje,mini-pasteitje	1
apero Total				**3**
bakery	croissants	AOF-045	Koffiekoek, croissant	1
	mix viennoiseries	AOF-044	Brioche n.s. / Koffiekoekn.s.	1
		AOF-046	Brioche n.s. / Koffiekoekn.s.	1
	viennoiseries/couques	AOF-047	Brioche, croissant	1
		AOF-048	Brioche, withchocolatebars	1
		AOF-049	Brioche,"Suisse"/serpentinelong/round	1
		AOF-050	Koffiekoek n.s.	1
		AOF-051	Brioche, croissant	1
		AOF-052	Brioche, withchocolatebars	1
		AOF-053	Koffiekoek n.s.	1
		AOF-054	Brioche, withchocolatebars	1
		AOF-055	Brioche, with raisins	1
		AOF-056	Brioche,"Suisse"/serpentinelong/round	1
		AOF-057	Brioche, croissant	1
bakery Total				**14**
BEIGNET	Beignet	AOF-76	Oliebol	1
		AOF-77	Oliebol	1
	Beignet fait maison	AOF-80	Oliebol	1
	Churros	AOF-78	Doughnutball	1
		AOF-79	Doughnutball	1
	donut/beignet-fourré	AOF-058	donut	1
		AOF-059	Beignet, fruit-	1
		AOF-060	Berlijnse bol, metpatisseriecreme	1
		AOF-061	donut	1
		AOF-062	Beignet, fruit-	1
BEIGNET Total				**10**
BREAD	Pain à base de graine	AOF-181	Brood, Bioform-	1
		AOF-182	Brood, glutenvrij	1
		AOF-183	Brood volkoren-	1
		AOF-184	Brood, rogge-, gewoon	1
		AOF-185	Brood, spelt-	1
		AOF-186	Brood, meergranen	1
	pain pita	AOF-036	Pitabroodje, wit	1
		AOF-037	Pitabroodje, wit	1
		AOF-038	Pitabroodje, wit	1
		AOF-039	Pitabroodje, wit	1
	Pains spéciaux (pain	AOF-065	Brood, rogge-, gewoon	1
		AOF-066	Brood, rogge-, gewoon	1
		AOF-068	Brood, rogge-, gewoon	1
		AOF-069	Brood bruin, met (noten,rozijnen, ...)	1
	Tortilla	AOF-040	Tortilla brood ongevuld	1
		AOF-041	Tortilla brood ongevuld	1
		AOF-042	Tortilla brood ongevuld	1
		AOF-043	Tortilla brood ongevuld	1
		AOF-200	Bread Tortilla, unfilled	1
BREAD Total				**19**
BREAD_f	afsca/favv	AFSCA_0465	(blank)	1
		AFSCA_0474	(blank)	1
		AFSCA_0477	(blank)	1
		AFSCA_0480	(blank)	1
		AFSCA_0487	(blank)	1
		AFSCA_0498	(blank)	1
		AFSCA_0540	(blank)	1
		AFSCA_0670	(blank)	1
		AFSCA_0678	(blank)	1
		AFSCA_0707	(blank)	1
		AFSCA_0718	(blank)	1
		AFSCA_0722	(blank)	1
		AFSCA_0729	(blank)	1
		AFSCA_0736	(blank)	1
		AFSCA_0744	(blank)	1
		AFSCA_0756	(blank)	1
		AFSCA_0757	(blank)	1
		AFSCA_0774	(blank)	1
		AFSCA_0807	(blank)	1
		AFSCA_0810	(blank)	1

BREAD_f	afsca/favv	AFSCA_0823	(blank)	1
		AFSCA_0843	(blank)	1
		AFSCA_0963	(blank)	1
		AFSCA_0975	(blank)	1
		AFSCA_0980	(blank)	1
		AFSCA_1001	(blank)	1
		AFSCA_1002	(blank)	1
		AFSCA_1034	(blank)	1
		AFSCA_1049	(blank)	1
		AFSCA_1058	(blank)	1
		AFSCA_1059	(blank)	1
		AFSCA_1072	(blank)	1
		AFSCA_1090	(blank)	1
		AFSCA_1091	(blank)	1
		AFSCA_1092	(blank)	1
		AFSCA_1093	(blank)	1
BREAD_f Total				**36**
Bread_soft	pain au lait	AOF-034	Broodje zacht, wit, melk-	1
		AOF-035	Broodje zacht, wit, melk-	1
	pain burger	AOF-031	Broodje zacht, wit,hamburger-	1
		AOF-032	Broodje zacht, wit,hamburger-	1
		AOF-033	Broodje zacht, wit,hamburger-	1
Bread_soft Total				**5**
BREAD_SPEC	Pains spéciaux (pain	AOF-063	Brood wit, met (chocolade,rozijnen, suiker, ...) Breadwhite, with (chocolate,raisins, s	1
		AOF-064	Brood wit, met (chocolade,rozijnen, suiker, ...) Breadwhite, with (chocolate,raisins, s	1
		AOF-067	Brood wit, met (chocolade,rozijnen, suiker, ...) Breadwhite, with (chocolate,raisins, s	1
		AOF-070	Bread white, with(chocolate, raisins,sugar,…)	1
BREAD_SPEC Total				**4**
cacao	Fèves de cacao grille	AOF-161	Cacaopoeder en analoogproduct	1
		AOF-162	Cacaopoeder en analoogproduct	1
		AOF-163	Cacaopoeder en analoogproduct	1
		AOF-164	Cacaopoeder en analoogproduct	1
		AOF-165	Cacaopoeder en analoogproduct	1
cacao Total				**5**
CHIA	biscottes	AOF-193	Knackebrot, cracotte,crispbread, filled salty	1
		AOF-194	Knackebrot, cracotte,crispbread, filled salty	1
	Cookies	AOF-187	Koekje zonder chocolade,zanddeeg Biscuit withoutchocolate, shortbreadpastry	1
		AOF-188	Bar, cereal-, muesli based	1
		AOF-189	Bar, cereal-, muesli based	1
		AOF-190	Bar, cereal-, muesli based	1
		AOF-191	Biscuit without chocolate,shortbread	1
		AOF-192	Koekje basis/methaver(zemelen) Biscuitbase/with oat(bran)	1
		AOF-195	Bar, cereal-, muesli based	1
		AOF-196	Bar, cereal-, muesli based	1
		AOF-198	Bar, cereal-, muesli based	1
CHIA Total				**11**
CHIPS_f	afsca/favv	AFSCA_0633	(blank)	1
		AFSCA_0637	(blank)	1
		AFSCA_0638	(blank)	1
		AFSCA_0640	(blank)	1
		AFSCA_0642	(blank)	1
		AFSCA_0645	(blank)	1
		AFSCA_0661	(blank)	1
		AFSCA_0665	(blank)	1
		AFSCA_0676	(blank)	1
		AFSCA_0677	(blank)	1
		AFSCA_0741	(blank)	1
		AFSCA_0748	(blank)	1
		AFSCA_0749	(blank)	1
		AFSCA_0754	(blank)	1
		AFSCA_0755	(blank)	1
		AFSCA_0783	(blank)	1
		AFSCA_0784	(blank)	1
		AFSCA_0788	(blank)	1
		AFSCA_0793	(blank)	1
		AFSCA_0794	(blank)	1
		AFSCA_0821	(blank)	1
		AFSCA_0829	(blank)	1
		AFSCA_0830	(blank)	1
		AFSCA_0834	(blank)	1
		AFSCA_0835	(blank)	1

CHIPS_f	afsca/favv	AFSCA_0836	(blank)	1
		AFSCA_0845	(blank)	1
		AFSCA_0846	(blank)	1
		AFSCA_0847	(blank)	1
		AFSCA_0873	(blank)	1
		AFSCA_0874	(blank)	1
		AFSCA_0875	(blank)	1
		AFSCA_0892	(blank)	1
		AFSCA_0897	(blank)	1
		AFSCA_0938	(blank)	1
		AFSCA_0943	(blank)	1
		AFSCA_0944	(blank)	1
		AFSCA_0945	(blank)	1
		AFSCA_0991	(blank)	1
		AFSCA_0992	(blank)	1
		AFSCA_0993	(blank)	1
		AFSCA_0994	(blank)	1
		AFSCA_1030	(blank)	1
		AFSCA_1042	(blank)	1
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		AFSCA_1064	(blank)	1
		AFSCA_1075	(blank)	1
		AFSCA_1088	(blank)	1
		AFSCA_1089	(blank)	1
		AFSCA_1109	(blank)	1
		AFSCA_1113	(blank)	1
		AFSCA_1114	(blank)	1
		AFSCA_1115	(blank)	1
		AFSCA_1116	(blank)	1
		AFSCA_1125	(blank)	1
CHIPS_f Total				**57**
CHIPS_grain	Chips	AOF-199	Crisps n.s.	1
	Cookies	AOF-197	Crisps n.s.	1
	Snacks de céréales (I	AOF-101	Corn crisps/tortilla chips	1
		AOF-102	Chipsachtigen, "mais"	1
		AOF-103	Chipsachtigen, "mais"	1
		AOF-104	Corn crisps/tortilla chips	1
		AOF-105	Chipsachtigen, "mais"	1
		AOF-106	"Crisps" n.s.	1
		AOF-110	Chipsachtigen, "mais"	1
		AOF-112	Corn crisps/tortilla chips	1
		AOF-113	Corn crisps/tortilla chips	1
		AOF-114	Corn crisps/tortilla chips	1
		AOF-115	"Crisps" corn	1
		AOF-117	Rice cracker, rice basedand other grains	1
		AOF-118	"Crisps" n.s.	1
CHIPS_grain Total				**15**
CHIPS_other	chips de légumes	AOF-137	"Crisps" n.s.	1
		AOF-138	"Crisps" n.s.	1
CHIPS_other Total				**2**
CHIPS_vegi	chips de légumes	AOF-131	Crisps n.s.	1
		AOF-132	"Crisps" n.s.	1
		AOF-133	"Crisps" n.s.	1
		AOF-134	"Crisps" n.s.	1
		AOF-135	"Crisps" n.s.	1
		AOF-136	"Crisps" n.s.	1
CHIPS_vegi Total				**6**
chips_vegi_f	afsca/favv	AFSCA_1012	(blank)	1
		AFSCA_1013	(blank)	1
		AFSCA_1014	(blank)	1
		AFSCA_1015	(blank)	1
chips_vegi_f Total				**4**
cof_chic_f	afsca/favv	AFSCA_0471	(blank)	1
		AFSCA_0473	(blank)	1
		AFSCA_0519	(blank)	1
		AFSCA_0537	(blank)	1
		AFSCA_0538	(blank)	1
		AFSCA_0545	(blank)	1
		AFSCA_0554	(blank)	1

cof_chic_f	afsca/favv	AFSCA_0557 (blank)	1
		AFSCA_0558 (blank)	1
		AFSCA_0571 (blank)	1
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		AFSCA_0801 (blank)	1
		AFSCA_0832 (blank)	1
		AFSCA_0861 (blank)	1
		AFSCA_0896 (blank)	1
		AFSCA_0911 (blank)	1
		AFSCA_0924 (blank)	1
		AFSCA_0946 (blank)	1
		AFSCA_1043 (blank)	1
		AFSCA_1046 (blank)	1
cof_chic_f Total			**25**
coffee_f	afsca/favv	AFSCA_0489 (blank)	1
		AFSCA_0493 (blank)	1
		AFSCA_0495 (blank)	1
		AFSCA_0496 (blank)	1
		AFSCA_0497 (blank)	1
		AFSCA_0503 (blank)	1
		AFSCA_0507 (blank)	1
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		AFSCA_0535 (blank)	1
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		AFSCA_0809 (blank)	1
		AFSCA_0811 (blank)	1
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		AFSCA_0822 (blank)	1
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		AFSCA_0899 (blank)	1
		AFSCA_0904 (blank)	1
		AFSCA_0907 (blank)	1
		AFSCA_0908 (blank)	1
		AFSCA_0909 (blank)	1

coffee_f	afsca/favv	AFSCA_0910	(blank)	1
		AFSCA_0913	(blank)	1
		AFSCA_0917	(blank)	1
		AFSCA_0923	(blank)	1
		AFSCA_0941	(blank)	1
		AFSCA_0959	(blank)	1
		AFSCA_0961	(blank)	1
		AFSCA_0964	(blank)	1
		AFSCA_0976	(blank)	1
		AFSCA_0978	(blank)	1
		AFSCA_0979	(blank)	1
		AFSCA_0981	(blank)	1
		AFSCA_0982	(blank)	1
		AFSCA_0983	(blank)	1
		AFSCA_0984	(blank)	1
		AFSCA_0995	(blank)	1
		AFSCA_1004	(blank)	1
		AFSCA_1016	(blank)	1
		AFSCA_1018	(blank)	1
		AFSCA_1019	(blank)	1
coffee_f Total				**72**
CRACKERS	maize crackers(galett	AOF-091	"Crisps" corn	1
		AOF-092	"Crisps" corn	1
		AOF-093	"Crisps" corn	1
		AOF-094	"Crisps" corn	1
		AOF-095	"Crisps" corn	1
		AOF-096	"Crisps" corn	1
		AOF-097	"Crisps" corn	1
		AOF-098	"Crisps" corn	1
		AOF-099	"Crisps" corn	1
		AOF-100	"Crisps" corn	1
	Rice crackers (galette	Add11	Wafel, rijst-, basis rijst	1
	rice crackers (galettes	AOF-081	Rice cracker, rice based	1
		AOF-082	Rice cracker, rice based	1
		AOF-083	Rice cracker, rice based	1
		AOF-084	Rice cracker, rice based	1
		AOF-085	Rice cracker, rice based	1
		AOF-086	Rice cracker, rice based	1
		AOF-087	Rice cracker, rice based	1
		AOF-088	Rice cracker, rice based	1
		AOF-089	Rice cracker, rice based	1
		AOF-090	Rice cracker, rice based	1
	Snacks de céréales (I	AOF-116	Toast small, mini cracker	1
CRACKERS Total				**22**
cracotte_f	afsca/favv	AFSCA_0470	(blank)	1
		AFSCA_0575	(blank)	1
		AFSCA_0576	(blank)	1
		AFSCA_0580	(blank)	1
		AFSCA_0581	(blank)	1
		AFSCA_0582	(blank)	1
		AFSCA_0585	(blank)	1
		AFSCA_0587	(blank)	1
		AFSCA_0588	(blank)	1
		AFSCA_0589	(blank)	1
		AFSCA_0592	(blank)	1
		AFSCA_0596	(blank)	1
		AFSCA_0602	(blank)	1
		AFSCA_0603	(blank)	1
		AFSCA_0604	(blank)	1
		AFSCA_0607	(blank)	1
		AFSCA_0610	(blank)	1
		AFSCA_0613	(blank)	1
		AFSCA_0614	(blank)	1
		AFSCA_0624	(blank)	1
		AFSCA_0639	(blank)	1
		AFSCA_0644	(blank)	1
		AFSCA_0649	(blank)	1
		AFSCA_0653	(blank)	1
		AFSCA_0654	(blank)	1
		AFSCA_0668	(blank)	1
		AFSCA_0669	(blank)	1

cracotte_f	afsca/favv	AFSCA_0671	(blank)	1
		AFSCA_0673	(blank)	1
		AFSCA_0679	(blank)	1
		AFSCA_0680	(blank)	1
		AFSCA_0685	(blank)	1
		AFSCA_0691	(blank)	1
		AFSCA_0699	(blank)	1
		AFSCA_0700	(blank)	1
		AFSCA_0703	(blank)	1
		AFSCA_0708	(blank)	1
		AFSCA_0709	(blank)	1
		AFSCA_0710	(blank)	1
		AFSCA_0712	(blank)	1
		AFSCA_0715	(blank)	1
		AFSCA_0733	(blank)	1
		AFSCA_0734	(blank)	1
		AFSCA_0740	(blank)	1
		AFSCA_0743	(blank)	1
		AFSCA_0745	(blank)	1
		AFSCA_0746	(blank)	1
		AFSCA_0759	(blank)	1
		AFSCA_0763	(blank)	1
		AFSCA_0769	(blank)	1
		AFSCA_0778	(blank)	1
		AFSCA_0791	(blank)	1
		AFSCA_0806	(blank)	1
		AFSCA_0812	(blank)	1
		AFSCA_0813	(blank)	1
		AFSCA_0814	(blank)	1
		AFSCA_0831	(blank)	1
		AFSCA_0840	(blank)	1
		AFSCA_0841	(blank)	1
		AFSCA_0842	(blank)	1
		AFSCA_0869	(blank)	1
		AFSCA_0880	(blank)	1
		AFSCA_0881	(blank)	1
		AFSCA_0886	(blank)	1
		AFSCA_0895	(blank)	1
		AFSCA_0905	(blank)	1
		AFSCA_0906	(blank)	1
		AFSCA_0933	(blank)	1
		AFSCA_0934	(blank)	1
		AFSCA_0935	(blank)	1
		AFSCA_0936	(blank)	1
		AFSCA_0937	(blank)	1
		AFSCA_0939	(blank)	1
		AFSCA_0940	(blank)	1
		AFSCA_0967	(blank)	1
		AFSCA_0969	(blank)	1
		AFSCA_0977	(blank)	1
		AFSCA_0990	(blank)	1
		AFSCA_0998	(blank)	1
		AFSCA_0999	(blank)	1
		AFSCA_1007	(blank)	1
		AFSCA_1009	(blank)	1
		AFSCA_1010	(blank)	1
		AFSCA_1011	(blank)	1
		AFSCA_1017	(blank)	1
		AFSCA_1020	(blank)	1
		AFSCA_1022	(blank)	1
		AFSCA_1051	(blank)	1
		AFSCA_1054	(blank)	1
		AFSCA_1087	(blank)	1
		AFSCA_1094	(blank)	1
		AFSCA_1095	(blank)	1
		AFSCA_1119	(blank)	1
		AFSCA_1120	(blank)	1
		AFSCA_1121	(blank)	1
		AFSCA_1122	(blank)	1
cracotte_f Total				**96**
croq_small	Pommes cubes	ADD2	Aardappel bolletjes	1

croq_small	Pommes cubes	ADD3	Aardappel bolletjes	1
		AOF-005	Aardappel bolletjes	1
		AOF-006	Aardappel bolletjes	1
	Pommes noisettes	AOF-021	Potato ball small and othershapes	1
		AOF-022	Potato ball small and othershapes	1
		AOF-023	Potato ball small and othershapes	1
		AOF-024	Potato ball small and othershapes	1
		AOF-025	Potato ball small and othershapes	1
croq_small Total				**9**
croquettes	Croquettes	AOF-011	Potato croquette	1
		AOF-012	Potato croquette	1
		AOF-013	Potato croquette	1
		AOF-014	Potato croquette	1
		AOF-015	Potato croquette	1
		AOF-016	Potato croquette	1
		AOF-017	Potato croquette	1
		AOF-018	Potato croquette	1
		AOF-019	Potato croquette	1
		AOF-020	Potato croquette	1
	Pomme duchesse	ADD4	Potato duchesse	1
		AOF-007	Potato duchesse	1
		AOF-008	Potato duchesse	1
		AOF-009	Potato duchesse	1
		AOF-010	Potato duchesse	1
	pommes de pin	Supp 15	Kroketten (aardappelen)	1
croquettes Total				**16**
Cruesli	Honey roasted muesl	AOF-122	Bar, cereal-, muesli based	1
		AOF-123	Bar, cereal-, muesli based	1
		AOF-124	Bar, cereal-, muesli based	1
		AOF-125	Bar, cereal-, muesli based	1
		AOF-126	Bar, cereal-, muesli based	1
		AOF-127	Bar, cereal-, muesli based	1
		AOF-128	Bar, cereal-, muesli based	1
		AOF-129	Bar, cereal-, muesli based	1
		AOF-130	Bar, cereal-, muesli based	1
Cruesli Total				**9**
CRUESLI_BAR_F	afsca/favv	AFSCA_0479	(blank)	1
		AFSCA_0500	(blank)	1
		AFSCA_0510	(blank)	1
		AFSCA_0732	(blank)	1
		AFSCA_0855	(blank)	1
		AFSCA_0866	(blank)	1
	Honey roasted muesl	AOF-121	Bar, cereal-, muesli based	1
CRUESLI_BAR_F Total				**7**
dish	Plat préparé	AOF-026	type 3	1
		AOF-027	type 3	1
		AOF-028	type 3	1
		AOF-029	type 3	1
		AOF-030	type 3	1
	Rosti	AOF-004	type 3	1
dish Total				**6**
dry_fruits	abricots secs	AOF-158	Raisin	1
	figues séchées	AOF-160	Fig	1
	pêches moelleuses	AOF-159	peach ???	1
	prunes	AOF-157	plum	1
	raisins secs	AOF-155	Raisin	1
		AOF-156	Raisin	1
dry_fruits Total				**6**
frites_f	afsca/favv	AFSCA_0467	(blank)	1
		AFSCA_0468	(blank)	1
		AFSCA_0472	(blank)	1
		AFSCA_0484	(blank)	1
		AFSCA_0485	(blank)	1
		AFSCA_0486	(blank)	1
		AFSCA_0488	(blank)	1
		AFSCA_0491	(blank)	1
		AFSCA_0492	(blank)	1
		AFSCA_0494	(blank)	1
		AFSCA_0501	(blank)	1
		AFSCA_0502	(blank)	1
		AFSCA_0505	(blank)	1

frites_f	afsca/favv	AFSCA_0506	(blank)	1
		AFSCA_0508	(blank)	1
		AFSCA_0512	(blank)	1
		AFSCA_0514	(blank)	1
		AFSCA_0516	(blank)	1
		AFSCA_0517	(blank)	1
		AFSCA_0518	(blank)	1
		AFSCA_0521	(blank)	1
		AFSCA_0522	(blank)	1
		AFSCA_0524	(blank)	1
		AFSCA_0525	(blank)	1
		AFSCA_0526	(blank)	1
		AFSCA_0527	(blank)	1
		AFSCA_0528	(blank)	1
		AFSCA_0539	(blank)	1
		AFSCA_0541	(blank)	1
		AFSCA_0550	(blank)	1
		AFSCA_0551	(blank)	1
		AFSCA_0553	(blank)	1
		AFSCA_0564	(blank)	1
		AFSCA_0572	(blank)	1
		AFSCA_0617	(blank)	1
		AFSCA_0622	(blank)	1
		AFSCA_0730	(blank)	1
		AFSCA_0737	(blank)	1
		AFSCA_0742	(blank)	1
		AFSCA_0747	(blank)	1
		AFSCA_0750	(blank)	1
		AFSCA_0751	(blank)	1
		AFSCA_0752	(blank)	1
		AFSCA_0753	(blank)	1
		AFSCA_0758	(blank)	1
		AFSCA_0760	(blank)	1
		AFSCA_0764	(blank)	1
		AFSCA_0767	(blank)	1
		AFSCA_0770	(blank)	1
		AFSCA_0771	(blank)	1
		AFSCA_0772	(blank)	1
		AFSCA_0775	(blank)	1
		AFSCA_0776	(blank)	1
		AFSCA_0779	(blank)	1
		AFSCA_0781	(blank)	1
		AFSCA_0782	(blank)	1
		AFSCA_0785	(blank)	1
		AFSCA_0786	(blank)	1
		AFSCA_0789	(blank)	1
		AFSCA_0790	(blank)	1
		AFSCA_0792	(blank)	1
		AFSCA_0795	(blank)	1
		AFSCA_0796	(blank)	1
		AFSCA_0797	(blank)	1
		AFSCA_0798	(blank)	1
		AFSCA_0799	(blank)	1
		AFSCA_0800	(blank)	1
		AFSCA_0804	(blank)	1
		AFSCA_0805	(blank)	1
		AFSCA_0817	(blank)	1
		AFSCA_0818	(blank)	1
		AFSCA_0820	(blank)	1
		AFSCA_0833	(blank)	1
		AFSCA_0848	(blank)	1
		AFSCA_0865	(blank)	1
		AFSCA_0868	(blank)	1
		AFSCA_1026	(blank)	1
		AFSCA_1032	(blank)	1
		AFSCA_1045	(blank)	1
		AFSCA_1050	(blank)	1
		AFSCA_1056	(blank)	1
		AFSCA_1062	(blank)	1
		AFSCA_1070	(blank)	1
		AFSCA_1073	(blank)	1

frites_f	afsca/favv	AFSCA_1076	(blank)	1
		AFSCA_1084	(blank)	1
		AFSCA_1086	(blank)	1
		AFSCA_1097	(blank)	1
		AFSCA_1098	(blank)	1
		AFSCA_1099	(blank)	1
		AFSCA_1104	(blank)	1
		AFSCA_1107	(blank)	1
		AFSCA_1110	(blank)	1
		AFSCA_1111	(blank)	1
frites_f Total				**94**
gingerbread_f	afsca/favv	AFSCA_0460	(blank)	1
		AFSCA_0651	(blank)	1
		AFSCA_0659	(blank)	1
		AFSCA_0664	(blank)	1
		AFSCA_0674	(blank)	1
		AFSCA_0681	(blank)	1
		AFSCA_0928	(blank)	1
		AFSCA_0929	(blank)	1
		AFSCA_0930	(blank)	1
		AFSCA_0931	(blank)	1
		AFSCA_0932	(blank)	1
		AFSCA_0953	(blank)	1
		AFSCA_0954	(blank)	1
		AFSCA_0955	(blank)	1
		AFSCA_0956	(blank)	1
		AFSCA_0957	(blank)	1
gingerbread_f Total				**16**
muesli_f	afsca/favv	AFSCA_0463	(blank)	1
		AFSCA_0627	(blank)	1
		AFSCA_0662	(blank)	1
		AFSCA_0682	(blank)	1
		AFSCA_0726	(blank)	1
		AFSCA_0727	(blank)	1
		AFSCA_0738	(blank)	1
		AFSCA_0739	(blank)	1
		AFSCA_0765	(blank)	1
		AFSCA_0766	(blank)	1
		AFSCA_0773	(blank)	1
		AFSCA_0887	(blank)	1
		AFSCA_0901	(blank)	1
		AFSCA_0921	(blank)	1
		AFSCA_0922	(blank)	1
		AFSCA_0997	(blank)	1
		AFSCA_1005	(blank)	1
		AFSCA_1025	(blank)	1
		AFSCA_1028	(blank)	1
		AFSCA_1031	(blank)	1
		AFSCA_1033	(blank)	1
		AFSCA_1035	(blank)	1
		AFSCA_1044	(blank)	1
		AFSCA_1055	(blank)	1
		AFSCA_1060	(blank)	1
		AFSCA_1065	(blank)	1
		AFSCA_1067	(blank)	1
		AFSCA_1071	(blank)	1
		AFSCA_1118	(blank)	1
muesli_f Total				**29**
NUTS	Graines oléagineuses	AOF-149	Zonnebloempitten	1
		AOF-150	Zonnebloempitten	1
		AOF-151	Sesamzaad	1
		AOF-152	Noten gemengde	1
		AOF-153	Zonnebloempitten	1
		AOF-154	Pindanoten	1
	Noix grillées	AOF-139	Peanut coated with starch,salt and spices(borrelnoten)	1
		AOF-140	Cashewnut	1
		AOF-141	Pistachionut	1
		AOF-142	Peanuts	1
		AOF-143	Peanut coated with starch,salt and spices(borrelnoten)	1
		AOF-144	Peanuts	1
		AOF-147	Mixed nuts	1

NUTS	Noix grillées	AOF-148	Macadamia	1
NUTS Total				**14**
olives	olives noires en boca	AOF-166	Olijven, zwart	1
		AOF-167	Olijven, zwart	1
		AOF-168	Olijven, zwart	1
		AOF-169	Olijven, ns	1
		AOF-170	Olijven, zwart	1
olives Total				**5**
OTHER	frites	Supp 12	Chipsachtigen n.s.	1
	Honey roasted muesl	Add15	Ontbijtgranen, krokantemuesli/cruesli	1
	Snacks de céréales (I	AOF-119	Shrimp crackers	1
		AOF-120	Shrimp crackers	1
OTHER Total				**4**
pancakes	crèpes	AOF-71	Pannenkoeken/flensjes,gewone	1
		AOF-72	Pannenkoeken/flensjes,gewone	1
		AOF-73	Pannenkoeken/flensjes,gewone	1
	pancakes	AOF-74	Pannenkoeken/flensjes,gewone	1
		AOF-75	Pannenkoeken/flensjes,gewone	1
pancakes Total				**5**
rosti	Rosti	ADD1	type 4	1
		AOF-001	type 4	1
		AOF-002	type 4	1
		AOF-003	type 4	1
rosti Total				**4**
snack_nuts	Noix grillées	AOF-145	Peanut coated with starch,salt and spices(borrelnoten)	1
		AOF-146	Borrelnoten	1
snack_nuts Total				**2**
snacks	Snacks de céréales (I	AOF-107	Salty biscuits, cracker,without cheese	1
		AOF-108	Salty biscuits, cracker, withcheese	1
		AOF-109	Salty biscuits puff pastry	1
		AOF-111	Salty biscuits n.s.	1
	Snacks de céréales (I	Add12	Salty biscuits, cracker, withcheese	1
snacks Total				**5**
speculaas_f	afsca/favv	AFSCA_0590	(blank)	1
		AFSCA_0591	(blank)	1
		AFSCA_0609	(blank)	1
		AFSCA_0705	(blank)	1
		AFSCA_0706	(blank)	1
		AFSCA_0761	(blank)	1
		AFSCA_0844	(blank)	1
		AFSCA_0853	(blank)	1
		AFSCA_0854	(blank)	1
		AFSCA_0919	(blank)	1
		AFSCA_1006	(blank)	1
		AFSCA_1008	(blank)	1
speculaas_f Total				**12**
subs_coffee	Substituts de café,aul	Add13	Koffie, surrogaat basis n.s.	1
		Add14	Koffie, surrogaat basis n.s.	1
		AOF-171	Koffie, surrogaat basis n.s.	1
		AOF-172	Koffie, surrogaat basis n.s.	1
		AOF-173	Koffie, surrogaat basis n.s.	1
		AOF-174	Koffie, surrogaat basis n.s.	1
		AOF-175	Koffie, surrogaat basis n.s.	1
subs_coffee Total				**7**
SW_POT	frites	ADD5	Bataat/sweet potato	1
		ADD6	Bataat/sweet potato	1
		ADD7	Bataat/sweet potato	1
SW_POT Total				**3**
sweets	caramel	AOF-178	Snoep, toffee/karamel,met chocolade	1
		AOF-179	Snoep, toffee/karamel,met chocolade	1
		AOF-180	Snoep, toffee/karamel,met chocolade	1
	nougat	AOF-176	Bouchee n.s.	1
		AOF-177	Nougat	1
sweets Total				**5**
Grand Total				**664**

Appendix C

Supplementary analytical data for PAHs in spices and dried herbs

Table C1: content in µg kg^{-1} for the four regulated PAHs and the ΣPAH4 for the individual spices & herbs samples

Sample	Spices herbs type	Group	BaA	CHR	BbF	BaP	∑PAH4
EH-19-26	Celery salt	Traditional spices	0,65	5,60	0,74	<LOQ	6,98
EH-19-27	Celery salt	Traditional spices	0,59	5,53	0,85	0,16	7,13
EH-19-62	Cloves	Traditional spices	1,75	9,55	1,06	0,69	13,0
EH-19-63	Cloves	Traditional spices	1,38	8,80	0,73	0,34	11,3
EH-19-01	Garlic	Traditional spices	3,14	5,29	4,47	4,06	17,0
EH-19-02	Garlic	Traditional spices	0,69	2,18	0,98	0,44	4,29
EH-19-03	Garlic	Traditional spices	0,73	1,48	0,70	0,37	3,28
EH-19-106	Garlic	Traditional spices	1,55	2,16	2,76	1,89	8,36
EH-19-111	Nutmeg	Traditional spices	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
EH-19-69	Nutmeg	Traditional spices	<LOQ	<LOQ	0,14	<LOQ	0,14
EH-19-70	Nutmeg	Traditional spices	0,93	1,46	0,49	0,42	3,30
EH-19-74	Nutmeg	Traditional spices	<LOQ	<LOQ	1,93	0,93	2,86
EH-19-06	Onion	Traditional spices	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
EH-19-109	Pepper	Traditional spices	13,4	15,0	7,55	4,82	40,7
EH-19-118	Pepper	Traditional spices	1,27	2,02	1,77	0,40	5,46
EH-19-128	Pepper	Traditional spices	0,24	0,78	0,62	0,28	1,92
EH-19-14	Pepper	Traditional spices	5,51	14,7	5,43	4,35	29,9

Continued on next page

Table C1: content in µg kg^{-1} for the four regulated PAHs and the ΣPAH4 for the individual spices & herbs samples (Continued)

EH-19-14	Pepper	Traditional spices	7,19	9,78	8,12	4,51	29,6
EH-19-15	Pepper	Traditional spices	0,46	0,54	0,23	0,18	1,40
EH-19-16	Pepper	Traditional spices	2,39	3,34	1,27	0,47	7,46
EH-19-17	Pepper	Traditional spices	2,18	4,17	1,30	0,48	8,13
EH-19-18	Pepper	Traditional spices	0,37	0,67	0,16	0,11	1,30
EH-19-20	Pepper	Traditional spices	3,02	4,17	1,36	<LOQ	8,56
EH-19-21	Pepper	Traditional spices	4,66	6,76	3,67	2,72	17,8
EH-19-22	Pepper	Traditional spices	4,40	8,50	4,56	2,65	20,1
EH-19-23	Pepper	Traditional spices	3,55	5,52	2,18	1,22	12,5
EH-19-24	Pepper	Traditional spices	3,36	7,82	2,41	1,35	14,9
EH-19-25	Pepper	Traditional spices	0,74	1,54	0,37	0,32	2,97
EH-19-105	4 spices mix	Exotic spices	7,17	12,1	4,60	2,68	26,6
EH-19-04	Cinnamon	Exotic spices	1,46	2,76	1,76	1,66	7,65
EH-19-05	Cinnamon	Exotic spices	0,91	2,32	1,29	0,58	5,10
EH-19-124	Cumin	Exotic spices	0,55	2,16	0,58	0,31	3,62
EH-19-65	Cumin	Exotic spices	0,55	2,23	0,53	0,29	3,60
EH-19-66	Cumin	Exotic spices	0,28	0,88	0,22	0,10	1,48
EH-19-100	Curry	Exotic spices	0,79	1,26	0,63	0,47	3,15
EH-19-116	Curry	Exotic spices	1,23	2,35	0,81	0,68	5,07
EH-19-131	Curry	Exotic spices	0,66	1,17	0,52	0,44	2,79
EH-19-68	Curry	Exotic spices	1,61	3,22	1,07	0,86	6,76
EH-19-72	Curry	Exotic spices	1,37	3,29	1,31	0,99	6,97
EH-19-86	Curry	Exotic spices	0,34	0,93	0,29	0,17	1,74
EH-19-43	Fennel	Exotic spices	1,00	6,14	0,50	<LOQ	7,64

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Table C1: content in µg kg^{-1} for the four regulated PAHs and the ΣPAH4 for the individual spices & herbs samples (Continued)

EH-19-103	Garam Masala	Exotic spices	3,56	5,47	2,42	1,73	13,2
EH-19-129	Garam Masala	Exotic spices	3,49	5,08	2,32	1,57	12,5
EH-19-101	Ginger	Exotic spices	0,34	0,66	0,48	0,32	1,80
EH-19-121	Ginger	Exotic spices	0,41	0,64	0,73	0,41	2,19
EH-19-127	Ginger	Exotic spices	0,72	1,08	0,65	0,53	2,98
EH-19-64	Ginger	Exotic spices	0,43	0,94	0,12	<LOQ	1,49
EH-19-85	Ginger	Exotic spices	0,93	4,36	0,97	0,43	6,68
EH-19-07	Paprika	Exotic spices	1,49	2,35	1,34	1,85	7,03
EH-19-08	Paprika	Exotic spices	1,40	2,26	1,33	0,85	5,84
EH-19-09	Paprika	Exotic spices	1,40	3,05	1,47	1,96	7,87
EH-19-10	Paprika	Exotic spices	2,05	3,21	1,81	1,35	8,42
EH-19-110	Paprika	Exotic spices	0,49	2,09	0,67	0,26	3,51
EH-19-114	Paprika	Exotic spices	0,69	1,55	0,52	0,48	3,24
EH-19-123	Paprika	Exotic spices	1,80	3,92	2,26	1,15	9,13
EH-19-12	Pili-pili	Exotic spices	2,89	7,27	1,95	<LOQ	12,1
EH-19-13	Pili-pili	Exotic spices	0,99	4,15	0,97	0,41	6,52
EH-19-133	Ras el hanout	Exotic spices	2,95	5,19	2,07	1,48	11,7
EH-19-71	Ras el hanout	Exotic spices	10,8	17,5	11,7	10,3	50,3
EH-19-71	Ras el hanout	Exotic spices	10,4	9,91	12,1	11,2	43,6
EH-19-112	Tumeric	Exotic spices	1,31	6,05	4,24	2,00	13,6
EH-19-117	Tumeric	Exotic spices	1,34	1,89	0,66	0,58	4,46
EH-19-125	Tumeric	Exotic spices	0,39	0,79	0,35	0,28	1,81
EH-19-67	Tumeric	Exotic spices	0,32	0,72	0,21	0,15	1,40
EH-19-75	Tumeric	Exotic spices	0,90	1,99	0,55	0,52	3,95
EH-19-107	Basil	Dried herbs	1,04	3,97	1,25	0,46	6,72
EH-19-119	Basil	Dried herbs	0,59	1,88	1,24	0,44	4,16
EH-19-120	Basil	Dried herbs	0,65	2,63	0,80	0,39	4,47
EH-19-126	Basil	Dried herbs	0,77	4,45	1,44	0,38	7,04
EH-19-29	Basil	Dried herbs	<LOQ	0,33	<LOQ	<LOQ	0,33
EH-19-30	Basil	Dried herbs	1,23	4,04	0,71	0,81	6,78
EH-19-31	Basil	Dried herbs	0,79	4,26	1,06	<LOQ	6,11
EH-19-32	Basil	Dried herbs	0,88	5,98	2,73	<LOQ	9,59

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Table C1: content in µg kg^{-1} for the four regulated PAHs and the ΣPAH4 for the individual spices & herbs samples (Continued)

EH-19-33	Bouquet garni	Dried herbs	3,37	12,1	7,53	3,89	26,9
EH-19-34	Bouquet garni	Dried herbs	0,19	0,43	1,97	7,10	9,69
EH-19-35	Chives	Dried herbs	1,07	3,61	2,05	0,34	7,07
EH-19-36	Chives	Dried herbs	0,28	0,33	<LOQ	<LOQ	0,61
EH-19-37	Chives	Dried herbs	0,29	0,86	<LOQ	<LOQ	1,15
EH-19-38	Chives	Dried herbs	0,78	4,32	1,25	0,52	6,88
EH-19-28	Coriander	Dried herbs	0,36	0,84	<LOQ	<LOQ	1,20
EH-19-39	Coriander	Dried herbs	0,81	2,13	1,42	0,85	5,21
EH-19-78	Dill	Dried herbs	0,40	2,47	0,51	0,78	4,16
EH-19-79	Dill	Dried herbs	0,28	2,83	0,69	0,07	3,87
EH-19-46	Italian herbs	Dried herbs	1,90	7,51	3,28	2,13	14,8
EH-19-73	Italian herbs	Dried herbs	5,23	7,42	2,24	1,16	16,1
EH-19-108	Laurel	Dried herbs	48,2	50,0	22,3	34,0	154
EH-19-115	Laurel	Dried herbs	1,00	6,30	1,48	<LOQ	8,78
EH-19-48	Laurel	Dried herbs	8,92	36,9	7,57	4,86	58,2
EH-19-48	Laurel	Dried herbs	8,44	19,8	7,83	4,91	41,0
EH-19-80	Marjoram	Dried herbs	1,28	3,80	2,88	<LOQ	7,96
EH-19-81	Marjoram	Dried herbs	0,83	2,92	1,07	<LOQ	4,82
EH-19-113	Origano	Dried herbs	0,29	0,70	0,39	0,49	1,87
EH-19-77	Origano	Dried herbs	0,89	1,77	1,64	<LOQ	4,29
EH-19-82	Origano	Dried herbs	0,67	2,76	0,87	0,32	4,62
EH-19-83	Origano	Dried herbs	0,31	0,89	<LOQ	<LOQ	1,20
EH-19-84	Origano	Dried herbs	2,55	5,60	2,01	1,32	11,5
EH-19-49	Parsley	Dried herbs	0,21	0,36	0,32	<LOQ	0,88
EH-19-50	Parsley	Dried herbs	0,24	0,73	0,46	<LOQ	1,43
EH-19-51	Parsley	Dried herbs	0,51	1,26	1,20	0,71	3,68
EH-19-52	Parsley	Dried herbs	0,18	0,31	0,07	0,43	0,99
EH-19-44	Provençal herbs	Dried herbs	0,59	2,10	<LOQ	<LOQ	2,69
EH-19-45	Provençal herbs	Dried herbs	1,41	8,57	2,73	1,18	13,9
EH-19-47	Provençal herbs	Dried herbs	0,98	3,55	1,64	1,56	7,74

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Table C1: content in µg kg^{-1} for the four regulated PAHs and the ΣPAH4 for the individual spices & herbs samples (Continued)

EH-19-122	Rosemary	Dried herbs	0,13	0,64	0,38	<LOQ	1,15
EH-19-53	Rosemary	Dried herbs	0,21	<LOQ	<LOQ	<LOQ	0,21
EH-19-54	Rosemary	Dried herbs	0,19	<LOQ	<LOQ	<LOQ	0,19
EH-19-55	Rosemary	Dried herbs	0,28	1,24	<LOQ	<LOQ	1,52
EH-19-87	Saffron	Dried herbs	0,22	0,71	0,16	0,07	1,16
EH-19-56	Sage	Dried herbs	0,92	2,97	<LOQ	<LOQ	3,88
EH-19-11	Smoked paprika	Dried herbs	51,5	48,8	33,2	31,0	164
EH-19-57	Spaghetti mix	Dried herbs	1,12	2,35	1,54	1,14	6,15
EH-19-76	Spaghetti mix	Dried herbs	1,77	3,09	0,91	0,58	6,35
EH-19-104	Tarragon	Dried herbs	0,78	4,15	1,19	0,63	6,75
EH-19-132	Tarragon	Dried herbs	0,49	1,54	0,70	0,36	3,09
EH-19-40	Tarragon	Dried herbs	0,74	2,27	2,74	<LOQ	5,75
EH-19-41	Tarragon	Dried herbs	0,37	1,32	<LOQ	<LOQ	1,68
EH-19-42	Tarragon	Dried herbs	0,18	0,83	0,27	<LOQ	1,28
EH-19-102	Thyme	Dried herbs	0,44	4,63	0,92	<LOQ	6,00
EH-19-130	Thyme	Dried herbs	1,73	6,54	3,25	1,82	13,3
EH-19-58	Thyme	Dried herbs	1,71	4,60	2,74	1,64	10,7
EH-19-59	Thyme	Dried herbs	4,94	23,9	7,35	3,88	40,1
EH-19-60	Thyme	Dried herbs	5,56	18,5	8,22	5,47	37,7
EH-19-61	Thyme	Dried herbs	23,7	43,2	32,9	26,4	126

Publications and Communications

1 Publications

Peer-Reviewed Articles

Ph. Szternfeld, V. Van Leeuw, M-L. Scippo, C. Vinkx, E. Van Hoeck, L. Joly, 2024. Characterisation of new sources of acrylamide in food marketed in Belgium. Food Additives & Contaminants: Part B. https://doi.org/10.1080/19393210.2024.2440362 **(article published)**

Ph. Szternfeld, C. Demoury, W. Brian, J-Y. Michelet, V. Van Leeuw, E. Van Hoeck, L. Joly, 2023. Modelling the pesticide transfer during tea and herbal tea infusions by the identification of critical infusion parameters. Food Chemistry. https://doi.org/10.1016/j.foodchem.2023.136893 **(article published)**

Ph. Szternfeld, D. Montalvo, J. Broos, K. Cheyns, L. Joly & C. Vanhee, 2022. Pesticides, trace elements, and pharmaceuticals in tea samples available in Belgian retail shops and the risk associated upon acute and chronic exposure. Food Additives & Contaminants: Part B, https://doi.org/10.1080/19393210.2022.2145617 **(article published)**

Ph. Szternfeld, A. Marakis, M-L. Scippo, E. Van Hoeck & L. Joly, 2022. Polycyclic aromatic hydrocarbons in spices and dried herbs and associated risk for the Belgian population. Food Additives & Contaminants: Part B, 15:4, 292-300. https://doi.org/10.1080/19393210.2022.2106518 **(article published)**

Ph. Szternfeld, J. Marchi, S.V. Malysheva, S.V. & L. Joly, 2019. Modular Method for the Determination of Polycyclic Aromatic Hydrocarbons in Spices and Dried Herbs by Gas Chromatography–Tandem Mass Spectrometry. Food Anal. Methods 12, 2383–2391. https://doi.org/10.1007/s12161-019-01579-4 **(article published)**

Popular science articles

Ph. Szternfeld, V. Van Leeuw, L. Joly. Is acrylamide also present in less-studied foods? AFSCA Publication for accredited laboratories (Lab info) (2021).

Ph. Szternfeld, C. Demoury, L. Joly. Are pesticides transferred in your tea? AFSCA Publication for accredited laboratories (Lab info) (2021).

Ph. Szternfeld, J. Alexandre. Acrylamidegehaltes onder de loep onderzoek naar ongewenste stof in levensmiddelen. VMTfood digitale magazine (2020).

2 Oral Presentations

Ph. Szternfeld. Grouping food for validation. DTU EURL on processing contaminants workshop, Copenhagen, Denmark, 19 September 2023.

Ph. Szternfeld, K. Cheyns, J. Broos, D. Montalvo, C. Vanhee. Exposure to pesticides, pharmacological residues, and toxic trace elements following tea consumption in Belgium. Chemical Friday, Ixelles, Belgium, 09 September 2022.

Ph. Szternfeld, C. Demoury, V. Van Leeuw, J-Y. Michelet, W. Brian, F. Scholz, L. Joly. Pesticides transfer from tea leaves to tea brew: identification of critical infusion parameters and database creation. EURL- pesticides joint workshop 2021, Almeria, Spain, 20 October 2021.

Ph. Szternfeld, V. Van Leeuw. Acrylamide in other foods. DTU EURL on processing contaminants workshop, Copenhagen, Denmark, 29 September 2021.

Ph. Szternfeld, C. Demoury, V. Van Leeuw, J-Y. Michelet, W. Brian, F. Scholz, L. Joly. Etude des facteurs d'infusion de résidus de pesticides présents dans le thé et les infusions. Groupe Français des pesticides 2021, Gembloux, Belgium, 27 of Mai 2021.

Ph. Szternfeld. Acrylamide Management – A Belgian point of view. DTU EURL on processing contaminants workshop, Copenhagen, Denmark, 14 November 2019.

Ph. Szternfeld, J. Marchi, S. Chao, J. Broos, L. Joly. How to deal with spices & dried herbs diversity during PAHs analysis. IRMM EURL on processing contaminants workshop, Geel, Belgium, 14 November 2018.

Ph. Szternfeld, J. Marchi, S. Chao, J. Broos, L. Joly. A modular method for PAHs determination in spices and dried herbs by GC-MS/MS. Dioxin 2018, Krakow, Poland, 30 August 2018.

3 Posters

Ph. Szternfeld, V. Van Leeuw, L. Joly. Grouping food for validation. Chemical Reaction in Food IX, Prague, Czech Republic, 12-15 September 2023.

Ph. Szternfeld, V. Van Leeuw, L. Joly. Analytical and validation strategy for the determination of acrylamide in a wide variety of foodstuffs. 2nd ADVANCES IN SEPARATION SCIENCE: from extraction to chromatographic separation, Gembloux, Belgium, 28-29 June 2023.

Ph. Szternfeld, K. Cheyns. Exposure to pesticides and metals following tea consumption in Belgium. 4th IMEKOFOODS, Tervuren, Belgium, 16-18 September 2019.

Ph. Szternfeld. Investigation of the Polycyclic Aromatic Hydrocarbons contamination in spices and dried herbs available on the Belgian market. 4th IMEKOFOODS, Tervuren, Belgium, 16-18 September 2019. **(Poster Awards 2019)**

Ph. Szternfeld, J. Marchi, S. Chao, J. Broos, L. Joly. Analysis of Polycyclic Aromatic Hydrocarbons in spices and dried herbs by GC-MS/MS. Euroanalysis 2015, Bordeaux, France, 6-10 September 2015.