Chemical hazards in leafy vegetables on the Dutch market

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Summary

A risk-based approach helps focus on the most relevant food safety hazards for human health to be included in a monitoring program. These can be identified based on information on the occurrence and severity of the hazards. For this purpose, a long list of hazards that may occur was established and prioritised based on frequency of detection and levels found. When this prioritised list is combined with information on the severity of a hazard (which is based on toxicological information) and Dutch consumption data, a short list of hazards can be compiled to include in a monitoring program.

This report describes the results of a literature study performed to identify chemical hazards in leafy vegetables. The report is part of a series of reports on chemical hazards in food crops for which previously mushrooms, fruits and cereals, seeds and nuts were investigated.

Long list of chemical hazards that might occur in leafy vegetables
A long list of chemical hazards possibly present in leafy vegetables was established by performing a literature study using pre-set search terms in Scopus and Web of Science. In total, 420 hits were evaluated for their relevancy resulting in 169 papers that were read in full and for which information was extracted in an Excel document. Additionally, a Google search was performed in four websites to obtain reports on the occurrence of chemical hazards in leafy vegetables, resulting in 31 relevant hits that were also summarised in the Excel document. Furthermore, for hazard groups that retrieved a limited number of papers, an additional literature search was performed resulting in 27 relevant papers. Based on the papers obtained, a long list of chemical hazards was extracted, which contained heavy metals, nitrate and nitrite, polycyclic aromatic hydrocarbons (PAHs), pharmaceuticals, personal care products and endocrine disrupting compounds, flame retardants, perfluor compounds, radionuclides, nanoparticles, plant protection products (PPPs), mycotoxins, plant toxins, cyanotoxins, processing contaminants, and cleaning agents and disinfectants. The majority of the papers described experimental studies on the possible uptake and accumulation of chemical hazards from the environment into leafy vegetables and most information was available for lettuce followed by cabbage and spinach.

Intermediate list of prioritised chemical hazards in leafy vegetables
Those hazards that were frequently found in leafy vegetables, detected above (EU) legal limits, or reported to lead to exceedances of health based guidance values were included on the intermediate list. The following substances were included on the intermediate list from the heavy metals and elements section: aluminium, cadmium, copper and lead. These substances either exceeded the EU maximum limit (ML) or leafy vegetables were seen as major contributor to the substance's acceptable daily intake (ADI). Manganese (Mn) was identified as knowledge gap, since an experimental study, in which lettuce was hydroponically grown in Mn contaminated medium, resulted in an exceedance of the US RDI. However, no occurrence data were found on manganese in leafy vegetables. Nitrate was added to the intermediate list, since levels were found above EU MLs for spinach, chard, lettuce, and rocket. PAHs were included on the intermediate list, since high levels may be found when leafy vegetables are grown in urban or industrial areas. PPPs are used during the cultivation of leafy vegetables, and residues may be found at harvest. In total, 42 PPPs were included on the intermediate list since they were detected above the maximum residue level (MRL) or demonstrated to cause exceedances of the acute reference dose (ARfD). However, the list of PPPs included on the intermediate list is solely based on literature results. It is recommended to check the relevance of the identified PPPs using Dutch monitoring data as some of these pesticides may not have been detected above EU MRLs in the Netherlands and others may have been missed in the literature review. If the identified PPPs are included in the multi-methods used within the national monitoring program, a further prioritization may not be needed. The literature revealed that acrylamide and 5-hydroxymethylfurfural (HMF) may be formed at levels of concern in coffee substitutes containing chicory. As such, these processing contaminants were added to the intermediate list. The disinfectant by-products chlorate and perchlorate were added to the intermediate list as they were either detected above the EU MRL or were seen as a potential human health risk by EFSA. Benzalkonium chloride was also added to the intermediate list since an MRL exceedance was found.

The nanoparticle TiO_2 and the cyanotoxin MC-LR were identified as knowledge gaps as experimental studies showed a possible uptake from the soil into leafy vegetables resulting in high levels (TiO_2) or the tolerable daily intake (TDI) was exceeded using the concentrations found in the study (MC-LR). Another knowledge gap identified was for *Alternaria* toxins as these toxins were found in two studies, but its relevance for human health was not established. The plant toxins pyrrolizidine alkaloids (PAs) were identified as knowledge gap, since high levels have been detected in ready-to-eat salads when weeds, such as coltsfoot and common groundsel, are accidentally mixed with lettuce like rocket during harvest.

All substances on the intermediate list should be evaluated further to determine their possible risk to human health by combining concentrations found and consumption data in comparison to health based guidance values. This report provides the building blocks to perform such an evaluation. Substances with a possible human health risk should be included on the short list. That list should then be included in risk based monitoring programs for leafy vegetables.

Trends in the supply chain of leafy vegetables

The evaluation of trends in leafy vegetables showed that the increasing circularity of cultivation practices may result in the use of treated wastewater and sludge in open field cultivation. This may lead to the uptake of, e.g., pharmaceuticals and is, thus, a relevant trend to monitor. Another relevant trend is the increased temperature in the summer leading to increased levels of cyanotoxins in irrigation water, which may be taken up in leafy vegetables.

Samenvatting

Een risicogebaseerde aanpak helpt om de meest relevante voedselveiligheidsgevaren te identificeren die in een monitoringsprogramma opgenomen zouden moeten worden. Hiervoor is informatie nodig over het vóórkomen en de ernst van de gevaren. Als start kan een zogenaamde 'long list' van chemische gevaren opgesteld worden, die kunnen voorkomen in de voedselketen. Deze lijst kan verder geprioriteerd worden op basis van de frequentie waarin de gevaren gevonden worden en de concentraties van de chemische gevaren. Als deze geprioriteerde lijst wordt gecombineerd met informatie over de ernst van het gevaar (toxicologische informatie) en Nederlandse voedselconsumptiegegevens kan een zogenaamde 'short list' van voedselveiligheidsgevaren worden opgesteld, die opgenomen zouden moeten worden in een monitoringsprogramma.

Dit rapport beschrijft de resultaten van een literatuuronderzoek dat is uitgevoerd om de chemische gevaren in bladgroente te kunnen identificeren. Het rapport is onderdeel van een serie rapporten over chemische gevaren in voedselgewassen; eerder zijn paddenstoelen, fruit en granen, zaden en noten onderzocht.

De long list van chemische gevaren die kunnen voorkomen in bladgroenten
Een literatuurstudie is uitgevoerd op basis van vooraf gedefinieerde zoektermen in Scopus en Web of Science om de long list van chemische gevaren in bladgroenten te kunnen opstellen. In totaal werden 420 hits geëvalueerd op hun relevantie, wat resulteerde in 169 artikelen die volledig zijn doorgelezen. Informatie uit deze artikelen is verwerkt in een Excelbestand. Daarnaast is in vier websites gezocht naar rapporten over het voorkomen van chemische gevaren in bladgroenten m.b.v. de Google zoekmachine. Dit resulteerde in 31 relevante hits die ook zijn samengevat in het Excelbestand. Indien er een beperkt aantal referenties werd gevonden voor een bepaalde gevarengroep, is een additioneel literatuuronderzoek uitgevoerd, wat resulteerde in 27 relevante artikelen. Op basis van de verkregen artikelen is een long list van chemische gevaren opgesteld, die de volgende chemische gevaren bevat: zware metalen, nitraat en nitriet, polycyclische aromatische koolwaterstoffen (PAK's), residuen van geneesmiddelen en persoonlijke verzorgingsproducten, hormoonverstorende componenten, vlamvertragers, perfluorverbindingen, radionucliden, nanodeeltjes, gewasbeschermingsmiddelen, mycotoxinen, planttoxinen, cyanotoxinen, procescontaminanten, en reinigings- en desinfectiemiddelen. De meeste artikelen beschreven experimentele studies waarin de mogelijke opname van chemische gevaren in bladgroenten werd beschreven. Verder werd de meeste informatie gevonden voor sla, gevolgd door kool en spinazie.

De intermediate list van geprioriteerde chemische gevaren in bladgroenten
Gevaren die regelmatig gevonden werden in bladgroenten, boven (Europese) wettelijke limieten werden aangetroffen of waarvoor de concentraties leiden tot overschrijding van de gezondheidskundige richtwaarden, zijn opgenomen op de intermediate list. Voor zware metalen en sporenelementen, waren dit de volgende chemische gevaren: aluminium, cadmium, koper en lood. Voor deze stoffen werden ofwel de EU maximumlimieten (ML's) overschreden of bladgroenten leveren een belangrijke bijdrage aan de ADI van de stof. Mangaan (Mn) werd aangemerkt als kennisleemte, aangezien een experimentele studie, waarin sla in hydrocultuur besmet met Mn werd gekweekt, aangaf dat de Amerikaanse RDI overschreden werd. Er zijn echter geen surveys gevonden van mangaan in bladgroente. Nitraat werd opgenomen op de intermediate list, aangezien concentraties in spinazie, snijbiet, sla en rucola boven de EU ML's werden gevonden. Polyaromatische koolwaterstoffen (PAK's) werden op de intermediate list opgenomen, aangezien hoge concentraties gevonden werden in bladgroenten geteeld in stedelijke of industriële gebieden. Tijdens de teelt van bladgroenten worden gewasbeschermingsmiddelen gebruikt. In totaal werden 42 gewasbeschermingsmiddelen op de intermediate list opgenomen, aangezien deze boven de EU MRL werden gevonden of omdat aangetoond is dat de acute referentiedosis (ARfD) werd overschreden. Deze lijst is echter alleen gebaseerd op literatuuronderzoek. Er wordt daarom aanbevolen om de lijst met gewasbeschermingsmiddelen te controleren aan de hand van Nederlandse monitoringsdata, aangezien sommige pesticiden in Nederland misschien niet boven de EU MRL's zijn

gevonden en andere wellicht gemist zijn in het literatuuronderzoek. Indien de geïdentificeerde gewasbeschermingsmiddelen zijn opgenomen in multi-methodes die momenteel gebruikt worden binnen het nationaal plan, is verdere prioritering niet noodzakelijk. Het literatuuronderzoek liet verder zien dat acrylamide en 5-hydroxymethylfurfural (HMF) gevormd kunnen worden in koffiesurrogaten met cichorei in concentraties die gezondheidseffecten kunnen veroorzaken. Deze procescontaminanten werden daarom ook opgenomen op de intermediate list. Chloraat en perchloraat, die als bijproducten van gechloreerde desinfectiemiddelen kunnen ontstaan, werden ook opgenomen op de intermediate list, aangezien deze boven de EU MRL's werden aangetroffen en door EFSA worden gezien als potentieel volksgezondheidsgevaar. Benzalkoniumchloride is opgenomen op de lijst aangezien MRL-overschrijdingen werden gevonden.

Het nanodeeltje TiO_2 en het cyanotoxine MC-LR werden als kennisleemtes aangemerkt, aangezien experimentele studies lieten zien dat deze stoffen vanuit de bodem kunnen worden opgenomen in bladgroenten en er vervolgens hoge concentraties werden gevonden (TiO_2) of de toelaatbare dagelijkse inname (TDI) werd overschreden (MC-LR) bij de concentraties die in de studies werden gebruikt. *Alternaria* toxinen werden ook aangemerkt als kennisleemte, aangezien deze toxinen in twee studies werden gevonden, maar de relevantie voor de humane gezondheid is niet vastgesteld. De planttoxinen pyrrolizidine alkaloïden (PA's) werden aangemerkt als kennisleemte, aangezien hoge concentraties werden aangetroffen in kant-en-klare salades indien onkruid, zoals hoefblad en klein kruiskruid, tijdens de oogst per ongelijk vermengd waren met sla, zoals rucola.

Alle stoffen op de intermediate list dienen verder geëvalueerd te worden om hun risico voor de humane gezondheid vast te kunnen stellen, door de gevonden concentraties te combineren met consumptiegegevens en te vergelijken met gezondheidskundige richtwaarden. Dit rapport geeft de bouwstenen voor een dergelijke evaluatie. Stoffen die een mogelijk humaan risico kunnen opleveren moeten worden opgenomen op de zogenaamde 'short list'; deze lijst kan worden opgenomen in een risicogebaseerd monitoringsprogramma voor bladgroenten.

Trends in de bladgroenteketen
Een evaluatie van de trends in bladgroente liet zien dat er een trend naar een meer circulaire economie, wat ertoe kan leiden dat gezuiverd afvalwater en slib gebruikt gaat worden in de open teelt. Hierdoor zouden bij voorbeeld geneesmiddelen vanuit deze bronnen opgenomen kunnen worden in bladgroenten. Dit is dus een relevant trend om in de gaten te houden. Een andere belangrijke trend is de stijgende temperatuur in de zomer, wat kan leiden tot verhoogde concentraties cyanotoxinen in irrigatiewater, die vervolgens kunnen worden opgenomen in bladgroenten.

1 Introduction

The main task of the Netherlands Food and Consumer Product Safety Authority (NVWA) is to protect human and animal health. For this purpose, potential human and animal health hazards are monitored in food and consumer products. Since not all products can be tested, a prioritization of activities is needed.

Risk-based monitoring will help to identify the most important food and feed safety hazards. Within risk-based monitoring, both the probability of a hazard occurring in the product and the effects of this hazard on human health are taken into account. In order to prioritise hazards, information on both the occurrence and on possible human health effects is needed. For this purpose, previously, the red meat chain, dairy chain, poultry chain, and egg chain have been assessed. Currently, the fruit and vegetable chain is being evaluated. This food supply chain is divided into seven sub-chains:

1. Fruits
2. Nuts, cereals, and seeds
3. Mushrooms
4. Leafy vegetables
5. Fruiting vegetables
6. Bulb, tuber (except potatoes), and root vegetables
7. Other vegetables

Sub-chain 4, leafy vegetables, is the focus of this report[1]. The aim was to make an inventory of possible chemical hazards that may occur in leafy vegetables on the Dutch market and to identify those hazards that are frequently found in leafy vegetables and/or found above legal limits as based on scientific literature. Together with toxicological information, the NVWA Office for Risk Assessment and Research (Bureau Risicobeoordeling & onderzoek; BuRO) can use this information to assess the human health risks in leafy vegetables.

The project consisted of the following tasks:

- A literature review on chemical hazards that may occur in leafy vegetables resulting in a long list of chemical hazards (section 3.1 and 3.2).
- Establishing an intermediate list of chemical hazards that are frequently found and/or above legal limits (section 3.3) based on the literature review (3.2).
- Gathering toxicological information (such as health based guidance values (HBGV)) of the chemical hazards on the intermediate list (section 3.4) as identified in section 3.3.
- An evaluation of trends and developments within the leafy vegetable chain within the next 5 years that may influence the occurrence of chemical hazards (section 3.5).

[1] A list of English to Dutch terms has been provided in Annex 1 as a guide to the reader.

2 Methods

2.1 Project description and demarcation

A literature study was performed to identify chemical hazards that may occur in leafy vegetables. Scientific literature and reports were screened for relevancy as described in 2.4.1. The relevant articles were retrieved, evaluated in detail (e.g., parts of the plant, concentrations reported, the main message of the study), and summarized. For those chemical hazards that were frequently found in leafy vegetables and/or found above legal limits (the intermediate list, see section 2.4), toxicological information was gathered such as HBGVs (see section 2.5).

For this study, leafy vegetables like lettuce, broccoli, cabbage, chicory, artichokes, and Brussel sprouts were selected. In some reports, e.g. by EFSA, the term leafy vegetables refers to vegetables such as lettuce and spinach, whereas the term *Brassica* vegetables refers to vegetables such as kale, cabbage and kohlrabi. In the current report, we refer to leafy vegetables as including both groups of vegetables. Details on the product search strings used for the scientific literature study on leafy vegetables can be found in section 2.4.1. Some leafy vegetables were excluded from this study; these included fermented vegetables like sauerkraut as well as leafy herbs like basil and parsley. Also, vegetable sprouts like bean sprouts and alfalfa were not a part of this study. These vegetables were excluded because both types of vegetables will be part of a separate literature review.

Also, an evaluation of the trends in leafy vegetable supply chain was performed using information from grey literature and results obtained through questionnaires (see section 2.5). Each step of the study is outlined below.

2.2 Product commodity

Since leafy vegetables include a wide variety of product groups, the first step was to define the leafy vegetable search terms to be used in the product commodity search string of the literature study (section 2.3). The NVWA Chain Classes for Fruits and Vegetables (Ketenklassen GF) as provided to WFSR on 4 September 2018 was used as a basis for the product classification supplemented with The European Food Safety Authority (EFSA) categories coming from the 2013 report on foods of non-animal origin (EFSA, 2013b). The classification was complemented with the Standards for fresh fruits and vegetables from the United Nations Economic Commission for Europe (UNECE) (https://www.unece.org/trade/agr/standard/fresh/ffv-standardse.html).

The details on the results and choices made for the final classification of vegetables in the leafy vegetables group are indicated in Annex 2.

2.3 Eurostat imports

To prioritize the references in the heavy metals and pesticide residue groups extracted during the literature screening (see section 2.3.1, stage 4), the NVWA extracted EUROSTAT import data for the Netherlands for 2017.

Four customs codes (indicators) were used to be able to encompass a broad range of leafy vegetables. These indicators included (i) fresh or chilled cabbage lettuce (70511); (ii) fresh or chilled lettuce (excluding cabbage lettuce) (70519); (iii) fresh or chilled witloof chicory (70521); and (iv) fresh or chilled globe artichokes (70991). Data was provided as the quantity in 100 kg. A separation between the EU-28 and non-EU was made for each of the indicators, while the total for these four indicators was also determined.

2.4 Literature study

2.4.1 Scientific literature

After selecting the product commodities to be included on the list of leafy vegetables, we searched for relevant scientific literature. Two databases were used for the scientific literature study: Scopus and Web of Science.

Several search combinations were performed that made use of combined keywords (see Annex 2 for details). These were analogous to previous chain studies performed for the NVWA-BuRO. Searches were further defined with exclusion terms to help obtain relevant papers. Finally, option 18 (Annex 2, Table A2.1) was extracted to Endnote using the following search terms limited to the years 2009-2019. The search strings used were as follows:

> #1 product commodity in Title:
> brocco* OR cauliflower* OR sprout* OR cabbage* OR chicory OR spinach* OR "turnip top*" OR "turnip green*" OR kale OR chard OR lettuce* OR endive OR escarole* OR "leafy vegetable*" OR "green vegetable*" OR "leafy vegetable*" OR salad OR choi OR choy OR artichoke OR arugula OR "beet green" OR bitterleaf OR celery OR celtuce OR "collard green*" OR *cress* OR epazote OR "garden rocket" OR komatsuna OR "mizuna greens" OR "mustard green*" OR "leaf mustard*" OR radicchio OR rapini OR tatsoi OR chaya OR chickweed OR "Chinese mallow" OR Chrysanthemum OR "fat hen" OR "fluted pumpkin" OR samphire OR "Greater plantain" OR "jute plant" OR Karkalla OR "Lagos bologi" or orache OR purslane OR rucola OR sculpit OR stridolo OR soko OR "spleen amaranth")
>
> AND #2 Chemical hazards in Title, abstract or keywords:
> "Food contamination" OR "Chemical pollutant*" OR "chemical hazard*" OR contamina* OR toxin* OR "toxic substance*" OR "toxic compound*" OR pollutant* OR "agricultural chemical*" OR "chemical compound*" OR "chemical substance*" OR residu*
>
> AND #3: Public Health In Title, abstract or keywords:
> "Public health" OR "HACCP" OR "Consumer protection" OR Consumer* OR "Food safety" OR "risk assessment*" OR "risk analys*" OR "hazard analys*" OR "Human health*" OR "Health impact" OR "health risk*"
>
> AND NOT #4 in Title:
> pathogen* OR streptococcus OR listeria OR *virus* OR bacillus OR salmonella OR clostridium OR staphylococcus OR outbreak OR "foodborne disease*" OR fung* OR campylobacter OR "Escherichia coli" OR "E. coli" OR model* OR analytic* OR microbio* OR bacteri* OR virol* Or nutri*

Once uploaded into Endnote, the duplicate hits were removed, and the remaining references were evaluated for relevancy in four stages.

The four stages of determining the relevancy of the references were primarily based on the title, keywords, and/or abstract. Details on the search topics are provided in Annex 3; these are described for each of the following four stages.

i. In stage 1, the title and, when necessary (e.g., for reviews), the abstract were screened. Here, the irrelevant references ("not relevant") were separated, and the remaining references were categorized based on potential hazard groups. Relevance was based on the focus of the study, namely the paper should be focused on chemical hazards in leafy vegetables. Annex 3 indicates topics that were regarded as not relevant.

ii. Next, in stage 2, the references that were considered relevant were further evaluated by reading the abstract and by screening the keywords. These references were separated into "not relevant," "maybe relevant," or "yes relevant," the latter two of which were categorized based on potential hazard groups. The literature of the "yes relevant" references were then downloaded. When articles were written in another language than English, they were then considered "maybe relevant."

iii. Then in stage 3, a limited number of the total references were peer reviewed (Annex 4) by two scientists to check for consistency. Inconsistencies were discussed, and the evaluation was aligned.

iv. Finally, since two hazard groups, heavy metals and pesticide residues, had >35 "yes relevant" hits each, another selection criterion was applied to the "yes relevant" references of these groups. When the location was specified in the title, abstract, or materials and methods section to be outside the European Union (EU) (see section 2.3.2), then the article was included on the "maybe relevant" list. Annex 3 provides further details on the separation of the yes and maybe relevant references.

2.4.2 Google search

To further support the literature search on possible chemical hazards in leafy vegetables, additional searches were performed using the advanced search feature in Google. In total, four websites from research or food safety institutes were selected for further screening for relevant literature. These four websites were selected because they were European, and they resulted in relevant hits. Further details on the selection of the following four websites are described in Annex 5.

The four websites used:
1. EFSA (http://www.efsa.europa.eu);
2. Federal Agency for the Safety of the Food Chain (FAVV) (http://www.afsca.be);
3. German Federal Institute for Risk Assessment (BfR) (https://www.bfr.bund.de); and
4. Swedish National Food Agency (Livsmedelsverket) (https://www.livsmedelsverket.se/).

Since the Google advanced search does not allow for an extensive list of search strings, the approach using separate search terms for individual leafy vegetables as done for Scopus and WoS (section 2.3.1) could not be followed. It was decided to use a broad approach using the general search term "vegetable" rather than "leafy vegetable." The following three search strings were performed on these four websites:
1. Vegetable AND "food safety"
2. Vegetable AND (contaminant OR residue)
3. Vegetable AND "risk assessment"

When an individual search resulted in more than 200 hits, the last 10 years were selected (2009-2019). If this still resulted in more than 200 hits, only the file type 'pdf' was selected.

The retrieved hits were first screened to determine if the reference concerned leafy vegetables and if it concerned a chemical hazard (i.e., not microbiological or physical hazards). Afterward, all the selected references were read, and the information on the hazards in leafy vegetables was extracted. A reference with valuable information, such as concentrations of nitrate in spinach, was selected to be included in this report. At this point, references with general statements, like: 'spinach is rich in nitrate,' were not selected for inclusion. Despite complying with the previously mentioned prerequisites, references could still be excluded if there were too many references for the same chemical hazard, such as for the heavy metals. Since the obtained hits were all originating from Europe, another selection criterion was used based on publication year. Only recent information (publications > 2015) was then included.

2.4.3 Additional searches

The literature searches, as described in sections 2.4.1 and 2.4.2, sometimes resulted in only a few relevant hits for certain chemical hazards or only experimental studies were found. In order to check whether information on the occurrence of these hazards in leafy vegetables was available, additional searches were performed in Scopus. Additional searches were performed for acrylamide, since the literature search resulted in only 2 papers and the Google search did not result in any hits. Flame retardants were further searched since the literature study gave no hits with the Google search and the literature search only obtained 1 paper. For mycotoxins, only three papers were obtained in the literature search and no hits in the Google search. For nanoparticles only 2 papers were found in the literature search and no hits in the Google search. The literature search obtained three papers on PAHs, one of which was relevant and no hits in the Google search. An additional search was performed for pharmaceuticals to find occurrence data (only experimental studies were found in the literature search). The additional search on pharmaceuticals, revealed a paper describing a high level of norfloxacin in leafy vegetables. This was investigated further by retrieving papers specifically on norfloxacin and leafy vegetables. Only limited information was found for plant toxins. One paper describing a technique to detect plant toxins and one report from BfR indicating the possible risk of plant toxins when gathering plants grown in the wild. For the heavy metals mercury, nickel and platinum group metals only a single paper was found describing the possible uptake in leafy vegetables. An additional search was performed to obtain information on the occurrence of these metals in leafy vegetables on the Dutch market.

The following searches were performed in title, abstract or keywords:
- ("leafy vegetable*" AND acrylamide)
- ("leafy vegetable*" AND flame retardant*)
- ("leafy vegetable*" AND (mycotoxin* OR aflatoxin* OR fumonisin* OR DON OR deoxynivalenol))
- ("leafy vegetable*" AND (nano-particle* OR micro-particle*))
- ("leafy vegetable*" AND (polycyclic aromatic hydrocarbon* OR PAH*))
- ("leafy vegetable*" AND (pharmaceutical* OR antibiotic* OR antimicrobial* OR steroid* OR endocrine*) AND NOT resistance OR resistant OR antioxida*)
- ("leafy vegetable*") AND norfloxacin
- ("leafy vegetable*" AND (plant toxin* OR pyrrolizidine alkaloid*))
- ("leafy vegetable*"AND (mercury OR nickel OR platinum group metal*)

When a search term containing leafy vegetable* resulted in no relevant hits or only one relevant hit, the search was extended with the words 'spinach,' 'lettuce,' 'cauliflower,' and 'broccoli.' This was done for the chemical hazards acrylamide, flame retardants, mycotoxins, nano-particles, and plant toxins.

The following topics are examples that were considered not to be relevant:
- Locally consumed vegetables from Africa, such as mutete and omboga;
- Anti-carcinogenic properties of vegetables;
- Pharmaceutical properties of vegetables;
- Phytotoxicity towards the plant itself;
- Plant extracts as medicine.

For those hazards that were included on the intermediate list, EFSA and RIVM reports were retrieved by searches in Google in order to obtain toxicological information for these hazards and their contribution to the dietary intake. In case additional information was obtained from these reports, this was also included in section 3.2.

2.5 Prioritization

The literature study indicated the chemical hazards that may occur in leafy vegetables. These hazards were included on a so-called long list of possible chemical hazards (see section 3.2). Based on the information from the retrieved literature that referred to concentrations in leafy vegetables, an intermediate list was established containing those hazards that were frequently mentioned in the literature to be present in leafy vegetables and/or which exceeded (EU) legal limits or for which human health problems were reported (e.g., as exceeding the HBGVs or when leafy vegetables were indicated as the main contributor to a substance's HBGV).

Furthermore, in some cases, experimental studies indicated possible human health risks, but no survey data from vegetables available on the market were found. These substances were identified as knowledge gaps. For the substances on the intermediate list and for the identified knowledge gaps, EFSA opinions and RIVM reports were consulted to establish information on the toxicity of the hazards. This information can be used by NVWA-BuRO to come to a short list of chemical hazards that may impact human health.

2.6 Evaluation of trends

Wageningen Food & Biobased Research (WFBR) performed an inventory of developments in leafy vegetables in the coming five years (2020-2024) as little difference was expected between leafy vegetables and other vegetables in future trends. The Innova database (see below) was consulted specifically for leafy vegetables. Information sources used were:

- Expert reports from international institutions and organizations such as the World Health Organization (WHO), Food and Agricultural Organization (FAO) and Rabobank.
- A Google search to find popular literature on future trends.
- A Google Scholar search to find scientific literature about future trends.
- Magazines and blogs on food and retail to find popular literature on future trends.
- Innova database to find new product introductions. Innova Market Insights collects all new product introductions, collecting all information available on the package into the Innova Database (www.innovamarketinsights.com). Information about the product that is not mentioned on the package is consequently not in the database, nor is registered how long the product is, was, or has been available on the market. The overview the database provides shows the trend in products in the past years. In the database, products can be sorted based on the type of ingredients, packaging type, year, country, etc. Furthermore, the team from Innova makes regular updates on trends they note in several of the categories. An Innova search was performed on introductions in the Netherlands between 2009 and 2018.
- Expert interviews (n = 5) and written responses of experts in the field concerning a questionnaire that was sent by email were used. The consulted experts had different positions in the food crop supply chain; they were involved in branch organizations (n = 3) and in processing and/or import companies (n = 2). Participants were informed beforehand that the results were going to be used for a research project for BuRO/NVWA. See Annex 6 for the questionnaire used for the interviews. Responses to the interviews or written questionnaires were processed anonymously.

3 Results

3.1 Results literature study and import data

3.1.1 Results from Scopus and Web of Science

The literature search resulted in 626 hits, of which 352 hits were from Scopus and 274 hits from Web of Science. From these, duplicates (206) were removed, resulting in a total of 420 references to review. Details on how the references were deemed relevant per each of the four stages are outlined in Annex 3.

The 420 references were evaluated in stage 1 (based on the title and if needed the abstract) to select relevant references as described in section 2.4.1; this resulted in 266 relevant references and 154 'not relevant' references. The majority of the papers included lettuce in the title (122 hits), followed by cabbage (68 hits), and spinach (52 hits). After stage 2 (based on abstract and keywords), 169 relevant references were left. References were separated into 16 hazard groups (antibiotics and steroids, metals, cyanotoxins, etc.), based on the literature found. During stage 3, 42 references (10% of the total references excluding duplicates) were peer reviewed for their relevancy. A comparison of the evaluated references is presented in Annex 4, Table A4.1. This evaluation between the two scientists showed that 69% of the references were evaluated in the same way, i.e., 'not relevant,' 'maybe relevant,' or 'yes relevant.' For the remaining references (13), the scientists discussed the final decision in order to align the evaluation and further sharpen the relevance criteria. After stage 3, there were 136 relevant references. From these 136 references, 37 were in the hazard group metals and 50 in the hazard group pesticide residues. To further prioritize the literature from these two hazard groups, the relevant references were further screened, and references that were non-EU focused were included in 'may be relevant' as we primarily import from EU countries (see section 3.1.2). After stage 4, there were 70 'yes relevant' references; 186 'may be relevant' references; and 165 'not relevant' references. The sum of which is 421, due to one overlapping reference that was 'yes relevant' for hazard category PAHs and 'maybe relevant' for the hazard category metals.

3.1.2 Results from Eurostat

The production volume of fresh vegetables (including melons) in the EU-28 in 2017 was 64.8 million tonnes. In the Netherlands, agricultural production of fresh vegetables in 2017 was 595 thousand tonnes, making up 0.9% of the total share from EU-28 countries (Eurostat, 2018).

Imported fresh or chilled vegetables (from cabbage lettuce, lettuce, chicory, and globe artichokes) to the Netherlands in 2017 showed that Spain (58%), Germany (14%), Belgium (10%), Italy (8%), and France (4%) were the top five EU countries for these four groups. Other EU countries and non-EU countries made up 3% each of the total imports to the Netherlands in 2017 for these four fresh or chilled vegetable groups (Figure 1). Imports for cabbage and lettuce were highest from Spain. Imports for chicory were highest from Belgium, while for globe artichokes, this was from France.

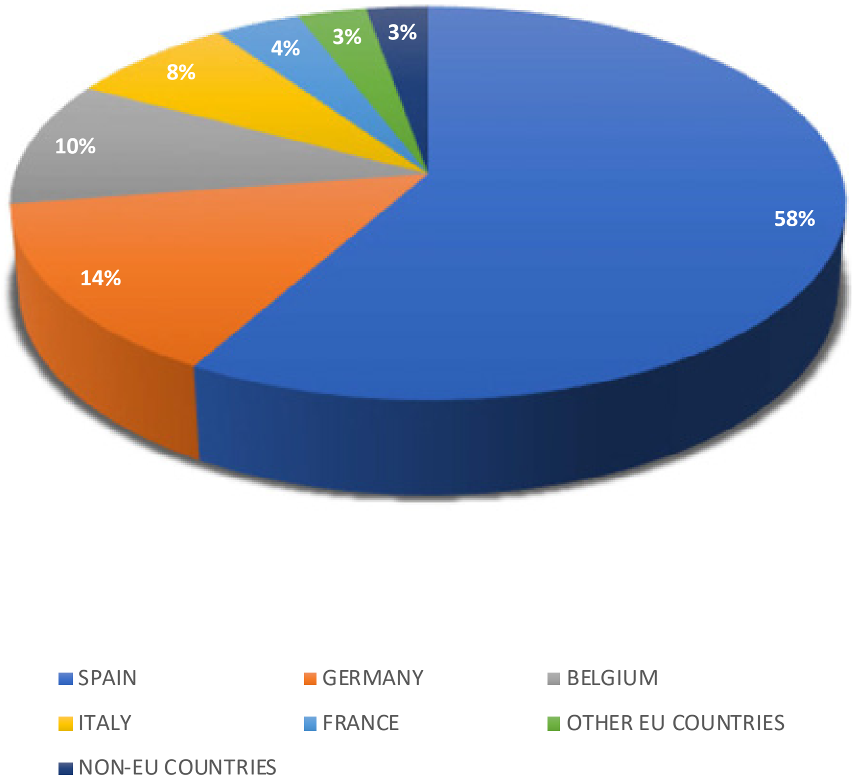

Figure 1 *Imports of fresh or chilled vegetables to the Netherlands (2017).*

The results from the import data were used to prioritize hazards groups with more than 35 relevant references. Namely, the hazard groups heavy metals and pesticide residues were refined to include literature from EU-28 countries.

3.1.3 Results from Google search

The advanced search in Google resulted in 31 relevant hits (EFSA: 13; FAVV: 10; BfR: 7; Livsmedelsverket: 1). The total hits found in these websites are indicated in Annex 5.

The following topics (examples) were considered to be not relevant:
- Vegetable oils;
- Microbiological hazards;
- List of stakeholders;
- Agenda of meetings, without valuable information given;
- Vegetables in general, without any relationship to the specific group 'Leafy vegetables';
- Phytotoxic hazards that were not relevant for human health;
- Hazards for bees;
- Environmental hazards;
- Catalogue of project ideas;
- Hygiene in the kitchen;
- Policy agenda;
- List of laboratories;
- Chemical analysis of food, not focusing on hazardous substances;
- Management guideline for food labelling.

3.1.4 Results from the additional literature research

Additional searches were performed in case limited information was found for a hazard group (see section 2.3.4). Furthermore, snowballing was used in case the full text evaluation referred to potentially relevant references. In total, 61 papers were found that might be relevant for the study based on title, keywords, and abstract. After reading the full texts, 28 papers were regarded as relevant and included in the literature overview as described in section 3.2. Two papers were added on perchlorate, one on flame retardants, three papers on mycotoxins, one paper on perfluor compounds, two papers on plant protection products, eight papers on pharmaceuticals, five papers on polycyclic aromatic hydrocarbons, three papers on acrylamide, one paper on cyanotoxins and two papers on radionuclides. Although the literature search retrieved many relevant papers on heavy metals (37 in total), there was only 1 paper on mercury, nickel and platinum group metals. An additional search on these heavy metals resulted in one study describing mercury uptake from the soil, which was included in section 3.2.1.1. Furthermore, one survey on the presence of nickel in supermarket samples of leafy vegetables was found, which is added to 3.2.1.2.

3.2 Overview of chemical hazards in leafy vegetables

Chemical hazards may enter the supply chain of leafy vegetables via various routes during primary production and processing. They may be naturally present in leafy vegetables, such as nitrate or can be contaminated with naturally present substances due to the production of toxins by fungi or algae, such as mycotoxins and cyanotoxins. Furthermore, plant toxins may end up in the final product when leafy vegetables are accidentally harvested together with some weeds. When plant protection products or biocides are used in the supply chain, residues may occur in leafy vegetables. Chemical hazards may also enter the supply chain during processing, for example when chicory root, which serves as a coffee substitute, is heated. The majority of the chemical hazards, however, end up in leafy vegetables during the cultivation stage. Heavy metals, polycyclic aromatic hydrocarbons, pharmaceuticals, flame retardants, perfluor compounds, radionuclides and nanoparticles can be taken up by the plants via aerial deposition, through uptake via the soil or due to the use of irrigation water that contains residues of these chemical hazards. Open field cultivation is, therefore, more vulnerable to this environmental contamination than cultivation in greenhouses as there the environmental conditions are more controlled. For example, in open field cultivation various water sources may be used for irrigation, whereas in greenhouses usually rain or tap water is used for this purpose. The literature review revealed the possible presence of chemical hazards due to the use of treated waste water and sludge, which is currently not used in Dutch agriculture. However, since these practices are applied in other EU countries from which we import leafy vegetables, this information is included in the report. The sections below give an overview of the chemical hazards that may be present in leafy vegetables (the so-called long-list) and their possible contamination routes.

3.2.1 Heavy metals and other elements

3.2.1.1 Uptake in leafy vegetables

Heavy metals are naturally present in the environment and can result from contamination through the use of fertilizers, contaminated sediment, or from atmospheric deposition, for instance, in industrial areas. The uptake of metals by plants can occur through the transfer from the soil to the root (and to the shoot), but also from irrigation water (Margenat et al., 2018) or through atmospheric deposition (Uzu et al., 2010; Folens et al., 2017; Margenat et al., 2018). The transfer of (heavy) metals from the roots to the leaves has shown to occur at different rates (Zorrig et al., 2013; Przybysz et al., 2017). Factors affecting plant uptake of heavy metals include the plant's species, plant growth medium such as soil type, root growth, vegetative growth, the bioavailability of the metal, as well as interactions with other elements and substances in water (Eregno et al., 2017).

Heavy metal uptake from the soil to the plant was studied in China by growing Chinese cabbage in polluted soil obtained near a mining area. Among the five heavy metals tested (cadmium (Cd), chromium (Cr), mercury (Hg), lead (Pb) and arsenic (As)), Cd was most readily taken up. Levels in the roots were higher than in the aerial part of the Chinese cabbage. Average values in the leaves were 0.61, 0.16, 0.00, 0.39 and 0.14 mg/kg for Cd, Cr, Hg, Pb and As respectively. Thus, the EU ML was exceeded for Cd and Pb. Cr, Hg and As did not exceed the Chinese limits of 0.5, 0.01 and 0.5 mg/kg, respectively (Mi et al., 2019).

The effect that atmospheric exposure during cultivation, e.g., when near urban or industrialized sites, has on the uptake of lead (Pb), among other heavy metals, in lettuce was demonstrated in many studies. An experimental study performed in France by Uzu et al. (2010) explored the pathways for foliar uptake of Pb in lettuce cultivated in an area of atmospheric exposure. Even after washing, the Pb average concentration after 43 days of exposure was 335 ± 50 mg/kg dry weight (DW) (= 30.8 mg/kg FW based on a DW of 10.85%), while the control treatment was 5 ± 3 mg/kg DW. Results showed that the EU maximum concentration of 0.3 mg Pb/kg fresh weight (FW) was reached after 0.5 day of exposure (Uzu et al., 2010). It was observed that Pb was found on the leaf surface and within the leaves. (Uzu et al., 2010). In a more recent study, Margenat et al. (2018) assessed the occurrence of several pollutants during the cultivation of lettuce at peri-urban farms in Spain, reporting the concentrations (mg/kg fresh weight (FW)) of 16 trace elements in lettuce (leaves) cultivated during summer and winter seasons. Authors observed Pb concentrations exceeding the EU ML in the edible portions of the lettuce (minimum-maximum concentration of 0.13 – 0.45 mg/kg FW) at one farm plot during summer cultivation (Margenat et al., 2018). Another study by Folens et al. (2017) explored the effect that an urban environment could have on the concentrations of heavy metals and platinum group metals (PGMs) in lettuce grown through domestic horticulture in Belgium. Results revealed that the cultivation substrate (either peat-based or natural) affected the variability in metal levels; namely, more variability in heavy metal levels was observed for lettuce cultivated in natural soils. For example, the average concentration for arsenic (As) was 5.8 times higher in natural soil versus peat-based soil ($p < 0.0001$) (Folens et al., 2017).

A couple of studies investigated the effects of heavy metal uptake when leafy vegetables were cultivated with treated wastewater (i.e. water that has been processed to remove contaminants from wastewater (any water that has been used by humans or sewage). Eregno et al. (2017) examined the reuse of treated greywater (i.e. relatively clean waste water that comes from kitchen and bathroom sinks, showers, and laundry) with added urine during hydroponic cultivation of three lettuce varieties, assessing the health risk of heavy metal contamination. Authors found the bioaccumulation of zinc (Zn), manganese (Mn), and copper (Cu) was relatively higher when compared to the other elements analysed (As, Cd, Cr, nickel (Ni), Pb, and Hg). Cu and As had the highest concentrations in the control (without urine in the irrigation water), of 9.9 – 43.0 mg/kg and 0.2 – 0.3 mg/kg, respectively, for the three lettuce varieties tested (Eregno et al., 2017). Authors concluded that the risk to human health from heavy metals from lettuce produced in such a system was not significant. Nonetheless, health risk-minimizing strategies were suggested like improving the removal efficacy of contaminants in greywater treatments and selecting plants varieties with a reduced potential for heavy metal bioaccumulation (Eregno et al., 2017). Another study investigated the health effects of globe artichoke consumption when cultivated with treated wastewater, finding that heavy metal content (of aluminium (Al), Cd, cobalt (Co), Cr, Cu, iron (Fe), Ni, Pb, Zn, and Mn) in the artichoke heads were lower than international thresholds from the FAO/WHO (mg/kg FW) of, respectively: no prescribed limits, 0.1, 50, 2.3, 73, 425, 67, 0.3, 100, and 500 (Gatta et al., 2018) after irrigation with secondary treated wastewater (i.e. wastewater from the second step during sewage or wastewater treatment, where dissolved and suspended biological matter is removed) and tertiary treated wastewater (i.e. water from the third and final step during sewage or wastewater treatment that improves the quality before reuse, recycling, or entry to the environment; this step can remove inorganic compounds among other substances like nitrogen and phosphorus.). Given low bioaccumulation factors, the authors suggested that the heavy metals did not accumulate in the edible part of the artichoke. Overall, the authors calculated that the health risk with exposure to heavy metals (singly and collectively) was negligible (Gatta et al., 2018).

Cd uptake in plants has been reported to be affected by three main factors, those being related to the soil environment, plant physiology, and season-specific growing factors like weather (Tack, 2017). In order to better estimate the probability that Cd concentrations exceed EU limits, Tack (2017) examined the effect that a watering regime (a seasonal/weather factor) had on Cd uptake in spinach. This study used a fertilized loamy soil from an agricultural region in Belgium with Cd content of 0.45 mg/kg dw (a typical concentration for loam soils in Flanders) (Tack, 2017). Results showed that the drier the conditions, the higher the uptake of Cd, which would thereby increase the probability to exceed the EU ML (Tack, 2017). The study also estimated the probability to exceed the ML for all watering regimes (from dry to wet circumstances), reporting that with an estimated Cd concentration of 0.12 mg/kg FW lettuce, the possibility to exceed the EU ML would be about 4% (Tack, 2017). The results of this study demonstrated the increased Cd uptake in spinach under dry circumstances. In another study, the uptake capacity of Cd in two lettuce varieties (Divina and Melina) to the roots and shoots were investigated by Zorrig et al. (2013). Results showed that Cd concentrations were two-fold higher in roots than shoots (Zorrig et al., 2013).

One study reported the effects of Mn on lettuce and concerns for human health. Przybysz et al. (2017) examined the uptake of the transition metal Mn in shoots of two lettuce cultivars (Satine and Locarno). Lettuce was hydroponically cultivated in growing medium containing varying Mn concentrations (0.5, 5, 25, and 50 mg/L). Results showed an increasing relationship between the concentration in the growing medium and that detected in the lettuce leaves (Przybysz et al., 2017). Authors indicated that at 25 mg/L, the concentrations in a whole lettuce plant would lead to an exceedance of the recommended daily intakes (RDI) presented by the National Academy of Sciences (USA) for Mn, of 2.3 and 1.8 mg/day, respectively for adult men and woman. They also advocated for monitoring Mn levels in water during, e.g., hydroponic cultivation (Przybysz et al., 2017).

3.2.1.2 Occurrence data

The following sections indicate the information found in the literature on the occurrence of individual metals in leafy vegetables. An additional search on nickel, mercury and platinum group metals (PGMs) did not result in papers describing the presence of these metals in leafy vegetables produced or imported in the Netherlands. In the EU, maximum levels are set for the heavy metals Cd and Pb in “leafy vegetables” of, respectively, 0.2 mg/kg FW and 0.3 mg/kg FW as described in Regulation (EC) 1881/2006. From the 10 scientific articles determined to be relevant for metals, 7 reported on Cd.

Aluminium (Al)
Between 2011 and 2016, Al was found with a median concentration of 2.5 mg/kg in both lettuce and spinach in Belgium. Vegetables are among the main contributors to the dietary exposure of Al. Using the concentrations found and European consumption data, a part of the European population surpasses the tolerable weekly intake (TWI) of 1 mg/kg body weight (bw) as set by the EFSA (FAVV, 2018b).

Arsenic (As)
European monitoring data showed that As is found in leafy vegetables and *Brassica* vegetables with upper bound (UB) mean concentrations 0.0235 and 0.0108 mg/kg, respectively. Leafy vegetables are frequently contaminated through direct atmospheric deposition (EFSA, 2009d). Another common pathway of contamination of food with As is through the irrigation of food crops with As-contaminated groundwater (Concha et al., 2013). However, EFSA indicated that rice and algae contribute most to the As exposure (EFSA, 2009d).

Cadmium (Cd)
In Belgium, the exposure of Cd in 2% of the adults is higher than the TWI of 2.5 mg/kg bw. Mean concentrations of Cd in samples of celery, lettuce, spinach, endive, and cabbage on the Belgian market between 2006 and 2007 were 0.044, 0.022, 0.079, 0.009, and 0.005 mg/kg, respectively (Vromman et al., 2010). When comparing different crops grown in the field, leafy vegetables accumulated Cd the most, followed by root vegetables and then grain crops (Concha et al., 2013). Amongst vegetables, spinach is one of the highest contributors to the TWI, 0.9%. Nevertheless, potatoes are contributing 9.03% to the TWI for Cd (Vromman et al., 2010). The EFSA noted exceedances of TWI for Cd in several European countries. In monitoring data collected by EFSA, mean concentrations of spinach and other leafy vegetables were 0.0615 and 0.0231 mg/kg. EFSA recognized leafy vegetables as significant contributors to the dietary exposure of Cd since this substance generally accumulates in the leaves of plants (EFSA, 2009a).

Chromium
Cr can be found in vegetables, but it is important to distinguish which isotope of Cr is of concern. Cr^{3+} may be present in vegetables in higher concentrations than that of Cr^{6+}, but Cr^{3+} has low toxicity and would, therefore, not be a concern to human health. The presence of Cr^{6+} in food would be a concern to health since this isotope is carcinogenic and genotoxic. According to FAVV, it is unlikely that Cr^{6+}

will be present in vegetables because it will be broken down into Cr^{3+}. Furthermore, frozen vegetables have higher concentrations of total Cr than boiled or washed vegetables, which suggests that Cr can leach out under such circumstances. The FAVV established action limits for Cr^{3+}, which are not exceeded in Belgium and EU samples. Measurements on Cr^{6+} are less reliable since this rapidly converts to Cr^{3+} (FAVV, 2018a).

Copper
Cu is found in several leafy vegetables with mean concentrations of 0.95 mg/kg for Chinese cabbage, kale, and baby leaf crop; 0.90 mg/kg for lamb's lettuce, lettuce, escarole, cress, land cress, rocket, spinach, purslane, and chard; and 0.76 mg/kg for watercress. The major contributor to the total dietary exposure of Cu was lettuce with a contribution of 8.2% to the acceptable daily intake (ADI) (EFSA, 2018b). Cherfi et al. (2016) performed a survey in supermarkets on the copper levels in salad and cabbage samples and found an average concentration of 4.35 mg/kg FW and 2.76 mg/kg FW, respectively. The estimated daily intake (EDI) for salad and cabbage was 0.71 µg/kg bw/day and 0.36 µg/kg bw/day for cabbage and salad respectively based on French consumption data, which is below the provisional tolerable daily intake (PTDI) of 500 µg/kg bw/day established by the FAO/WHO.

Nickel
A survey in three supermarkets in France revealed that the average nickel concentration in cabbage and salad was 0.58 mg/kg FW and 0.27 mg/kg FW, respectively. The EDI for the French population exceeded the PTDI of 5 µg/kg bw/day established by the FAO/WHO when all vegetables examined were included (i.e. root vegetables, fruiting vegetables and leafy vegetables). However, the EDI for leafy vegetables was below the PTDI as the EDI resulting from the consumption of salad was 2.06 and for cabbage 0.51 µg/kg bw/day (Cherfi et al., 2016).

3.2.1.3 Effects of processing
Folens et al. (2017) found that washing lettuce decreased the average concentration of all elements studied, with significant differences between before and after washing observed for As, Pb and palladium. Washing decreased the percentage of samples surpassing the EU limit of 0.3 mg/kg for Pb from 33% to 5%. Overall, crop selection, cultivation substrate, and washing before consumption were recommended measures to support safe horticulture in urban environments (Folens et al., 2017).

3.2.1.4 Conclusion
Heavy metals and other elements can be present in leafy vegetables due to uptake from the soil, atmospheric deposition (to the edible shoots), and irrigation water. Several factors can affect the uptake of heavy metals in edible leafy vegetables, such as the plant's species, plant growth medium and watering regime. One study estimated Cd uptake to the roots and leaves of lettuce at a 2:1 ratio. Irrigation with treated wastewater did not result in heavy metal levels higher than the FAO/WHO thresholds. A part of the European population surpasses the TWI of 2.5 mg/kg bw for Cd and the TWI of 1 mg/kg bw for Al. An experimental study showed that the US RDI was exceeded for Mn when lettuce was hydroponically cultivated with water containing > 25 mg/L. Furthermore, EU ML exceedances were reported for Cd and Pb. Finally, lettuce was reported as the main contributor to the Cu intake via the diet.

3.2.2 Nitrate and nitrite

3.2.2.1 Uptake in leafy vegetables
Nitrate can be taken up from the soil and accumulate in leafy vegetables like spinach, chard, or lettuce. Croitoru et al. (2015) reported that previous surveys showed that lettuce is a main source of nitrate, while monitoring data on retail samples detected nitrate and nitrite in spinach and lettuce; the details of which are summarized below (Iammarino et al., 2014). The nitrate concentrations in the leaves are higher than that of roots and fruits (Menal-Puey and Asensio, 2015). Influencing factors include the plant (e.g., variety, species, maturity) and the environment or season (e.g., temperature, humidity, light exposure, use of fertilizers or herbicides, water used, soil) (Iammarino et al., 2014; Croitoru et al., 2015; Menal-Puey and Asensio, 2015). For instance, winter-grown leafy vegetables have been reported to have higher nitrate concentrations than summer-grown leafy vegetables (Menal-Puey and Asensio, 2015; Margenat et al., 2018). This difference can be explained by the fact

that a breakdown of nitrate in the plant is effectuated by high temperature and high light intensity. In contrary, dryness promotes accumulation in the plant. Vegetables cultivated in greenhouses usually have higher concentrations of nitrate than vegetables cultivated in the field (BfR, 2005). Also, Croitoru et al. (2015) indicated that previous studies have shown that lettuce grown in hydroponic conditions have significantly higher amounts of nitrate than those grown in soil.

High concentrations of nitrate in soil occur especially after fertilization, meaning the absorption in the roots can be elevated and exceed a plant's capacity to assimilate nitrogen leading to nitrate accumulation in plants (Yusof et al., 2016). Another possible entry of nitrate in the food chain is contaminated water due to livestock production, sewage discharge, and intensive cultivation methods (EFSA, 2017b). The reduction of the exposure of nitrate should be achieved primarily through sound cultivation and harvesting methods according to BfR (BfR, 2013a).

3.2.2.2 Occurrence data

A summary of the relevant literature for nitrate and nitrite is presented below by crop type. In terms of human health, the *in vivo* conversion of nitrate to nitrite is of concern since the latter is shown to have toxic effects in humans (Croitoru et al., 2015; Menal-Puey and Asensio, 2015; Yusof et al., 2016). Nitrate is naturally present in leafy vegetables, whereas nitrite exposure is primarily through the conversion of nitrate into nitrite. EFSA confirmed the ADI for nitrate as set by JECFA of 3.7 mg/kg bw/day (EFSA, 2008b).

Spinach

Monitoring of 75 retail spinach samples from Italy, of which 21 were fresh, 18 were IV range (meaning ready-to-eat fresh-cut vegetables) and 36 frozen, were analysed by Iammarino et al. (2014). Results showed that nitrate concentrations ranged from 182.5 – 2291.8 mg/kg, 15.2 – 2252.0 mg/kg, and 103.0 – 2978.1 mg/kg, respectively, for the three types. Four spinach samples (*i.e.,* 5.3% of the total samples), of which 1 was from the IV range, and 3 were frozen, had nitrate concentrations above the EU legal limits (Iammarino et al., 2014). Nitrite concentrations ranged from, respectively, 9.5 – 28.5 mg/kg, 12.0 – 197.5 mg/kg, and 9.6 – 106.8 mg/kg for fresh, IV range and frozen spinach. Also, the authors discussed the presence of non-negligible concentrations of nitrite in spinach, advising for a maximum admissible level in legislation (Iammarino et al., 2014).

Chard

The study by Menal-Puey and Asensio (2015) evaluated nitrate from edible portions of chard (*Beta vulgaris cycla*) found in Spanish retail, reporting mean concentrations of 2293 mg/kg and 1930 mg/kg, respectively, for samples cultivated in winter (n = 28) and summer (n = 28). Authors estimated the dietary intake of chard resulted in an exposure at 3.5% and 1.7% of the ADI, respectively, for adult and children, while for extreme consumers this was 79% of the ADI (Menal-Puey and Asensio, 2015).

Lettuce

In total, 75 lettuce samples obtained from retailers in Italy, consisting of 17 Iceberg, 6 Butterhead, 6 Canasta, 9 Trocadero, 20 Mixed, 9 Romana, and 8 Scarola, were analysed by Iammarino et al. (2014). Of these 75 lettuce samples, 15 were of the IV range: 5 Iceberg, 8 Mixed, and 2 Scarola. Results showed that nitrate concentrations ranged from 35.3 mg/kg – 5101.0 mg/kg, depending on the type of sample. Five lettuce samples (*i.e.,* 6.7% of the total samples), of which 1 was Butterhead, 1 was Trocadero, 2 were Romana, and 1 was Scarola lettuce, had nitrate concentrations above the EU legal limit of 3000 mg/kg for lettuce grown in the open air in summer (Iammarino et al., 2014). Nitrite was only found in the Romana lettuce (66.5 mg/kg). Authors concluded that given the amount of non-compliant samples, control is needed (Iammarino et al., 2014).

Croitoru et al. (2015) examined the difference in hydroponic growing conditions to try and obtain "nitrate free" lettuce. Authors found that growing lettuce on nutrient solutions with low nitrate content resulted in a significant decrease in nitrate, reported as 1741 mg/kg versus 39 mg/kg. Moreover, replacing nitrate with ammonium resulted in a decrease in lettuce nitrate concentration, from 1896 mg/kg to 14 mg/kg. These results indicated that changing the growing media (environment) is one possible way to reduce nitrate concentrations in lettuce.

Furthermore, while assessing lettuce quality parameters, concentrations of nitrates (mg/kg) have been reported to range from 628 – 1854 mg/kg (Margenat et al., 2018). Authors reported concentrations of nitrate in lettuce during open field cultivation to be below EU limits of 4000 mg/kg and 3000 mg/kg, respectively, for winter and summer (Margenat et al., 2018).

Rocket

Rocket has shown to have a high degree of accumulation of nitrate. In a survey of 350 rocket samples in Germany, approximately half of them contained nitrate concentrations above 5000 mg/kg. This means that rocket could contribute for a great part to the ADI of 3.7 mg/kg body weight (BfR, 2005).

3.2.2.3 Effects of processing

Yusof et al. (2016) examined the effects of processing with vacuum impregnation (VI) as a means to reduce nitrate content in spinach leaves. Since foods like spinach can have high levels of nitrate, e.g., up to 3000 mg/kg FW, consumption of 100 g of spinach would exceed the ADI (Yusof et al., 2016). Results showed that sucrose feeding with VI significantly reduced nitrate concentrations in baby spinach leaves; for example, after 72h of VI exposure, levels of nitrate reduced by 70% compared to only 24% for non-VI. After 72 h, nitrite did not accumulate given impregnation with sucrose solutions (Yusof et al., 2016).

Food preparation also influences nitrate exposure. For example, removing the stems and veins of the lettuce and spinach, nitrate content thereof can be decreased by 30 – 40%. Further, cooking methods, like boiling, can reduce the amount of nitrate in vegetables (FAVV, 2014).

3.2.2.4 Conclusion

Several studies reported the uptake of nitrate or nitrite in leafy vegetables like spinach, chard, and lettuce. Plant characteristics and the environment can affect the uptake of nitrate. In spinach and lettuce samples, MLs for nitrate were occasionally exceeded. In chard, the dietary intake of nitrate from chard for extreme consumers was reported as 79% of the ADI.

3.2.3 Polycyclic aromatic hydrocarbons (PAHs)

3.2.3.1 Uptake in leafy vegetables

PAHs are substances that are formed during the incomplete combustion or pyrolysis of organic matter as can occur during forest or brush fires or in other industrial processes and can lead to environmental contamination. PAHs have showed to vary in their toxicity and can bioaccumulate in the food chain. They can also be formed during food preparation, e.g., due to frying, smoking, drying, barbecuing, grilling, baking, or broiling.

PAH uptake from the soil to the plant (e.g., through root uptake and translocation) is a source and entry of PAH into the food chain. PAH contamination in food crops is not often found (Deng et al. 2018). The authors showed that root uptake was the main pathway for PAH in pak choi and that accumulation occurred in the plant tissues. For instance, PAH content was 174.1 µg/g and 10.8 µg/g, respectively, in roots and shoots, with dominant PAHs being naphthalene (22.1 µg/g in roots and 2.5 µg/g in shoots); benz[a]anthracene (28.0 µg/g in roots and 1.5 µg/g in shoots); and dibenz[a,h]anthracene (265.9 µg/g in roots and 0.4 µg/g in shoots) (Deng et al., 2018). When pak choi was exposed to both PAHs and heavy metals, PAH concentrations in the roots and shoots were significantly lower versus that of PAH-only exposed plants ($p < 0.05$), except for PAHs + Cd and PAHs+ Zn treatments. Cd was shown to increase PAH content in roots slightly, while Zn had no significant impact ($p > 0.05$) (Deng et al., 2018). Authors reported that the soil-to-root or water-to-root pathways contributed to the main source of PAHs in pak choi tissues. (Deng et al., 2018). In contrast, Paris et al (2018) indicated that although contamination via the soil may occur, contamination via air is the main source of PAH contamination of leafy vegetables.

3.2.3.2 Occurrence data

Literature from the Google search resulted in no hits related to PAHs in leafy vegetables. An additional search was performed for PAH in leafy vegetables, which resulted in 3 surveys on PAHs in leafy vegetables, 2 in China and 1 in the Netherlands and 1 review paper. The results of which are described below.

PAHs were detected in pak choi in a Chinese survey. Together with Chinese cabbage (n = 11) and spinach (n = 14), pak choi (n = 15) was sampled in the years 2012 and 2017. The concentrations of the sum of PAH16 tested were 38, 40 and 47 µg/kg for Chinese cabbage, spinach and pak choi, respectively. It was concluded that the presence of PAHs was higher in leafy vegetables than in root vegetables (Wang et al., 2018a). Five samples of each cauliflower, Chinese cabbage and green vegetables, purchased in May 2015 in supermarkets in China, were tested on the presence of PAHs. Concentrations for PAH16 were 198 µg/kg for cauliflower, 312 µg/kg for Chinese cabbage and 89.9 µg/kg for green vegetables. Fruit vegetables and root vegetables were tested on the presence of PAHs as well, but showed lower concentrations than the leafy vegetables. Furthermore, a risk assessment was performed with the help of the calculation of a margin of exposure for PAH4. For most population groups, the margin of exposure was under the value of 10,000, which indicates a potential cancer risk (Wu et al., 2016). Another Chinese survey in 2014 was conducted in areas close by industrial zones in Shanghai. Here, the presence of PAHs was identified in samples of Chinese cabbage (n = 8), lettuce (n = 8), Romaine lettuce (n = 5) and Shanghai green cabbage (n = 8). The mean concentrations of PAH16 in the products mentioned were 261 µg/kg DW, 205 µg/kg DW, 321µg/kg DW and 239 µg/kg DW, respectively. In the latter survey, the highest concentrations were also found in leafy vegetables, followed by stem vegetables, seed/pod vegetables and rhizome vegetables (Jia et al., 2018).

The presence of PAH16 was monitored during the period 2004-2013 in kale and spinach cultivated on a field close by waste incinerators in The Netherlands. The highest mean concentrations were measured in 2004 with 315 µg/kg DW spinach and 310 µg/kg DW kale(Van Dijk et al., 2015).

Paris et al (2018) recently published a review on PAH levels in fruits and vegetables. Levels found largely depend on the environment of the crop cultivation. They found lower PAH levels in rural areas than in urban or industrial arias. Lighter PAHs (based on molecular weight; for example, napthalene) were found at higher concentrations than the heavy PAHs. The latter are seen as more toxic. In general, PAH concentrations in fat products, dairy products and meat are higher than in vegetables. Meat and meat products and oils and fats contribute to 54 – 71% and 8 – 12% to the dietary PAH intake of adults, respectively. Nevertheless, depending on consumption patterns and the occasionally high levels found in vegetables, vegetables may be a significant source of PAH intake. The authors summarised the sum of the 8 heavy PAHs (PAH8, i.e. benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, chrysene, dibenz[a.h]anthracene, and indeno[1.2.3-cd]pyrene) as an indicator of the presence of genotoxic and carcinogenic PAHs, the most relevant PAHs for toxicity. PAH8 levels found range between 0.03 – 11.15 µg/kg FW for cabbage, 0.08 – 6.47 µg/kg FW for cauliflower, 0.36 – 17.08 µg/kg FW for celery, 0.77 – 4.14 µg/kg FW for kale, 0.05 – 29.51 µg/kg FW for lettuce, 0.6 – 6.57 µg/kg FW for spinach and 0.37 – 8.66 for turnip (Paris, 2018).

3.2.3.3 Conclusion

PAHs in the soil and atmospheric deposition can result in presence of PAHs in the edible parts of the plants. When leafy vegetables are grown in urban or industrial areas, high levels (> 5 µg/kg) were found for PAH8.

3.2.4 Pharmaceuticals, personal care products and endocrine disrupting compounds

3.2.4.1 Uptake in leafy vegetables

Pharmaceutical and personal care products (PPCPs) and endocrine disrupting compounds (EDCs) can be introduced during the primary production of leafy vegetables. This introduction can occur from the application of either manure or biosolids (i.e., organic solids derived from sewage treatment processes) or the use of reclaimed wastewater (i.e. previous wastewater that has been further treated to remove solids and impurities with the end purpose of reuse e.g., for irrigation purposes) on the field, meaning compounds present in these sources can enter the food chain through uptake in the plants when grown in open field resulting in its potential exposure to humans (Tian et al., 2019). PPCPs and EDCs can also be found in surface water impacted by wastewater treatment plant effluent (i.e. final product from wastewater treatment plant) and in groundwater (Dodgen et al., 2013b). Also, pharmaceuticals such as antibiotics can end up in (leafy) vegetables when they are grown in the vicinity of animals treated with veterinary drugs (Caban et al., 2018).

Experimental studies have been performed to analyse the uptake of pharmaceuticals like antibiotics, including the metabolites thereof, and EDCs like steroids and phthalate esters in leafy vegetables. According to Berendonk (2018), experimental results have shown that the uptake of these substances by plants decreased as follows: leafy vegetables > root vegetables > cereal and food crops > fruit vegetables. Lettuce (*Lactuca sativa*) had often been chosen as an experimental crop since it absorbs about half of its weight in water, so the exposure from a contaminated environment (in particular water), makes it a vulnerable crop. Also, since it can be grown in both the summer and winter there is a longer cultivation season in which the crop could potentially be contaminated (Shargil et al., 2015; Shargil et al., 2016) and, furthermore, it is a widely cultivated crop (Adeel et al., 2018).

Antibiotics

Experimental studies on the uptake of the antibiotics clarithromycin (CLA), sulfadiazine (SDZ), and sulfathiazole (STZ) have reported uptake in the roots and leaves of lettuce. Tian et al. (2019) found eight metabolites of CLA and two metabolites of sulfadiazine in both the leaves and roots of lettuce grown under hydroponic conditions after exposure to nutrient solutions containing 1 mg/L CLA or SDZ. Concentrations in lettuce leaves were 1629 μg/kg and 683 μg/kg, respectively for CLA and SDZ, while concentrations in roots were reported to be higher for both antibiotics with concentrations of 4977 μg/kg and 24,599 μg/kg, respectively. This indicates that uptake in the roots is higher than in the leaves. The authors advocated that in addition to evaluating parent compounds, a risk assessment of the metabolites of antibiotics in edible vegetables should be done to be able to better conclude on the consequence of long-term exposure (Tian et al., 2019). Contrary, another experimental study showed that antibiotics were mostly translocated to the leaves, followed by the stem and the roots (Yu et al., 2018b).

Caban et al. (2018) indicated that the application of green manure (hairy vetch, *Vicia villosa*) significantly reduced the uptake of STZ to 5.1% given the application; authors concluded that this practical solution could be used to give extra time to degrade antibiotics and/or have them be absorbed into the soil, thereby reducing their uptake by the plant (Caban et al., 2018).

Steroids

Steroidal estrogens may constitute a high probability of entering the food chain given the release into the environment (soil and water) via, e.g., human and livestock excreta (Adeel et al., 2018). In the second half of the last century, it was already discovered that not all hormones are fully biodegraded and can still be present in water after purification (Colon and Toor, 2016). Adeel et al. (2018) determined the potential uptake of estrogens in lettuce, finding that absorption of both ethinylestradiol and 17β-estradiol (17β-E2) in the roots and leaves occurred. Ethinylestradiol uptake was very low (3% and 0.5% of the spiked concentration of 2000 μg/L in roots and leaves respectively) during week 1 and increased over time up to 12% in roots and 8% in leaves. For 17β-E2, uptake was mainly to the roots after week 2 (13%), and by the end of the study was 10% in the leaves (Adeel et al., 2018). Bioaccumulation of estrogens in lettuce roots and leaves were higher at the spiked concentration of 50 μg/L versus that of 500 or 2000 μg/L. At the spiked concentration of 50 μg/L, bioaccumulation of ethinylestradiol and 17β-E2 was 23% and 27%, respectively, in leaves (Adeel et al., 2018). Overall, the plants (roots and leaves) accumulated 17β-E2 at higher concentrations than ethinylestradiol. Estrogen concentrations in plants were compared to the Joint FAO/WHO Expert committee on food additives (JECFA) who established a recommended daily intake for 17β-E2 of 3 μg/day and were found to exceed the RDI for certain individuals, indicating a human health concern (Adeel et al., 2018).

Other experimental studies for leafy vegetables researched the uptake of steroids like corticosterone, estrone, and testosterone in lettuce. Irrigation water was reported to be the main factor contributing to the absorption of hormones by lettuce. There was a positive, significant correlation between the concentration of the steroids corticosterone (Shargil et al., 2016), as well as estrone and testosterone (Shargil et al., 2015) with the plant’s fresh weight in winter grown lettuce.

Phthalate esters

Phthalates or phthalate esters (PAEs) are EDCs that can occur in the environment and are used in many industrial products. They are mainly used as plasticizers and can be taken up by agricultural products when cultivated with contaminated soil or water, or polluted air. The use of plastic films in greenhouse production may also be a source of exposure during primary production (Zhao et al., 2015; Ma et al., 2018), along with the use of wastewater irrigation and sewage sludge in fields (Zhao et al., 2015). Leafy vegetables may also become contaminated during processing (e.g., packing, canning, drying) or storage depending on the materials used as is the case for all food products that come into contact with plastic films (Cao, 2010).

Concentrations of di-n-butyl phthalate (DnBP) and di (2-ethyl hexyl) phthalate (DEHP), two PAE additives of concern, were reported to be >32 mg/kg in urban areas (in China) (Ma et al., 2018). Authors found that soil conditions like total protein, total soluble sugar, and free amino acid content were positively correlated with DnBP and DEHP concentrations in lettuce. The changes in these soil conditions and the effect on pH values in the soil influenced the toxicity of PAEs and thus, the safety of the vegetables (Ma et al., 2018).

Another study by Zhao et al. (2015) found that DnBP and DEHP accumulation in Chinese flowering cabbage varied between the 28 cultivars studied with significant differences ($p < 0.05$) in the PAE concentrations of the shoots. Authors observed a positive correlation between both PAE additives, meaning simultaneous uptake of these PAEs could occur. They also found a lower PAE translocation from roots to shoots and more accumulation in cells walls and organelles of the roots (Zhao et al., 2015).

Other pharmaceuticals and personal care products

Hurtado et al. (2016) investigated the possible uptake of a wide range of emerging organic contaminants (EOCs) in lettuce: BPA, CBZ, propranolol, tonalide, sulfamethazine, TCS, caffeine, and ibuprofen. These substances were chosen, given their detection and occurrence in all types of water and effects on humans (Hurtado et al., 2016). Results showed that the concentrations in the lettuce leaves were on average between 0.5 and 110 times lower than in roots; concentrations of CBZ (ranging from 233 – 2054 ng/g dw) were much higher than the other contaminants measured (Hurtado et al., 2016).

A recent experimental study by González García et al. (2018) looked at the accumulation of an anticonvulsant, carbamazepine (CBZ) and an anti-inflammatory agent, diclofenac (DCF) in three lettuce varieties. These drugs have been reported to be found in surface water and groundwater in many EU countries, including the Netherlands, and may be found in treated wastewater to be used for irrigation water during crop production. Authors found that highly hydrophobic drugs (e.g., DCF) were readily absorbed by the roots and showed a slow translocation process to the leaves. Meanwhile, CBZ (an intermediate hydrophobic compound) more easily translocated and accumulated in the leaves (González García et al., 2018). Bhalsod et al. (2018) found that most pharmaceuticals quickly dissipated in soils, except for CBZ, which was very persistent in soil and hyper accumulated in lettuce shoots. Moreover, this study found that the use of overhead irrigation water, versus that, e.g., of soil-surface irrigation, resulted in a greater concentration of pharmaceuticals (antibiotics) like trimethoprim, monensin sodium, and tylosin in lettuce shoots (Bhalsod et al., 2018). Hence, pharmaceutical compounds can be absorbed in different rates, e.g., based on their physicochemical properties, in different plant compartments (González García et al., 2018). This reasoning is also supported by another study which observed the uptake of CBZ and its transformation product 10,11-epoxycarbamazepine in lettuce leaves of peri-urban farms in Spain (Margenat et al., 2018).

Another study explored the uptake of three common pharmaceuticals (fluoxetine hydrochloride (Prozac®), ibuprofen, and 17α-ethinylestradiol (EE2) in celery (Schroeder et al., 2015). Authors found that the uptake of these in celery occurred at different rates; these were thought to be due to the soil organic carbon-water partitioning coefficient (Koc). Given higher Koc values, like that for EE2 and fluoxetine HCl, the pharmaceutical took more time to be taken up in the celery stalk, while for lower Koc values, like for ibuprofen, it was taken up quickly and metabolized (Schroeder et al., 2015).

Wu et al. (2013) studied the translocation of 20 pharmaceuticals and personal care products including 16 pharmaceuticals, 3 personal care products (i.e. *N,N*-diethyl-*meta*-toluamide (DEET), triclosan, triclocarban and 1 herbicide (diuron) in vegetables. Triclocarban, fluoxetine, triclosan, and diazepam were primarily found in the roots of the vegetables. Mebrobamate, primidone, CBZ, phenytoin (also known as dilantin) and diuron were mostly distributed to the stems and/or leaves of the plant. For this reason, these substances are expected to be present in higher values in leafy vegetables rather than in other vegetables (Wu et al., 2013).

In an experimental study on lettuce and collards, the uptake and accumulation of bisphenol A (BPA), diclofenac sodium (DCL), naproxen (NPX), and 4-nonylphenol were measured. The accumulation of these compounds in lettuce and collards decreased as follows: BPA>NP>DCL>4-nonylphenol. It was expected that vegetables, which are hydroponically cultivated, accumulate these pharmaceuticals and EDCs to a greater extent than vegetables that are grown in soil. Moreover, it was concluded that the accumulation was higher in roots than in stems and leaves (Dodgen et al., 2013a).

3.2.4.2 Occurrence data

The literature study primarily resulted in experimental studies; the results of which are indicated above (section 3.2.4.1). An additional search on pharmaceuticals resulted in one survey from Jordan and two surveys from China. In Jordan, a survey was conducted between October 2014 and May 2015 to determine several pharmaceuticals and other micropollutants (25 substances) in field-grown vegetables irrigated with treated municipal wastewater. The highest concentrations of total pharmaceuticals (25 substances) tested were found in leafy vegetables with a range of 247 – 533 µg/kg DW, followed by root vegetables with concentrations of 73 – 126 µg/kg DW and fruiting vegetables of 5 – 76 µg/kg DW. The authors conducted a risk assessment using Spanish consumption data, which resulted in a daily human exposure to the substances analysed of 0.18-7.56 ng/kg bw/day in the leafy vegetables tested (cabbage, lettuce and ruccola) meaning that exposure through consumption of food crops is low. Furthermore, they used the Threshold of Toxicological Concern (TTC) approach and estimated that a minimum daily consumption of 9 kg per day is needed to reach the TTC of class III compounds (e.g., carbamazepine, trans-DiOH-CBZ, and gabapentin) (Riemenschneider et al., 2016).

In a Chinese survey, the antibiotics enrofloxacin (ENR), ciprofloxacin (CIP), norfloxacin (NOR) were widely detected in organic Chinese cabbage, lettuce, and mustard. Concentrations measured were 15 µg/kg for Chinese cabbage, 15 µg/kg for lettuce, and 20 µg/kg for mustard. Here, the concentrations of the leafy vegetables were the lowest and fruiting vegetables showed the highest concentrations. Notably, the average concentrations of CIP and NOR in greenhouse soils were lower than those in open-field soils (Wu et al., 2014). Another survey in China tested the presence of the same antibiotics in samples of celery, lettuce, and spinach. In this study, only spinach had detectable concentrations of the antibiotics with mean concentrations of 411.0 µg/kg for NOR, 17.6 µg/kg for CIP, and 24.1 µg/kg for ENR. The study concluded that NOR had a higher ability to be taken up by the plants than the antibiotics ENR and CIP. Nevertheless, fruiting vegetables showed higher transfer of the antibiotics than the leafy vegetables (Li et al., 2014b). An additional search on the presence of NOR in leafy vegetables did not confirm the high mean concentration of NOR found.

3.2.4.3 Conclusion

Primarily experimental studies were found indicating a possible uptake of PPCPs and ECDCs in leafy vegetables such as lettuce and cabbage. Uptake to the edible shoots (leaves) occurred when cultivated with e.g., contaminated soil or irrigation water. One survey from Jordan was found on the uptake of several pharmaceuticals. Results indicated human exposure to these compounds via consumption of leafy vegetables is low (< 8 ng/kg bw/day). Two Chinese surveys were found on the antibiotics ENR, CIP, and NOR. Levels of ENR and CIP were low compared to the EU MLs for other food products. One of the Chinese studies reported a high NOR level, which was not confirmed in an additional literature search.

3.2.5 Flame retardants

3.2.5.1 Uptake in leafy vegetables

Flame retardants are compounds that have been used in products, like plastics, textiles, and electronics, to inhibit combustibility. They can enter into the environment through air, water, or soils, or from waste streams. For leafy vegetables, the source of flame retardants may be attributed to the soil used for cultivation, thus soil is potentially a route for further transfer along the food chain (Hyland et al., 2015).

The literature on the presence of flame retardants in leafy vegetables was limited. An experimental study by Hyland et al. (2015) investigated the uptake of three organophosphate flame retardants, tris(2-chloroethyl)-phosphate (TCEP), tris(2-chloroisopropyl)-phosphate(TCPP), tris(1,3-dichloro-2-propyl)-phosphate (TDCPP) in lettuce. TCEP and TCPP were found to have a linear correlation in lettuce to their concentration in the applied irrigation water. The accumulation of TCEP and TCPP was suggested, by the authors, to be affected by soil organic carbon where the plants were grown. Authors reported that ionizable contaminants (e.g., chemicals like diphenhydramine and trimethoprim) might have less accumulation potential to the edible portions of the lettuce than non-ionized contaminants like organophosphate flame retardants (Hyland et al., 2015). The paper showed that exposure to tested flame retardants after consumption of contaminated strawberries and lettuce was estimated to be 0.62 and 9.17 ng/kg bw/day for TCEP and TCPP respectively, which is well below the indicated maximum acceptable daily intakes of 2200 ng/kg per day and 8000 ng/kg per day, respectively, for TCEP and TCPP (Hyland et al., 2015).

An experiment by Navarro et al. (2017) tested the effect of amending biosolids to the soil on vegetables. Authors detected in spinach a sum of polybrominated diphenyl ethers (PBDEs) of 0.27 μg/kg DW in the control, 0.48 μg/kg DW from soil added with anaerobically digested thermal drying sludge and fortified with deca-BDE and 0.43 ϑg/kg DW from soil added with anaerobically digested municipal solid waste compost and fortified with deca-BDE (Navarro et al., 2017).

3.2.5.2 Occurrence data

The literature study and the additional search did not result in papers describing the occurrence of flame retardants in leafy vegetables on the market. Literature from the Google search resulted in no hits related to flame retardants in leafy vegetables. However, flame retardants accumulate in fat and as a result are primarily found in animal products.

3.2.5.3 Conclusion

Flame retardants like TCEPs, TCPPs, and PBDEs can be taken up in the edible portions of leafy vegetables such as lettuce and spinach. Levels found did not result in an exceedance of the ADI. No papers were found reporting the presence of these substances in leafy vegetables on the market.

3.2.6 Per- and polyfluoroalkyl substances

3.2.6.1 Uptake in leafy vegetables

Perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA) are examples of per- and polyfluoroalkyl substances (PFASs). These substances have been used for several decades in industrial and household products; they are of public health concern given their potentially adverse risk to human health (Xiang et al., 2018; Yu et al., 2018a). PFASs like PFOS can make their way into soils through land-application of biosolids, pesticide use, and irrigation with waste water (Yu et al., 2018a). Studies have shown that lettuce can accumulate PFASs to the edible parts of the plant (Xiang et al., 2018; Yu et al., 2018a). Three experimental studies were found that evaluated the factors affecting the presence of PFASs in leafy vegetables; these are briefly summarized below.

Yu et al. (2018a) studied the accumulation and translocation of PFOS among 26 cultivars of lettuce. Results showed that PFOS concentrations in shoots and roots along with bioaccumulation and translocation factors varied among the lettuce cultivars. For example, concentrations in roots ranged from 0.5 – 1.3 and 4.5 – 5.9 mg/kg dw, respectively, for PFOS levels tested at 0.2 and 1.0 mg/kg. For shoots, this was 0.04 – 0.08 and 0.62 – 0.83 mg/kg dw, respectively. The authors found that proteins in the lettuce played a role in the carrier and transpiration of PFOS uptake, highlighting that the function of the proteins was cultivar-specific (Yu et al., 2018a).

Another study, by Xiang et al. 2018, studied the accumulation and translation of PFOA among 20 cultivars of lettuce when cultivated in PFOA-contaminated soils showing differences in PFOA uptake between cultivars. The concentration of PFOA was significantly lower ($p < 0.05$) in the shoots of loose-leaf cultivars than that of romaine or head lettuce cultivars.

An experimental study, performed by Navarro et al. (2017) in Spain, showed a concentration of 1.72 µg/kg DW for PFASs in spinach grown in the control soil. Soils amended with two types of biosolids resulted in levels of 5.33 µg/kg DW and 0.99 µg/kg DW (Navarro et al., 2017).

3.2.6.2 Occurrence data

The literature search and Google search did not result in papers describing the occurrence of PFASs in leafy vegetables. The additional literature search resulted in an EFSA report on PFAS in food (EFSA, 2018c). In their study, EFSA collected data on PFOS and PFOA in various food products. For PFOS, the mean LB and UB concentrations both for leafy and *Brassica* vegetables were 0.002 µg/kg and 0.11 µg/kg, respectively. For PFOA, the mean LB and UB concentrations for leafy vegetables were, respectively, 0.007 µg/kg and 0.14 µg/kg, while for *Brassica* vegetables this was 0.002 µg/kg and 0.15 µg/kg. They concluded that fish and other seafood contributed most (86%) to the LB chronic PFOS exposure, followed by meat (products) and egg (products). For PFOA, milk and dairy products (although limited data), drinking water, and fish and seafood contributed most to the LB mean chronic exposure (EFSA, 2018c).

3.2.6.3 Conclusion

Experimental studies showed the possible uptake of PFASs like PFOS and PFOA in lettuce cultivars and spinach. However, levels reported by EFSA in leafy vegetables on the market are low and leafy vegetables were not considered major contributors to PFAS intake.

3.2.7 Radionuclides

3.2.7.1 Uptake in leafy vegetables

Radionuclides are known for their high carcinogenicity to humans. Sources of radionuclides in the environment can occur due to nuclear waste disposal, testing nuclear weapons, or from accidents involving nuclear power generation or manipulation with nuclear fuel (Šuňovská et al., 2012). The pathway of radionuclides towards food can occur through root uptake, direct deposition to above-ground parts of the plant, or resuspension in contaminated soil. Leafy vegetables may also take up radionuclides via the air (Šuňovská et al., 2012). Extrinsic characteristics, like climate, soil type, and vegetation influences the transfer factors of radionuclides into plants, and therefore, transfer factors are location specific (Al Attar et al., 2015). Also plant characteristics such as the physiological activity of the plant at the time of exposure, the capacity of root uptake of the radionuclide, and its mobility through the plant could influence the transfer to the plant (Al Attar et al., 2015).

In August 2008, a nuclear incident occurred in Fleurus, Belgium. The installation at the National Institute of Radio elements was discarding Iodine-131 (^{131}I) in the environment. This incident was noticed three days later, and the Belgian Federal Agency for Nuclear Control switched off the installation. As a result, the installation had been leaking the radionuclides for at least three days. ^{131}I is a health hazard to humans as it accumulates in the thyroid. Samples were taken from surrounded cultivation areas by the Belgian Food Agency for the Safety of the Food Chain (FAVV). Several samples of lettuce, cabbage, spinach, celery, endive, and Brussels sprouts were tested for ^{131}I. The concentration of the radionuclide was lower than the EU limit of 2000 Bq/kg for all samples, and thus, the FAVV concluded and also informed stakeholders that the incident did not endanger food safety (FAVV, 2009).

More recently, a study evaluated the influence of transfer factors of ^{90}Sr and ^{137}Cs from soil to lettuce during different growing stages, finding that soil contaminated with ^{90}Sr at early stages was associated with the highest transfer rates (c.a. 1.2) to the lettuce (Al Attar et al., 2015). For ^{137}Cs, the germination stage showed the lowest transfer factor (0.066), which then increased more than three times when contamination occurred during leaf development (Al Attar et al., 2015).

Another study conducted field studies near a former uranium mine, exploring the effects on two lettuce varieties grown in fall and summer when exposed to varying uranium concentrations (Abreu et al., 2014). Results showed that lettuce grown in uranium-contaminated soil (9.2 – 14.5 mg/kg) and irrigated with uranium-contaminated water (0.94 – 1.14 mg/L) had uranium concentrations in the leaves (2.13 and 5.36 mg/kg DW) and roots (4.64 and 28.2 mg/kg DW), respectively, for the Marady and Romana varieties (Abreu et al., 2014). The higher concentrations of uranium in the leaves and roots of in the summer-grown variety Romana were due to the higher frequency and amount of irrigation water used. Authors estimated the intake of uranium from such consumed lettuce to be low related to the WHO tolerable daily intake of 0.6 mg uranium/kg body weight per day, meaning that such uranium-contaminated lettuce was not seen as a health concern (Abreu et al., 2014).

3.2.7.2 Occurrence data

The additional search yielded two papers reporting radionuclides in foods. Out of the 48 vegetable samples (not specified which vegetables), only 1 sample was positive for ^{137}Cs. This sample concerned wild mushroom at a level below the EU limit (RIVM, 2016). An overview of samples taken between 2010 and 2013 showed that only 7 out of 585 vegetable samples (not specified which vegetables) contained radionuclides. Again these positive samples concerned wild mushrooms with levels below the EU limit (Brandhoff et al., 2016).

3.2.7.3 Conclusion

The transfer of radionuclides towards leafy vegetables can differ, e.g., based on the location, cultivation conditions, or growing stage. Leafy vegetables that were grown in contaminated areas were shown to take up radionuclides. However, the levels found were either below the EU limit (for ^{131}I) or did not result in an exceedance of the TDI (for uranium). Occurrence data on vegetables showed that if radionuclides were detected, the vegetable samples concerned other vegetables like mushrooms with levels below the EU limit.

3.2.8 Nanoparticles

3.2.8.1 Uptake in leafy vegetables

Nanoparticles (NPs) are particles between 1 – 100 nm, among which silver nanoparticles (AgNPs) and titanium dioxide (TiO_2-NPs) are some examples. One pathway for the exposure of AgNPs in the environment is via wastewater streams, with the majority of AgNPs then being retained by biosolids. Consequently, the application of biosolids to the soil is a potential source of AgNPs in food (Doolette et al., 2015). Similarly, for TiO_2-NPs, these can enter the environment via wastewater streams, accumulating in sewage sludge, but can also be released from paints and concretes or into the atmosphere upon abrasion (e.g., while sanding the outside of buildings). A concern is their eventual presence and uptake in food crops, for example, via fertilizers or plant protection products (Larue 2014).

Two experimental studies were found that evaluated the effects of nanoparticles on leafy vegetables; these are briefly summarized below.

Doolette et al. (2015) studied the bioavailability of silver (Ag) to lettuce amended with biosolids containing silver sulphide nanoparticles ($Ag2_S$-NPs) at environmentally relevant concentrations (0.3-2.2 mg Ag/kg). Results showed very low concentrations in the edible parts of the lettuce shoots of <0.02% of the total Ag concentration for treatments. Overall, the probability of AgNPs in soils is low; for instance, for plants with the highest Ag concentrations, only 0.06% of added Ag was found in the edible parts of the lettuce shoots in terrestrial systems (Doolette et al., 2015).

In another experimental study, Larue et al. (2014) examined the foliar transfer pathway of TiO_2-NPs in lettuce finding that pristine TiO_2-NPs and TiO_2-NPs (and TiO_2 microparticles) from aged paints were internalized in lettuce leaves. Results showed that foliar exposure to 1250 nmol/g TiO_2-NPs was significantly higher than the control (p = 0.009) with concentrations reaching 630 nmol Ti/g FW

(= 83.9 µg/g FW) (Larue et al., 2014). Also, particles larger than 100 nm in diameter have been shown to transfer to plant tissues, meaning microparticles, along with NPs can be a concern (Larue et al., 2014)

3.2.8.2 Occurrence data

The literature review as well as additional searches and the Google search resulted in no hits related to the natural occurrence of nano- (and micro-) particles in leafy vegetables.

3.2.8.3 Conclusion

Based on two experimental studies, the uptake of NPs to the edible portions of leafy vegetables like lettuce is possible. Bioavailability of silver nanoparticles in the soil and uptake in the edible shoots of lettuce was reported to be only 0.06%. For TiO_2, high levels (84 µg/g FW) were found in lettuce after exposure to these substances. No papers were found reporting the presence of these compounds in leafy vegetables during processing (e.g., from packaging) or on the market.

3.2.9 Plant Protection Products (PPPs)

Pesticides are agents that aim to reduce harmful organisms such as microorganisms, insects or pests. The term pesticide is often interchangeably used with plant protection products (PPPs) although the term pesticides is broader and also includes biocides. Initially, the hazard group 'pesticides' was used in the evaluation of the literature (as indicated in section 2.3.1). This group contained 37 relevant hits. For the results section, the hazard group pesticides was split in PPPs and biocides. This section describes the literature found for PPPs. Results for biocides are described in the section on cleaning agents and disinfectants (section 3.2.14).

3.2.9.1 Uptake and degradation in leafy vegetables

Residues of plant protection products (PPPs) may be a health concern. In the EU, maximum residue levels (MRLs) for several pesticide residues have been established; they indicate the maximum levels that are legally allowed when pesticides are applied correctly (e.g. with Good Agricultural Practices). The literature study retrieved two papers describing the uptake of PPPs in leafy vegetables, which are summarised below. In general, uptake and degradation are described in the dossiers submitted for authorisation of PPPs.

Uptake

Margenat et al. (2018) evaluated the occurrence of various pollutants during lettuce cultivation at peri-urban farms, finding that several fungicides (dimetomorph, carbendazim, and methylparaben) had been taken up into the edible parts of the lettuce. Authors suggested that the presence of these could be due to pesticide applications but can also be due to biosynthesis of the plant itself as has been demonstrated for methylparaben (Margenat et al., 2018).

The uptake and translocation of azole compounds, which are widely used as fungicides in agriculture, was studied with hydroponically grown lamb's lettuce (García-Valcárcel et al., 2016). Three azole compounds, clotrimazole, fluconazole, and propiconazole, were selected as levels of these compounds have been reported in water at high levels of up to 8650 ng/L for clotrimazole; 369 ng/L for fluconazole; and 4500 ng/L for propiconazole (García-Valcárcel et al., 2016). Results showed that the highest concentrations in the leaves were taken up by fluconazole, follow by propiconazole. The physiochemical properties of the azoles were observed to be the main factors that influenced uptake into the plant, e.g., azoles with low K_{ow} values (like fluconazole) readily transported from the roots and accumulated in aerial parts (García-Valcárcel et al., 2016). Authors estimated the human exposure for fluconazole, propiconazole, and clotrimazole, respectively, as 0.192 – 0.692, 0.048 – 0.085, and 0.038 – 0.051 µg/kg body weight-day; however, given the ADIs for these azoles, the human risk was considered negligible (García-Valcárcel et al., 2016).

Degradation

Two studies reported the degradation of PPPs in leafy vegetables. In an experimental study, Wołejko et al. (2016) assessed the effect of yeasts and microorganisms on the degradation of four fungicides azoxystrobin, boscalid, pyraclostrobin, and iprodione in lettuce. Based on the levels found, they performed a chronic health risk of these four pesticide residues. Calculated hazard quotients (EDI/ADI x 100%) for individual pesticides ranged from 0.4 – 64.8% (day 1) and by day 14 were 0.0 – 20.9% (Wołejko et al., 2016). Furthermore, the work of Kocourek et al. (2017) evaluated the degradation rate (over time) of several pesticide residues in three types of *Brassica* vegetables: Chinese cabbage (20 pesticide residues); head cabbage (17 pesticide residues), and cauliflower (18 pesticide residues). The rate of pesticide degradation was fastest among head cabbage, followed by Chinese cabbage and cauliflower. The half-lives ranged from 1.5-5.3 days for Chinese cabbage, 0.5 – 6.5 days for head cabbage, and 1.9 – 7.2 days for cauliflower, respectively (Kocourek et al., 2017).

3.2.9.2 Occurrence data

In a risk assessment on the pesticide flupyradifurone, EFSA concluded that it poses a risk to consumers of lettuce that is grown in greenhouses. Before drafting a conclusion on the risk of this active substance in lettuce grown in the field, EFSA recognises that more information is needed in order to perform a risk assessment (EFSA, 2015c). Based on the risk assessment on the pesticide glyphosate, EFSA concluded that glyphosate is unlikely to be carcinogenic. Furthermore, no other consumer risk had been identified with regards to the use of glyphosate as a pesticide (EFSA, 2015d).

EFSA regularly publishes reports on pesticide residues in food products. The EFSA report from 2015 reveals that spinach type vegetables and leafy vegetables have the most MRL exceedances. For the vegetables head cabbage, leek, and lettuce imported from outside the EU, 5.7% of the samples exceeded the MRLs in 2013. The MRLs were exceeded in 1.4% of the products coming from within the EU. The residues of the pesticides boscalid and dithiocarbamates were most frequently identified in these vegetables. Noteworthy, EFSA mentions that the findings of residues of the latter pesticide can be due to natural compounds and thus might be false positive findings. For broccoli, MRL exceedances were found for chlorpyriphos, chlorantranilprole, fenvalerate, thiophonate-methyl, carbendazim, tebuconazole, dimethoate, mandipropamid, and trifloxystrobine (EFSA, 2015a). A more recent EFSA report on pesticide residues (EFSA, 2018a) indicates MRL exceedances in celery (13%), celery leaves (16%), chards (5.3%), chicory (6.3%), Chinese cabbage (7.7%), kale (4.9%), lamb's lettuce (4.8%), rocket (10.8%), spinach (5.1%), broccoli (4.3%), and Brussels sprouts (5%). Multiple pesticide residues were detected in over 50% of samples (number of samples not specified) of lettuce, rocket, celery, and Brussels sprouts. EFSA performed dietary intake assessment and found exceedances of the ARfD for chlorpyrifos in head cabbage (2 samples), iprodione (25 lettuce and 1 head cabbage sample), lambda-cyhalothrin in lettuce (5 samples), and deltamethrin in lettuce (4 samples). The report indicates the results of 987 cabbage samples. The most frequently detected pesticides were difenoconazole (3.8% of the samples), azoxystrobin (3.7%), and boscalid (3.5%). MRL exceedances were found for chlorpyrifos (6 samples), difenoconazole (4 samples), propamocarb (1 sample), indoxacarb (1 sample), pyraclostrobin (1 sample), and propyzamide (1 sample). Out of 1188 lettuce samples, bromide ion was most frequently found (24%), followed by boscalid (19%), imidacloprid (15%), and propamocarb (14%). MRL exceedances were found for 19 pesticides: boscalid, propamocarb, dithiocarbamates, cyprodinil, acetamiprid, deltamethrin, propyzamide, chlorpyrifos, chlorothalonil, carbendazim, acrinathrin, triadimenol, tihophanatae-methyl, linuron, methiocarb, formetanate, dimethoate, chlorpyrifos-methyl, and vinclozolin (EFSA, 2018a). The EFSA report published in 2019 reports pesticide residues measured in 2017. In this report, chlorpyrifos is identified as most frequently found pesticide residue on cauliflower. However, none of these quantifications showed levels above the MRL for chlorpyrifos. MRL exceedances in cauliflower were measured for the pesticides chlorpropham, dimethoate, etofenprox, methomyl, propiconazole, pyrimethanil and thiophanate-methyl in only one sample each. These samples were all originating from EU Member States (Cyprus, Germany, The Netherlands, Poland and Spain). When assessing the acute risk, exceedances of ARfD were not seen for cauliflower. Nevertheless, in the scenario where the exposures of both dimethoate and omethoate are cumulated, one cauliflower sample from The Netherlands exceeded the ARfD. Tentative risk-assessment for the long-term exposure did not identify concern for pesticide residues on cauliflower (EFSA, 2019).

Apart from EU wide surveys, occurrence data were reported for individual countries. The RIVM (National Institute for Public Health and the Environment) reported monitoring data between 2013-2017 for pesticide residues on products on the Dutch market. MRL exceedances in leafy vegetables were most frequently found in celery (4.3%), Chinese cabbage (5%), and spinach (6.3%), all with a

Dutch or European origin. Dietary intake assessments showed ARfD exceedances for indoxacarb and iprodion in head lettuce (28 samples from the Netherlands, 18 from the EU). Overall, both the number of MRL and the ARfD exceedances have declined since 2010. Products originating from outside the EU exceeded the MRLs and ARfDs more frequently than products from within the EU. In 2016, the number of MRL exceedances was 7.2% for products outside the EU. For products originating from within the EU this number was 2.4%. Exceedances with the ARfDs were especially the case for babies. For this population group the difference in the exceedances ARfDs in products from within the EU and outside the EU was approximately 1%(RIVM, 2019).

Claeys et al (2011) performed a risk assessment on pesticide residues in leafy vegetables in Belgium, including cabbages, cauliflower, celery, endive, lamb's lettuce, leek, lettuce, rocket and spinach. Amongst these, lettuce contributes the most to the ADI for iprodione (0.12% of ADI), also for the exposure of dithiocarbamates and lamda-cyhalothrin, lettuce was one of the highest contributors. Overall, adults and young adults (>15 years) are at negligible risk, since the exposure was calculated to be approximately 100 times lower than the ADI for most of the pesticides. However, some concerns are identified with regards to the pesticide exposure in the population group 2-5 years (Claeys et al., 2011). The Federal Agency for the safety in the food chain (FAVV) found MRL exceedances in 2014 for celery (11.5%), rocket (6.7%), spinach (4.1%), escarole (4.0%), lamb's lettuce (2.7%), cauliflower (2.7%), and lettuce (2.0%) (FAVV, 2015). In 2015, notifications for celery, head cabbage, and escarole with residues of dimethoate, fluazifop-P-butyl, and methiocarb, respectively, entered RASFF. While in 2017, celery, head cabbage, and lettuce with residues of dithiocarbamates, fluazifop and fluopyram, respectively, were notified in RASFF. All these products were from Belgian origin (FAVV, 2016, 2018c). Santarelli et al. (2018) assessed the concentrations of pesticide residues in raw leafy vegetable vegetables (n = 300) at the retail level in Italy (2013 – 2015). Authors founds that 53% of the samples were positive for pesticides residues, with pre-cut vegetables being more often contaminated (59%) then uncut vegetables (48%) (Santarelli et al., 2018). Nonetheless, only a few samples were found to be non-compliant for MRLs for tested pesticide residues. Namely, EU MRLs were exceeded for the fungicide paclobutrazol and the insecticide tau-fluvalinate in two multi-residual samples (overall non-compliance prevalence 0.67%) of uncut romaine lettuce (*L. sativa* var. longifolia) (Santarelli et al., 2018).

3.2.9.3 Effects of processing

Camara et al. (2017) focused on establishing processing factors of six pesticides during fresh-cut lettuce preparation and assessed the risk of ingestion of these pesticides given lettuce consumption. MRLs were indicated as 2, 10, 2, 3, 0.5, and 15 mg/kg for respectively, imidacloprid, tebufenozide, cypermethrin, metalaxyl, tebuconazole, and azoxystrobin (Camara et al., 2017). The applied doses (in grams active ingredient per hectare) was, respectively, 31.5, 81.6, 37.5, 100, 87.5, and 112.5. Authors observed that processing factors ranged from 0.34 for tebuconazole to 0.53 for imidacloprid, meaning that processing showed to reduce residues in processed lettuce versus that of fresh lettuce (Camara et al., 2017). Moreover, risk quotients (EDI/ADI) ranged from 0.04% for imidacloprid to 0.52% for cypermethrin, and with processing factors these decreased to 0.02% for imidacloprid to 0.24% for cypermethrin. For all six pesticides, dietary exposure of fresh-cut lettuce showed to be of no concern to public health (Camara et al., 2017).

3.2.9.4 Conclusion

The literature study revealed that leafy vegetables can contain PPP residues, which was demonstrated in surveys on leafy vegetables. In some cases, MRL exceedances or RASFF notifications were mentioned and dietary intake assessments showed ARfD exceedances for several pesticides. Processing was shown to decrease residue levels of some PPPs in leafy vegetables.

3.2.10 Mycotoxins

3.2.10.1 Uptake in leafy vegetables

Leafy vegetables can be affected by pathogenic fungi that lead to quality and yield losses of the crops. *Alternaria* is a common genus that encompasses several species that are pathogenic to (leafy) vegetables. For example, cruciferous plants, such as cabbage and cauliflower, have been reported to be damaged by *Alternaria* spp. like *A. brassicicola*, and *A. brassicae*, and sometimes *A. alternata*

(Siciliano et al., 2017). Also, *A. japonica* has been reported on rocket, Chinese cabbage, and turnip (Siciliano et al., 2015). In addition, some fungi can produce mycotoxins, which are toxic secondary metabolites that can cause serious adverse health effects in humans and animals.

Siciliano et al. (2015) investigated 28 *Alternaria* isolates from rocket and cabbage to determine their potential mycotoxin production. Results revealed that more than 80% of the isolates were capable of producing at least one mycotoxin. *In vivo* results showed that only two strains produced tenuazonic acid (TeA): 70 ± 15.1 µg/kg on cultivated rocket and 475 ± 56.7 µg/kg on cauliflower (Siciliano et al., 2015). Similarly, tentoxin (TEN) was detected in only a few strains: with 20.5 ± 4.28 µg/kg on cauliflower as well as 1.86 ± 0.08 µg/kg and 3.98 ± 0.94 µg/kg for two isolates on cabbage (Siciliano et al., 2015). Alternariol (AOH), alternariol monomethyl ether (AME), and altenuene (ALT) were found in some rocket samples and all tested cabbage and cauliflower samples (Siciliano et al., 2015).

More recently, Siciliano et al. (2017) looked at the variability within 29 *Alternaria* isolates for different plants and contaminated seeds of rocket, cabbage, and cauliflower. Results showed than 80% of the isolates were able to produce at least one mycotoxin, with TeA being the main mycotoxin produced (Siciliano et al., 2017). AOH, AME, and ALT were found in more than 50% of the samples, while TEN was found in only four isolates, three from cultivated rocket and one from cauliflower (Siciliano et al., 2017).

3.2.10.2 Occurrence data

The literature search and Google search resulted in no hits related to the occurrence of mycotoxins in leafy vegetables. The additional search resulted in an EFSA report on the intake assessment of alternariol. The occurrence data reported in this report, however, does not include leafy vegetables (EFSA, 2016b). Two surveys with leafy and *Brassica* vegetables conducted in India were found in the additional search. Hariprasad et al. (2013) studied the natural occurrence of aflatoxin in spinach and cabbage samples (n=81), taken in 2011. The highest concentrations were measured to be 15.3 µg/kg for spinach and 26.0 µg/kg for cabbage (Hariprasad et al., 2013).

In the other survey (also from India), the mycotoxins zearalenone (ZEN) and deoxynivalenol (DON) were detected. Both mycotoxins were identified in 25% of the samples of dried cauliflower. Concentrations ranged from 238 ± 30 µg/kg to 632 ± 70 µg/kg for ZEN and from 250 ± 50 µg/kg to 2120 ± 40 µg/kg for DON (Sodhi and Sumbali, 2012). The moisture content of the dried cauliflower was not specified so levels in fresh weight could not be established.

3.2.10.3 Conclusion

Two papers reported the potential production of *Alternaria* toxins in leafy vegetables like rocket, cabbage, and cauliflower. However, no occurrence data on *Alternaria* toxins could be found. In India, one survey reported high values of naturally occurring aflatoxins in spinach (15.3 µg/kg) and cabbage (26.0 µg/kg) compared to EU MLs available for other food products. ZEN and DON were found in 25% of the dried cauliflower in another Indian survey. The Netherlands, currently, does not import leafy and *Brassica* vegetables from India.

3.2.11 Plant toxins

3.2.11.1 Uptake in leafy vegetables

Plant toxins are toxic secondary plant metabolites that can occur in animal feed, weeds, ornamental plants, and foods including leafy vegetables. Pyrrolizidine alkaloids (PAs) are a widespread group of secondary metabolites, known for their toxicological and pharmacological properties, which can occur in species relevant for human consumption.

The literature on the presence of plant toxins in leafy vegetables was limited. One study evaluated the capacity for fourier transform infrared (FTIR) spectroscopy to differentiate rocket (*Eruca sativa* and *Diplotaxis tenuifolia*) salad with common groundsel (*Senecio vulgaris*) leaves, suggesting it as a possible technique to distinguish the two and for future use to control safety (Kokalj et al., 2016). They indicated that research in Germany showed the contamination of ready-to-eat rocket salads and salad mixtures with common groundsel. One sample of 45 g contained 2606 µg PA, which is seen as a very high level for a single dose. This reference motivates to investigate PAs in leafy vegetables (Kokalj et al., 2016).

The German Institute for Risk Assessment (BfR) reported a trend for gathering other plants grown in the wild to be used for salads and green smoothies. Nonetheless, expertise is needed to distinguish leafy vegetables from weeds such as coltsfoot, which may contain 1,2-unsaturated PAs (BfR, 2018b).

3.2.11.2 Occurrence data

Apart from the studies indicated above, which describe an occasional finding of PAs in leafy vegetables, no literature was found describing a survey on the presence of PAs in leafy vegetables. Also, the Google search did not result in additional information on occurrence data.

3.2.11.3 Conclusion

PAs may be present in leafy vegetables when harvested together with weeds such as coltsfoot and common groundsel. No occurrence data were found on plant toxins in leafy vegetables.

3.2.12 Cyanotoxins

3.2.12.1 Uptake in leafy vegetables

Cyanobacteria (blue-green algae) can produce toxins in quantities, especially during booms, which are adverse to human health. Some cyanotoxins reported in the literature for leafy vegetables included β-methylamino-L-alanine (BMAA), cylindrospermopsin (CYN), and microcystin-LR (MC-LR), which are described in further detail below.

β-methylamino-L-alanine (BMAA)
BMAA is a neurotoxin that can be synthesized by cyanobacteria. It plays a role in neurodegenerative diseases like ALS/PDC or Alzheimer’s, among others, making its potential presence in the food chain of a human health concern. BMAA has been found in cyanobacteria from natural waters; the migration and biomagnification of it along the food chain in aquatic ecosystems is a concern. Likewise, authors have suggested that overfertilization can facilitate BMAA transfer and accumulation in the soil, soil organisms, and crops (Li et al., 2019). These authors investigated the potential uptake of BMAA in Chinese cabbage, finding that BMAA transferred from the soil to the roots, stem, and leaves during growth. Final concentrations of BMAA observed in the edible portions were higher than initial levels in the soil and that of roots, namely 13.8 μg/g in leaves and 4.7 μg/g in stems (Li et al., 2019).

Cylindrospermopsin (CYN)
CYN is a cyanobacteria secondary metabolite that can be found globally in bodies of water and is associated with cyanobacterial blooms. CYN can adversely affect human health. Human exposure to CYN can be direct (e.g., swimming, aquatic sports, use of contaminated potable water) and indirect via diets through the bioaccumulation in tissues of aquatic organisms or from irrigated crops (Cordeiro-Araújo et al., 2017). The latter has received limited consideration. Cordeiro-Araújo et al. (2017) evaluated the bioaccumulation and depuration of environmentally relevant concentrations of CYN in leaf tissues of lettuce and arugula after irrigation with contaminated water as well as estimated the amount of CYN consumed daily from these sources. Authors observed that lettuce and arugula irrigated with CYN contaminated water represented a key exposure pathway for humans. Total daily intake was below a recommended <0.03 mg/kg per day. They estimated the total daily intake given the concentrations found in the experiment, which ranged in lettuce between 2.05-5.53 ng CYN/kg bw/day for adults and between 1.24 and 3.32 ng CYN/kg bw/day for children. For arugula the EDI ranged between 3.73 and 7.67 ng CYN/kg bw/day for adults and between 2.20 and 4.60 ng CYN/kg bw/day. These levels were all well below the recommended 30 ng/kg bw per day (Cordeiro-Araújo et al., 2017).

Microcystin-LR (MC-LR)
MC-LR is a toxin produced by cyanobacteria; it is a toxic, common microcystin (MC) variant found in aquatic systems. In humans, MCs can adversely affect health resulting in certain types of cancers given chronic exposure; deaths related to MCs concern haemodialysis patients exposed to contaminated water have also been reported (Cordeiro-Araújo et al., 2016). MCs can occur in plant

tissues, e.g., through the use of contaminated irrigation water, but limited information is known about their accumulation in vegetables (Cordeiro-Araújo et al., 2016). Cordeiro-Araújo et al. (2016) studied the bioaccumulation and depuration kinetics of microcystin-LR in leafy tissues of lettuce and arugula that had been exposed to contaminated water. Authors found that the bioaccumulation in lettuce depended on the exposure (i.e., the concentration and time), while MC-LR accumulation in arugula was not found (Cordeiro-Araújo et al., 2016). Finally, the study evaluated human exposure to MC-LR through contaminated lettuce, finding that 40 g of MC-LR contaminated lettuce would expose adults to levels higher than the TDI set by the WHO (0.04 µg MC-LR/kg) (Cordeiro-Araújo et al., 2016).

Levizou et al. (2017) evaluated the effect that MC-rich irrigation water had during the cultivation of lettuce during 4 developmental stages: seed, cotyledon (embryonic leaf with first leaves), 2 leaves, and 4 leaves, and authors estimated the daily intake to determine if TDIs were exceeded. Authors tried to reproduce natural conditions given the use of water from reservoirs (Lake Karla, Greece) with prolonged cyanobacterial blooms and tried to mimic agricultural irrigation practices. The study found that irrigation of lettuce with MC-rich irrigation water constituted a serious public health risk, especially when lettuce was exposed during early stages of cultivation (e.g., the seed or cotyledon) (Levizou et al., 2017). EDIs for the treatments with seeds and cotyledon found that the WHO TDI was exceeded by a factor 6; the TDIs were also exceeded at later stages of development, 2 leaves (by a factor 4) and 4 leaves (by a factor 2) (Levizou et al., 2017).

3.2.12.2 Occurrence data

The literature search and the Google search did not result in papers describing surveys on leafy vegetables on the market.

3.2.12.3 Conclusion

Limited research on the uptake of BMAA in leafy vegetables was found; however, one study did report that BMAA could transfer from the soil to the edible portions of Chinese cabbage. CYN can be taken up by lettuce and arugula although levels do not reach the recommended daily intake. Moreover, MC-LR is another cyanotoxin of concern that can accumulate in lettuce, e.g., from the use of contaminated water, and was reported in two different studies to exceed the WHO TDI, meaning it is a public health risk. However, no occurrence data were found for cyanotoxins in leafy vegetables on the market.

3.2.13 Processing contaminants

3.2.13.1 Uptake in leafy vegetables

Processing contaminants such as acrylamide can be formed during processing as a result of the Mallard reaction, e.g., of heat treated, carbohydrate-rich foods. This may occur with plant-origin food rich in carbohydrates that are then exposed to high temperatures, e.g., through frying and roasting (Mroczek et al., 2014). It can also occur via environmental exposure through polymer manufacture and use. Polyacrylamides (PAMs) are used when treating wastewater, sewage, and drinking water. Monomeric acrylamide (AMD), which is a carcinogenic and mutagenic compound, can also migrate from PAM-containing packaging into food. For edible plants, a concern lies with the potential transfer of residual AMD from sewage sludge used for organic fertilization (Mroczek et al., 2015).

One study checked the mobility of AMD in the environment during the hydroponic cultivation of lettuce, finding that lettuce cultivated with representative AMD-based flocculants (containing 176 and 763 mg/kg of AMD) absorbed AMD nutrient solutions into their leaves. Average AMD content in lettuce ranged between 10 – 30 µg/kg (Mroczek et al., 2014), which is low compared to levels found in potato chips and French fries (530-3700 and 200-1900 µg/kg, respectively) (Becalski et al., 2003). Mroczek et al. (2015) investigated the migration of AMD (0.5 – 3.0 mg/dm^3 of peat substrate) to lettuce grown in organic medium (peat substrate), finding that lettuce plants absorbed AMD in the leaves between 0 – 21 mg/kg. Authors stated that when PAM is applied to crop land, it should be strictly controlled given the potential for AMD uptake to the edible parts of leafy vegetables like lettuce (Mroczek et al., 2015).

Apart from environmental exposure, acrylamides may be formed during heating. Roasted chicory root, a coffee substitute, is known to contain high levels of acrylamide. Therefore, Loaëc et al. (2014b) investigated the effect of chicory variety, agronomic characteristics and drying on the amount of free asparagine, which together with sugars causes the formation of acrylamide during heating. The study revealed that the concentration of free asparagine varied between varieties and between years (ranging from 444 to 2786 mg/kg). The application of nitrogen in the soil increased the levels of asparagine. Drying before roasting showed to significantly decrease the acrylamide levels during roasting.

3.2.13.2 Occurrence data

The additional search resulted in two papers describing the levels of neoformed contaminants (acrylamide, 5-Hydroxymethylfurfural (HMF) and Ne-carboxymethyllysine (CML) in coffee substitutes containing chicory. Acrylamide levels found ranged between 47.4 and 493.7 µg/100g. Two out of the 24 tested samples exceeded the indicative level of 4000 µ/kg as set by the European Commission. HMF ranged between 74.3 and 1299.1 mg/100g and CML between 1.0 and 24.4 mg/100g. According to the authors, the CML intake via coffee substitutes only contributes 0.5% of the total CML intake via food. A regular consumption of coffee substitutes will contribute to the HMF dietary exposure (Loaëc et al., 2014a). EFSA reported median acrylamide level in coffee substitutes of 351, 786, 1223, and 870 µg/kg in 2007, 2008, 2009, and 2010, respectively. The maximum levels found each year exceeded the indicative level of 4000 µg/kg (EFSA, 2012c).

3.2.13.3 Conclusion

Two experimental studies reported the possible uptake of AMD in lettuce cultivated under hydroponic conditions and with organic mediums. Levels found were, however, low compared to food known for their acrylamide levels (potato chips and French fries). Acrylamide levels found after roasting of chicory roots showed to exceed the EU indicative level for coffee substitutes. A regular consumption of coffee substitutes will contribute to the HMF intake.

3.2.14 Cleaning agents and disinfectants

3.2.14.1 Uptake in leafy vegetables

In the Netherlands, irrigation water and water used for processing of leafy vegetables is not treated with disinfectants. However, in other European countries, disinfectants are used to reduce bacterial loads in water. When importing leafy vegetables from these countries, residues of the disinfectants used may be present on the products. The following sections describe the uptake of disinfection by-products from the use of chlorine compounds in water as found in this literature study. In a currently running WFSR research project, the disinfectants used in amongst others leafy vegetables is investigated resulting in a long list of around 40 disinfectants. Based on this list, an intermediate list of disinfectants that are relevant to include in national monitoring will be established We refer to the report of that project for further information (Banach et al., in preparation).

Several research studies from Spain investigated the likelihood for <u>chlorate</u> residues as disinfection by-product when leafy vegetables (spinach and lettuce) were irrigated or washed with water disinfected with chlorinated based products (e.g., sodium hypochlorite, chlorine dioxide, electrolyzed water to produce free chlorine).

During primary production, López-Gálvez et al. (2018b) evaluated the effects on baby spinach when disinfecting the irrigation water with low levels (< 1 mg/l) of chlorine dioxide. Results for levels of <u>chlorate</u> in baby spinach (leaves) were 0.49 ± 20 mg/kg and 0.99 ± 0.40 mg/kg for each treatment trial, respectively (López-Gálvez et al., 2018b). Levels exceeded the EU MRL of 0.01 mg/kg. (López-Gálvez et al., 2018b). Since levels of chlorate residues in leafy vegetables exceed the default MRL of 0.01 mg/kg, the EU level is still under discussion.

Gil et al. (2016) investigated the possibility of <u>chlorate</u> residues in the lettuce (and water) when sodium hypochlorite (1 – 80 mg/L) was used as a water disinfectant during washing of fresh-cut iceberg lettuce. Chlorate residues in the lettuce were observed at concentrations reaching 4.5 – 5.0 mg/kg. However, chlorate residues on the lettuce were below the limit of quantification (0.0024 mg/kg) once fresh-cut lettuce was rinsed for 1 min with tap water (Gil et al., 2016). This study motivated the need for control measures during processing that minimize the presence of chlorates.

In another study, which looked at both primary production and processing, López-Gálvez et al. (2018a) elucidated the effects on baby lettuce (of Oak Leaf and Red Batavia cultivars) when disinfecting the irrigation water with electrolyzed water (to produce free chlorine), followed by processing and storage. Results showed a significant increase in chlorate concentrations, with chlorate concentrations in lettuce exceeding the EU MRL of 0.01 mg/kg; for Red Oak leaf 63.9% and for Red Batavia 38.9% of the samples were above the MRL (López-Gálvez et al., 2018a). Processing (i.e. washing with cold tap water) and storage were not observed to significantly influence chlorate concentrations in the lettuce, except for the third harvest that showed significantly higher chlorate concentrations after storage. Authors suggested that this observation may be due to the presence of chlorate in the substrate (parameter was not quantified) or due to the fact that the lettuce was deteriorated (noted by the low visual quality score) resulting in an increased extraction of chlorates into the leaves post-storage (López-Gálvez et al., 2018a).

López-Gálvez et al. (2018a) investigated the concentration of trihalomethanes (THMs) as possible by-products of electrolysed water. Only low concentrations were observed in treated water (95.3 ± 5.1 µg/kg) (López-Gálvez et al., 2018a). Similarly, low concentrations of THMs were found for lettuce, ranging from 0.66 – 1.56 and 0.71-1.68 µg/kg, respectively, for Red Oak leaf and Red Batavia cultivars (López-Gálvez et al., 2018a). Van Haute et al. (2013) confirmed these results showing that THM accumulated up to the 125 µg/L water, depending on the carbon oxygen demand (COD) of the water tested. Results in the lettuce showed that the total THMs was < 6.3 µg /g given treatments with tap water and processing waters with CODs of 500 and 1000 mg O_2 /L. Authors showed that after the rinsing step, THM presence on the lettuce was not detected.

Lonigro et al. (2017) evaluated the effects of using chlorinated water (0, 0.2, 10, and 40 mg Cl/L as Cl_2) for irrigation of lettuce (grown in sandy or silty-clay soils) on the presence of halogenated organic compounds (EOX). Results showed that the EOX concentrations in the lettuce leaves varied from 400 – 2330 µg Cl/kg (DM), based on samples irrigated with 0.2 and 40 mg Cl/L, respectively. Moreover, the results showed that the EOX accumulated in both soil types. The EOX value in chlorine treated irrigation water being generally lower in the silty-clay soil (46-58 µg/kg of dry soil) versus that of silty-clay soil (130 - 230 µg/kg of dry soil) as a result of the higher organic matter found in the silty-clay soil (23 g/kg versus 9.6 g/kg in the sandy soil). Overall, authors motivated that the use of chlorinated water for irrigation purposes results in the accumulation of organo-halogenated compounds in the soil and bioaccumulation to the edible parts of the lettuce.

3.2.14.2 Occurrence data

Disinfection by-products from the use of chlorine products
Chlorate is no longer approved as a pesticide and as a result, a default MRL of 0.01 mg/kg has been set. However, chlorate can also be present in food products due to other sources, e.g. due to the presence in drinking water. Therefore, the NVWA applies an intervention level of 0.25 mg/kg for leafy vegetables (www.nvwa.nl). EFSA recognizes chlorate as food safety risk after concentrations were collected in the EU. Mean concentrations using LB and UB were as follows; 351 µg/kg and 358 µg/kg for broccoli, 23 µg/kg and 28 µg/kg for other *Brassica* vegetables, 240 µg/kg and 253 µg/kg for celery, 138 µg/kg and 144 µg/kg for lettuce and 44 µg/kg and 48 µg/kg for other leafy vegetables (EFSA, 2015f). EFSA is currently working on an action plan to reduce dietary intake. The MRLs for food may be adjusted in the future. In a joint risk assessment, EFSA and BfR found that frozen broccoli has one of the highest concentrations of chlorate in food (EFSA, 2015b). Hereafter, BfR concluded that the high presence of chlorate in this type of food could be the result of glazing or washing of the products with water containing chlorate (BfR, 2018a).

Another by-product of chlorinated water is perchlorate. In Germany, mean concentrations of 14.7 and 110.2 µg/kg for *Brassica* vegetables and leafy vegetables, respectively were found (BfR, 2013b). Monitoring data in the EU showed the presence of perchlorate in celery, lamb’s lettuce, lettuce, other *Brassica* vegetables and other leafy vegetables with mean concentrations using MB of 16 µg/kg, 43 µg/kg, 120 µg/kg, 10 µg/kg, 43 µg/kg, respectively (EFSA, 2014c). A more recent report indicates mean concentrations using MB of 10 µg/kg for *Brassica* vegetables and 42 µg/kg for leafy vegetables. Within these groups, rocket salad and spinach contained the highest mean MB concentrations of

75 µg/kg and 132 µg/kg, respectively (EFSA, 2017a). According to EFSA, perchlorate has the potential of being a food safety risk for particularly the high consumers in younger age groups and for iodine deficient individuals. The latter can be due to the fact that perchlorate inhibits the iodine intake in humans (EFSA, 2014c). In a risk assessment performed by Vejdovszky et al. (2018), it was noticed that spinach and other leafy vegetables are contaminated the most in means of positive samples as well as in concentrations (Vejdovszky et al., 2018).

Quaternary ammonium compounds (quats)
In 2012, BfR performed a health assessment on residues of the disinfectant benzalkonium chloride (BAC). In this assessment, none of the 115 leafy vegetable samples tested were quantifiable. Out of 40 *Brassica* vegetable samples tested, 1 sample showed a concentration above the limit of quantification with 0.11 mg/kg. This sample surpassed the default MRL of 0.01 mg/kg, set for benzalkonium chloride in food (BfR, 2012a).

Also, for didecyldimethylammonium chloride (DDAC), a health assessment was performed by BfR. In this assessment, 1 sample of leafy vegetables (n=114) contained 0.041 mg/kg and 1 sample of *Brassica* vegetables (n=38) contained 0.011 mg/kg of the chemical compound. BfR indicates that DDAC is unlikely to pose a risk to human health, having in mind the ADI and ARfD of 0.1 mg/kg bw (BfR, 2012b).

Hydrogen peroxide (H_2O_2)
Hydrogen peroxide can function as a disinfectant and its use in the disinfection of washing water for ready-to-eat leafy vegetables has been explored. Therefore, FAVV issued an advice on the risks of this use in 2017. This advice concludes that there would not be a food safety risk in case residual concentrations of hydrogen peroxide are below or equal to 1.5 mg/kg, which is the current detection limit for hydrogen peroxide. However, FAVV recognizes that establishing a legal limit would be hardly manageable in practice as hydrogen peroxide is highly reactive. The reactivity and instability mean that the compound is rapidly broken down. Therefore, it is unlikely to be present at the time of consumption. Noteworthy, it is important to mention that in some cases, one makes use of additives to stabilize hydrogen peroxide. Since not all additives used are known, FAVV could not establish a risk assessment for these additives (FAVV, 2017).

3.2.14.3 Conclusion
The use of chlorinated water during primary production and processing of leafy vegetables may result in disinfection by-products (DPBs) such as chlorate, EOX, THM, and perchlorate. The levels of perchlorate found in leafy vegetables showed to result in food safety concerns for children and iodine deficient people. Also, for chlorate, leafy vegetables showed high concentrations (above the current MRL). For BAC, an exceedance of the EU MRL was reported for *Brassica* vegetables. For DDAC and hydrogen peroxide no health concerns were indicated by the competent authorities.

3.2.15 Allergens

EFSA indicated in their *scientific opinion on the evaluation of allergenic foods and food ingredients for labelling purposes* that celery can cause allergic reactions due to the presence of Api g 2, Api g 4, and Api g 6. If people are allergic to celery, the intake of celery root may trigger severe anaphylactic reactions (EFSA, 2014b).

3.2.16 Other chemical hazards

Two papers were found describing other residues found in leafy vegetables.

3.2.16.1 Uptake in leafy vegetables
Margenat et al. (2018) reported the presence of 2-Mercaptobenzothiazole (2MBT) and bisphenol F in the edible portions of lettuce cultivated at peri-urban farms. Authors suggested that the presence of these could be attributed to the use of plastic materials during agriculture practices on the field, e.g., from pipelines, plastic mulch, or film.

Moreover, the authors reported the presence of surfynol 104 in lettuce, being taken up from the water, with concentrations between <4.07 and 8.10 ng/g FW lettuce (Margenat et al., 2018). Surfynol® appears to be a commercial name for the chemical substance 2,4,7,9-tetramethyl-5-decyne-4,7-diol (TMDD), an industrial defoaming agent; The presence of this substance has been detected in rivers in Germany (up to 2.5 µg/L) (Guedez et al., 2010).

3.2.16.2 Occurrence data

The literature study and Google search did not reveal data on the presence of the above mentioned substances in leafy vegetables on the market.

3.2.16.3 Conclusion

Substances from plastic material such as bisphenol F and 2MBT may be taken up in leafy vegetables. When the water contains TMDD, this can also be taken up by leafy vegetables during cultivation. However, no occurrence data of the above mentioned substances in leafy vegetables on the market were found.

3.3 Long list and intermediate list

Based on the literature review, a list of hazards that may occur in leafy vegetables was established, the so-called long list. This list contains environmental contaminants such as heavy metals, radionuclides, pharmaceuticals, flame retardants, nanoparticles, PAHs and PFAS. Besides environmental contamination, leafy vegetables may be contaminated through naturally occurring chemical hazards such as mycotoxins, plant toxins and cyanotoxins. Pesticides are applied during the cultivation of leafy vegetables. At harvest, residues of these pesticides may be present, sometimes exceeding the EU MRLs. Processing contaminants such as acrylamide, HMF and CML can be found during the roasting of chicory. Finally, chlorine-based disinfectants are used in other countries, whose leafy vegetables are imported to the Netherlands. These disinfectants are used to reduce microbial loads in the irrigation water or wash water during processing. Residues (of by-products) of these may be found in leafy vegetables. Those hazards that were frequently found in leafy vegetables according to the literature or were found above legal limits were included on the intermediate list. The literature study revealed that many substances (such as PPPs and EDCs) can be taken up from the environment and end up in leafy vegetables. Occurrence data for these substances in most cases were not found. Therefore, a definite conclusion for these substances cannot be drawn. However, in some cases, the levels found indicated human health risks may arise. These substances were included on the intermediate list as knowledge gap (Table 1).

Table 1 *Long list and intermediate list of chemical hazards in leafy vegetables.*

Long list	Intermediate list	Knowledge gaps	Rationale for inclusion/exclusion on intermediate list
Heavy metals and other elements (section 3.2.1)			
Aluminium (Al)	Al		Al concentrations found in lettuce and spinach resulted in an exceedance of the TWI.
Arsenic (As)	-		As was found at low concentrations (0.0235 and 0.0108 mg/kg for *Brassica* and leafy vegetables, respectively. Furthermore, leafy vegetables were not identified as main contributors for As intake (EFSA, 2009d).
Cadmium (Cd)	Cd		Cd was shown to accumulate in the leaves of vegetables. Levels above the ML are found and leafy vegetables were reported as significant contributor to total intake.
Chromium (Cr)	-		The toxic compound Cr6+ rapidly degrades to Cr3+; levels of which were not found above Belgium action limits
Cobalt (Co)	-		Levels of Co were not found above the FAO/WHO international threshold for artichoke (50 mg/kg FW). No information was found for other leafy vegetables.
Copper (Cu)	Cu		Lettuce was reported as main contributor of Cu intake (8.2% of the ADI) by EFSA.
Iron (Fe)	-		Levels of Fe were not found above the FAO/WHO international threshold for artichoke (425 mg/kg FW). No information was found for other leafy vegetables.
Lead (Pb)	Pb		Studies showed that the EU ML was sometimes exceeded in lettuce.
Manganese (Mn)		Mn	One experimental study showed that the US RDI was exceeded for Mn when lettuce was hydroponically cultivated. No occurrence data were found for Mn.
Mercury (Hg)	-		Two studies investigated the uptake of mercury from the soil or treated grey water. Levels found were low (Mi et al., 2019) and would not raise a human concern according to Eregno et al. (2017).
Nickel (Ni)	-		Only 1 experimental study was found on an experiment using treated greywater. An additional search resulted in 1 supermarket survey showing that levels in salad and cabbage did not exceed the PTDI.
Platinum group metals (PGMs)	-		Only 1 paper found mentioning these substances. No concentrations were reported. No literature was found on presence in leafy vegetables on the market.
Zinc (Zn)	-		Levels of Zn were not found above the FAO/WHO international threshold for artichoke (100 mg/kg FW). No information was found for other leafy vegetables.

Long list	Intermediate list	Knowledge gaps	Rationale for inclusion/exclusion on intermediate list
Nitrate and nitrite (section 3.2.2)			
Nitrate	Nitrate		Nitrate levels above the EU MLs were found for spinach, chard, lettuce and rocket.
Nitrite	-		Exposure to nitrite is primarily due to conversion of nitrate into nitrite. Nitrite levels are occasionally found in leafy vegetables at concentrations much lower than nitrate levels.
Polycyclic Aromatic Hydrocarbons (section 3.2.3)			
PAHs	PAHs		A literature review revealed that levels may be high (> 5 µg/kg) when leafy vegetables are grown in urban or industrial areas.
Pharmaceuticals and other environmental contaminants (section 3.2.4)			
Antibiotic residues (clarithromycin (CLA), sulfadiazine (SDZ), and sulfathiazole (STZ), enrofloxacin (ENR), ciprofloxacin (CIP), norfloxacin (NOR))	-		Experimental studies showed a possible uptake in lettuce leaves. Two Chinese surveys were found indicating ENR and CIP levels were low compared to EU MLs for other food products. The mean NOR concentration in spinach (411 µg/kg) was high compared to the other fluoroquinolones. This finding was not confirmed in an additional literature search and since we currently do not import from China, this hazard was not included on the intermediate list.
Steroids (ethinylestradiol, 17α-ethinylestradiol (EE2), corticosterone, estrone, testosterone)	-		Experimental studies showed a possible uptake in lettuce leaves. One survey from Jordan was found indicating that levels found were not a human health concern.
Phthalate esters (di-n-butyl phthalate (DnBP) and di (2-ethyl hexyl) phthalate (DEHP))	-		Experimental studies showed a possible uptake in lettuce leaves. However, no literature was found on presence in leafy vegetables on the market
Others (bisphenol A, Carbamazepine (CBZ), Diclofenac (DCF), fluoxetine HCl, ibuprofen, mebrobamate, primidone, phenytoin, diuron, Diclofenac sodium (DCL), Naproxen (NPX), 4-nonylphenol)	-		Experimental studies showed that residues may be found when crops are grown in contaminated area. However, literature did not indicate a frequent presence of these substances in leafy vegetables.
Flame retardants (section 3.2.5)			
Tris(2-chloroisopropyl)-phosphate (TCPP)	-		One experimental study was found showing the possible uptake of TCPP. However, the ADI was not exceeded.
Tris(2-chloroethyl)-phosphate TCEP	-		One experimental study was found showing the possible uptake of TCEP. However, the ADI was not exceeded.
PBDEs	-		One experimental study was found showing the possible uptake of PBDEs. No occurrence data on PBDEs were found. However, these substances are primarily found in animal products.

Long list	Intermediate list	Knowledge gaps	Rationale for inclusion/exclusion on intermediate list
PFASs (section 3.2.6)	-		PFOS and PFOA levels reported by EFSA are low and leafy vegetables were not considered major contributors to PFAS intake
Perfluorooctanesulfonate (PFOS)	-		
Perflurooctanoic acid (PFOA)	-		
Radionuclides (section 3.2.7)	-		Radionuclides are only found at low levels (< EU legal limits) and were shown not to exceed the TDIs.
Caesium-137 (^{137}Cs)	-		
Iodine-131 (^{131}I)	-		
Strontium-90 (^{90}Sr)	-		
Uranium	-		
Nanoparticles (section 3.2.8)			
Silver nanoparticles (AgNPs)	-		One experimental study showed that uptake in lettuce was low (0.06% of the added AgNPs)
Titanium dioxide (TiO_2-NPs)	-	TiO_2-NPs	An experimental study showed elevated levels in lettuce (84 µg/g FW). The relevance of this finding should be further investigated.
PPPs (section 3.2.9)			
Overview of all PPPs found see section 3.2.1	Acetamiprid		MRL exceedances in lettuce
	Acrinathrin		MRL exceedances in lettuce
	Azoxystrobin		most frequently detected in cabbage
	Boscalid		Most frequently detected in cabbage, leek and lettuce and MRL exceedances in lettuce
	Bromide ion		Most frequently detected in lettuce. However, it should be noted that bromide ion also occurs naturally in plants and as such is not an unambiguous maker for the use of the pesticide methyl bromide
	Carbendazim		MRL exceedances in broccoli and lettuce
	Chlorantranilprole		MRL exceedances in broccoli
	Chlorothalonil		MRL exceedances in lettuce
	Chlorprofam		MRL exceedance in cauliflower
	Chlorpyrifos		Detected > EU MRL in broccoli, lettuce and cabbage and ARfD exceedance in head cabbage
	Chlorpyrifos-methyl		MRL exceedances in lettuce
	Cyprodinil		MRL exceedances in lettuce
	Deltamethrin		MRL and ARfD exceedance in lettuce
	Difenoconazole		most frequently detected and MRL exceedances in cabbage

Long list	Intermediate list	Knowledge gaps	Rationale for inclusion/exclusion on intermediate list
	Dimethoate		MRL exceedances in broccoli
	Dithiocarbamates		Most frequently detected in cabbage, leek and lettuce, although might be naturally present. MRL exceedances in lettuce and lettuce is main contributor to the ADI. RASFF notifications for celery
	Etofenprox		MRL exceedance in cauliflower
	Fenvalerate,		MRL exceedances in broccoli
	Fluazifop-P-butyl		RASFF notifications for cabbage
	Fluopyram		RASFF notifications for lettuce
	Flupyradifurone		According to EFSA, a possible risk is identified for greenhouse grown lettuce
	Formetanate		MRL exceedances in lettuce
	Imidacloprid		most frequently detected in lettuce
	Indoxacarb		MRL exceedance in cabbage and ARfD exceedance in lettuce
	Iprodione		ARfD exceedance in lettuce and head cabbage and lettuce contributes most to the ADI for iprodione
	Lambda-cyhalothrin		ARfD exceedance in lettuce and lettuce is main contributor to the ADI.
	Linuron		MRL exceedances in lettuce
	Mandipropamid		MRL exceedance in broccoli
	Methiocarb		MRL exceedances in lettuce and RASFF notifications for escarole
	Methomyl		MRL exceedance in cauliflower
	Paclobutrazol		MRL exceedances for lettuce
	Propamocarb		MRL exceedances in cabbage and lettuce and most frequently detected in lettuce
	Propiconazole		MRL exceedance in cauliflower
	Propyzamide		MRL exceedances in cabbage and lettuce
	Pyraclostrobin		MRL exceedance in cabbage
	Pyrimethanil		MRL exceedance in cauliflower
	Tau-fluvalinate		MRL exceedances for lettuce
	Tebuconazole		MRL exceedances in broccoli
	Thiophonate-methyl		MRL exceedances in broccoli, lettuce and cauliflower
	Triadimenol		MRL exceedances in lettuce
	Trifloxystrobine		MRL exceedances in broccoli
	Vinclozolin		MRL exceedances in lettuce

Long list	Intermediate list	Knowledge gaps	Rationale for inclusion/exclusion on intermediate list
Mycotoxins (section 3.2.10)			
Alternaria toxins (TeA, TEN, AOH, AME, ALT)		*Alternaria* toxins	Two papers indicated the possible presence of *Alternaria* toxins. The relevance of this should be further explored.
Aflatoxins	-		One paper from India showed aflatoxin levels in spinach and cabbage > 10 µg/kg. This finding was not confirmed in an additional literature search and since we currently do not import from India, this hazard was not included on the intermediate list.
Deoxynivalenol (DON)	-		One paper from India showed DON levels in dried cauliflower. Since we currently do not import from India, this hazard was not included on the intermediate list.
Zearalenone (ZEA)	-		One paper from India showed ZEA levels in dried cauliflower. Since we currently do not import from India, this hazard was not included on the intermediate list.
Plant toxins (section 3.2.11)			
1,2-Unsaturated pyrrolizidine alkaloids (PAs)		PAs	Although not frequently found in the literature, PAs may be present at high levels in ready-to-eat salads in case weeds are accidentally included. No occurrence data were found.
Cyanotoxins			
b-Methylamino-L-alanine (BMAA)	-		Only one experimental study reported the possible presence of BMAA in Chinese cabbage due to uptake from the soil. However, no literature was found on presence in leafy vegetables on the market.
Cylindrospermopsin (CYN)	-		An experimental study showed the possible uptake of CYN in lettuce and arugula. Levels found however resulted in EDI below the recommended intake of 30 ng CYN/kg bw/day.
Microcystin-LR		MC-LR	Two experimental studies confirmed the possible uptake of MC-LR in lettuce and arugula. When using realistic levels, the final concentrations in the studies leafy vegetables exceeded the WHO TDI. No literature was found on MC-LR levels in leafy vegetables on the market.
Processing contaminants (section 3.2.13)			
Monomeric Acrylamide (AMD)	-		Two experimental studies indicated a possible uptake of AMD in lettuce cultivated under hydroponic conditions and with organic mediums. Levels found were however low (between 10 and 30 µg/kg).
Acrylamide	Acrylamide		Acrylamide levels were shown to exceed the EU indicative level of 4000 µg/kg in coffee substitutes based on chicory roots.
5-Hydroxymethylfurfural (HMF)	HMF		HFM was found at high levels (between 74 and 1299 mg/100g) in coffee substitutes with chicory and a regular consumption of these coffee substitutes will contribute to the HMF intake.

Long list	Intermediate list	Knowledge gaps	Rationale for inclusion/exclusion on intermediate list
Nε-carboxymethyllysine (CML)	-		CML was found at low levels in coffee substitutes, not significantly contributing to the daily intake.
Cleaning agents and disinfectants (section 3.2.14)			
Disinfection by-products (DPBs)	Chlorate, perchlorate		The use of chlorine based disinfectants (e.g. ClO_2, NaOCl), which are used in other countries, may lead to the production of by-products. Chlorate residues were detected above the EU MRL. According to EFSA, perchlorate is a potential human health risk.
Quaternary ammonium compounds (BAC, DDAC)	BAC		BfR reported an MRL exceedance for BAC in *Brassica* vegetables. DDAC levels found in *Brassica* and leafy vegetables did not result in exceedances of the ADI or ARfD.
Hydrogen peroxide (H_2O_2)	-		H_2O_2 is rapidly broken down into H_2O and O_2.
Allergens (section 3.2.15)			
Celery	-		Food allergen labelling is enforced to help sensitive consumers avoid adverse reactions as a result of the presence of celery in food products.
Other chemical hazards (section 3.2.16)			
2-Mercaptobenzothiazole (2MBT)	-		One experimental study showed the possible uptake of 2MBT in leafy vegetables. No literature was found on the occurrence of this substance in leafy vegetables.
Bisphenol F	-		One experimental study showed the possible uptake of Bisphenol F in leafy vegetables. No literature was found on the occurrence of this substance in leafy vegetables.
2,4,7,9-Tetramethyl-5-decyne-4,7-diol (TMDD)	-		One experimental study showed the possible uptake of TMDD in leafy vegetables. No literature was found on the occurrence of this substance in leafy vegetables.

3.4 Information on the health based guidance values and contributors to the dietary intake of the prioritised hazards

This section provides information on the health based guidance values of the prioritised hazards on the intermediate list (Table 2) as well as on the main contributors to the dietary intake of these hazards. Information on the prioritised pesticides can be found in Table 3.

Table 2 *Health based guidance values of the prioritised hazards.*

Prioritised hazards	ML (mg/kg FW)	Chronic effect (µg/kg bw/day)	Acute effect (µg/kg bw)
Heavy metals and other elements (section 3.2.1)			
Aluminium (Al)		TWI: 1000 (EFSA, 2008)	NA
Cadmium (Cd)	0.2	TWI: 2.5 (EFSA, 2009b)	NA
Copper (Cu)		ADI: 150 (EFSA, 2018b)	NA
Lead (Pb)	0.3	$BMDL_{01}$: 0.5 (for young children) $BMDL_{01:}$ 1.5 µg/ kg bw /day (for cardiovascular effects in adults) $BMDL_{10}$: 0.63 µg/ kg bw /day (for and nephrotoxicity in adults) (EFSA, 2012b)	NA
Manganese (Mn)		NA	NA
Nitrate and nitrite (section 3.2.2)			
Nitrate	2000-7000	ADI: 3700 (EFSA, 2008a).	NA
Polycyclic Aromatic Hydrocarbons (section 3.2.3)			
PAHs		BMDL10: Benzoapyrene: 0.07 () PAH2: 0.17 PAH4: 0.34 PAH8: 0.49 (EFSA, 2008d)	NA
Nanoparticles (section 3.2.8)			
Titanium dioxide (TiO_2-NPs)		NA	NA
Mycotoxins (section 3.2.10)			
Alternaria toxins		TTC: 1500 ng/kg bw/day for TeA and TEN, 2.5 ng/kg bw/day for AOH and AME (EFSA, 2016a).	NA
Plant toxins (section 3.2.11)			
1,2-Unsaturated pyrrolizidine alkaloids (PAs)		$BMDL_{10}$: 70 (EFSA, 2011a)	NA
Cyanotoxins (section 3.2.12)			
Microcystin-LR (MC-LR)		TDI: 0.04 or 0.16 (depending on the PoD*) (EFSA, 2016c).	Acute no-effect dose: 2.5
Processing contaminants (section 3.2.13)			
Acrylamide		$BMDL_{10}$: 430 peripheral neuropathy and 170 for neoplastic effects (EFSA, 2015g).	NA
5-Hydroxymethylfurfural (HMF)		NA	NA
Cleaning agents and disinfectants (section 3.2.14)			
Disinfection by-products (DPBs) Chlorate, perchlorate		TDI (Chlorate): 3 (EFSA, 2015f)	ARfD: 36 (EFSA, 2015f)
Quaternary ammonium compounds (BAC, DDAC)		ADI (BAC and DDAC): 100 (EFSA, 2014a).	ARfD (BAC and DDAC): 100 (EFSA, 2014a).

* PoD; point of departure.

Table 3 *Information for the prioritised pesticides based on the EU pesticides database.*

Pesticides	Most relevant leafy vegetables[1]	EU MRLs (mg/kg)	ADI (mg/kg bw/day)	ARfD (mg/kg bw)	EU approval
Acetamiprid	lettuce	1.5	0.025	0.025	Yes
Acrinathrin	lettuce	0.02*	0.01	0.01	Yes
Azoxystrobin	head cabbage	5	0.2	NA[2]	Yes
Boscalid	head cabbage, leek, lettuce	5, 9, 50 (resp.)	0.04	NA[2]	Yes
Methyl bromide (Bromide ion)	lettuce	50.0	0.001	0.003	Not approved
Carbendazim	broccoli, lettuce	0.1*, 0.1* (resp.)	0.02	0.02	No
Chlorantranilprole	broccoli	1	1.56	NA[2]	Yes
Chlorothalonil	lettuce	0.01*	0.015	0.05	No
Chlorpropham	cauliflower	0.01*	0.05	0.5	No
Chlorpyrifos	broccoli, lettuce, head cabbage	0.01*, 0.1*, 0.1* (resp.)	0.001	0.005	Yes
Chlorpyrifos-methyl	lettuce	0.01*	0.01	0.1	Yes
Cyprodinil	lettuce	15	0.03	NA[2]	Yes
Deltamethrin	lettuce	0.5	0.01	0.01	Yes
Difenoconazole	head cabbage	0.3	0.01	0.16	Yes
Dimethoate	broccoli	0.02	NE[3]	NE[3]	No
Dithiocarbamates	head cabbage, leek, lettuce, celery	3 (mancozeb), 3 (mancozeb, metiram, ziram), 5 (maneb, mancozeb), 0.1* (resp.)	NE[3]	NE[3]	NA[2,4]
Etofenprox	cauliflower	0.4	0.03	1	Yes
Fenvalerate	broccoli	0.05	0.0125	NA[2]	No
Fluazifop-P-butyl	head cabbage	0.01*	0.01	0.017	Yes
Fluopyram	lettuce	15	0.012	0.5	Yes
Flupyradifurone	lettuce	5	0.064	0.15	Yes
Formetanate	lettuce	0.01*	0.004	0.005	Yes
Imidacloprid	lettuce	2	0.06	0.08	Yes
Indoxacarb	lettuce	3	0.006	0.125	Yes
Iprodione	lettuce, head cabbage	0.01*, NE[2] (resp.)	0.02	0.06	No
Lambda-cyhalothrin	lettuce	0.15	0.0025	0.005	Yes
Linuron	lettuce	0.01*	0.003	0.03	No
Mandipropamid	broccoli	2	0.15	NA[2]	Yes
Methiocarb	lettuce, escarole	1.0, 0.1* (resp.)	0.00025	0.0005	No
Methomyl	cauliflower	0.1*	0.0025	0.0025	No
Paclobutrazol	lettuce	0.01*	0.022	0.1	Yes
Propamocarb	head cabbage, lettuce	0.7, 40	0.29	1	Yes
Propiconazole	cauliflower	0.01*	0.04	0.1	No
Propyzamide	head cabbage, lettuce	0.01*, 0.6 (resp.)	0.05	0.13	Yes
Pyraclostrobin	head cabbage	0.4	0.03	0.03	Yes
Pyrimethanil	cauliflower	0.01*	0.17	NA[2]	Yes
Fluvalinate	lettuce	0.7	0.005	0.05	Yes
Tebuconazole	broccoli	0.15	0.03	0.03	Yes
Thiophonate-methyl	broccoli, lettuce, cauliflower	0.1*, 0.1*, 0.1* (resp.)	0.08	0.2	Yes
Triadimenol	lettuce	0.01*	0.05	0.05	No
Trifloxystrobin	broccoli	0.5	0.1	0.5	Yes
Vinclozolin	lettuce	0.01*	0.005	0.06	No

* Indicates lower limit of analytical determination.

1 Most relevant leafy vegetables are those vegetables in which the pesticides were frequently found and/or in which MRL exceedances were found (see Table 1).

2 NA= not applicable.

3 NE= not established.

4 dithiocarbamates comprises a group of substances including maneb, mancozeb, metiram, propineb, thiram and ziram.

3.4.1 Aluminium (Al)

Food is the major route of exposure to Al for the general population. Al may persist for a long time in the human body before it is excreted via the urine. Al has been shown to be neurotoxic and nephrotoxic. Neurotoxicity and nephrotoxicity studies were used to derive a NOAEL, which was used to set a TWI of 1 mg/kg bw/week. The estimated daily dietary exposure in Europe is 0.2-2.3 mg/kg bw/day, so the TWI is likely to be exceeded in a significant part of the population. Most unprocessed foods contained less than 5 mg/kg of Al, while higher mean concentrations of 5-10 mg/kg were often found in among others leafy vegetables, such as spinach, swiss chard, and lettuce. Vegetables were considered as one of the major contributors to the dietary Al exposure, but no detailed conclusion on for example the contribution of specific leafy vegetables could be made, because of lack of detailed information in the used total diet studies (EFSA, 2008e).

3.4.2 Cadmium (Cd)

EFSA concluded that the main source of Cd exposure for the non-smoking general population is food. Cd is toxic to the kidney, especially to the proximal tubular cells, where Cd accumulates (half-life: 10-30 years) and may cause renal dysfunction. This can progress after prolonged or high exposure to renal failure. Cd is also classified as human carcinogen Group 1 IARC. The TWI of Cd was set at 2.5 µg/kg bw/week, which corresponds to a TDI of 0.357 µg/kg bw/day (EFSA, 2009c).
Based on detailed individual food consumption data, EFSA made a better estimation of dietary intake of Cd in 2012 (EFSA, 2012a). Foods that are consumed in larger quantities have the greatest impact on the dietary Cd exposure. This was the case for the broad food categories of grains and grain products (26.9%), vegetable and vegetable products (16%) and starch roots and tubers (13.2%). For the more detailed food categories, across age groups, potatoes (13.2%), bread and rolls (11.7%) and fine bakery wares (5.1%), chocolate products (4.3%), leafy vegetables (3.9%) and water molluscs (3.2%) contributed the most to the dietary Cd exposure.
An average weekly dietary exposure of Cd was estimated at 2.04 µg/kg bw per week and a high exposure (P95) was estimated at 3.66 µg/kg bw per week. This review confirmed that the 95^{th} percentile exposure could exceed the TWI. There is a small margin between the dietary exposure and the TWI. Although the risk for adverse effects on kidney function is low, EFSA concluded that the current exposure to Cd should be reduced at population level (EFSA, 2012a).
RIVM concluded in 2015 that the median daily intake in the Netherlands exceeded the TDI up to the age of about 10. However, the life-long Cd exposure estimates were very low, therefore the risk of developing kidney failure due to life-long exposure was regarded as negligible for the general population. Cereals and potatoes were important contributors to the exposure of Cd due to the high consumption of these food products. Vegetables and fruits were also important contributors, in which spinach and pineapple had the highest contribution. The refined model used in this RIVM study showed that the long-term intake was lower than calculated by EFSA in 2012 (RIVM, 2015).

3.4.3 Copper (Cu)

Cu can endogenously occur in soil and plants; it can also be a pesticide active substance. EFSA has derived an ADI for pesticide Cu compounds of 0.15 mg/kg bw/day in 2008 and confirmed this ADI in 2018. Cu is also an essential micronutrient, therefore, EFSA has also derived adequate intakes for Cu and an UL of 5 mg/day.
Most important chronic effects of Cu in humans are effects on the liver function. While acute effects are mainly in the gastrointestinal tract, as a local irritation in the intestine.
Cu is found in several leafy vegetables with mean concentrations of 0.95 mg/kg for Chinese cabbage, kale, and baby leaf crop; 0.90 mg/kg for lamb's lettuce, lettuce, escarole, cress, land cress, rocket, spinach, purslane, and chard; and 0.76 mg/kg for watercress. The major contributor to the total dietary exposure of Cu was lettuce with a contribution of 8.2% to the acceptable daily intake (ADI) (EFSA, 2018b).
RIVM concluded in 2010 that previously reported consumption data of Cu in children overestimated the intake in children. Based on new dietary intake values, the intake of Cu for children is not exceeding the UL. This is only the case for 7-9% percent of children of 2-3 years. According to the RIVM, vegetables were not one of the main contributors to the dietary intake of Cu in children (RIVM, 2010).

3.4.4 Lead (Pb)

The major exposure route to Pb is via food. Pb can accumulate in the skeleton of the human body; the half-life in bones is 10-30 years. In blood, the half-life of Pb is approximately 30 days. The main target organ of Pb toxicity is the central nervous system. Neurotoxicity associated with Pb can affect the short-term verbal memory, fine motor skills, information processing and can cause psychiatric symptoms. In 2010, EFSA established a new health based guidance value as the previously established PTWI was concluded to be no longer appropriate. A 95th percentile lower confidence limit of the benchmark dose of 1% extra risk ($BMDL_{01}$) of 0.5 µg/kg bw/day for developmental neurotoxicity in young children was identified. The broad food categories contributing the most to Pb exposure are: grains and grain products (16.1 %), milk and dairy products (10.4%), non-alcoholic beverages (10.2%) and vegetables and vegetable products (8.4 %). In the vegetable and vegetable products category, leafy vegetables contained the highest mean concentrations of Pb after tea and herbs for infusion, coffee imitates and fungi. The mean level found in leafy vegetables was 41 µg/kg. The highest mean concentrations were found in beet leaves (152 µg/kg), endive (73 µg/kg), vine leaves (70 µg/kg), fresh spinach (59 µg/kg) and lamb's lettuce (47 µg/kg).
The $BMDL_{01}$ of 0.5 µg/ kg bw /day is lower than the estimated mean exposure for young children. For adults, the respective BMDLs for cardiovascular effects ($BMDL_{01}$ of 1.5 µg/ kg bw /day) and nephrotoxicity ($BMDL_{10}$ of 0.63 µg/ kg bw /day) were not exceeded by the estimated mean exposure for adults (EFSA, 2012b).
RIVM concluded that because of the dietary intake of Pb in the Netherlands detrimental health effects cannot be excluded for children up to 7 years of age, pregnant women and adults. The food groups contributing the most to the dietary intake of Pb were the same as the groups mentioned by EFSA, but additionally fruits were mentioned in the RIVM report. These food groups contributed 61-74 % of the total contribution. Data from other countries was also used because of limited Dutch data for some food products. Within the group of vegetables, brassica and leafy vegetables were the main contributors. For children (2-6 years) brassica contributed with 23% and leafy vegetables with 22% to the contribution of vegetables (RIVM, 2017).

3.4.5 Manganese (Mn)

Food is the major route of manganese exposure for the general population. The concentrations in food can vary widely, but in most food products, the concentrations are below 5 mg/kg. Grain, rice and nuts contain levels up to 10-30 mg/kg. Furthermore, high concentrations have been found in tea. Mn is an essential micronutrient, therefore, EFSA has also derived an adequate intakes for Mn. There is no sufficient data available to derive an average requirement, therefore an adequeate intake has been proposed based on estimated intake {EFSA, 2013 #346}.
However, high oral intake of Mn has been shown to cause neurotoxic effects. Some groups of the population are more susceptible to the effects of Mn. These are very young neonates due to increased absorption of Mn, elderly people with liver disease and people with iron deficiency anaemia. However, because of the limited data, no UL has been set by EFSA. EFSA indicates that the margin between oral intake levels and oral effect levels in human and animal experiments is very small. Therefore, oral exposure to Mn higher than normal levels present in food and beverages could pose a risk for human health (EFSA, 2006).

3.4.6 Nitrate

Vegetables are the main contributors to human exogenous exposure to nitrate; to a lesser extent, exposure also occurs via other food products and water. Nitrate is also endogenously formed. Nitrate is relatively non-toxic. However metabolites and reaction products, such as nitrite, nitric oxide and N-nitroso compounds have been shown to give adverse health effects. The adverse effects are among others methaemoglobinaemia and some evidence for carcinogenic effects.
The type of vegetables consumed and the corresponding nitrate levels have a high impact on the dietary exposure, more than the total amount of vegetables consumed. The highest nitrate values were found for leafy vegetables. Within this group, the highest level was found in oak-leaf lettuce of 19,925 mg/kg and the highest median concentration was found for rucola (4,800 mg/kg).

An ADI of 3.7 mg/kg bw/day has been derived by EFSA and was also confirmed by JECFA. EFSA estimated that the ADI was not exceeded in a conservative case of consumption of 400 grams mixed vegetables per day. For a small part of the population, which consumes only leafy vegetables, such as rucola, in high amounts, the ADI of nitrate could be exceeded. Overall, the EFSA concluded that the estimated exposures are unlikely to pose a risk for human health (EFSA, 2008a).
In 2010, EFSA published a statement on the possible risks of nitrates in leafy vegetables for infants and young children. It was concluded that nitrate exposure at the maximum levels found for cooked spinach is unlikely to be a health concern for infants (0-1 year), although risks cannot be excluded for infants consuming more than one spinach meal per day. For children, it was also concluded that there was no health concern at the maximum levels. However, nitrate levels in spinach have the potential to increase over time so during storage, which indicates that a concern cannot be excluded for some young children. Only about 5% of the spinach samples exceeded the maximum levels (EFSA, 2010).

3.4.7 Polycyclic aromatic hydrocarbons (PAHs)

PAHs can be considered mutagenic, genotoxic, and carcinogenic to humans (EFSA, 2008c; International Agency for Research on Cancer (IARC), 2018). For non-smokers, the major route of exposure is via food. EFSA used the margin of exposure (MOE) approach considering $BMDL_{10}$ values to evaluate potential concerns for human health. For high end consumers (P97.5) only, the margin of exposure (MOE) was around 10,000, which indicates a potential concern for human health (EFSA, 2008d). Cereals and cereal products together with seafood and seafood products have the highest contribution to consumer PAH exposure (median value of 67 and 36 ng BaP/day, respectively). Vegetables or leafy vegetables were not indicated as contributors to the dietary PAH intake (EFSA, 2008d).

3.4.8 Titanium dioxide nanoparticles

Titanium dioxide (E171) is used as a food additive in various food products. A small fraction of titanium dioxide consists of nanoparticles. It has been shown that excretion of these nanoparticles is very slow, which could potentially lead to accumulation in the human body. Computer modelling of RIVM showed that adverse effects on the liver of the titanium dioxide nanoparticles could not be excluded. EFSA re-evaluated to use of titanium dioxide as a food additive, potential risks of nanoparticles were also included in this evaluation. EFSA concluded that there is no human health concern, while RIVM is more cautious in their conclusion considering the possible accumulation in organs of titanium dioxide nanoparticles (RIVM, 2018).

3.4.9 Alternaria toxins

Alternaria toxins can be produced by fungi that can grow on cereals, oilseeds and fruits and vegetables. The exposure of four *Alternaria* toxins was estimated by EFSA: AOH, AME, TeA and TEN. A TTC approach was used by EFSA to assess the level of concern for human health, because of very limited toxicity data available. For the non-genotoxic *Alternaria* toxins, TeA and TEN, the TTC is 1,500 ng/kg bw per day and for the genotoxic *Alternaria* toxins, AOH and AME, the TTC value is 2.5 ng/kg bw per day. Among the four assessed *Alternaria* toxins, the highest intakes were reported for TeA (100-1,614 ng/kg bw per day). Products with the highest levels varied between the different toxins. The highest mean values of TA were found in paprika powder (8.8 mg/kg) and in four samples of mulberries (5.7 mg/kg). For infants, the main contributor to the dietary exposure of TeA was cereal-based food for infants and young children. In the adult population, fruiting vegetables (mainly tomatoes and tomato-based products) were the main contributors to the exposure. EFSA concluded that the dietary exposure to TeA is unlikely to be a human health concern (EFSA, 2016a). For the four *Alternaria* toxins in general, relatively high levels were reported for tomato-based products, tree nuts, oil seeds, grains and fruits. The highest dietary exposure was found for toddlers and other children (EFSA, 2011c). Leafy vegetables were not mentioned as contributors to the dietary exposure of Alternaria toxins.

3.4.10 Pyrrolizidine alkaloids (PAs)

PAs are secondary metabolites that can be produced by a wide variety of plants. PAs have genotoxic and carcinogenic properties, and are therefore unwanted substances in food. It is not appropriate to establish a TDI for genotoxic and carcinogenic compounds, therefore a MOE approach was used by EFSA, and a $BMDL_{10}$ of 70 µg/kg bw/day was set. Monitoring results were gathered for animal food products, such as milk, eggs and meat, and plant-based products such as teas and food supplements. Furthermore, an evaluation on honey has been done. No data on (leafy) vegetables was available (EFSA, 2011a, 2015e). Therefore, the contribution of leafy vegetables to the PA dietary intake was not established.

3.4.11 Microcystin-LR

The most studied cyanotoxin is MC-LR for which a provisional TDI could be derived of 0.04 µg/kg bw/day based on the available animal data. Furthermore, a reference value for subchronic toxicity of 0.16 µg MC-LR/kg bw/day has been set. An acute no-effect dose of 2.5 µg/kg bw could be set as well. EFSA reported that in one study, microcystins in edible crops were reported in Europe. This was in lettuce (94-2487 µg/kg dw) due to contaminated water. Occurrence data of microcystins showed that most of de data related to fish and other seafood (83%). There was no reliable data on microcystins in edible crops to perform a exposure scenario (EFSA, 2016c).

3.4.12 Acrylamide

Acrylamide can be naturally formed during high temperature processing, like frying, baking, roasting or industrial processing of starchy food. Because data from human studies were inadequate, EFSA selected $BMDL_{10}$ values of 0.43 mg/kg bw per day for peripheral neuropathy in rats and 0.17 mg/kg bw per day for neoplastic effects in mice as starting points in the risk assessment.
Acrylamide is present in high amounts in solid coffee substitutes and coffee, and in fried potato products. For coffee substitutes, higher levels were observed in substitute coffee based on chicory roots (average 2947 µg/kg), than based on cereals (510 µg/kg). The main contributors to dietary exposure of acrylamide were fried potato products (except potato crisps and snacks). Leafy vegetables in general were not mentioned as major contributors to the dietary intake of acrylamide.
Highest estimated total dietary intakes were up to 1.9 µg/kg bw per day (mean) and 3.4 µg/kg bw per day (P95). EFSA concluded that the dietary exposure to acrylamide is not of concern for the non-neoplastic effects. Acrylamide has not been demonstrated to be a human carcinogen. However, the MOE for neoplastic effects indicated a concern based on animal evidence, because the MOEs calculated are substantially lower than 10,000 (EFSA, 2015g).
The main contributors to the dietary intake of acrylamide for (young) adults in the Netherlands were French fries, coffee, crisps and Dutch spiced cake as reported by RIVM. RIVM concluded that acrylamide has a high priority in risk management, because of the small MOE (RIVM, 2011).

3.4.13 5-hydroxymethyl-2-furfural (HMF)

No toxicity information was found on the production of HMF during processing as described in 3.2.13. However, EFSA did evaluate 5-hydroxymethyl-2-furfural as a food additive. In vitro, HMF has been shown to be genotoxic. However, EFSA concluded that no genotoxicity or carcinogenicity is expected in humans. Nevertheless, it is recommended that the levels should be reduced as much as technologically feasible (EFSA, 2011b).

3.4.14 Chlorate

Chlorate can occur in food due to the use of chlorinated water during processing or due to disinfection of processing equipment. The critical effect for chronic exposure is inhibition of iodine uptake. A TDI of 3 µg/kg bw has been established by EFSA (2015f). The ARfD was established at 36 µg/kg bw. Chronic exposure of adolescent and adult age classes did not exceed the TDI. However, the TDI was exceeded at P95 intake in infants and toddlers. Chronic exposures are thus of concern for the human health of the younger age groups. The ARfD was not exceeded by the estimated acute exposures for all age groups. Overall, the main contributor to dietary exposure was drinking water (EFSA, 2015f). Concentrations of chlorate found in leafy vegetables as found by EFSA are described in paragraph 3.2.14.

3.4.15 Quaternary ammonium compounds (quats)

EFSA evaluated monitoring data on residues of the quaternary ammonium compounds DDAC and BAC in food. Overall, the highest mean values were found in animal products and fruits and nuts. Around 2% of the vegetable samples analysed were positive. The compounds found were mostly detected in leafy vegetables and fresh herbs, legumes and solanacea (tomatoes and peppers). The highest mean value found in leafy vegetables and fresh herb was 0.3 mg/kg (EFSA, 2013a).
Furthermore, EFSA concluded based on dietary risk assessments for DDAC and BAC that the proposed temporary MRL of 0.1 mg/kg for all food commodities is expected to be sufficiently protective for the general population in Europe (EFSA, 2014a). The Netherlands currently applies an MRL of 0.5 mg/kg expressed for cetryltrimethylammoniumchloride (Warenwetregeling residuen van bestrijdingsmiddelen). EFSA used ADI and ARfD values for their dietary risk assessment that were derived by the BfR; ADI for both DDAC and BAC: 0.1 mg/kg bw per day, ARfD for both compounds: 0.1 mg/kg bw (EFSA, 2014a).

3.5 Trends in leafy vegetables

This chapter evaluates the trends in the vegetable chain that may influence food safety in relation to the presence of chemical hazards in leafy vegetables. The information was obtained by consulting experts and from grey and scientific literature. The Innova database search was performed with search terms specific for leafy vegetables. Furthermore, from the expert interviews information for vegetables in general was obtained. In total, five experts working in the vegetable supply chain were interviewed or filled in the questionnaire. These experts were involved in two branch organizations (n = 3) and processing and/or import (n = 2).

3.5.1 Consumer trends

Evaluation of the Innova database revealed that for the ‘Fruit and Vegetables’ category, convenience, health, and ethical (either packages that could be recycled, increased sustainability or animal welfare) are most used reasons for product placements on the market. Growth is mainly seen in ethical positioning as well as ‘choice’ (a category that includes vegetarian, vegan, kosher and GMO free). This connects to the consumer trends of increasing awareness of health and increasing conscientiousness of sustainability.

3.5.1.1 Convenience
The high demand for convenience products also opens the opportunity for new products. Lately, new products for satisfying the demand of convenience products have been successfully introduced. A couple of examples are complete packages for one meal, mostly with recipes included, fresh-cut vegetables which are ready-to-cook or ready-to-eat, conserved vegetables, frozen vegetable mixes, and portion packages for smoothies (Figure 2).

Figure 2 *Complete pack for vegetable soup with spinach.*

3.5.1.2 Health

Consumption of vegetables is recognized as having positive health effects (Colapinto et al., 2018). However, although consumers are more and more conscious about health (expert opinion) (CBI; Insights, 2018; Rijswick, 2018b), vegetable consumption has not increased between 2000 and 2013 (Baselice et al., 2017), except for children (Vereecken et al., 2015). Nevertheless, with the increased attention to health, vegetable consumption is expected to increase (Rabobank, 2017a). This change is already observed in snacks, where consumers are searching for more healthy (or healthy sounding) variants (Innova Market Insights, 2018c), such as ready to eat pickles (Innova Market Insights, 2019b) (fitting in the convenience trend), extruded snacks or crisps with either vegetable content or vegetable flavour (Innova Market Insights, 2018a, 2018b). Dried vegetables may be another future possibility (Huang and Zhang, 2012). The trend towards convenience in combination with freshness (expert opinion) is clear in the fresh cut fruit and vegetables, where a double-digit growth was observed from the 1980s (Baselice et al., 2017). Lettuce has a prominent role in the fruit and vegetable market and counts for 50% thereof (Baselice et al., 2017).

Vegetables, as being seen as foodstuffs high in nutrients, do fit fine in several diets. An example hereof is low-carbohydrate diet, where consumers tend to replace products rich in carbohydrates and reduce the intake thereof. This has led to pasta and rice substitutes based on vegetables, such as zucchini and cauliflower (Innova Market Insights, 2019a), respectively (Figure 3). Also, bread or pizza base with part of the flour replaced by vegetables have been found (Figure 4) (Innova Market Insights, 2018c).

Figure 3 *Cauliflower and broccoli rice.*

Figure 4 *Cauliflower pizza crust.*

3.5.1.3 Product introductions

For the category of flowers and flower buds of leafy vegetables, the Innova database distinguishes artichoke, broccoli, and cauliflower and variants of these vegetables. Over the last ten years, 714 new product introductions in the database included artichoke, broccoli, or cauliflower (Figure 5a). Over the years, the amount of introductions clearly increased. Most of the introductions were in the 'ready meals and side dishes' category, with products like steam meals, frozen meals, soup boxes, vegetable rice, and quiches. Other important categories for these products were 'Fruit and Vegetables' and 'Supplements' (Figure 5b).

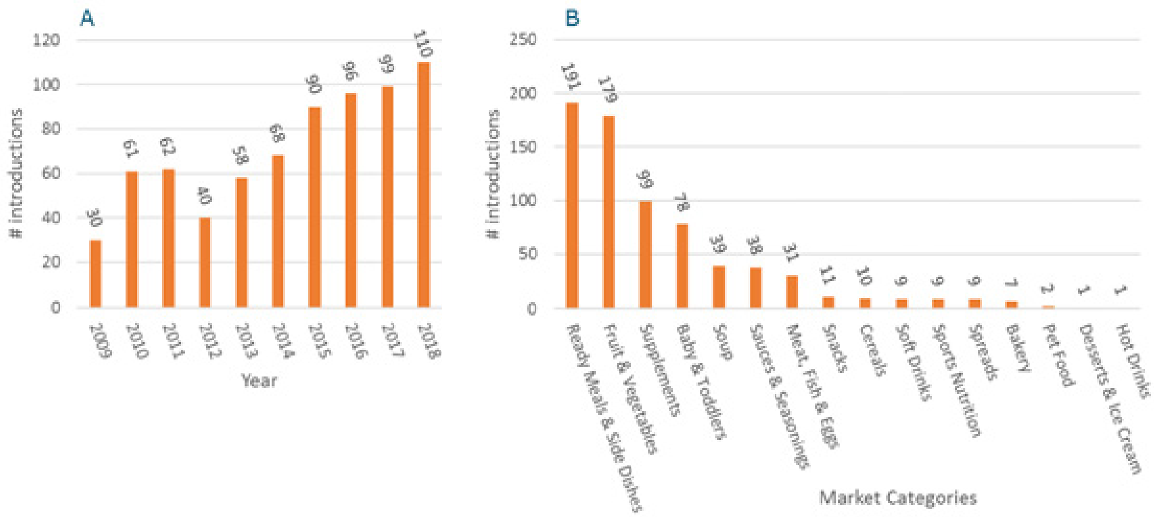

Figure 5 *Annual (a) and Category of market introductions (b) for flowers and flower buds of leafy vegetables.*

The 'Fruit & Vegetables' category contains vegetable products like ready-to–prepare packages to make a stew or soup, frozen vegetable products, puree or vegetable rice. The amount of introductions per year in this category fluctuates. The limited growth in the market category of fruits and vegetables is probably also connected to the limited convenience (relatively difficult while on-the-go) of the products in this category. The products are marketed mainly as convenient, for health and ethical as indicated previously. Not much change is seen in these marketing positions. Only the number of product introductions with 'no additives' and 'vegan' claims increased over the years.

3.5.1.4 Conclusions for chemical hazards

Increased consumption of leafy vegetables, such as lettuce may result in an increased exposure to chemical hazards, such as heavy metals and plant protection products.

3.5.2 Sustainability

Sustainability and circularity is increasing in importance as a driver for consumer behaviour (expert opinion) (CBI) and is high on the agenda of many companies (Rabobank, 2018a). In 2012, an initiative for sustainable fruit and vegetable production was initiated by 13 Dutch retailers and traders (CBI - Ministry of Foreign Affairs, 2018b; Sifav). Multiple other sustainability initiatives are running as well (CBI - Ministry of Foreign Affairs, 2018a). The consumer trend towards sustainability is seen, among others, in the increase in organic produce. Also, experts note that the production and processing of vegetables is increasingly sustainable (expert opinion). Interest is growing on the use of effluent and sludge products for the purpose of irrigation fields with food crops, in view of circular economy. The use of effluent and sludge in food cultivation increases the concentrations of organic and inorganic contaminants. It is, therefore, important to avoid their presence in the food chain. Along these lines, the advantages of agricultural circularity, e.g., the use of sludge for nutrients and as a solution to waste disposal, should be realized in line with the need for safe food production. Besides, no effluent should be applied in the field for vegetables within 10 months before harvesting (FAO, ND). The application of effluent in the cultivation of food crops in France, Spain, UK and scarcely in Belgium is reported (Kirchmann et al., 2017).

Food waste

One of the examples in reducing food waste is the introduction of complete packages for one meal as described in section 3.5.1. In the complete packages and mixes can be made use of rest products, which can help in the reduction of food waste. This reduction of food waste can contribute to the success of the complete packages and mixes (expert opinion). The increased initiatives towards reduction of food waste is one of the expressions of the move towards a more sustainable world. A large recent initiative, organized by a group of Dutch stakeholders, is called 'Samen tegen

Voedselverspilling' (Samen tegen voedselverspilling, 2019). It includes 18 partners, varying from supermarkets to ingredient suppliers, from packaging to banks and from catering to research. Examples of initiatives to reduce food waste are to remove 'use before' dates from AGF (potato, vegetable and fruit) products (Rabobank, 2018b), Experts note that parts of the plants not previously consumed are now used by the consumers (expert opinion). Examples are the leaves of radishes and the green of carrots or the use of cauliflower stumps to make the now popular cauliflower rice (expert opinion). This may result in risks, because of the presence of plant toxins (expert opinion).

Increased collaboration in the production chain is decreasing food waste as well. Monitoring and smart use of data help in optimizing the supply (and reducing food waste). The company Hessing is, for instance, only supplying the quantity of products that have been sold (Rabobank, 2018b). Other technological advancements, such as sensors, robotics, genome editing, plant based proteins, urban farming, solar powered cold rooms, temperature tracing devices, artificial intelligence, block chain can also help increasing sustainability (expert opinion) (Rabobank, 2018a). Block chain may also help in sharing (part of) information (expert opinion).

Packaging

Packaging is important to keep products fresh and prevent damage, but also to inform and persuade the consumer to purchase the product (Pinela and Ferreira, 2017). The increasing attention towards sustainability and prevention of food waste stimulates packaging research (Rabobank, 2018b). With the trend towards minimally processed food and hurdle technology to increase food safety, the importance of packaging in food safety increases (Duan et al., 2013; Bodbodak and Rafiee, 2016), while at the same time the amount of packaging is expected to decrease for sustainability reasons (expert opinion). Research is performed on several types of packaging. Lowered temperature and controlled pressure are effective in increasing shelf life (Duan et al., 2013). Vacuum packaging and modified atmosphere packaging (MAP) have also already been applied for some time and their application is expected to increase (Duan et al., 2013; Pinela and Ferreira, 2017). Edible coatings can also be considered a form of packaging that decreases oxygen at the product while it may in the future also incorporate active ingredients (Duan et al., 2013). Furthermore, biodegradable packaging may be used to reduce packaging waste.

More innovative forms of packaging are for example active packaging that can help in removing compounds such as ethylene, oxide or moisture and adding compounds such as CO_2, antioxidants, and preservatives (Bodbodak and Rafiee, 2016; Pinela and Ferreira, 2017). A step further is intelligent packaging, where sensors and other innovative devices are incorporated in the package. Smart packaging combines active and intelligent packaging (Pinela and Ferreira, 2017). The role of this innovative packaging is expected to increase (expert opinion) (Bodbodak and Rafiee, 2016).

3.5.2.1 Conclusions for chemical hazards

New innovations in biodegradable packaging require extensive assessment, also with regards to the shelf-life as the material can be degraded. Current manners of assessment for conventional food contact materials may not be sufficient to assess biodegradable materials with respect to their use as food contact material. Applying effluent and sludge in the cultivation of food crops result in increased concentrations of environmental contaminants, such as pharmaceuticals, personal care products and endocrine disrupting compounds in leafy vegetables.

3.5.3 Production

Horticulture is increasing in the EU (Rabobank, 2017a) and is expected to change in the future from the current group of very diverse, small producers towards larger collectives (expert opinion) (Rabobank, 2017a) and/or growth of more value added produce (Rabobank, 2017a). In this section of agriculture especially, data-gathering and use is expected to play an important role (Rabobank, 2017a). The horticultural sector in the Netherlands does not have the largest land area, but is frontrunner both in yield and in the use of high tech methodologies (expert opinion) (Rabobank, 2017b; Rijswick, 2018a; Graber and Twilley, 2019). Therefore, the Netherlands may play an important role in the change towards data driven production and innovations (Rabobank, 2017b; Rijswick, 2018a). Some examples of emerging technology in agriculture are drones and block chain modelling (Rocha and Ducasse, 2018).

A growing demand for vegetables and higher quality standards require innovation in the production of vegetables; this results in the need to increase production scales and capacity. However, extreme weather circumstances can lead to losses during production, followed by gaps in yield. These circumstances combined with a lack of human labour capacity require producers to act innovatively and make more use of technology (expert opinion). Climate change influences production as temperatures are expected to increase. This enhances the presence of blue algae in stagnant water. These algae can produce cyanotoxins, which can be taken up by horticulture produce via irrigation water (see section 3.2.12). As a result, the Dutch government set a limit of 1 µg/l for these toxins in irrigation water (https://www.rijksoverheid.nl/). In the dry summer of 2018, levels above this limit were detected (data not shown).

Organic production is a trend that keeps increasing worldwide (Dorais and Alsanius, 2015; Rabobank, 2017c; CBI). Despite the higher production costs and increased risk to obtain lower yields, the margins are such that even with possible future price decrease, production will stay profitable (Rabobank, 2017c). The global organic horticulture doubled from 2003 to 2013 (Dorais and Alsanius, 2015), which is even faster than growth of organic production in general. Most of the organic vegetable area is used for root vegetables, followed by pulses and fruiting and leafy vegetables. *Brassica*s have a far lower organic area (Dorais and Alsanius, 2015). The most popular crops grown organically are potatoes, followed by lettuce, carrots and peas (Dorais and Alsanius, 2015). The Netherlands has, however, a lower percentage of organic sales compared to other countries in Western Europe with a similar income level (Rijswick, 2018b). Still, retail sales show a steady growth, to a current level of about 5% of fruit and vegetables in the organic sector (CBI - Ministry of Foreign Affairs, 2018b; Rijswick, 2018b). Further change towards organic or other 'greener' production methods is expected (expert opinion) due to the reluctant attitude towards the use of pesticides in food. Contradictory to this, consumers' acceptance towards foreign particles, such as insects and weeds in vegetables has decreased. More products are purchased for the purpose of ready-to-eat and the consumers' knowledge on how fresh products are produced has declined (expert opinion). This results in a challenge for producers, since pesticides are a tool for pest control in fresh produce. Since the use of pesticides and foreign particles are less accepted, producers face the challenge to innovate cultivation processes with the help of new technologies. In conventional production, experts foresee changes towards crops that are more resistant, requiring less crop protection measures (expert opinion), although these may need GMO labelling. The introduction in Europe is expected to be slow (expert opinion). Furthermore, healthier soil is also aimed to increase plant health (expert opinion). The trend towards a decrease of chemical crop protection will continue (expert opinion), thereby, increasing the need for technologies such as precision farming (expert opinion). Experts also mentioned changes to other types of cultivation (such as strip cultivation) and other cultivation regions (expert opinion). High tech solutions are expected for weeding; these would be necessary when less chemical crop protection is applied (expert opinion). The structurally low price of vegetables combined with the changes described above threatens the continuity of the business (expert opinion).

3.5.3.1 Conclusions for chemical hazards

Organic products, produced without pesticides, may contain more foreign particles, such as insects and weeds. Without good separation of these foreign particles, consumers of these products can unintentionally be exposed to chemical hazards, like plant toxins (weeds).
Furthermore, climate change may result in warmer summers with increased levels of cyanotoxins in the irrigation water that may be taken up in leafy vegetables.

3.5.4 Trade

With digitalization, more foodstuffs are purchased online (expert opinion). The wish for convenience is not only recognizable in products, but also in service. In the Netherlands, supermarkets have online-delivery services for all types of groceries, such as Jumbo and Albert Heijn, but also new companies have developed services. PicNic, for example, delivers all kinds of groceries in small electric vans. Marley Soon and HelloFresh are examples of delivery services that include a box with fresh products and recipes for meals. The products are directly delivered at home from the distribution centers. This can impact the food chain, as retail then diminishes and the food chain shortens. Furthermore, foodstuffs purchased online are delivered in conditioned temperature contrary to conventional shopping, where the consumer purchases and transports the products. This realizes a closed cool chain, which can result in fewer food safety risks.

Following digitalization is globalization. Trade has brought product diversity to the market and products can be shipped worldwide; hence trade has become more broad and complex. Global trade has allowed access to products not common to market; for instance, for Dutch consumers that of Chinese pak choi. With this trade complexity, traceability is key to be able to assure food safety. Besides, consumers want more information on the background and the origin of the product and, thus, transparency is required. Labelling on food packaging makes it possible to communicate information towards the consumer. Furthermore, labelling is a tool, which can support traceability, for example, information such as the origin of the product and its pathway through the food chain. Besides labelling, a new tool has emerged in means of intelligent packaging. The Pasteur-sensor tag won the Food Valley Award in 2013 and is designed to reduce food waste and increase traceability. This tag cannot only display information with regards to the origin and the ways of distribution, but it can also measure temperature and gas compositions during distributions. This makes it possible to calculate the shelf-life based on the conditions within the packaging (Vanderwerff, 2013).

3.5.4.1 Conclusions for chemical hazards

Increased traceability using intelligent labelling will reduce food safety risks, for instance with respect to allergens. With proper labelling, one can make informed choices and is aware of potential hazards in food. Further, labelling can be of importance for the preparation of food, which can also impact food safety.

3.5.5 Legal and policy aspects

Legislation and policy affects the trade of foodstuffs. In the EU, imports from third countries also have to comply with EU legislation. Regulation (EC) No 669/2009 implements regulation for the purpose of official controls on imports from third countries. In Annex I of said regulation is a list of foods where the official control is increased from third party countries; for example, Chinese celery (*Apium graveolens*) from Cambodia are more frequently checked at the EU border for pesticide residues.
In terms of EU policies, one example is to make the diets of EU citizens healthier. The focus is on reducing salt and sugar in processed food. This is relevant for canned leafy vegetables such as collard greens and turnip greens and producers should make efforts to reduce salt and sugar contents.
In 2020, a new set of European regulations on organic produce will be implemented, which aims to guarantee fair competition and improve consumer confidence, although experts also expect that especially small suppliers will not be able to comply (CBI, 2018).

3.5.5.1 Conclusions for chemical hazards

Pesticide regulation helps to decrease the exposure to possible residues in imported leafy vegetables as products at risk are specified and obtain an increased import control. The EU policy regarding reduced salt and sugar may impact microbial hazards but no impact on chemical hazards is foreseen.

4 Conclusions

This report gives an overview of chemical hazards that may occur in leafy vegetables. The literature study performed showed that most of the papers described experimental studies in which the possible uptake of chemical hazards, for example upcoming hazards such as pharmaceuticals, was examined from the soil or the water into the leafy vegetables. The number of papers indicating occurrence data was limited compared to the experimental studies found.

The literature study revealed that most information was available for lettuce followed by cabbage and spinach. The <u>long list</u> of chemical hazards for leafy vegetables contains the following hazard groups:

- Environmental contaminants, which are primarily important for open filed cultivated leafy vegetables. Hazards on the long list for leafy vegetables are heavy metals, nitrate, PAHs, pharmaceuticals and endocrine disruptors, flame retardants, perfluor compounds (PFASs), radionuclides and nanoparticles.
- Naturally occurring chemical hazards such as mycotoxins, plant toxins and cyanotoxins.
- Plant protection products, which are applied during the cultivation of leafy vegetables, residues of which may be found at harvest.
- Processing contaminants, which can be found during the roasting of chicory roots as coffee substitutes.
- Water disinfection agents, which are applied during cultivation and further processing of leafy vegetables. Although this is currently not applied in the Netherlands, disinfection of water is used in other EU countries. Through imports, consumers may be exposed to possible residues of the (by-products of) agents used.

Those hazards that were frequently found in leafy vegetables or detected above (EU) legal limits or reported to exceed HBGVs were included on the intermediate list. The following substances were included on the <u>intermediate list</u>:

- Aluminium, cadmium, copper and lead from the heavy metals and elements section. These substances either exceeded the EU ML or leafy vegetables were seen as major contributor to the substance's ADI.
- Nitrate was added since levels were found above EU MLs for spinach, chard, lettuce and rocket. Vegetables cultivated in greenhouses usually have higher concentrations of nitrate than vegetables cultivated in the field.
- PAHs were included on the intermediate list, since levels above 5 µg/kg were found when leafy vegetables were grown in urban or industrial areas.
- Plant protection products are used during the cultivation of leafy vegetables. As a result, residues may be found at harvest. In total, 42 PPPs were included on the intermediate list, since they were detected above the MRL or demonstrated to cause exceedances of the ARfD. However, the list of PPPs included on the intermediate list is solely based on literature results. It is recommended to check the relevance of the identified PPPs using Dutch monitoring data as some of these pesticides may not have been detected above EU MRLs in the Netherlands and others may have been missed in the literature review. If the identified PPPs are included in the multi-methods used within the national monitoring program, a further prioritization may not be needed.
- The literature revealed that acrylamide and HMF may be formed at levels of concern in coffee substitutes containing chicory. As such, these processing contaminants were added to the intermediate list. Whether this is indeed relevant for the Netherlands depends on the consumption levels of coffee substitutes.
- The disinfectant by-products chlorate and perchlorate were added to the intermediate list as they were either detected above the EU MRL or were seen as a potential human health risk by EFSA. BAC was added to the intermediate list, since an MRL exceedance was found. Currently, a WFSR project is running identifying active ingredients of cleaning agents and disinfectants that are relevant from a food safety perspective. That study may reveal additional disinfectants to be included in the national monitoring of leafy vegetables.

Some knowledge gaps were identified for chemical hazards that were shown to be present in leafy vegetables at levels of human health concern in experimental setups. For these hazards, occurrence data are missing, and more information is needed to establish their relevance for leafy vegetables on the Dutch market. The following knowledge gaps were identified during the literature study:

- Manganese was added as knowledge gap, since an experimental study indicated that the US RDI was exceeded. However, no occurrence data on manganese in leafy vegetables were found.
- The nanoparticle TiO_2, since high levels were found (TiO_2) in an experimental study but no information on the presence of these substances in leafy vegetables on the Dutch market was found.
- The cyanotoxin MC-LR, since the TDI was exceeded using the concentrations found in the experimental study. However, no occurrence data for MC-LR were found for leafy vegetables on the Dutch market.
- *Alternaria* toxins as these toxins were found in two studies, but its relevance for human health was not established.
- Pyrrolizidine alkaloids (PAs), since these plant toxins have been detected at high levels in ready-to-eat salads. Weeds, such as coltsfoot and common groundsel, may accidentally be mixed with lettuce like rocket during harvest. Since these weeds can contain PAs, this may result in human health risks. However, no occurrence data on PAs in leafy vegetables were found, so these hazards were also identified as knowledge gaps.

Several trends and developments regarding leafy vegetables could be observed:

- An increased consumption of leafy vegetables as part of the trend towards a healthier diet. Increased consumption of leafy vegetables, such as lettuce, may result in an increased exposure to the identified chemical hazards on the intermediate list, such as heavy metals.
- An increased interest in sustainability, for example, aiming at closing nutrient loops. In some EU countries, treated wastewater and sludge are applied during open field cultivation. Since this fits to the trend to reduce waste, these practices might also be applied in the Netherlands in the future. As a result, chemical hazards that are present in treated wastewater and sludge, such as pharmaceuticals and endocrine disruptors, may end up on the land. Experiments showed that these substances are taken up by the plants. It is, thus, recommended to follow this trend and once applied, analyse leafy vegetables on these substances.
- Increased temperatures related to climate change. This may result in increased levels of cyanotoxins in irrigation water. The literature study revealed that these cyanotoxins can be taken up in leafy vegetables. This is, thus, also a trend that should be closely followed.

All substances on the intermediate list should be evaluated further to determine their possible risk to human health by combining concentrations found with Dutch consumption data. Substances with a possible human health risk should be included on the short list; the list that should be included in risk based monitoring programs for leafy vegetables.

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Annex 1 English-Dutch terms

English	Abbreviations	Dutch
17α-ethinylestradiol	EE2	17α-ethinylestradiol
17β-estradiol	17β-E2	17β-estradiol
2-Mercaptobenzothiazole	2MBT	2-Mercaptobenzothiazool
2,4,7,9-tetramethyl-5-decyne-4,7-diol	TMDD	2,4,7,9-tetramethyl-5-decyne-4,7-diol
5-Hydroxymethylfurfural	HMF	5-Hydroxymethylfurfural
β-methylamino-L-alanine	BMAA	β-methylamino-L-alanine
Acceptable daily intake	ADI	Aanvaardbare dagelijkse inname
Acute Reference Dose	ARfD	Acute referentiedosis
Allergen		Allergeen
Altenuene	ALT	Altenueen
Alternariol	AOH	Alternariol
Alternariol monomethyl ether	AME	Alternariol monomethyl ether
Aluminum	Al	Aluminium
Analytical method		Analysemethode
Arsenic	As	Arseen
Artichoke		Artisjok
Arugula (also rucola or (garden) rocket)		Rucola
Baby leaf crop		Jonge bladsla
Beet green (also chard)		Snijbiet
Benz[a]anthracene		Benz[a]anthracene
Benzophenone	BZP	Benzophenone
Biocides		Biociden
Bisphenol A	BPA	Bisfenol A
Bisphenol F		Bisfenol F
Bitterleaf		Bitterblad (*Vernonia amygdalina*)
Body weight	bw	Lichaamsgewicht
Brassica vegetables		Koolsoorten
Broccoli		Broccoli
Brominated flame retardants	BFRs	Broomhoudende vlamvertragers
Cabbage		Kool
Caesium	Cs	Cesium
Caffeine		Cafeïne
Carbamazepine	CBZ	Carbamazepine
Carbon oxygen demand	COD	Chemisch ZuurstofVerbruik (CZV)
Carbon-water partitioning coefficient	Koc	Koolstof-water-partitiecoëfficiënt
Cauliflower		Bloemkool
Celery		Selderij
Celtuce		Stengelsla
Chard (also beet green)		Snijbiet
Chaya		Chaya (*Cnidoscolus aconitifolius*)
Chemical substances/hazards		Chemische stoffen/gevaren
Chickweed		Vogelmuur
Chicory		Cichorei
Chinese mallow		Krulmalva
Choi/choy		Paksoi
Chromium	Cr	Chroom
Chrysanthemum		Chrysant
Ciprofloxacin	CIP	Ciprofloxacin
Clarithromycin	CLA	Clarithromycin
Cleaning agent		Schoonmaakmiddel
Nε-carboxymethyllysine	CML	Nε-carboxymethyllysine
Cobalt	Co	Kobalt

English	Abbreviations	Dutch
Collard green		Eeuwig moes, splijtkool
Coltsfoot		Hoefblad
Common groundsel		Klein kruiskruid (*Senecio vulgaris*)
Contaminants		Contaminanten
Contaminants of emerging concern	CECs	Opkomende gevaren
Copper	Cu	Koper
Cotyledon		Cotyledon
Cress		Tuinkers
Cylindrospermopsin	CYN	Cylindrospermopsin
Deoxynivalenol	DON	Deoxynivalenol
Depuration		Uitspoeling
Detection limit		Dectectielimiet
Di (2-ethyl hexyl) phthalate	DEHP	Di (2-ethyl hexyl) phthalate
Dibenz[a,h]anthracene		Dibenz[a,h]anthracene
Diclofenac	DCF	Diclofenac
Diclofenac sodium	DCL	Natriumdiclofenac
Di-n-butyl phthalate	DnBP	Di-n-butyl phthalate
Disinfectant		Desinfectiemiddel
Dry matter	DM	Droge stof
Dry weight	DW	Drooggewicht
Endive		Andijvie
Endocrine disrupting compounds	EDCs	Hormoonontregelende verbindingen
Enrofloxacin	ENR	Enrofloxacin
Escarole		Escarole
Estimated daily intake	EDI	Aanvaardbare dagelijkse inname
Ethinylestradiol		Ethinylestradio
European Food Safety Authority	EFSA	Europese Voedselautoriteit
European Statistical Office	EUROSTAT	Europees Bureau voor de Statistiek
European Union	EU	Europese Unie
Exposure		Blootstelling
Fat hen		Melganzenvoet, witte ganzenvoet (*Chenopodium album*)
Federal Agency for the Safety of the Food Chain (Belgium)	FAVV	Federaal Agentschap voor de veiligheid van de voedselketen (België)
Fertilizer		Meststof
Fluoxetine hydrochloride (Prozac®)		Fluoxetine hydrochloride (Prozac®)
Fluted pumpkin		Geribbelde kalebas, geribbelde pompoen (*Telfairia occidentalis*)
Food and Agricultural Organization of the United Nations	FAO	Voedsel- en Landbouworganisatie van de Verenigde Naties
Fourier transform infrared spectroscopy	FTIR spectroscopy	Fourier getransformeerde infrarood (FTIR) spectroscopie
Fresh weight	FW	Versgewicht
Garden rocket (also arugula, rucola, rocket)		Rucola
German Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung)	BfR	Duits federale instituut voor risicobeoordeling
Greater plantain		Grote weegbree
Green vegetable		Bladgroente
Halogenated organic compounds	EOX	Gehalogeneerde organische verbindingen
Health-based guidance values	HBGVs	Gezondheidskundige richtwaarden
Heavy metals		Zware metalen
Ibuprofen		Ibuprofen
Iodine	I	Jodium
Iron	Fe	IJzer
IV range		Kant-en-klaar, versgesneden groente
Joint FAO/WHO Expert Committee on Food additives	JECFA	Comité van deskundigen voor Levensmiddelenadditieven van de FAO/WHO

English	Abbreviations	Dutch
Jute plant		*Corchorus olitorius* (Nederlandse naam onbekend)
Kale		Boerenkool
Karkalla		*Carpobrotus rossii* (Nederlandse naam onbekend)
Komatsuna		Komatsuna
Lagos bologi		Surinaamse postelein (*Talinum fruticosum)*
Lamb's lettuce		Veldsla
Lead	Pb	Lood
Leaf mustard		Bladmosterd (Brassica juncea)
Leafy vegetables		Bladgroenten
Leafy greens		Bladgroenten
Lettuce		Sla
Livsmedelsverket (Swedish National Food Agency)		Zweedse levensmiddeleninspectiedienst
Lower bound	LB	Ondergrens
Norfloxacin	NOR	Norfloxacin
Manganese	Mn	Mangaan
Maximum limit	ML	Maximumlimiet
Maximum residue level	MRL	Maximumwaarde voor residuen
Mercaptobenzothiazole	2MBT	Mercaptobenzothiazol
Mercury	Hg	Kwik
Microcystin	MC	Microcystine
Microcystin-LR	MC-LR	Microcystine-LR
Mizuna greens		Japanse bladmosterd
Monensin sodium		Natriummonensine
Monomeric acrylamide	AMD	Monomere acrylamide
Mustard greens		Bladmosterd
Mycotoxins		Mycotoxinen
Nanoparticles	NPs	Nanodeeltjes
Naphthalene		Naftaleen
Naproxen	NPX	Naproxen
National Academy of Sciences (USA)		Nationale Academie voor Wetenschappen
Netherlands Food and Consumer Product Safety Authority	NVWA	Nederlandse Voedsel- en Warenautoriteit
Nickel	Ni	Nikkel
Non-compliant		Niet-conform
Office for Risk Assessment and Research	BuRO	Bureau Risicobeoordeling & onderzoek
Orache		Tuinmelde
PAH8		Benzo[a]pyreen, benzo[a]anthraceen, benzo[b]fluorantheen, benzo[k]fluorantheen, benzo[ghi]peryleen, chryseen, dibenz[a.h]anthraceen, and indeno[1.2.3-cd]pyreen
PAH16		Benzo[c]fluoreen, benzo[a]antraceen, cyclopenta[c,d]pyreen, chryseen, 5-methylchryseen, benzo[b]fluoranteen, benzo[k]fluoranteen, benzo[j]fluoranteen, benzo[a]pyreen, indeno[1,2,3,c,d]pyreen, dibenzo[a,h]antraceen, benzo[g,h,i]peryleen, dibenzo[a,l]pyreen, dibenzo[a,i]pyreen en dibenzo[a,h]pyreen
Palladium		Palladium
Per- and polyfluoroalkyl subtances	PFASs	Per- en polyfluoralkylverbindingen
Perfluorooctanesulfonate	PFOS	Perfluoroctaansulfonaat
Perfluorooctanoic acid	PFOA	Perfluoroctaanzuur
Pesticides		Pesticiden
Pharmaceutical and personal care products	PPCPs	Farmaceutische producten en producten voor persoonlijke verzorging
Phthalate esters	PAEs	Ftalaatesters
Plant toxins		Planttoxinen
Platinum group metals	PGMs	Platinum group metals
Polyacrylamides	PAMs	Polyacrylamides

English	Abbreviations	Dutch
Polybrominated Diphenyl Ethers	PBDEs	Polybroomdifenylethers
Polycyclic Aromatic Hydrocarbons	PAHs	Polycyclische aromatische koolwaterstoffen
Propranolol		Propranolol
Provisional tolerable daily intake	TDI	Voorlopige toelaatbare dagelijkse inname
Purslane		Postelein
Radicchio		Radijs
Radionuclide		Radionuclide
Rapini		Rapini
Risk based		Risicogebaseerd
Risk quotient		Risicoquotiënt
Risks		Risico's
Rocket		Rucola
Rucola		Rucola
Salad		Salade
Samphire		Zeekraal
Sculpit		Blaassilene (*Silene inflata*)
Shoots		Scheuten
Silver	Ag	Zilver
Silver nanoparticles	AgNPs	Zilver nanodeeltjes
Silver sulfide nanoparticles	Ag_2S-NPs	Zilversulfide nanodeeltjes
Soil organic carbon-water partitioning coefficient	Koc	Organisch koolstof genormaliseerde verdelingscoëfficiënt bodem-water
Soko		Afrikaanse spinazie
Spinach		Spinazie
Spleen amaranth		Chinese spinazie (*Amaranthus dubius*)
Sprouts		Spruiten
Stridolo		Blaassilene (*Silene inflata*)
Strontium-90	^{90}Sr	Strontium-90
Sulfadiazine	SDZ	Sulfadiazine
Sulfamethazine		Sulfamethazine
Sulfathiazole	STZ	Sulfathiazool
Surfynol 104		Surfynol 104
Tatsoi		Tatsoi
Titanium dioxide	TiO_2-NPs	Titaniumdioxide
Tolerable daily intake	TDI	Toelaatbare dagelijkse inname
Tolerable weekly intake	TWI	Toelaatbare wekelijkse inname
Tonalide		Tonalide
Tricolsan	TCS	Tricolsan
Trihalomethane	THMs	Trihalomethaan
Trimethoprim		Trimethoprim
Tris(1,3-dichloro-2-propyl)-phosphate	TDCPP	Tris(1,3-dichloro-2-propyl)-fosfaat
Tris(2-chloroethyl)-phosphate	TCEP	Tris(2-chloroethyl)-fosfaat
Tris(2-chloroisopropyl)-phosphate	TCPP	Tris(2-chloroisopropyl)-fosfaat
Turnip greens		Raapstelen
Turnip tops		Raapstelen
Tylosin		Tylosine
United Nations Economic Commission for Europe	UNECE	Economische Commissie voor Europa van de Verenigde Naties
Upper bound	UB	Bovengrens
Upper limit	UL	Maximumwaarde
Uranium		Uranium
Wageningen Food & Biobased Research	WFBR	-
Wageningen Food Safety Research	WFSR	-
Wash water disinfectant		Desinfectiemiddel voor het waswater
Watercress		Waterkers
World Health Organization	WHO	Wereld Gezondheidsorganisatie
Zinc	Zn	Zink
Zearalenon	ZEN	Zearalenon

Annex 2 Search terms leafy vegetables

Background
Several standards for fresh fruits and vegetables have been published by the United Nations Economic Commission for Europe (UNECE) (https://www.unece.org/trade/agr/standard/fresh/ffv-standardse.html).

A list of products standards used to preliminary search leafy vegetables is listed below. Broader terms (e.g., kale for ruvo kale) were included in the search string. These terms were provided, along with some other general search terms related to leafy vegetables, in search string 1.

1. **UNECE STANDARD FFV-48 concerning the marketing and commercial quality control of BROCCOLI**

broccoli
Calabrese broccoli

2. **UNECE STANDARD FFV-11 concerning the marketing and commercial quality control of CAULIFLOWERS (2017)**

cauliflowers

3. **UNECE standard FFV-08 concerning the marketing and commercial quality control of Brussels sprouts**

Brussels sprouts

4. **UNECE Standard FFV-09 concerning the marketing and commercial quality control of Headed cabbages**

cabbages
red cabbages
pointed cabbages
savoy cabbages

5. **UNECE STANDARD FFV-44 concerning the marketing and commercial quality control of CHINESE CABBAGE (2017)**

Chinese cabbage(s)

6. **UNECE Standard FFV-38 concerning the marketing and commercial quality control of Chicory**

chicory
witloof chicory
red chicory

7. **UNECE Standard FFV-58 concerning the marketing and commercial quality control of leafy vegetables (2017)**

watercress
rocket
spinach
turnip tops
turnip greens
brococoli raab
ruvo kale
kale
chard

8. UNECE Standard FFV-60 concerning the marketing and commercial quality control of Lambs Lettuce

lambs lettuce

9. UNECE STANDARD FFV-22 concerning the marketing and commercial quality control of LETTUCES, CURLED-LEAVED ENDIVES AND BROAD-LEAVED (BATAVIAN) ENDIVES (2017)

lettuce
endive
escaroles

Additionally search terms, which were not already included, were extracted from the EFSA categories, coming from the 2013 report on risks posed by pathogen in foods of non-animal origin, see EFSA (2013b) for details.

These included the following groups:

- *Brassica* vegetables - leaves - Other leaves: 4 products
- *Brassica* vegetables - 23. flowers and flower bulbs - blank: 9 products
- Leaf vegetables, herbs and edible flowers - leaves - 15. leafy vegetables eater raw as salads: 31 products

Total: 44

The following search strings were then tested in the Scopus database. Changes were made to be able to narrow down and focus the hits. Option 16 and 18 were also tested in the WOS database. Option 18 was utilized in the scientific literature study (section 2.3). A description of the terms searched per option is described below, with a summary of the preliminary results provided in Table A2.1.

Option 1:

#1: Leafy vegetables

Searched in TITLE-ABS-KEY:

broccoli OR "Calabrese broccoli" OR cauliflower* OR "brussels sprouts" OR cabbage* OR "red cabbage*" OR "pointed cabbage*" OR "savory cabbage*" OR "Chinese cabbage*" OR chicory OR "witloof chicory" OR "red chicory" OR watercress OR rocket OR spinach OR "turnip top*" OR "turnip green*" OR "broccoli raab" OR "ruvo kale" OR kale OR chard OR "lambs lettuce" OR lettuce* OR endive OR escarole* OR "leafy vegetable"

AND #2: Chemical hazards:

In title, abstract or keywords:
"Food contamination" OR "Chemical pollutant*" OR "chemical hazard*" OR contamina* OR toxin* OR "toxic substance*" OR "toxic compound*" OR pollutant* OR "agricultural chemical*" OR "chemical compound*" OR "chemical substance*" OR residu*

Option 2:

=option 1 with **#1 only in Title**

Option 3:

=option 1 added with search strings on public health

#1 And #2 in TITLE-ABS-KEY
AND #3: Public Health:

In title, abstract or keywords:
"Public health" OR "HACCP" OR "Consumer protection" OR Consumer* OR "Food safety" OR "risk assessment*" OR "risk analys*" OR "hazard analys*" OR "Human health*" OR "Health impact" OR "health risk*"

Option 4:

=option 3 **Limited to 10 years:**

(LIMIT-TO (PUBYEAR , 2019) OR LIMIT-TO (PUBYEAR , 2018) OR LIMIT-TO (PUBYEAR , 2017) OR LIMIT-TO (PUBYEAR , 2016) OR LIMIT-TO (PUBYEAR , 2015) OR LIMIT-TO (PUBYEAR , 2014) OR LIMIT-TO (PUBYEAR , 2013) OR LIMIT-TO (PUBYEAR , 2012) OR LIMIT-TO (PUBYEAR , 2011) OR LIMIT-TO (PUBYEAR , 2010) OR LIMIT-TO (PUBYEAR , 2009))

Option 5:

=option 4 with adapted search strings for leafy vegs:
#1 in TITLE-KEY-ABS:
broccoli OR "Calabrese broccoli" OR cauliflower* OR "brussels sprouts" OR cabbage* OR "red cabbage*" OR "pointed cabbage*" OR "savory cabbage*" OR "Chinese cabbage*" OR chicory OR "witloof chicory" OR "red chicory" OR watercress OR rocket OR spinach OR "turnip top*" OR "turnip green*" OR "broccoli raab" OR "ruvo kale" OR kale OR chard OR "lambs lettuce" OR lettuce* OR endive OR escarole* **OR "leafy vegetable" OR "green vegetable*"**

Option 6:

The added search strings in option 5 included separately:
#1 in TITLE-KEY-ABS:
"leafy vegetable*" OR "green vegetable"

Option 7:

= option 5 **only in TITLE**

Option 8:

=option 7 with exclusion terms
#1 AND #2 AND #3
AND NOT #4 in TITLE:
pathogen* OR streptococcus OR listeria OR virus OR bacillus OR salmonella OR clostridium OR staphylococcus OR outbreak OR "foodborne disease*" OR fung* in Title

Option 9:

=option 8 with **#4 in Title-Abs-Key**

Option 10:

=option 8 with extended exclusion terms
And NOT #4 in TITLE:
pathogen* OR streptococcus OR listeria OR virus OR bacillus OR salmonella OR clostridium OR staphylococcus OR outbreak OR "foodborne disease*" OR fung* **OR campylobacter OR "Escherichia coli" OR "E. coli" OR model OR "microb* contamin*"**

Option 11:

=Option 10 with **#4 in Title-Abs-Key**

Option 12:

Extension of search terms for leafy vegs:

#1 in TITLE:

broccoli OR "Calabrese broccoli" OR cauliflower* OR "brussels sprouts" OR cabbage* OR "red cabbage*" OR "pointed cabbage*" OR "savory cabbage*" OR "Chinese cabbage*" OR chicory OR "witloof chicory" OR "red chicory" OR watercress OR rocket OR spinach OR "turnip top*" OR "turnip green*" OR "broccoli raab" OR "ruvo kale" OR kale OR chard OR "lambs lettuce" OR lettuce* OR endive OR escarole* OR "leafy vegetable" OR "green vegetable*" **OR "pak choy" OR "pok choy" OR "pak choi" OR "pok choi" OR artichoke OR broccoflower OR "broccoli romanesco" OR "Chinese broccoli" OR "courgette flower" OR "squash blossom" OR "wild broccoli" OR arugula OR "beet green" OR bitterleaf OR "bok choy" OR celery OR celtuce OR "ceylon spinach" OR "collard greens" OR cress OR epazote OR gardencress OR "garden rocket" OR komatsuna OR "lamb's lettuce" OR landcress OR "mizuna greens" OR mustard OR "New Zealand spinach" OR radicchio OR rapini OR tatsoi OR "water spinach" OR "wrapped heart mustard cabbage"**

AND #2 AND #3 in TITLE-ABS-KEY And NOT #4 (from option 10) in Title

Option 13:

=option 12 with **#4 (from option 10) in Title-Abs-KEY**

Option 14:

=option 13 with **#1 in Title-Abs-Key**

Option 15:

Compressed search terms for leafy vegs:

#1 in TITLE:

*brocco** OR *cauliflower** OR *sprout** OR *cabbage** OR *chicory* OR *spinach** OR *"turnip top*"* OR *"turnip green*"* OR *kale* OR *chard* OR *lettuce** OR *endive* OR *escarole** OR *"leafy vegetable*"* OR *"green vegetable*"* OR "leafy vegetable*" OR *choi* OR *artichoke* OR *arugula* OR *"beet green"* OR *bitterleaf* OR *celery* OR *celtuce* OR *"collard green*"* OR **cress** OR *epazote* OR *"garden rocket"* OR *komatsuna* OR *"mizuna greens"* OR "*mustard* green*" OR "leaf mustard*" OR *radicchio* OR *rapini* OR *tatsoi*

AND #2 AND #3 in TITLE-ABS-KEY And NOT #4 (from option 10) in Title

Option 16:

Extended search terms for leafy vegs and extended exclusion terms

#1 in TITLE:

*brocco** OR *cauliflower** OR *sprout** OR *cabbage** OR *chicory* OR *spinach** OR *"turnip top*"* OR *"turnip green*"* OR *kale* OR *chard* OR *lettuce** OR *endive* OR *escarole** OR *"leafy vegetable*"* OR *"green vegetable*"* OR "leafy vegetable*" OR salad OR *choi* OR choy OR *artichoke* OR *arugula* OR *"beet green"* OR *bitterleaf* OR *celery* OR *celtuce* OR *"collard green*"* OR **cress** OR *epazote* OR *"garden rocket"* OR *komatsuna* OR *"mizuna greens"* OR "*mustard* green*" OR "leaf mustard*" OR *radicchio* OR *rapini* OR *tatsoi* **OR sauerkraut OR chaya OR chickweed OR "Chinese mallow" OR Chrysanthemum OR "fat hen" OR "fluted pumpkin" OR samphire OR "Greater plantain" OR "jute plant" OR Karkalla OR "Lagos bologi" or orache OR purslane OR rucola OR sculpit OR stridolo OR soko OR "spleen amaranth")**

AND #2 AND #3 in TITLE-ABS-KEY

AND NOT #4 in Title:

*pathogen** OR *streptococcus* OR *listeria* OR **virus** OR *bacillus* OR *salmonella* OR *clostridium* OR *staphylococcus* OR *outbreak* OR *"foodborne disease*"* OR *fung** OR *campylobacter* OR *"Escherichia coli"* OR *"E. coli"* OR *model** **OR analytic* OR *microbio** OR bacteri* OR virol* Or nutri***

Option 17:

=option 16 with #4 (from option 16) in TITLE-ABS-KEY

Option 18:

=option 16 without sauerkraut

Table A2.1 *Overview of results for the various search options.*

Search nr.	Scopus	Details	Number of hits
1	#1 and #2	Title-abs-key	10534 Scopus
2	idem	#1 Title #2 Title-abs-key	3331 Scopus
3	#1 and #2 and #3	Title-abs-key	1860 Scopus
4	#1 and #2 and #3	Title-abs-key Limited to 2009-2019	1408 Scopus
5	#1 and #2 and #3	Adapted search strings in #1 Title-abs-key Limited to 2009-2019	1422 Scopus
6	#2 and #3	And only "leafy vegetable*" OR "green vegetable" in Title-abs-key	170 Scopus
7	#1 (option 5) and #2 and #3	#1Title #2 and #3 in Title-abs-key	470 Scopus
8	#1 (option 5) and #2 and #3 and NOT #4	Exclusion terms added #1 Title #2 and #3 in Title-abs-key And NOT **#4 in TITLE**	391 Scopus
9	#1 (option 5) and #2 and #3 and NOT #4	#1 Title #2 and #3 in Title-abs-key And NOT **#4 in Title-Abs-Key**	252 Scopus
10	#1 (option 5) and #2 and #3 and NOT #4	Extended exclusion terms #1 Title #2 and #3 in Title-abs-key And NOT **#4 in TITLE**	293 Scopus
11	#1 (option 5) and #2 and #3 and NOT #4	#1 Title #2 and #3 in Title-abs-key And NOT **#4 in Title-Abs-Key**	191 Scopus
12	#1 (extended) and #2 and #3 and NOT #4 (option 10)	Extension of #1 #1 Title #2 and #3 in Title-abs-key And NOT **#4 in TITLE**	331 Scopus
13	#1 (option 12) and #2 and #3 and NOT #4 (option 10)	#1 Title #2 and #3 in Title-abs-key And NOT **#4 in Title-Abs-Key**	222 Scopus
14	#1 (option 12) and #2 and #3 and NOT #4 (option 10)	#1 **in Title-abs-key** #2 and #3 in Title-abs-key And NOT #4 in Title-Abs-Key	808 Scopus
15	#1 (compressed) and #2 and #3 and NOT #4 (option 10)	Compressed #1 #1 Title #2 and #3 in Title-abs-key And NOT #4 in TITLE	316 (Scopus)
16	#1 (extended) and #2 and #3 and not #4 (extended)	Extended #1 and #4 #1 Title #2 and #3 in Title-abs-key And NOT #4 in TITLE	355 (Scopus) *only 5 reviews 274 (WOS)
17	#1 (option 16) and #2 and #3 and not #4 (option 16) and not #4 (option)	#1 Title #2 and #3 in Title-abs-key And NOT **#4 in Title-abs-key**	187 (Scopus)
18	#1 (- sauerkraut) and #2 and #3 and not #4 (option 16)	Option 16 without sauerkraut #1 Title #2 and #3 in Title-abs-key And NOT #4 in TITLE	352 (Scopus) 274 (WOS) 206 duplicates **Total: 420**

Annex 3 Relevancy of scientific literature

Stage 1: Relevance title and (if necessary) abstract

Not relevant

- Pathogenic bacteria
- Parasites
- Decontamination of produce (ozone, active edible coating, irradiation, plasma, E-beam, electrolyzed water, ultra sonication, vaporized ethyl pyruvate)
- Cost-benefit analysis
- Crop management (e.g., against pests such as IPM)
- Biodiesel phytotoxicity
- Microbial quality at retail level, restaurant,
- Treating wastewater
- Determining geographical origin
- Non-relevant sprouts (e.g., soybean, buckwheat)
- Quality (visual, post-processing, yield, shelf-life, packaging)
- Food Safety Management Systems (FSMS)
- Ecological risks
- Antimicrobial activity (e.g., essential oils)
- Handling practices and training (e.g., restaurants)
- Aquaculture production
- Risk communication
- Hyperspectral fluorescence imaging (e.g., for fecal contamination)
- Effects of glucosinolates
- Plant growth with organic amendments
- Biosynthesis of compounds/metabolic engineering
- Foliar surface free energy
- Non-relevant product (e.g., soy bean sprouts, tuna salad, salad dressing, *Glyceria maxima,* tea, tomatoes)
- Flavonoid identification
- Effects of nitrogen application on leaf
- Nitrogen replacement soil
- Willingness to pay
- Method development (Point raman scanning method)
- Bioaccessibility of polyphenols

Stage 2 and 3: Relevance abstract and keywords, including suggestions of peer-review

Not relevant (continued)

- For Ab and steroids
  - Validation of analytical method (No. 44)
  - Cleanup strategy for analytical method (No. 151)
- For Metals and other elements:
  - Hydrogen sulfide role in mediating stress during vegetable growth
  - Cd trophic transfer along soil-plant-snail system
  - Vegetables for soil or water phytoremediation
- For pesticide residues:
  - Development and validation of methods (see exceptions in may be relevant)
  - Effect on parasites

May be relevant

- Questions on relevance of product type and location cultivated (e.g. No. 530)
- Use of shrimp farming effluents for lettuce cultivation
- Effect on plant growth (e.g., No. 28, No. 40)
- Validation methods of novel insecticide (e.g., No. 36, No. 495)
- Quantification of pesticide (e.g., No. 78, No. 24)
- EFSA modification of MRLs or setting import tolerances
  - Pesticide residues
    1. No. 358
    2. No. 370
    3. No. 395
    4. No. 421
    5. No. 422
    6. No. 423
    7. No. 427
    8. No. 428
    9. No. 433
    10. No. 459
    11. No. 473
    12. No. 477
    13. No. 507
    14. No. 512
    15. No. 513
    16. No. 516
    17. No. 523
- Pot experiments
  - Metals, examples include:
    1. No. 55
    2. No. 87 (also under Other elements and compounds group)
    3. No. 113
    4. No. 163
    5. No. 221
    6. No. 237
    7. No. 265
    8. No. 266
    9. No. 292
    10. No. 320
    11. No. 569
- Relevant article; location was specified in abstract (e.g. representative soil samples or field studies) and may not be relevant
  - Antibiotics and steroids
    - Ghana (No. 44)
  - PAHs
    - Nigeria (No. 127)
  - POPs, pesticide residues
    - South Africa (No. 2)

Stage 4 (a): Relevance for study outside the EU, based on title or abstract text
"Yes relevant" references from the hazard groups metals and pesticide residues, where the location was specified in title or abstract (e.g. from research on representative soil samples or field studies), were then considered "may be relevant". These are as follows:

- Metals, other elements and compounds
  1. Argentina (No. 11)
  2. Argentina (No. 37)
  3. Bangladesh (No. 252)
  4. Brazil (No. 112)
  5. Brazil (No. 20)

6. Brazil (No. 493)
7. Cambodia (No. 352)
8. Chile (No. 56)
9. China (No. 110)
10. China (No. 114)
11. China (No. 132)
12. China (No. 147)
13. China (No. 211)
14. China (No. 217)
15. China (No. 228)
16. China (No. 239)
17. China (No. 246)
18. China (No. 249)
19. China (No. 295)
20. China (No. 315)
21. China (No. 328)
22. China (No. 330)
23. China (No. 344)
24. China (No. 441)
25. China (No. 48)
26. China (No. 599)
27. China (No. 67)
28. China (No. 69)
29. Columbia (No. 162)
30. Egypt (No. 364)
31. Egypt (No. 413)
32. Ethiopia (No. 66)
33. Ghana (No. 176)
34. Ghana (No. 44)
35. India (No. 119)
36. India (No. 210)
37. Iran (No. 19)
38. Iran (No. 274)
39. Iran (No. 380)
40. Iran (No. 51)
41. Iran (No. 68)
42. Korea (No. 29)
43. Malaysia (No. 160)
44. Malaysia (No. 64)
45. Mexico (No. 212)
46. Nigeria (No. 303)
47. Nigeria (No. 332)
48. Nigeria (No. 343)
49. Pakistan (No. 141)
50. Pakistan (No. 248)
51. Pakistan (No. 290)
52. Pakistan (No. 310)
53. Pakistan (No. 522)
54. South China (No. 23)
55. Sri Lanka (No. 193)
56. Taiwan (No. 538)
57. Uganda (No. 279)
58. Uganda (No. 313)

- Pesticide residues
  1. Bolivia (No. 448)
  2. Chile (No. 101) ***118 samples**
  3. China (climate conditions) (No. 47)

4. China (No. 174) ***300 samples**
5. China (No. 283)
6. China (No. 306)
7. China (No. 319)
8. China (No. 71)
9. Ghana (no. 116)
10. Ghana (No. 207)
11. Ghana (No. 208)
12. India (No. 220)
13. India (No. 294)
14. India (No. 81)
15. Pakistan (No. 115)
16. South Africa (No. 297)
17. South Korea (No. 173) ***8496 samples**
18. Thailand (No. 189)
19. Thailand (No. 86)

Stage 4(b): Relevance for study outside the EU, based on EUROSTAT data
Remaining "yes relevant" references from the hazard groups metals and pesticide residues, where the research was conducted or pertained to products outside the EU, were separated in the following way.

- Metals, other elements and compounds (Yes, **relevant**)
  1. Belgium (No. 79)
  2. France & Tunisia (No. 268)
  3. France (No. 326)
  4. Italy (No. 398)
  5. Norway (No. 93)
  6. Poland (No. 118)
  7. Portugal (No. 75)
  8. Spain (No. 377)
- Metals, other elements and compounds (No, **may be relevant**)
  1. Australia (No. 45)
  2. Australia and Pakistan (No. 65)
  3. Brazil (No. 175)
  4. Brazil (No. 305)
  5. China (No. 100)
  6. China (No. 102)
  7. China (No. 15)
  8. China (No. 18)
  9. China (No. 269)
  10. China (No. 280)
  11. China (No. 3)
  12. China (No. 346)
  13. China (No. 388)
  14. China (No. 98)
  15. China and USA (No. 92)
  16. Pakistan (No. 12)
  17. Pakistan (No. 139)
  18. Pakistan (No. 14)
  19. Pakistan (No. 494)
  20. Pakistan, Germany, and France (study in Pakistan) (no. 91)
  21. Pakistan and USA (No. 190)
  22. Turkey (No. 96)
  23. Uruguay (No. 165)
  24. USA (No. 233)
  25. USA (No. 329)
  26. USA and Canada (No. 204)
  27. USA and China (No. 145)

28. USA and China (No. 150)
29. USA and Pakistan (No. 453)

- Pesticide residues (Yes, **relevant**)
  1. Czech Republic (No. 460)
  2. France (No. 215)
  3. Italy (No. 52)
  4. Poland (No. 155)
  5. Romania (No. 276)
  6. Spain (No. 152)
  7. Spain (No. 153)
  8. Spain (No. 41)
  9. Spain (No. 42)
  10. Spain (No. 77)
- Pesticide residues (No, **may be relevant**)
  1. Brazil (No. 32)
  2. China (No. 10)
  3. China (No. 134)
  4. China (No. 137)
  5. China (No. 143)
  6. China (No. 144)
  7. China (No. 149)
  8. China (No. 166)
  9. China (No. 202)
  10. China (No. 213)
  11. China (No. 219)
  12. China (No. 22)
  13. China (No. 226)
  14. China (No. 24)
  15. China (No. 243)
  16. China (No. 25)
  17. China (No. 257)
  18. China (No. 274)
  19. China (No. 289)
  20. China (No. 502)
  21. China (No. 90)
  22. China and USA (No. 46)
  23. Ghana (No. 157)
  24. India (No. 187)
  25. India (No. 247)
  26. India (No. 258)
  27. India (No. 260)
  28. India (No. 261)
  29. India (No. 277)
  30. India (No. 322)
  31. India (No. 350)
  32. India (No. 420)
  33. India (No. 61)
  34. Japan (No. 224)
  35. South Korea (No. 80)
  36. South Korea, Egypt and China (No. 59)
  37. South Korea, Egypt and China (No. 62)
  38. Turkey (No. 206)
  39. USA (No. 133)

Annex 4 Peer review of scientific literature

Table A4.1 *References reviewed and judgement of peer review.*

No.	Reference	Scientist 1	Scientist 2	Remarks	Final decision
1	(Abakari et al., 2018)	Not relevant	Not relevant	Paper on microbiology	Not relevant
2	(Abbasi et al., 2013)	May be relevant	May be relevant	Paper on risk assessment of heavy metals, but focused on wild leafy vegetables in a specific region in Pakistan	May be relevant
3	(Abdourahime et al., 2019)	Relevant	May be relevant	Modification of the MRL for trifloxystrobin	May be relevant
4	(Abougrain et al., 2010)	Not relevant	Not relevant	Paper on parasites	Not relevant
5	(Abreu et al., 2014)	Relevant	Relevant	Uranium concentrations in lettuce when grown near former uranium mines	Relevant
6	(Broad et al., 2009)	Not relevant	Not relevant	New production system for broccoli that reduces insecticide use	Not relevant
7	(Cao et al., 2018)	Relevant	May be relevant	Effect of copper and microcystin on lettuce growth	May be relevant
8	(Dala-Paula et al., 2018)	May be relevant	May be relevant	Heavy metal concentrations in lettuce grown in urban agriculture in a specific region in Brazil	May be relevant
9	(Danyluk and Schaffner, 2011)	Not relevant	Not relevant	Paper on microbial risks	Not relevant
10	(Dodgen et al., 2013b)	Relevant	Relevant	Uptake and accumulation of pharmaceutical and personal care products in leafy vegetables	Relevant
11	(Doolette et al., 2015)	Relevant	Relevant	Bioavailability of Ag nanoparticles in lettuce	Relevant
12	(Everard et al., 2016)	Not relevant	Not relevant	Method to detect fecal contamination of spinach	Not relevant
13	(França et al., 2017)	May be relevant	Relevant	Heavy metal contamination of lettuce due to traffic emissions	Relevant
14	(Gautam and Fomsgaard, 2017)	Relevant	Not relevant	Analytical method to detect azoxystrobin	May be relevant
15	(Gómez et al., 2009)	Not relevant	Not relevant	Paper on microbial loads in lettuce	Not relevant
16	(He et al., 2018)	May be relevant	May be relevant	Plant experiment using ercapto-grafted palygorskite to reduce Cd uptake in pak choi	May be relevant
17	(Iheshiulo et al., 2017)	Not relevant	Not relevant	Plant experiments using different oil types and effects on plant growth	Not relevant
18	(Ismail, 2016)	Not relevant	Not relevant	Paper on parasites	Not relevant
19	(Jouquand et al., 2017)	Not relevant	Relevant	Acrylamide formation in roasted chicory	Relevant
20	(Khan Achakzai et al., 2011)	May be relevant	May be relevant	Plant experiments: Uptake of heavy metals by lettuce from different irrigation sources	May be relevant
21	(Lara-Viveros et al., 2014)	Not relevant	Not relevant	Paper on plant diseases	Not relevant
22	(Li et al., 2014a)	May be relevant	May be relevant	Plant experiments: uptake of heavy metals from soil to lettuce	May be relevant
23	(Li et al., 2018)	Relevant	May be relevant	Experiment with uptake of neonicotinoids in komatsuna	May be relevant
24	(Liu et al., 2010)	May be relevant	May be relevant	Experiment with heavy metal uptake by different cabbage cultivars	May be relevant
25	(Luan et al., 2012)	Relevant	Not relevant	Plant experiment on effect of alginate cerium on reduction of chlorpyrifos on spinach	May be relevant

No.	Reference	Scientist 1	Scientist 2	Remarks	Final decision
26	(Marcussen et al., 2009)	May be relevant	May be relevant	Heavy metal concentrations in water spinach due to use of wastewater inlets in a lake in Cambodia	May be relevant
27	(Martínez-Sánchez et al., 2018)	Not relevant	Not relevant	Paper on microbial quality	Not relevant
28	(Menal-Puey and Asensio, 2015)	Relevant	Relevant	Study on nitrate levels on supermarket chard samples from Spain	Relevant
29	(Neres et al., 2011)	Not relevant	Not relevant	Paper on parasites	Not relevant
30	(Nwadinigwe et al., 2015)	May be relevant	Not relevant	Paper on leaves of fluted pumpkin	May be relevant
31	(Oluwatosin et al., 2010)	May be relevant	May be relevant	Heavy metal concentrations in leafy vegetables from an urban valley in Nigeria	May be relevant
32	(Pérez et al., 2009)	May be relevant	May be Relevant	Pesticide residues in broccoli. Relevant topic, but paper is in Spanish.	May be relevant
33	(Ray et al., 2013)	Relevant	Not relevant	Plant experiment on uptake of Zn in Indian spinach	May be relevant
34	(Santos et al., 2012)	Not relevant	Not relevant	Paper on pathogens	Not relevant
35	(Siddamallaiah et al., 2016)	Relevant	May be relevant	Paper on an analytical method to detect spiromesifen	May be relevant
36	(Sun et al., 2018)	Relevant	Not relevant	Description of an IR method to qualitatively detect pesticide residues	May be relevant
37	(Tunc and Sahin, 2017)	Relevant	May be relevant	Effect of using reclaimed wastewater on heavy metals in cauliflower	May be relevant
38	(Tyokumbur and Okorie, 2011)	May be relevant	May be relevant	Bio concentration factors for trace elements cultivated with effluent water in Nigeria	May be relevant
39	(Wang et al., 2018b)	Relevant	Relevant	Risk assessment of a new pesticide Flonicamid	Relevant
40	(Yang et al., 2011)	May be relevant	May be relevant	Plant experiment on uptake of Cd in cole and celery grown on polluted soil	May be relevant
41	(Yusof et al., 2016)	Relevant	Relevant	Effect of processing (vacuum impregnation) on nitrate levels in lettuce	Relevant
42	(Zorrig et al., 2013)	Relevant	May be relevant	Plant experiment on Cd uptake in lettuce	Relevant

Annex 5 Results advanced Google search

Before performing the advanced search in Google, a preliminary search of various websites from research or food safety institutes were explored with the aforementioned three search strings. Below, is a complication of those other websites and a justification for excluding them further from the advanced Google search.

FAO

Vegetables AND "food safety" site:http://www.fao.org	11100 hits
Id., 2009-15/04/2019	1540 hits
Id., only PDF	1270 hits
Id., 2014-15/04/2019	750 hits
Id., 2016-15/04/2019	526 hits

Vegetables AND (contaminant OR residue) site: http://www.fao.org	1130 hits
2008-now	134 hits

Vegetables AND "risk assessment" site: http://www.fao.org 1860 hits	1130 hits
2008-now	151 hits

Excluded from further search, because of many irrelevant hits, such as guidelines for developing countries.

Nébih

Vegetable "food safety" site: https://portal.nebih.gov.hu/	163 hits

Excluded from further search, because of choice to select authority from a country closer to The Netherlands.

FSANZ

Vegetable "food safety" site: www.foodstandards.gov.au/	1730 hits
Id., 2009-15/04/2019	318 hits
Id., only PDF	108 hits

Vegetables residue OR contaminant site: www.foodstandards.gov.au/	1240 hits
Id., 2009-15/04/2019	180 hits

Vegetables "risk assessment" site: www.foodstandards.gov.au/	1540 hits
Id., 2009-15/04/2019	170 hits

Excluded from further search, because it is a non-EU authority, plus many references concerned guidelines and reports on microbiological hazards.

WHO

Vegetable "food safety" site: https://www.who.int/	488 hits
Id., 2009-15/04/2019	165 hits

Excluded from further search, because of many irrelevant hits, such as leaflets on how to handle food hygienically.

An overview of the number of hits with the three search strings and corresponding search criteria for the four websites is provided in Table A5.1. The total number of hits per website is specified, followed by the total relevant hits and, in parentheses, the total number of hits included in the report.

Table A5.1 *Number of hits in advanced Google search.*

Searches	EFSA[a]	FAVV[b]	BfR[c]	Livsmedelsverket[d]
1. Vegetable AND “food safety”	1510	**112**	**56**	**77**
Id., 2009-15/04/2019	306			
Id., only PDF	**140**			
2. Vegetable AND (contaminant OR residue)	832	**144**	**55**	**69**
Id., 2009-15/04/2019	**144**			
3. Vegetable AND “risk assessment”	1910	**131**	282	**87**
Id., 2009-15/04/2019	323		**66**	
Id., only PDF	**180**			
total hits	**464**	**387**	**177**	**233**
total relevant hits	**13**	**10**	**7**	**1**

[a] https://www.efsa.europa.eu/

[b] https://www.afsca.be/

[c] https://www.bfr.bund.de

[d] https://www.livsmedelsverket.se/

Annex 6 Questionnaire for evaluating trends in leafy vegetables (in Dutch)

1. Welke veranderingen en ontwikkelingen verwacht u de komende 5 jaar in de bladgroenten sector?
    a. Verwacht u veranderingen in import? (bijv. meer/minder, andere soorten, uit andere landen)
    ....

    b. Verwacht u specifieke ontwikkelingen? (bijv. groei biologische markt, groei van een specifieke soort)
    ....

    c. Verwacht u veranderingen in de ketenstructuur (bv complexere keten door meer tussenhandelaren, of juist minder kleine bedrijven door fusies etc.)?
    ....

2. Welke trends verwacht u in de primaire sector? (bijv. andere oogstmethoden, andere bestrijdingsmethoden, precisielandbouw, automatisering)
    ....

3. Welke trends verwacht u de verwerkende industrie? (bijv. nieuwe producten, andere technologieën, nieuwe droogprocessen)
    ....

4. Zijn er bepaalde consumententrends die relevant zijn voor de bladgroenten sector? (bijv. meer convenience food, andere soorten, superfood trend, meer plantaardige voeding)
    ....

5. Verwacht u beleidsmaatregelen in de nabije toekomst die een effect kunnen hebben op de bladgroenten productie (bijv., subsidies voor biologische landbouw, of strengere wetgeving m.b.t. pesticiden, etc.)
    ....

6. Welke microbiologische gevaren vindt u het belangrijkst in bladgroenten
    a. Kunt u aangeven welke microbiologische gevaren van belang zijn in welke producten en waarom?
    ....

7. Welke chemische gevaren vindt u het belangrijkst in bladgroenten
    a. Kunt u aangeven welke chemische gevaren van belang zijn in welke producten en waarom?
    ....

8. Zijn er nog andere aspecten die u hier zou willen toelichten?
    ....

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WFSR report 2019.013

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WFSR report 2019.013

The mission of Wageningen University & Research is "To explore the potential of nature to improve the quality of life". Under the banner Wageningen University & Research, Wageningen University and the specialised research institutes of the Wageningen Research Foundation have joined forces in contributing to inding solutions to important questions in the domain of healthy food and living environment. With its roughly 30 branches, 5,000 employees and 10,000 students, Wageningen University & Research is one of the leading organisations in its domain. The unique Wageningen approach lies in its integrated approach to issues and the collaboration between different disciplines.