MICROBIOLOGICAL RISK ASSESSMENT SERIES

14

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Microbiological hazards in fresh leafy vegetables and herbs

MEETING REPORT

WHO

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WORLD HEALTH ORGANIZATION

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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Declarations of interest

All participants completed a Declarations of Interest form in advance of the meeting. Five of the experts that participated in the meeting declared a specific consulting, investment and/or intellectual property interest in the topic under consideration.

- Silvia Estrada-Flores has provided consulting services in some of the areas under consideration and received support for her research.
- Franz Eelco has received support for research in some of the areas under consideration.
- Victor Garcia Moreno has provided consulting services in some of the areas under consideration.
- Karin Goodburn has provided consulting services in some of the areas under consideration, and just prior to the meeting was employed by an association representing producers of chilled prepared foods.
- Jeffery LeJeune has received support for research in some of the areas under consideration.
- Robert Premier has provided consulting services in some of the areas under consideration and received support for her research.
- Joaquín Vicente Baños has provided consulting services in some of the areas under consideration and received support for her research.

Upon detailed review of these declarations it was considered that they did not present a conflict of interest and therefore all experts participated fully in the deliberations of the meeting. Nevertheless, for the purposes of transparency, these declarations were made known to all participants at the beginning of the meeting. Experts participated in their individual capacity and not as representatives of their country, government or organizations.

Foreword

Members of the Food and Agriculture Organization of the United Nations (FAO) and of the World Health Organization (WHO) have expressed concern regarding the level of safety of food at both national and international level. Increasing foodborne disease incidence over recent decades seems, in many countries, to be related to an increase in disease caused by micro-organisms in food. This concern has been voiced in meetings of the Governing Bodies of both Organizations and in the Codex Alimentarius Commission. It is not easy to decide whether the suggested increase is real or an artefact of changes in other areas, such as improved disease surveillance or better detection methods for microorganisms in patients or foods. However, the important issue is whether new tools or revised and improved actions can contribute to our ability to lower the disease burden and provide safer food. Fortunately, new tools that can facilitate actions seem to be on their way.

Over the past decade, risk analysis—a process consisting of risk assessment, risk management and risk communication—has emerged as a structured model for improving our food control systems, with the objectives of producing safer food, reducing the number of food-borne illnesses and facilitating domestic and international trade in food. Furthermore, we are moving towards a more holistic approach to food safety, where the entire food chain needs to be considered in efforts to produce safer food.

As with any model, tools are needed for the implementation of the risk analysis paradigm. Risk assessment is the science-based component of risk analysis. Science today provides us with in-depth information on life in the world we live in. It has allowed us to accumulate a wealth of knowledge on microscopic organisms, their growth, survival and death, even their genetic make-up. It has given us an understanding of food production, processing and preservation, and of the link between the microscopic and the macroscopic world, and how we can benefit as well as suffer from these microorganisms. Risk assessment provides us with a framework for organizing these data and information and gaining a better understanding of the interaction between microorganisms, foods and human illness. It provides us with the ability to estimate the risk to human health from specific microorganisms in foods and gives us a tool with which we can compare and evaluate different scenarios, as well as identify the types of data necessary for estimating and optimizing mitigating interventions.

Microbiological risk assessment (MRA) can be considered as a tool that can be used in the management of the risks posed by foodborne pathogens, including the elaboration of standards for food in international trade. However, undertaking an MRA, particularly quantitative MRA, is recognized as a resource-intensive task requiring a multidisciplinary approach. Nevertheless, foodborne illness is one of the most widespread public health problems, creating social and economic burdens as well as human suffering., it is a concern that all countries need to address. As risk assessment can also be used to justify the introduction of more stringent standards for imported foods, a knowledge of MRA is important for trade purposes, and there is a need to provide countries with the tools for understanding and, if possible, undertaking MRA. This need, combined with that of the Codex Alimentarius for risk-based scientific advice, led FAO and WHO to undertake a programme of activities on MRA at international level.

The Nutrition and Consumer Protection Division (FAO) and the Department of Food Safety, Zoonoses and Foodborne Diseases (WHO) are the lead units responsible for this initiative. The two groups have worked together to develop MRA at international level for application at both

national and international level. This work has been greatly facilitated by the contribution of people from around the world with expertise in microbiology, mathematical modelling, epidemiology and food technology, to name but a few.

This Microbiological Risk Assessment series provides a range of data and information to those who need to understand or undertake MRA. It comprises risk assessments of particular pathogen–commodity combinations, interpretative summaries of the risk assessments, guidelines for undertaking and using risk assessment, and reports addressing other pertinent aspects of MRA.

We hope that this series will provide a greater insight into MRA, how it is undertaken and how it can be used. We strongly believe that this is an area that should be developed in the international sphere, and the work to date clearly indicates that an international approach and early agreement in this area will strengthen the future potential for use of this tool in all parts of the world, as well as in international standard setting. We would welcome comments and feedback on any of the documents within this series so that we can endeavour to provide member countries, the Codex Alimentarius and other users of this material with the information they need to use risk-based tools, with the ultimate objective of ensuring that safe food is available for all consumers.

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Abbreviations

ACC	Aerobic Colony Count
AcEW	Acidic electrolyzed water.
AU	Arbitrary units per millilitre
CAC	Codex Alimentarius Commission
CCFH	Codex Committee on Food Hygiene
cfu	Colony forming unit
DNA	Deoxyribonucleic Acid
EO	Electrolyzed oxidizing water.
EPA	Environmental Protection Agency [USA]
ERS	Economic Research Service
FAO	Food and Agriculture Organization of the United Nations
FSA	Food Standards Agency
GAP	Good Agricultural Practice
GHP	Good Hygiene Practice
GMP	Good Manufacturing Practice
MAP	Modified atmosphere packaging
MPN	Most Probable Number
ND	Not done / not reported
NFT	Nutrient Film Technique
RTE	Ready-to-eat
STEC	Shiga-toxigenic *Escherichia coli*
TCB	Thermotolerant Coliform Bacteria
TVC	Total Viable Count
USDA	United States [of America] Department of Agriculture
WHO	World Health Organization

Executive summary

This FAO/WHO Expert meeting was convened on 5-9 May 2008 in Bangkok, Thailand, to address the request for scientific advice received from the 39th Session of the Codex Committee on Food Hygiene (CCFH) on the microbiological hazards associated with leafy vegetables and herbs. In responding to the questions posed by the CCFH, the meeting addressed the pathways for contamination, survival and persistence of microbiological hazards associated with leafy vegetables and herbs, and the potential management options from primary production through to the consumer. Consideration was given to all aspects of the farm to fork continuum, i.e. including pre-harvest and post-harvest.

Production systems for leafy vegetables and herbs fall into two broad categories; open field and protected culture systems. Within these two categories there can be wide variation in terms of inputs, size, location, environmental conditions, productivity and target markets. Such variation from one production site to another highlights the difficulty of providing very specific guidance. Knowing and understanding a particular production environment is critical to the identification and implementation of appropriate mitigations. The meeting highlighted the disconnect that often exists between knowledge of the production environment and knowledge of what constitutes a hazard. The variability that exists between production environments means that the capacity to identify hazards within a system is critical to identifying and applying relevant and effective mitigations.

Pre-harvest of leafy vegetables and herbs

In addressing the production environment of leafy vegetables and herbs, the meeting considered the potential role of wildlife, livestock, human activity, topography and climate, flooding, seed and crop selection, and prior land use in the microbial contamination of leafy vegetables and herbs.

Although indistinguishable pathogens have been recovered from animals and leafy vegetables implicated with disease outbreaks it has not been possible to conclusively determine if the animals were indeed the source of the product contamination or a sentinel of broader environmental contamination that infected the animals and contaminated the crop simultaneously. Animals in crop production environments may be incidental, or they may be attracted to leafy vegetable production sites. Effective and acceptable measures to minimize wildlife intrusion in crops require knowledge of both the type of wildlife and the reason for their intrusion. Sites for growing fresh produce and the produce itself can be contaminated indirectly as well as directly by domestic animals and wildlife. While direct contamination can occur as a result of animals entering the growing fields, indirect contamination may occur from livestock production and feeding facilities via faecal waste, water, aerosols and dust. In addition wildlife may play a role in the dispersal of pathogens from other sources such as landfill and wastewater treatment sites to horticulture fields. The extent to which these sources contribute to the contamination of product are dependent upon other factors such as climate, topography, hydrology and weather.

In addition the meeting sought to address the situation of a recognized contamination event, such as flooding. The meeting highlighted the importance of assessing the risks associated with such events and implementing measures to reduce the risk of pathogens on the produce (e.g. delayed harvesting, heat treatment of produce) or to assure appropriate disposal of contaminated

product. Although most of the data on seeds as a source of contamination relates to seeds for sprouting, there is evidence to indicate that transfer of a microbial contaminant from a seed to a plant is possible, though caution is required in the extrapolation of such data. Although there are no data available in the literature, new crops or prior use of land were considered to have the potential to introduce new hazards and therefore this is an area where on-site assessment of factors that can contribute to increased risk needs to be undertaken. An important conclusion relating to environmental contamination from, for example, wildlife or flooding was that if effective practical mitigation measures were not feasible, then the growing of crops for raw consumption in that particular location should be reconsidered.

Farm workers may also be sources or vehicles for contamination of produce in the growing field. Foodborne outbreaks have been attributed to poor hygiene practices of food handlers. Machinery and equipment were also considered to have the potential to transfer microbial hazards from contaminated areas to growing fields.

Soil amendments, fertilizers and water are very important inputs to productive horticultural systems, but at the same time are potential sources of microbial contamination. Organic wastes play an important role in providing nutrients to crops and improving overall soil quality, and their use in horticulture also provides a means of managing animal, human and plant wastes. However, pathogens associated with these manures may survive for extended periods, and while there has been a substantial amount of research in this area, uncertainties regarding pathogen behaviour remain. Pathogen reduction can be achieved through composting and several countries have established regulatory requirements for composting in their risk management programmes. However, composting is not universally applied and may not always be feasible. Very limited studies have been undertaken on fertilizers derived from composted plant waste. The evidence available indicates that bacterial foodborne pathogens are able to survive for extended periods in a variety of these soil amendments, and some will support growth to high levels. To the extent possible, the meeting addressed the differences in risk associated with different manure types and possible ways to minimize risk.

In noting the potential role of irrigation water in contamination of leafy vegetables and herbs, the meeting identified the water sources at greatest risk of contamination, and a number of practical and cost-effective mechanisms for reducing the risk. Potable supplies or rainwater stored in closed containment systems were considered safest for the production of leafy vegetables and herbs, provided they are delivered to crops through well maintained distribution systems. In contrast, the microbiological quality of waters derived from surface or subsurface sources is highly variable. The potential for spread of microbial contamination through different irrigation strategies (overhead sprays, drip irrigation systems or flooding of fields through furrows) was considered, and it was agreed that subsurface irrigation lowers the risk of pathogen transfer from water to growing plants. The use of contaminated water in the preparation of insecticide, herbicide and fungicide solutions for application to the surfaces of leafy vegetables was also considered to present a risk. However, the current lack of data prevents the elaboration of science-based advice on the time interval between final irrigation and harvesting needed to minimize risk. Existing guidelines and criteria for water used in agriculture were considered, and the meeting noted inconsistencies in the sampling strategies between jurisdictions, and also questioned the applicability and validity of coliforms and generic *Escherichia coli* as indices for the microbiological quality of water used to irrigate crops.

Post-harvest of leafy vegetables and herbs

Harvesting either by machine or manual labour each has their own risks. There is substantial information available on virus transfer via manual harvesting. Much less scientific data is available on machine harvesting, although the potential it presents for contamination is well recognized. This was identified as an area where more data is needed. Some of the more recently introduced practices at time of harvest, such a coring of lettuce heads, were highlighted as needing particular attention to ensure they do not lead to increased contamination of product.

Particular concerns were raised regarding post-harvest processes where there is potential for altering leaf structure and forcing pathogens into the plant cells (infiltration or internalization), for example as a result of mechanical injuries during harvest, application of water under pressure or vacuum, and during washing.

Post-harvest operations can be very varied, from simple open-air packing to more sophisticated washing, drying and processing steps. The meeting addressed the less complex processes separately from the more sophisticated ones in an effort to identify hazards and risks relevant to each approach.

For the more sophisticated operations particular attention was given to the washing and sanitization steps, where the efficacy of sanitizers and other interventions aimed at reducing pathogen levels were considered. The meeting concluded that while some reduction can be achieved there is a lack of significantly effective options other than heat or irradiation. While the latter can result in several log reductions of even colonizing or internalized pathogens, there remains a need for further research focusing on both fundamental attachment mechanisms and inactivation of the pathogens *in situ*. Thus, while processing would appear to be the one step with the potential for the reduction of microbial risks (e.g. disinfection), provide control of amplification of risks (e.g. chilling) and protect the product from further exposure (e.g. packaging), given the current state of knowledge and technology the meeting concluded that if a product is contaminated there is little that can be done to completely remove the contaminant, although a reduction can be achieved and actions taken to prevent exacerbation of a problem.

Temperature is the single most important factor contributing to bacterial growth and survival. Therefore, temperature control and maintenance of adequate cold chain conditions are critical to food safety. However, the existence and role of the cold chain in distribution of leafy vegetables and herbs was considered to vary extensively, and depended to a certain extent on the form of the end product. While low temperatures will not reduce risk, they will prevent an increase in risk during storage and distribution. Shelf life is implicitly linked with the cold chain, and the meeting noted that there were many different scenarios around shelf life, packaging and the cold chain, which could affect product safety positively or negatively. It was also noted that many leafy vegetables are distributed in the absence of a cold chain, but if marketed and used immediately this would not necessarily present a greater risk.

The role of education, training and awareness was considered critical all along the chain in order to improve product safety. It was considered that education needed to be used as a real risk mitigation measure, by primarily applying it in situations where it would have the greatest impact and then monitoring, and evaluating its impact on a regular basis and reviewing as and when needed. As it was considered to play such a critical role in leafy vegetables to be eaten raw, it is important to apply and use education and training in the structured manner in which other mitigations are implemented. Consumers are also an important target group for information and education on how to handle fresh and fresh-cut leafy vegetables and herbs safely, and need to understand their roles and responsibilities in protecting these products from

contamination and deterioration, and in preventing foodborne illness.

The diversity of production systems around the world make it difficult to provide very specific recommendations. Therefore the meeting sought to use the available science to identify the specific issues that need to be considered when developing guidance in this area. Through the discussions it became very obvious that it is critical to know and understand the production and processing system of concern, and to marry that with information on possible hazards and risks. Thus, for example, the need to undertake an assessment of a production site in terms of the potential of factors such as wildlife, domestic animals, human activity, proximity to urban areas, climate, topology, weather, hydrology, prior land use and geographical features to contribute to an increased risk of microbiological contamination of leafy vegetables and herbs during the growing phase is emphasized. Similarly, the differences in post-harvest practices are highlighted in terms of risks and mitigations. The meeting re-emphasized the importance of implementing the recommendations of the existing Codex Code of Hygienic Practice for Fresh Fruits and Vegetables and the Codex Recommended International Code of Practice – General Principles of Food Hygiene. In addition, through its review and summary of the available information and scientific data, the meeting sought to highlight the value and utility of the existing knowledge in identifying and implementing further measures to minimize pathogens on leafy vegetables and herbs to the extent possible. At the same time, by identifying the gaps that exist in the data base, the meeting emphasized the need for further research in certain areas to facilitate improved risk management in the future.

MEETING REPORT

1. Introduction

1.1 Background

Problems linked with pathogens in fresh produce, including the associated public health and trade implications, have been reported in a number of countries worldwide, Therefore the 38th Session of the Codex Committee on Food Hygiene (CCFH), through the Codex Alimentarius Commission (CAC), in 2006 requested FAO and WHO to provide scientific advice to support the development of commodity-specific annexes for the Codex Alimentarius "Code of Hygienic Practice for Fresh Fruits and Vegetables" (CAC, 2003b). The Committee highlighted the need to address, in more detail, aspects related to the control of specific hazards of concern, in particular fresh fruit and vegetable products, and provided terms of reference as guidance to the type of scientific advice needed (CAC, 2006).

The terms of reference for scientific advice was extensive, identifying the need for advice on eight types of products and eight different pathogens, and answers to approximately 40 questions spanning the whole food chain. Given the need to provide advice in a timely manner (the request specified an 18-month timeframe), FAO and WHO decided it was necessary to address the various tasks in a prioritized manner, including the specific pathogen–commodity combinations identified.

A step-wise process was applied to the provision of scientific advice on these products. The first step was to issue a call for data. This was issued in the form of the Codex Circular Letter (CAC, 2007) to all Codex members, and was also circulated via other routes, such as the FAO and WHO Web pages, newsletters and food safety networks. A call for experts was issued at the same time.

The second step was to call for an Expert Meeting to review the available data and, in particular, to prioritize the issues to be addressed. This meeting was convened on 19–21 September 2007.

The 2007 FAO/WHO Expert Meeting agreed to a set of six criteria, which should be used to rank the commodities of concern as identified by the CCFH and by member countries. The criteria were as follows:

- Frequency and severity of disease.
- Size and scope of production.
- Diversity and complexity of the production chain/industry.
- Potential for amplification of foodborne pathogens through the food chain.
- Potential for control.
- Extent of international trade and economic impact.

The available information was reviewed in light of these criteria, which enabled the identified commodities to be ranked into the following three priority groupings.

Level 1 Priorities – leafy vegetables (including herbs).

Leafy vegetables (including herbs) were accorded the highest priority based on the ranking criteria. The available data varied by completeness, but the meeting concluded that there was sufficient information to indicate that, from a global perspective, leafy vegetables currently presented the greatest concern in terms of microbiological hazards. Leafy vegetables are grown and exported in large volume, have been associated with multiple outbreaks with high numbers of illnesses in at least three regions of the world, and are grown and processed in diverse and complex ways, ranging from in-field packing to pre-cut and bagged product. Such post-harvest activities contribute to the possibility of amplification of foodborne pathogens.

Level 2 Priorities – berries, green onions, melons, sprouted seeds, tomatoes.

These commodities were identified as being the second-highest concern. Given the available knowledge, berries, green onions, melons and tomatoes were considered to be similarly problematic, and it was not possible to rank them from a global perspective. However, it was clear that regional differences exist and therefore it would be easier to rank these commodities in order of priority from a regional perspective.

Sprouted seeds were considered somewhat apart from the other four in this group as a Codex guideline for the hygienic production and packaging of sprouted seeds already exists. However, sprouted seeds continue to be implicated in outbreaks and therefore the meeting considered that the existing code should be reviewed in light of the available information to determine if any revisions are necessary.

Level 3 Priorities.

This is the largest group, and includes carrots, cucumbers, almonds, baby corn, sesame seeds, onions and garlic, mango, paw paw, celery and maimai. These were considered to be the lowest priority of the identified commodities of concern. While all these commodities have been implicated in cases or outbreaks of foodborne illness, the public health impact was considered to be low based on information available for the meeting. Also, there are limited data available for most of these commodities and, in several cases, the associated problems have been recognized only recently. However, these may be emerging problems and therefore the meeting recommended that problems linked to these commodities be noted and the commodities be monitored for further problems. As more information becomes available the ranking of these commodities will need to be re-evaluated.

Based on the above, the meeting made the following recommendations.

- Leafy vegetables should be considered the highest priority in terms of fresh produce safety from a global perspective, and that FAO and WHO should focus its efforts to develop scientific advice on this commodity grouping.
- The CCFH should take into account the outcome of the ranking exercise and the priority rankings assigned to the different commodities when selecting their work priorities.
- The annex to the Codex code of hygienic practice for fresh fruits and vegetables which addresses sprouted seeds should be reviewed for adequacy.
- The ranking should be reviewed in the future and revised when substantial new information is

available.

In addition, the meeting made a number of recommendations to FAO, WHO and Codex to be taken into consideration in the elaboration of scientific advice and risk management guidance, and to governments and institutions working on these issues.

The report of the meeting (FAO/WHO, 2008a) was presented to the 39th session of the CCFH in 2007 (CAC, 2008). The 39th session of the CCFH took this report into consideration when prioritizing and agreeing new work for the Committee. The CCFH agreed to begin work on a commodity-specific annex to the existing "Code of Hygienic Practice for Fresh Fruits and Vegetables" for leafy vegetables including leafy herbs. In doing so, the Committee also confirmed to FAO and WHO that it required scientific advice on the microbiological hazards on leafy vegetables, including leafy herbs, in accordance with the terms of reference and timeframe provided by the 38th Session of the Committee in 2006 (CAC, 2006).

In order to provide the necessary scientific advice, FAO and WHO convened an expert meeting in Bangkok, Thailand, 5–9 May 2008. In responding to the questions posed by the CCFH, this meeting addressed the microbiological hazards associated with leafy vegetables and herbs, the pathways for contamination, survival and persistence of microbial hazards, and the potential management options from primary production through to the consumer.

1.2 Objectives

The objectives of the meeting were as follows:

- To review the current information and data on fresh and fresh-cut leafy vegetables and herbs from production through to consumption. Specifically the meeting addressed:
    - Primary production, including environmental hygiene, water for primary production and field packing, use of soil amendments and fertilizers, personnel health and hygiene and sanitary facilities.
    - Processing, including packing establishments, field packing operations and other post-harvest handling facilities, particularly key aspects of hygiene control systems, such as post-harvest water use, worker health and hygiene, disinfection processes, cleaning and sanitization of equipment and facilities.
    - Distribution and cold chain maintenance.
    - Consumer use and behaviour.
- To use the available information to identify and rank factors that contribute to the microbiological contamination of leafy vegetables and herbs.
- To identify potential mitigations and interventions for control and management of microbiological contamination of leafy vegetables and herbs, and provide information on their potential impact.
- To advise on possible risk assessment approaches for leafy vegetables and herbs.

2. Fresh and fresh-cut leafy vegetables and herbs

2.1 Scope

The FAO/WHO Expert Meeting agreed that leafy vegetables and herbs include all vegetables and herbs of a leafy nature and of which the leaf (and core) is intended to be consumed raw, e.g. lettuce (all varieties), spinach, cabbages, chicory, leafy herbs (e.g. cilantro, basil, parsley) and watercress (FAO/WHO, 2008a). "Leafy greens" is a term used also for this group. However, it is not used in this text as some varieties may be colours other than green, and this term may be misleading and result in the exclusion of some varieties. Green onions are not included under leafy vegetables and herbs as they differ in morphology from the above-mentioned vegetables. A list with examples of leafy vegetables and herbs that meet this definition is presented in Annex 1.

2.2 International production and trade

From 1980 to 2004, the global production per annum (p.a.) of fruit and vegetables grew by 94%. During that period the average yearly production growth of vegetables (4.2% p.a.) was almost twice that of fruits (2.2% p.a.) (EU, 2007). Human fruit and vegetable consumption increased by an average of 4.5% p.a. between 1990 and 2004 (EU, 2007). Global production figures for fruit and vegetables from 1979 to 2004 are provided in Figure 1 (FAOSTAT, 2008).

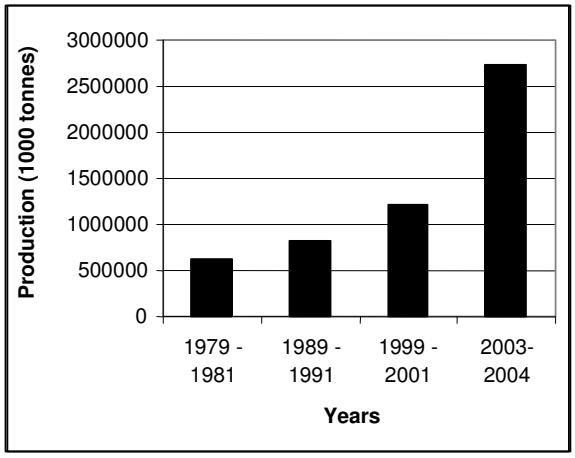

Figure 1. Global production of fruit and vegetables from 1979-2004
SOURCE: FAOSTAT, 2008.

The world harvest area and production of leafy vegetables such as lettuce, chicory and spinach, cabbages and other brassicas has increased progressively since the early 1990s (Figures 2 and 3., FAOSTAT, 2008). The harvest area for lettuce and chicory increased by 218% and that for spinach increased by 300% in the period 1986 to 2006. In 2006, the major producers of lettuce and chicory were China (50%) and the United States of America (20%). China also produced 84% of all the spinach produced globally (FAOSTAT, 2008).

The fresh market for leafy vegetables and herbs has increased in particular. For example, in the USA, where the consumption of spinach has increased, fresh spinach now accounts for about three-quarters of total spinach consumption (ERS-USDA, 2008). This is sold as triple-washed packaged spinach and, more recently, baby spinach, which has become one of the fastest-growing segments of the packaged salad industry.

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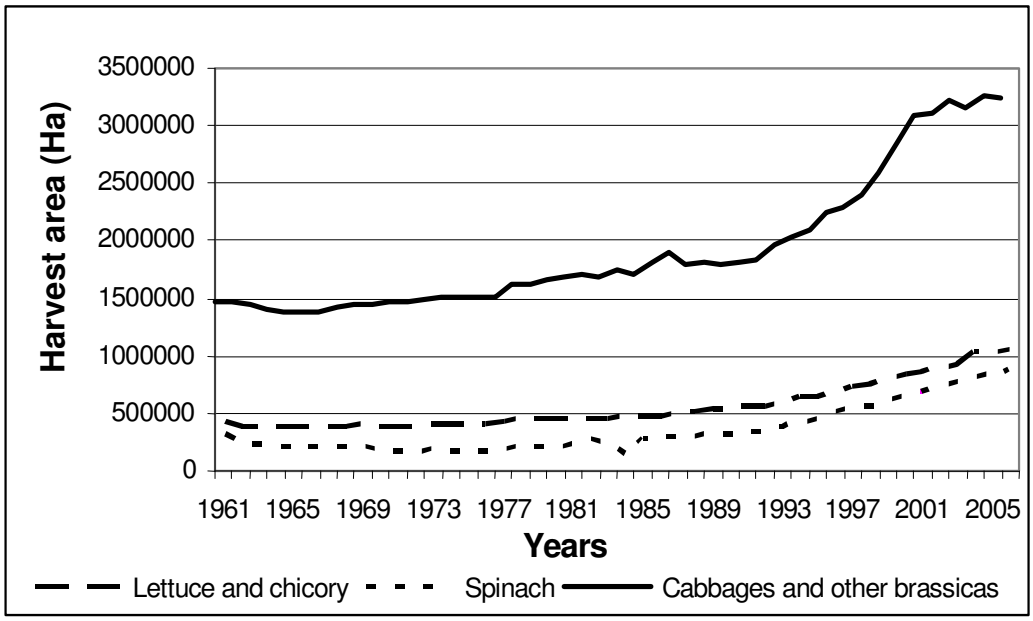

Figure 2. World harvest area of selected leafy vegetables, 1961–2005
SOURCE: FAOSTAT, 2008.

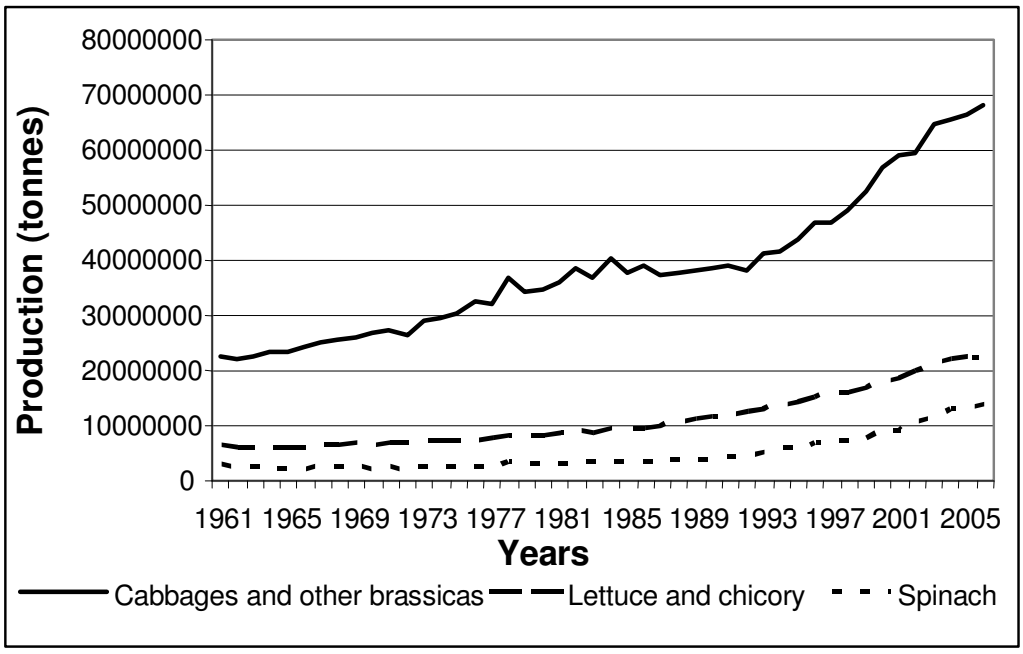

Figure 3. World production of selected leafy vegetables, 1961-2005
SOURCE: FAOSTAT, 2008.

Fruit and vegetables are an important component of a healthy diet and there is an international move to increase their consumption (FAO/WHO, 2005). Regular daily consumption of fruit and vegetables in sufficient amounts can help prevent major diseases such as cardiovascular diseases and certain cancers. All fruits and vegetables are considered likely to contribute to this benefit and leafy vegetables such as lettuce, spinach, chard, mustard greens and cabbage have been identified as making an important contribution (Hung et al., 2004; Link and Potter, 2004). Therefore the consumption—and thus the production—of leafy vegetables and herbs are expected to continue to increase in the future

2.3 Foodborne illness

2.3.1 Microbial hazards

Fresh produce at harvest has a natural epiphytic microflora much of which is non-pathogenic. During any of the steps in the farm-to-consumer continuum (growth, harvest, processing, packaging, transportation, handling, retail) further microbial contamination can occur from a variety of sources, e.g. environmental, animal or human. There is a risk that this may include pathogenic microorganisms. A review of major microbial pathogens contaminating fresh vegetables has been undertaken previously (WHO, 1998) and the report of the 2007 FAO/WHO meeting also provides an overview of the pathogens most commonly associated with fresh fruit and vegetables (FAO/WHO, 2008a; see also Table A2.1 of Annex 2).

Fresh vegetables and herbs, including those of the leafy variety, have been implicated as vehicles for the transmission of microbial foodborne disease worldwide (Beuchat, 2006). A list of microbial agents and leafy vegetable and herbs or their products implicated in outbreaks is presented in Table A2.3 in Annex 2.

2.3.2 Epidemiology

Data on the number of incidents of foodborne illness attributed to leafy vegetables and herbs is limited by several characteristics of this product group, and they are not directly comparable between countries (EU, 2002). Identifying the role of leafy vegetables and herbs in an outbreak can be difficult, especially when they are a component of a salad made up with a dressing and other foods that are equally suitable for transmission of the pathogen. Epidemiological reports often categorize the attributed food as a "salad", "green salad" or "coleslaw", so that is not possible to identify specifically the leafy vegetables and/or herbs and other ingredients. Specific attribution of these foods can be further complicated by the multiple and intermittent culinary use over a period of a single purchase of these products.

A food vehicle is more often implicated in an outbreak involving two or more persons than in sporadic cases. For this reason, available information has to be considered an underestimate, as most reports on the incidence of foodborne disease associated with leafy vegetables and herbs are based on outbreaks and do not include sporadic cases. Other factors that contribute to under-estimation of foodborne illness for this group are the lack of traceability of leafy vegetables and herbs in the past, and the incompleteness or lack of disease surveillance in some countries.

Reported outbreaks associated with leafy vegetables and herbs have been notable for the wide geographical distribution of the contaminated products and the high numbers of consumers exposed, and thus the large number of cases. The proportion of total outbreaks of foodborne disease attributed to leafy vegetables and herbs varies between countries (a summary of outbreaks for the period 1996 to 2006 is presented in Table A2.2 in Annex 2). Outbreaks attributed to leafy vegetables and herbs are often implicated among fresh produce outbreaks,

70% of the total fresh produce outbreaks in the USA (data from CSPI for the period 1998–2005) and 75% of the total fresh produce outbreaks in Brazil were attributed to leafy vegetables and herbs (Table A2.2 in Annex 2).

There are reports of increasing incidents of foodborne illness attributed to leafy vegetables and herbs. For example, in the USA between 1998 and 2002, vegetables were associated with 2.9% (192/6647) of the total foodborne outbreaks recorded (Lynch *et al.*, 2006). More recently, foodborne vegetable outbreaks specifically associated with leafy vegetables were analysed in the USA by Herman, Ayers and Lynch (2008). Between 1973 and 2006, 502 (4.8%) outbreaks, 18 242 (6.5%) illnesses and 15 (4.0%) deaths were associated with "leafy greens", described as lettuce, cabbage, mescalin mix, spinach or a salad item containing one or more of these leafy vegetables. Within this period both the consumption of leafy vegetables (based on per capita availability of leafy vegetables) and the proportion of outbreaks attributed to leafy vegetables increased (Table 1). The authors conclude that the increase cannot be explained simply by increased consumption, implying other production factors are involved.

The epidemiology will be influenced by the exposure of consumers to leafy vegetables and herbs. The consumption of leafy vegetables and herbs is not specific to any consumer group, although the very young may be less likely to be exposed to raw products. Similarly, their consumption is not specific to any geographical region. Although some varieties may be more common to a specific region, increasing international trade has made a wide variety of produce available all year round, particularly in developed countries.

Table 1. Increase in the consumption of "greens" (leafy green vegetables) and the proportion of outbreaks attributed to leafy greens in the USA (1986–2005). Data taken from Herman, Ayers and Lynch, 2008.

Period	% increase compared with previous decade	
	Consumption	Proportion of total outbreaks
1986–1995	17.2	59.6
1996–2005	9.0	38.6

2.3.3 Microorganisms and fresh leafy vegetables and herbs relevant to food safety

Leafy vegetables and herbs have a natural epiphytic flora and may be contaminated by various intentional or accidental inputs to the growing field environment (e.g. water, soil amendments, animals and birds), farm equipment and farm workers. Factors influencing the survival and growth of microbial hazards include the characteristics of the organism, the physiological state of the plant and its inherent resistance to microbial metabolic processes, the intrinsic factors of the plant environment (e.g. pH, water activity, atmospheric composition) and the effect of processing, if employed (de Roever, 1998). A summary of some studies of the microbiological analyses of leafy vegetables and herbs at various points on the food chain from farm-to-retail are presented in Table A2.4 of Annex 2. The availability of information in the scientific and peer-reviewed literature is limited, and does not provide a global picture. There are significant differences between studies in the sizes of the samples examined, the locations of the sampling, and the methodology used. Comparisons cannot therefore be made.

Much of the microbial flora associated with fresh plants is of no known public health significance, although pathogens may be present. The factors that influence the presence or level of pathogens at any point in time is multifactorial, and in addition to the inputs mentioned above, other factors such as the plant type, weather, floods or the prior use of the land can influence the microbial communities present (Brackett, 1999). In the studies cited (Table A2.4

of Annex 2), populations of mesophilic aerobic bacteria of up to 8 $\log_{10}$ cfu/g were commonly reported on produce at various points in the food chain from farm-to-retail. Coliforms are common members of these populations and reports of counts up to 8 $\log_{10}$ MPN/g were recorded; however, these counts varied between studies, plant varieties and point of sampling. Because fresh produce may have high levels of natural flora, genera of which are included in the coliform group and yet not considered foodborne pathogens, these indicators have little significance when considering food safety.

E. coli is a more specific indicator of faecal contamination. Mukherjee et al. (2004) found *E. coli* in 10.7% (9/84) of field samples of leafy vegetables, with 22.4% (12/49) lettuce, 10.2% (4/39) cabbages and 13.3% (2/15) bok choi contaminated. The average count recorded was 3.1 $\log_{10}$ MPN/g. In most studies cited (Table 2.4 in Annex 2), *E. coli* were detected and counts were typically ≤2 $\log_{10}$ cfu/g. Counts in the range 3–5 $\log_{10}$ cfu/g were recorded for only a small number of samples. Exposure to risk factors for faecal contamination was linked to higher rates of *E. coli* contamination. For example, leafy crops fertilized with inadequately composted manure and those fertilized with animal manure were found to have a higher risk of *E. coli* contamination (Mukherjee et al., 2004; Mukherjee, Speh and Diez-Gonzalez, 2007). A similar situation was noted for watercress irrigated with contaminated water (Edmonds and Hawke, 2004).

Microbial food safety hazards that have been associated with leafy vegetables and herbs are listed in Table A2.1 of Annex 2. Bacterial food safety hazards, if present in foods that are not implicated in an outbreak, are usually present in low numbers amongst large numbers of background microbial flora. There are certain limitations associated with the testing of foodstuffs for these hazards, as amplification or enrichment of the bacterial hazard is required. Thus test results are reported as either presence or absence of the bacterium, usually in a 25 g sample. Analyses for parasites and viruses are more complex, and in the case of viruses such as rotavirus and hepatitis A, may rely on the use of DNA-based or immunological assays, while parasite detection methods for cysts may not establish the viability of the cysts detected.

In 1998, Beuchat in reviewing studies of leafy vegetables and decontamination (WHO, 1998) reported that: (i) *Salmonella* was detected in less that 8% of samples (there were 2 exceptions, i.e. it was detected in 17% of cabbages and 68% of lettuces), (ii) *Campylobacter* was detected in 3.1% of lettuces (n=67), (iii) *E. coli* O157 was detected in 25% of cabbages, 19.5% of cilantro, and 20% of coriander samples, and (iv) *Listeria monocytogenes*, an environmental bacterium, was detected in cabbages (up to 7%), leafy vegetables (22.7%) and lettuce (20%). Lower detection rates were reported by Sagoo, Little and Mitchell (2003) in a study undertaken in the United Kingdom on open, ready-to-eat (RTE), prepared salad vegetables from catering or retail premises. *E. coli* O157, *Campylobacter* spp. and salmonellas were not detected in any of the samples examined (n=2950). One sample (<1%) was of unacceptable microbiological quality because of the presence of *Listeria monocytogenes* at 840 cfu/g. Other studies since 2000 presented in Table A2.4 of Annex 2 also show low detection rates of these pathogens in leafy vegetables (less than 1–2%), although the detection rates have been higher for leafy herbs such as cilantro, parsley and dill (1 – 20%). It is noted the sample sizes in these studies are variable with some much larger than others.

2.4 Overview of production systems for leafy vegetables and herbs

In recent years, increasing international trade has resulted in globalization of the food supply leading to, first, the intensification of crop production, and, second, the introduction of new crop

varieties to provide exotic varieties and year-round supplies to importing countries. This has influenced both the type of cultivation system and the distribution of cultivation areas.

Cultivation systems vary considerably between and within countries, but fall into two broad categories: open field, and protected cultivation systems (Maloupa, 2000). Within these categories there can be wide variation in terms of inputs, location, size, productivity, target market and the extent to which one is practiced rather than another. Protected cultivations can increase yield, provide year-round supply, and allow greater control of abiotic factors and pests compared to open field culture systems. However, protected cultivation systems require a higher level of inputs per hectare, thus concentration of contaminants may occur.

Cultivation systems can be further divided into soil and soilless culture systems (Johnson, 2008). Soilless systems are suited to produce with short cultural cycles and high plant density. They are often used for the production of high-value-added crops. Plant nutrition can be better controlled in these systems and soil contamination is avoided; however, the universal requirement for safe water and hygiene control of the aquatic systems remains. Plug systems (these begin with seedlings grown in plugs) offer a less labour intensive production system, with reduced use of agrochemicals. Numerous soilless culture systems are in place around the world, such as the Nutrient Film Technique (NFT) system, pot and sacs systems, aeroponics, ebb-and-flow system, and floatation systems (Johnson, 2008).

Further differences exist between organic and conventional production systems, i.e. differences exist in the use of natural fertilizers and the avoidance of the use of chemicals in the control of pests and disease.

With increasing urbanization and scarcity of land, fresh leafy vegetables and herbs are often grown in urban and peri-urban areas, or in areas close to other agricultural production systems, such as livestock production (Drescher, Nugent and de Zeeuw, 2000). This may lead to food safety risks arising from exposure (direct and indirect) to animal, human and industrial wastes. Other environmental factors that have an impact include the local wildlife ecology, and climatic and topographic features of the growing area (this is discussed in more detail in Section 3). The infrastructure to manage this varies between and within countries.

Management of food safety can vary with different supply chains and retail outlets (Martinez et al*.,* 2007). Retailers and supermarkets set food safety specifications, auditing and certification requirements that contrast with sales in local markets, where minimal or no regulation may exist. In some supply chains, a crop may be grown by many small producers who then supply a single processor or distributor. This renders risk management more complex and can affect the ability to trace product back to source.

3. Production environment of leafy vegetables and herbs

Leafy vegetables and herbs, regardless of the production system used, are grown in environments that have a wide range of accidental or intentional inputs that are potential sources of microbial foodborne hazards and may lead to contaminated produce (Beuchat, 2006; Brackett, 1999). The major potential inputs identified were wildlife, livestock, human activity and wastes, water, soil and soil amendments, seeds and plant stocks. Other inputs identified that may affect the risk of contamination were climate and flooding, topographical features of growing fields, and prior use of the growing field land. This section discusses the impact of these inputs on the contamination risk of leafy vegetables and herbs, and discusses approaches to risk mitigation.

3.1 Wildlife, livestock, human activity

3.1.1 Problem scope

Domestic and wild animals, as well as humans, are potential sources of microorganisms that are commonly associated with illness attributed to leafy vegetables and herbs (Table 2.1 in Annex 2). The major pathogens causing illness include:

- non-typhoid *Salmonella* and *E. coli* (enterohaemorrhagic types), which are common foodborne zoonoses,
- those specific to the human host, such as *Shigella*, hepatitis A virus, and various parasites common in communities (including farming) with poor sanitation and hygiene, and
- *Listeria monocytogenes*, which is an environmental and also a zoonotic bacterium.

3.1.2 Potential impact

Faecal waste, urine and hair from live animals and carcasses of dead animals in the field may directly contaminate produce while growing in the field. In addition, human waste may be a source of direct contamination if deposited in the growing field. Alternatively, environmental contamination with pathogens from these sources may be transferred indirectly to produce via contaminated water, insects, workers, or fomites such as dust, tools and equipment.

3.1.3 Available data

Microbial hazards from livestock and wildlife

A large number of infectious agents, including those most important to the microbiological safety of leafy vegetables and herbs, have been identified in domestic animals and wildlife. Foodborne pathogens may be present in the faeces of domestic and wild animals without causing outward signs of illness or disease, making it difficult, if not impossible, to determine by visual inspection if an animal is carrying a specific pathogen. To briefly describe infection dynamics, an infected animal population can be classed as either a maintenance or spillover host, depending on the dynamics of the infection. In a maintenance host, infection can persist by

intraspecies transmission alone, and may also be the source of infection for other species. In a spillover host, infection will not persist indefinitely unless there is re-infection from another species or the environment. Transmission from a spillover wild host to domestic livestock or other wild host may also occur, and vice versa, and therefore, maintenance and spillover hosts may both act as disease vectors (Morris, Pfeiffer and Jackson, 1994).

Risks posed by livestock and wild animals are dependant upon the prevalence, incidence, and magnitude of pathogen carriage in the animal hosts (Morris, Pfeiffer and Jackson, 1994), the degree of interaction between the animals and the growing environment (Jay *et al.,* 2007), animal behaviour and ecology (Carter et al., 2007). With respect to wildlife, the most abundant species in a particular region are of the greatest concern as the risk of faecal contamination by these animals is the highest. Most mammalian pests range fairly close to crops, whereas birds are particularly problematic because they have the ability to transmit pathogens over substantial distances and are difficult to control. Control of birds would require a regional plan or completely protected enclosures for a specific growing area.

E. coli O157 has become an important cause of illness attributed to leafy vegetables and herbs. Ruminant animals are among the most common reservoir species for this pathogen, with cattle being considered the primary maintenance reservoir host (Hancock et al., 2001). The prevalence of *E. coli* O157 in cattle may vary from 0 to over 50%, depending on location and season (Renter and Sargeant, 2002) and the number of cells excreted in faeces averages around 3.3 $\log_{10}$ cfu/g (Berg et al., 2004). In addition, *E. coli* O157 and other Shiga-toxigenic *Escherichia coli* (STEC) are present in a large variety of other ungulates (deer, sheep, goats) and numerous other domestic and wild animals, including horses, pigs, chickens, turkeys and dogs (Doane et al., 2007). In studies of free-ranging deer, the faecal prevalence of *E. coli* O157:H7 was estimated to range from zero to less than 3% (Sargeant et al., 1999; Fisher et al., 2001; Renter et al., 2001; Dunn et al., 2004; Branham et al., 2005). Among samples from feral pigs, 23% of faecal samples were positive for *E. coli* O157 in California, USA (Jay et al., 2007).

STEC have been isolated from other wildlife, including rodents, birds (gulls, geese, starlings and passerines), insects and molluscs, e.g. houseflies, beetles and slugs (Nielsen et al., 2004). These may be incidental hosts due to their proximity to ruminant hosts, or independent hosts. Near an English sheep farm, 0.2% of slugs were found to be carriers of STEC (Sproston et al., 2006) and 1.4 to 2.9% houseflies associated with cattle in Kansas, USA, were carrying the bacterium (Alam and Surek, 2004). Results from studies on rodents vary. Hancock, Besser and Rice (1998) did not detect *E. coli* 0157:H7 in 300 samples of rodents on cattle farms in the USA Pacific Northwest, whereas Nielsen et al. (2004) found 2 out of 10 rat samples carried other pathogenic forms of *E. coli* on farms in Denmark. Wild birds close to farm animals may play a possible role in STEC transmission. Nielsen et al. (2004) found 1.6% of these birds positive for STEC, and other studies have supported this. For example, *E. coli* O157 was detected in 2.9% of gulls from English natural areas and in 0.9% of gulls associated with landfills (Wallace, Cheasty and Jones, 1997). Furthermore, 1% of passerines and woodpeckers studied in Wisconsin, USA (Brittingham, Temple and Duncan, 1988), 1.6% of wild birds living close to cattle and pig farms in Denmark (Nielsen et al., 2004) and 0.5% wild birds on cattle ranches in the USA Pacific Northwest (Hancock, Besser and Rice, 1998) carried *E. coli* O157 or other pathogenic *E. coli*. The prevalence of transmission between ruminant hosts and associated incidental insects and pests is not certain. Experimental studies have been used to demonstrate that flies are capable of transmitting in excess of 3 $\log_{10}$ cfu at each landing (DeJusús et al., 2004). This suggests that if flies acquire the bacterium from a source such as cattle faeces, they would be capable of transmitting many bacteria to the next surface (e.g. vegetables leaves).

It is estimated that herd or flock prevalence of *Salmonella* in domestic animals varies between 0% and 90%, depending on the animal species and region (Forshell and Wierup, 2006). Among wild animals, an apparently low prevalence of *Salmonella* faecal shedding occurs (Renter et al., 2001), although *Salmonella* were detected in 8% of rumen samples from white-tailed deer (Renter et al., 2006). Prevalence of microbial pathogens such as *Salmonella*, associated with foodborne illness, is usually low among wild birds. Gulls have been found to carry *Salmonella*; Palmgren et al., (2006) found 4% positive and Fenlon (1981) reported 12.9% positive where they may have been associated with human waste.

Listeria monocytogenes is a saprophytic organism that is commonly present in the environment, especially soils enriched with plant matter (Weis and Seeliger, 1975). Domestic livestock, especially cattle and other small ruminants play an important role in the amplification and environmental dissemination of *L. monocytogenes*. Thirty percent of animals on individual farms may be shedding *L. monocytogenes* in their faeces (Nightingale et al., 2004). Furthermore, this pathogen has been isolated from the faeces of poultry, wild birds and a number of wildlife species, including deer, moose, otters and raccoons (Hellström et al., 2008; Lapen et al., 2007).

It is important to note that the list of prevalence studies of foodborne pathogens is not exhaustive, and studies have not been conducted in every species and every geographical region. Therefore the prevalence of pathogens in a particular region may differ significantly (lower or higher) from the values in the examples provided. Although they have not been reported as causes of foodborne disease outbreaks in leafy vegetables, a number of other organisms that may cause food poisoning, such as, *Yersinia pseudotuberculosis*, *Cryptosporidium*, *Giardia* and hepatitis E virus are occasionally present in the manure of domestic and wild animals. Moreover, it is possible that (i) pathogens may be present in animal species that have not been extensively studied, (ii) novel zoonotic pathogens may emerge as important causes of zoonoses (Bengis et al., 2004), or (iii) pathogens may emerge in species not previously infected with a specific organism. Although not evaluated, re-emergence of diseases is usually associated with areas where people are associated with animals and agriculture (e.g. this maybe of concern in pre-harvest areas where animal excreta may reach crops).

Since the Norovirus genus comprises viruses that infect humans, pigs, cattle and mice, the possibility for zoonotic transmission of infection exists. Recent findings highlight a possible route for indirect zoonotic transmission of noroviruses through the food chain, which could also involve leafy vegetable contamination (Mattison et al., 2007; FAO/WHO, 2008b).

Salmonella Typhi and hepatitis A are obligate human pathogens and are not found in animal reservoirs. Besides humans, *Shigella dysenteriae* may colonize non-human primates, but not other domestic or wild animals (Nizeyi et al., 2001).

Although indistinguishable pathogens have been recovered from animals and leafy produce implicated in disease outbreaks, it has not been possible to conclusively determine if the animals were indeed the source of the product contamination or a sentinel of broader environmental contamination that infected the animals and contaminated the crop simultaneously (Jay et al., 2007). Animals in crop production environments may be incidental, or animals may be attracted to leafy vegetable crop protection sites for various reasons, including:

- As a food source: slugs, insects, other animals.
- For shelter, either in crop or nearby in work storage sheds.
- To feed on other insects or animal nesting in or near crop.

- To seek associated water.
- Incidental or accidental when encroaching on habitat or when animals are moving between appropriate habitats, such as buffer zones.
- Livestock intentionally allowed to forage on crop waste.
- Animals used for work (horses, water buffalo, oxen).
- Free-range livestock.

Furthermore, specific animal behaviours may predispose increased crop contamination. Such factors include predictable and unpredictable events, such as animals moving in groups (Jay et al., 2007), migration and dispersion patterns, and territorial marking (Delahay et al., 2000).

Microbial hazard inputs from other sources

In addition to direct contamination of growing sites by animals, fields for growing leafy vegetables and product can be contaminated by indirect means, such as contaminated water, aerosols and dust from livestock production and feeding facilities and other human activities such as landfills and wastewater treatment sites. Wildlife may play a role in dispersal of pathogens from these sources to fields used for vegetable production. For example, landfills and wastewater treatment attract wildlife, the wildlife pick up pathogens from farms and landfills, and water near these facilities can become contaminated with microorganisms (Nesse et al., 2005). Finally, it should be noted that farm workers and contaminated equipment may also be vehicles by which pathogens are transferred from contaminated locations to the growing field.

3.1.4 Uncertainty and data gaps

Specific information is not currently available linking wild animal density, distribution and land use in a geographical region with the risk of produce contamination. However, there are several lines of evidence that support the hypothesis that animal density may be an important risk factor in the contamination of crops. Increased animal density

- increases the transmission of microorganisms between animals (Gortázar et al., 2006),
- increases faecal contamination of the environment (Acevedo et al., 2007), and
- may increase environmental contamination with pathogens and increase risk of environmentally acquired zoonotic infection (Efimov, Galaktionov and Galaktionova, 2003).

The status of maintenance or spillover host is significant in order to determine whether the management of diseases in an animal host would affect the risk of microbial contamination of leafy vegetables (Wobeser, 2007).

The extent to which domestic animal holding and slaughter operations, landfills, wastewater treatment facilities, urban development and human settlements contribute to the contamination of produce have not been described. Modelling of complex interactions among factors such as wildlife populations, climate, topography, hydrology and weather (as discussed in other sections of this document) and sanitary practices around industrial and residential activities is required to more precisely estimate the risk associated with these activities.

3.1.5 Mitigation recommendations

Control of wild animal populations may be difficult or restricted by specific animal protection guidelines in different regions of the world. However, to the extent feasible, where wildlife is a

concern, practices could be considered to deter or redirect wildlife to areas where crops are not destined for fresh produce markets. When low environmental impact strategies and traditional low cost deterrents are not successful, some invasive approaches, such as regulated harvest and culling, wildlife translocation or human relocation, may be necessary. However, aggressive depopulation activities may have negative environmental impacts. For example, removal of animals from one location may result in perturbations in animal behaviours, causing increased pathogen dispersion (Donnelly et al., 2005). If the above-mentioned methods are unsuccessful at reducing or eliminating risks, consideration could be given to growing alternative crops in the specific location or to avoid growing leafy vegetables and herbs at times that animal intrusion is expected to be unavoidable (during particular migration times, etc.). Crops that are considered less susceptible to contamination, such as vegetables that will be cooked, would have lower risk for human health.

Given the broad host range and the sporadic and unpredictable nature of carriage of foodborne pathogens by domestic and wild animals, all animals entering leafy vegetable and herb production areas should be considered as potential hazards. Animals should therefore be excluded and dissuaded from the production site. This is especially important as the crop nears the time of harvest. Several methods to achieve this goal have been suggested:

- As fresh manure presents a high risk for subsequent contamination, the grazing of animals on crop waste or stubble shortly before re-planting could be restricted.
- Good standards of waste management are important to avoid attracting wild animals to human settlements and to prevent wild populations being augmented and artificially sustained by human-induced food availability. Each stage of waste handling needs to be considered, from collection to transportation to disposal of carcasses (Tarsitano, 2006).
- Dissuasive feeding in forests to bait animals for easier removal and to distract them from agricultural fields.
- Physical barriers such as fencing may reduce crop damage by wildlife, however, this method can be counterproductive since it may promote high densities and aggregation of other species. Burrowing animals, for instance, breach the barrier and permit access to other species.
- Distress machines and substances, such as those emitting noise or calls (predator calls) in a large variety of formats, are available. For example, sonic fences harass the starlings when they land in a crop and cause them to fly away. Although they may land again, the cycle continues causing the starlings to use up valuable energy for no return. Ultrasonic (very high frequency) rodent repellers make rats and mice leave the immediate area completely and find a new residence elsewhere.
- Restoration of previous ecological situations, such as the re-introducing of predators that formerly inhabited the area (White and Garrott, 2005). However, this generates social conflicts that can easily outbalance the advocated benefits of this management decision.
- Recently, wildlife contraception has been considered, but there is still little information on the reliability of this method under field conditions (Ramsey et al., 2006).
- Buffers and areas cleared of natural vegetation may be used. There exists concern and controversy about the effectiveness and effects of this approach on wildlife conservation. For example, many rodents will not travel more than 50 m from their nest and living place. However, other animals, such as birds and larger animals, may travel many kilometres in

search of food.

Several lethal methods to reduce wildlife pest populations are available. All have both benefits and limitations. Increased hunting or hunting the target species can reduce wildlife numbers and their risks. Trapping pests is usually ineffectual and inefficient. Care during handling captured animals is needed in order to avoid zoonotic disease risks for personnel. Poisoning may not be very selective. Bait may not be effective in the presence of large amounts of available produce. Poisoning will always present a danger to livestock and humans.

Farmers should take a hazard analysis control approach and be aware of the hazards for their crops. They should be aware of indigenous wildlife and their behaviour in the growing area to know when mitigation might be most important. Region-specific information concerning microbiological hazards present in wildlife in the region, appropriate pest control strategies and environmental regulations should be available. Further information on Education and Training is provided in Section 9.

The meeting noted recent research indicating that leaf age influences bacterial colonization and population levels on lettuce, and that young lettuce leaves may be associated with a greater risk of contamination, thus indicating that control measures are also important when the crop is young or when baby leaves are being grown (Brandl and Amundson, 2008).

3.2 Topography and climate

3.2.1 Problem scope

Climate, weather, topology, hydrology and other geographical characteristics of the growing site may influence the magnitude and frequency of transfer of pathogenic microorganisms from environmental sources to the crop.

3.2.2 Potential impact

Topology

Growing sites that are located downstream from heavily industrialized or populated areas are more prone to potential contamination; therefore, run-off water from potentially contaminated sites must be a major consideration when examining the risks of growing leafy vegetables and herbs in these areas. Wind drift carrying contaminated dust is an important consideration. Farms located in valleys must consider potential contamination from higher elevations and distant sources (e.g. contaminated run-off water or dust circulation and drift).

Weather

Weather and particularly changes in expected weather patterns can be the reason for transfer of microbial contaminants to leafy vegetables and herbs. Dry periods can cause dust storms that settle dust particles on leafy vegetables. The correlation between dust as a carrier of microorganisms and the spread of contaminants has been demonstrated (Davies and Wray, 1996; Varma et al., 2003). Also the spread of contaminants through aerosols is well documented (Baertsch et al., 2007).

Increased temperatures can increase the rate of microbial growth. It may also influence the population of insects and pests found in and around farms that transfer human pathogens to leafy vegetables. Increased UV light in contrast may be responsible for the decrease of potential human pathogens in soil and on leaves (Zaafrane et al., 2004; Tannock and Smith, 1972). Relative humidity (RH) has been shown to have an effect on survival of human pathogens on

plant surfaces (Dreux et al., 2007).

Changes in weather (temperature, humidity, etc.) may also affect the growth and physiological conditions of leafy vegetables and thus their susceptibility to pathogen contamination.

Hydrology

A rising water table may act as a carrier for potential human pathogens, especially in areas of high population density. Low-lying areas are also important, as stagnant water may contribute to potential microbial contamination in leafy vegetables and herbs.

3.2.3 Available data

The concept that dust is a carrier of microbial contamination is well documented, although there is no direct scientific evidence that dust is responsible for foodborne outbreaks in the leafy vegetables and herb industries. The potential for survival of *Salmonella* in dust has been reported to be 26 months (Davies and Wray, 1996) and the survival of *E. coli* to be 10 months (Varma et al., 2003). The potential for dust to travel long distances (up to thousands of km) (Griffin et al., 2001) and the spread of aerosols by wind has been documented (Baertsch et al., 2007).

Human pathogens may travel by water over long distances. For example, in New Zealand, *Giardia* has been found in remote areas, transported downstream in rivers and creeks (Brown et al., 1992). Urban wastewater that runs off into streams influences stream water quality, and in peri-urban agriculture this increases the risk of contamination spread. Although urban and peri-urban agriculture has a positive impact on food supply and livelihoods, it has been shown to pose health risks for farmers and consumers (Keraita, Dreschel and Amoah, 2003).

Higher than expected temperatures may increase the growth of human bacterial pathogens that grow optimally at around 37°C (in environments capable of supporting growth). Increased temperatures may also influence the population of insects and pests associated with plant disease, permitting more severe infection (Epstein, 1995; FAO, 2005). It may also increase microbial growth on leafy vegetables and herbs post-harvest if the cold chain is not adequate. It is also known that high temperature affects the profile of human diseases, e.g. increases in disease notifications, particularly salmonellosis (D'Souza et al., 2004) and to a lesser extent campylobacteriosis (Kovats et al., 2005) are frequently preceded by weeks of elevated ambient temperature. However, the relationship between the increased risk of high microbial loading on leafy vegetables and herbs and increases in the incidence of foodborne disease is not well documented.

Increased UV light, in contrast, may be responsible for a decrease of potential human pathogens in soil and on leaves (Zaafrane et al., 2004., Tannock and Smith, 1972). Furthermore, low relative humidity (RH) has been proposed as one of the main factors limiting survival of bacteria on plant surfaces For instance, *Salmonella* Typhimurium populations decline rapidly under low RH on cilantro, bean and maize plants, whereas they are able to grow under humid conditions on cilantro leaves (O'Brien and Lindow, 1989; Brandl and Mandrell, 2002). Epiphytic bacterial populations usually decrease after prolonged periods of dry weather, but increase following rain and irrigation (Hirano and Upper, 2000). Under low RH and high inoculum, the rate of decline of *L. monocytogenes* populations on parsley leaves decreased after a few days (Dreux et al., 2007). This may be explained either by the settlement of *L. monocytogenes* in rare and more protected sites on the leaf surface, as suggested for *Pseudomonas syringae* (Wilson and Lindow, 1994) and/or by the presence in the inoculum of a

small proportion of cells better adapted to the stress conditions encountered on parsley leaves. Under non-saturated RH, *L. monocytogenes* declined on parsley leaves, but a clear relationship was not observed between RH and the rate of decline of *L. monocytogenes*.

3.2.4 Uncertainty and data gaps

Although there is circumstantial evidence that climate, topology, weather, hydrology and geographical features may contribute to an increase in microbial contamination in the leafy vegetable and herb industries, there is little direct evidence, and more research needs to be conducted in all of these areas.

Another area of uncertainty is the impact of climate and weather on wildlife populations, pathogen survival in soil, and insect populations that indirectly interact.

3.2.5 Mitigation recommendations

Climate, topology, weather, hydrology and geographical features can contribute to an increased risk of microbiological contamination of leafy vegetables and herbs; therefore a risk assessment should be conducted prior to farm establishment and planting.

Based on the available publications, a more precise recommendation cannot be made.

3.3 Flooding

3.3.1 Problem scope

Fields used for the growing leafy vegetables and herbs may periodically be subject to flooding from natural rainfall or from an accidental breakdown in the water storage site.

3.3.2 Potential impact

Flooding can affect the microbial contamination of leafy vegetables and herbs through the spread of faecal waste onto the growing area, or through contaminated soil and water. Because of compaction, flooding after drought can result in more severe run-off and can increase the risk of microbial contamination. Alternating periods of floods and drought can therefore aggravate the problem.

3.3.3 Available data

Excessive precipitation or flooding is often a factor triggering waterborne disease outbreaks. Curriero et al. (2001) noted that over half of all waterborne outbreaks occurring in the USA over the past 50 years were preceded by a period of excessive rainfall. These waterborne outbreaks were caused by bacteria (*E. coli* O157:H7) and parasitic protozoa (*Cryptosporidium* and *Giardia*). Furthermore, excess water taxes existing wells, septic systems and water and sewage treatment facilities, which are designed to operate within certain specifications for temperature, precipitation, etc. When water levels exceed these specifications, pathogens can enter the system with relative ease and the ability to remove or inactivate them by standard methods may be compromised (Charron et al., 2004). Water is critical at both pre-harvest and post-harvest stages (it is used for irrigation, application of chemicals, washing machinery, washing produce, etc.) and is essential for maintaining personal cleanliness, therefore, contaminated water may serve as a source of microbes entering the food supply (this is covered in more detail in Section 5).

Faecal contamination of agricultural soils has been shown to increase after environmental

flooding (Casteel, Sobsey and Mueller, 2006). For example, Hurricane Floyd and other storms in 1999 caused widespread and extensive flooding of eastern North Carolina (USA), with consequent environmental contamination with faecal wastes from municipal wastewater and livestock operations. Soil analysis revealed that a number of samples were positive for micro-organisms indicative of the presence of human or animal faeces, i.e. faecal coliforms, *E. coli* and coliphages. Furthermore, most samples were positive for total coliforms, and almost all samples contained high levels of *C. perfringens* spores. The levels of *C. perfringens* spores were significantly ($P<0.001$) higher in flooded soil (post-Hurricane Floyd) compared to pre-flood soil (Casteel, Sobsey and Mueller, 2006).

3.3.4 Uncertainty and data gaps

Further studies on the direct impact of flooding on the microbiological safety of leafy vegetables and herbs are required.

3.3.5 Mitigation recommendations

Additional or alternative measures may need to be considered to ensure (i) wells, septic systems and water and sewage treatment systems are capable of operating safely and effectively during periods of excessive rainfall; and (ii) crop growing areas are protected from faecal contamination.

Preparedness is essential, in particular the establishment of response plans to deal with the adverse effects of accidental or natural flooding. Consideration should be given to the whole food chain. When growing fields have been contaminated or damaged, assessments should be carried out to establish measures to reduce the risk of pathogens (e.g. delayed harvesting, heat treatment of produce) or to assure disposal (FAO, 2005; WHO, 2005). Proper disposal of food stocks found to be unfit for human consumption may need to be undertaken under the supervision of appropriate authorities (FAO, 2008).

3.4 Seeds and crop selection

3.4.1 Problem scope

Farmers may move to growing non-traditional leafy vegetable and herb crops to meet demands arising from new export markets (opened up with the increasing global trade) and customer demand for diversity and year-round supply.

Farmers may obtain seeds and seedlings for each new crop either from their own farm or from an external source. Seeds and seedlings, if contaminated, may be a means by which hazards can be introduced to the growing area for leafy vegetables and herbs.

3.4.2 Potential impact

New crops may be the source of microbial contamination if grown in new areas of production. This contamination may be due to different practices employed by farmers during the production chain or to different climatic condition (e.g. more heat and more water), which may allow the persistence of some contaminants on the leaves. However, the expert group could find no available data on changes in the risks of microbial contamination arising from the introduction of plants to new areas.

Contamination of seeds and seedlings can result in the presence of pathogens associated with plants. However, the probability of seed contamination is considered to be minimal when

produced under normal controlled conditions.

3.4.3 Available data

Sprouted seeds for human consumption have been implicated in foodborne illness and as a result much research has been undertaken to understand the role of the seed in the pathogen pathway during sprout production. However, caution is required in extrapolating from research undertaken on seeds used for sprouting, as the process is very different. Although research on decontamination processes for the elimination of *E. coli* from salad vegetables and herbs suggests that seeds are carriers of microbial contaminants, no evidence has been identified in the literature of internalization from seed to harvest in the leafy vegetables and herb industry. Just how these contaminants are retained during plant growth and carried through to harvest is not clear (Warriner et al., 2005).

3.4.4 Uncertainty and data gaps

No data to support the transfer of any seed contaminants through the growing phase of the plant was identified. No available data has been found on changes in the risks of microbial contamination arising from the introduction of plants to new areas.

3.4.5 Mitigation recommendations

When farmers change to non-traditional crops, a proper assessment of potential risk factors (climate, wildlife, topology, pathogen presence) is required to be undertaken in establishing the safety programme. Farming practices aimed at reducing microbial contamination of the new plant may need to be introduced and explained to workers in growing areas.

Due to lack of scientific information on the transfer of any seed contaminants through the growing phase of the plant, no recommendations are made.

3.5 Prior land use and assessment

3.5.1 Problem scope

Periodically farmers may change the use of their fields and new growing areas may be established. The prior use of the land is important as it may pose a risk of contamination for leafy vegetables and herbs subsequently introduced.

A recorded history of the land may be available. In addition, microbiological analysis of environmental samples could be considered a useful tool for assessment of safety. If the latter is used, prior consideration should be given to the sample site, sampling technique, sampling plan, interpretation of results and action to be taken in the event of unsatisfactory results.

3.5.2 Potential impact

The prior use of land to be used for cultivation of leafy vegetables and herbs is important. This is particularly important if the land was: (i) used to cultivate a different crop; (ii) supplemented with soil amendments; (iii) irrigated in an manner inappropriate for leafy vegetables and herbs that will not be cooked; or (iv) used for livestock production, as a wildlife habitat or for land fill for urban or industrial waste. All of these activities have the potential to contaminate the environment with foodborne hazards, which may subsequently be transmitted to plants.

If a pathogen is detected in an environmental sample, the exact species of pathogen or microbial contaminant, the sampling plan, the methodology used for analysis and its ecological

characteristics are important in making risk-based decisions on its significance. The level of pathogen or microbial contamination is also an important consideration, as is the distance of the environmental sample from the growing area, together with any potential means of transmission to plants. Contamination can be spread in a number of ways, e.g. through water, soil, dust, farm workers, farm vehicles, equipment, farm animals and other vectors. Contamination due to flooding from a nearby contaminated environmental source was thought to be the most important way for contamination to occur.

3.5.3 Available data

Pathogenic bacteria have the ability to survive for extended periods of time in manure and manured soil; this is addressed in Section 4 of this report. For example, studies on the persistence of enterohaemorrhagic *E. coli* O157:H7 in soil treated with contaminated manure composts or irrigation water found that *E. coli* O157:H7 persisted for >5 months after application of contaminated compost or irrigation water (Islam et al., 2004). Very little difference was observed in *E. coli* O157:H7 persistence based on compost type alone (i.e. contaminated poultry or bovine manure composts). The persistence of *E. coli* O157:H7 on leafy vegetables following cultivation on soils amended with contaminated composts has also been shown. This pathogen was detected on lettuce and parsley for up to 77 and 177 days, respectively, after seedlings were planted (Islam et al., 2004).

Foodborne viruses show varying resistance to different environmental stresses such as acid, heat, drying, pressure, disinfectants and ultraviolet radiation; they are generally tough-natured and survive well in the environment (FAO/WHO, 2008b). For example, in dried faeces, hepatitis A virus remained infectious for 30 days when stored at 25°C and 42% RH (Hollinger and Ticehurst, 1996). The persistence of Poliovirus 1 in soil irrigated with inoculated sewage sludge and effluent has also been shown experimentally (Tierney, Sullivan and Larkin, 1977). The longest period of survival of this virus in soil was 96 days during the winter and 11 days during the summer. Furthermore, the virus was recovered 23 days after irrigation from mature vegetables (lettuce and radishes).

3.5.4 Uncertainty and data gaps

No specific data on the impact of prior land use on the microbiological safety of leafy vegetables and herbs was found in the literature. No data was found on the recommended minimum distance between the contaminant and the growing area or on recommended levels of pathogen or microbial contamination allowed in the proximity of the farming area. Further research is essential in this area.

3.5.5 Mitigation recommendations

While cross-contamination of farm land with potentially contaminated nearby environmental sources should be avoided, the lack of data prevented the meeting from making any more specific recommendations.

4. Soil amendments and fertilizers

4.1 Problem scope

Organic fertilizers that might be contaminated with bacterial, protozoan and viral pathogens may promote the survival or proliferation of pathogens in the environment and on crops.

4.2 Potential impact

In many parts of the world organic wastes play an important role in providing nutrients to crops and improving overall soil quality. Furthermore, the use of organic wastes in agricultural applications is considered an ecologically important means of managing animal, human and plant wastes. Pathogens may survive for extended periods and may subsequently become associated with leafy vegetables and herbs grown in these soils. The manure type, method of application, application rate, frequency of application and time period between application and planting or harvesting may influence the associated risk of pathogen transfer from manure-amended soil to leafy vegetable and herb crops. In many vegetable production systems it is common practice to plough under crop waste left after harvest or from other sources.

4.3 Available data

The potential contamination of vegetables grown in soils enriched with contaminated manure will largely depend on the survival capabilities of the pathogen in manure and manure-amended soils.

4.3.1 Survival in manure

Pathogenic enteric bacteria like *E. coli* O157:H7 and *Salmonella* have the ability to persist for extended periods in manure, with survival times ranging from several weeks to several months, and even up to nearly 2 years (Jiang, Morgan and Doyle, 2002; Franz et al., 2005; Kudva, Blanch and Hovde, 1998). *Salmonella* generally survives longer than *E. coli* O157:H7. Survival in manure is generally shortened with higher pH (Park and Diez-Gonzalez, 2003; Franz et al., 2005), higher fibre content (Franz et al., 2005), higher temperature (Kudva, Blanch and Hovde, 1998; Himathongkham et al., 1999; Semenov et al., 2007), larger temperature fluctuations (Semenov et al., 2007), higher levels of native coliforms (Franz et al., 2007a) and higher levels of aeration (Kudva, Blanch and Hovde, 1998; Heinonen-Tanski, 1998; Shepherd et al., 2007; Semenov et al., 2008). Survival of *E. coli* O157:H7 and *Salmonella* Typhimurium in manure was significantly reduced when cattle were fed a low-energy high-fibre diet (straw), compared to a high-energy low-fibre diet (grass-maize silage) (Franz et al., 2005). Survival of *E. coli* O157:H7 and *S.* Typhimurium were considerably longer in dairy slurry compared to solid dairy farmyard manure (Hutchison et al., 2005; Nicholson, Groves and Chambers, 2005).

4.3.2 Survival in manure-amended soil

Enteric microorganisms that are pathogenic to humans can survive for extended periods in manure-amended soils. Reported survival times of *E. coli* O157:H7, *E. coli* O26, *Salmonella*, *Listeria*, *Campylobacter* and *Cryptosporidium* are up to 6 months, 3 years, 2 years, 20 days and 3 months respectively (Islam et al., 2004; Nicholson, Groves and Chambers, 2005; Fremaux et al., 2008). Survival in manure-amended soil is generally reduced by higher temperatures

(Fremaux et al., 2008), higher levels of native microflora (Jiang, Morgan and Doyle, 2002; Semenov et al., 2007), lower levels of easily available nutrients (Franz et al., 2007b; Habteselassie et al., 2007), increased levels of microbial diversity (van Elsas et al., 2007) and lower clay content (Mubiru, Coyne and Grove, 2000; Fenlon et al., 2000; Franz et al., 2007b). *L. monocytogenes* was shown to grow in manure-amended soil when the inoculum density was low, and reached higher levels in soils amended with solid chicken manure than in soils amended with either liquid hog manure or inorganic fertilizer (Dowe et al., 1997). High manure-to-soil ratios resulted in reduced survival of *E. coli* O157:H7, probably due to increased microbial activity (Jiang, Morgan and Doyle, 2002). Subsurface injection of organic wastes into soil was reported to reduce the risk of pathogen persistence, as compared to surface application (Avery et al., 2004). Preliminary results suggest that the risks of leafy vegetable contamination from the aerosolized spread of bacteria during slurry spreading by "rain gun" are low (Hutchison, Avery and Monaghan, 2008).

4.3.3 Survival of viruses

Human noroviruses are the predominant cause of foodborne gastroenteritis worldwide. Human norovirus sequences have been isolated from cattle and swine, indicating a possible route for leafy vegetable contamination via manure (Mattison et al., 2007). Little information is available on the fate of viruses in animal waste and manure-amended soils. The available literature suggests that enteric viruses can be even more persistent in manure and manure-amended soil than bacterial pathogens (Gessel, 2004), findings that are similar to what has been observed with human biosolids (see below). Manure application rate was positively correlated with the persistence of coliphages, but they did not relate to the survival of indicator organisms. Survival of viruses in semi-liquid cattle manure ranges from 1 week for herpesvirus to more than 6 months for rotavirus (Pesaro, Sorg and Metzler, 1995).

4.3.4 Human biosolids

Biosolids generated from the treatment of human wastes is a major environmental challenge throughout the world. One of the focal areas for addressing this problem has been to encourage the use of the waste as fertilizers and soil amendments for agricultural uses, e.g. for the production of foods, the enhancement of pastureland and the fertilization of parklands. The degree to which human biosolids can be used in agriculture applications and the degree of treatment that is required before such uses are permitted varies substantially from region to region. For example, within the United Kingdom the cropping intervals between the application of human biosolids and the planting of an edible crop is dependent on the class of crop being planted and the types of treatment that the human sludge has received (ADAS, 2001). This United Kingdom guidance recommends that after application of conventionally treated sludge, a 12-month crop interval is required for vegetables that are to be cooked and a 30-month interval is required for ready-to-eat salad vegetables such as lettuce. For sludge treated with enhanced interventions that ensure inactivation of bacterial pathogens, crop intervals can be reduced to 10 months for both classes of vegetables. The scientific basis for these values is unclear. Gale (2005) used linear inactivation rates in combination with risk assessment methodologies to compare the relative risk among foodborne pathogens after 12- and 30-month cropping intervals. The evaluation predicted that pathogenic protozoa (i.e. *Cryptosporidium parvum* and *Giardia*) would represent the greatest risk with the 12-month cropping interval. The study did not consider pathogenic nematodes nor the more environmentally resistant human viruses.

Only a limited number of studies have examined the relative contribution of factors affecting the inactivation of enteric bacteria in human biosolids. Factors affecting the inactivation of

generic *E. coli* in human-sludge-amended soil have been studied by Lang, Bellett-Travers and Smith (2007). They considered both conventional (de-watered, mesophilic anaerobic digestion) and enhanced (thermally dried digested sludge and composted sludge with green waste) treatment sludges. Sludges treated with enhanced treatments did not add any *E. coli* to the environment but did affect the rate of inactivation of indigenous *E. coli*. Conventionally treated sludge added significant levels of *E. coli*. Linear regression analysis indicated that the relative contributions of factors affecting inactivation of the bacterium were (1) high for the type of treatment and time, (2) moderate for soil temperature and soil moisture (though some regrowth did occur if dry soils were wetted), and (3) low for season and the use of plastic covers. Wachtel, Whitehand and Mandrell (2002) found that the levels of *E. coli* in the soil and attached to the roots of cabbage were increased after inadvertent irrigation with partially treated sewage waste-water, but levels were not increased on the leaves.

There has been ongoing concern that the amendment of soil with composted sewage sludge could lead to increased levels of human pathogens in the soil used to cultivate fresh vegetables, particularly leafy vegetables and herbs. Salmonellae have been shown to grow in sterile composted sewage sludge, but they appear to be normally suppressed by competing micro-organisms in non-sterile compost (Hussong, Burge and Enriki, 1985). *Salmonella* will grow in desiccated human biosolids and biosolids-amended soil after rain events, but the source of the salmonellae appears to be largely due to recontamination from animals and other sources in the farm environment (Zaleski et al., 2005).

The use of human biosolids as a soil amendment has additional risks over animal manures due to the potential presence of specific human viruses (e.g. hepatitis Type A (HAV), norovirus (NV), protozoa (e.g. *Cyclospora cayatenensis*) and nematode and trematode eggs (e.g. *Ascaris sumi*, *Taenia saginata*). Furthermore, a specific concern with sewage-related contamination is that it can result in the food becoming contaminated with multiple viruses (FAO/WHO, 2008b).

In general, non-enveloped viruses (most foodborne viruses) are more resistant to stresses such as acidic environments, desiccation, antimicrobial treatments and heating than vegetative bacteria. For example, HAV is highly resistant to desiccation of faeces (McCaustland *et al.,* 1982) and is considered more thermally resistant than *E. coli*. It is now known that some commonly used methods for sewage treatment may not be sufficient to effectively remove or inactivate viruses of public health concern. Various studies in Europe, Japan and the USA showed that treated sewage was still positive for human enteric viruses (van de Berg et al., 2005; Villar et al., 2007; Laverick, Wyn-Jones and Carter, 2004; Silva et al., 2007; Gregory, Litaker and Noble, 2006; la Rosa et al., 2007; Myrmel et al., 2006; Ueki et al., 2005). Enhanced treatments, such as thermal treatments (>60°C), are required to eliminate thermostable viruses (Spillman et al., 1987).

There has been a number of researchers who have suggested that viruses such as male-specific (F+) coliphages would make a better indicator of the effectiveness of different composting and inactivation technologies due to its increased resistance to stresses such as heating (Mocé-Llivina et al., 2003; Nappier, Aitken and Sobsey, 2006). While this bacteriophage appears to be a good indicator for many enteroviruses and *Ascaris sumi* (Nappier, Aitken and Sobsey, 2006), it is not clear that it would be a good predictor of the behaviour of HAV or NV. HAV has been shown to survive conventional sewage treatment (Graff, Ticehurst and Flehmig, 1993).

4.3.5 Plant biowaste

Biowaste materials derived from plant materials are widely used as soil amendments for leafy vegetables, for both the fertilizer and water-holding properties, and as an effective means of disposing of waste materials. This can include material derived from a variety of plant sources, such as culled fruits and vegetables, green waste, rice hulls and paper or cardboard. The addition of green waste to animal manure and human sludge is one means for enhancing the composting of these biosolids (Lang, Bellett-Travers and Smith, 2007). Again, the effects of these soil amendments on the inactivation, survival or growth of human pathogens has only been studied to a limited degree. Lemunier et al. (2005) did find that *Listeria monocytogenes*, *E. coli*, and particularly *Salmonella* Enteritidis, could survive for extended periods in biowaste composts made of different combinations of fruits and vegetables, paper and cardboard, green waste and water. They also noted that *S.* Enteritidis and *E. coli* grew to high levels in one of the composts and then survived at those levels for 3 months.

4.3.6 Pathogen association with leafy vegetables grown in manure-amended soil

A major issue with respect to the microbiological risks associated with leafy vegetables and herbs grown in soil enriched with organic waste is the ability of pathogens present in the amended soil to colonize the plant. Several experimental studies have shown associations between human pathogens and the surface of leafy vegetables grown in manure-amended soil (Islam et al., 2004; Natvig et al., 2002). *E. coli* O157:H7 persisted on lettuce and parsley for 77 and 177 days, respectively, after seedlings were planted in manure-amended soil (Islam et al., 2004). However, these studies typically were conducted with relatively high inoculum densities. With a laboratory-simulated lettuce production chain, where *E. coli* O157:H7 and *S.* Typhimurium were allowed to decline to levels of 10^2/g in manure-amended soil, both pathogens could not be detected on lettuce plants grown in this substrate (Franz et al., 2005). Similarly, the transmission of *E. coli* O157:H7 from natural and experimentally inoculated manure-amended soil to lettuce could not be demonstrated (Johannessen et al., 2004, 2005). There is considerable debate on the possibility of pathogens being present internally in leafy vegetables. Internalization of *E. coli* O157:H7 and *Salmonella* into the leaf tissue was demonstrated in seedlings grown in soil amended with inoculated non-composted fresh manure (Solomon, Yaron and Matthews, 2002; Franz et al., 2007c; Klerks et al., 2007). However, the internal presence of pathogens was never demonstrated in mature leafy vegetables (Jablasone, Warriner and Griffiths, 2005; Girardin et al., 2007).

4.4 Uncertainty and data gaps

There is a large amount of uncertainty associated with the contamination or colonization of leafy vegetables and herbs in the field. Laboratory studies cannot be directly extrapolated to field situations for several reasons:

1. No scientific data so far has shown pathogen association with leafy vegetables under more realistic, naturally occurring, pathogen densities. Naturally occurring densities are usually substantially lower than those used in laboratory experiments, with an average of around 2.5 log cfu/g (Zhao et al., 1995; Fegan et al., 2004a, b; Omisakin et al., 2003; Ogden, MacRae and Strachan, 2004). However, high naturally occurring densities (>7 log cfu/g) of *E. coli* O157:H7 have been reported to occur (Fukushima and Seki, 2004; Robinson et al., 2005) and it should be recognized that the sporadic occurrence of these high densities probably reflects the risk of contaminated crops to a significant extent.

2. Pathogens are likely to be distributed extremely heterogeneously throughout the soil.
3. Climatic factors will probably influence pathogen survival and plant susceptibility. Knowledge is lacking on the threshold pathogen density needed for plant colonization, the likelihood of infection and the subsequent level of colonization up to harvest. In addition, more research is needed on the effect of the manure type used as soil amendment on pathogen survival and the effect of different application methods.

4.5 Mitigation recommendations

Composting

Composting, if done properly, can be a practical and efficient method to inactivate human pathogens in manure (Jiang, Morgan and Doyle, 2003; Shepherd et al., 2007; Ceustermans et al., 2007). Several countries have established regulatory requirements for composting. For example, the USDA National Organic Program prescribes that composting temperatures should reach between 55° and 70°C for 3–15 days (depending on composting system), with periodic heap turning (USDA, 2008). A second example is the United Kingdom regulations that prescribe that manure heaps should be composted using a validated method such as turning at least 3 times to achieve internal minimum temperature of 55°C for 3 consecutive days after each turning, and stored for at least 3 months prior to use (CFA, 2007). Manure with a C:N ratio ranging from 20:1 to 40:1 are particularly suited for composting (Shepherd et al., 2007). The regular and thorough turning of compost heaps, so that all of the material will be exposed to elevated temperatures, is of high importance since pathogens can survive up to months on the heap surface (Shepherd et al., 2007). In addition to the benefits of pathogen elimination, composting benefits the environment because manure nutrients are converted to more stable forms and are less likely to reach groundwater or move in surface run-off. Moreover, there is a large body of literature detailing the benefits of compost as a plant disease suppressant when used in cropping systems (Noble and Coventry, 2005).

Time (cropping) intervals

A sufficient time interval between applying manure and planting the leafy vegetable and herb crops can result in a sufficient decline of pathogens. For example, in the USA, the USDA organic certification programme limits the application and incorporation of manure into the soil to no later than 90 days before harvesting an edible product that does not come into contact with the soil, and to at least 120 days before harvesting an edible product that does come into contact with the soil (USDA, 2008).

Based on the scientific literature, it can be deduced that some manure types and manure handling strategies imply higher risks than others. The risks of prolonged pathogen survival are likely to be reduced in (dairy) manures characterized by high fibre content, high pH and high levels and diversity of background flora, and which are stored under conditions characterized by elevated temperatures, temperature fluctuations and sufficient aeration. These characteristics and conditions are more associated with solid farmyard manure as compared to liquid manure (slurry). The subsurface application of manure is likely to result in a faster pathogen decline. Although factors like ammonia gas, desiccation and microbial antagonism are suggested to contribute to pathogen decline in bio-waste, the combination of time and temperature is generally thought to be the most effective in reducing or eliminating pathogen loads.

In contrast to water and plant material, the use of generic *E. coli* as an indicator organism for pathogen presence is questionable for substrates like soil and manure, where *E. coli* has a natural niche. Human pathogenic bacteria were reported to survive longer during composting

treatments compared to indicator organisms such as *E. coli* (Lemunier et al., 2005; Wery et al., 2008). The recording of physical parameters like temperature (55–70°C) and moisture content (60–65%) might be suitable alternatives in order to check the potential success of the composting process with respect to pathogen elimination. With non-thermal inactivation, higher levels of native coliforms in manure were shown to be associated with shorter survival times of *E. coli* O157:H7 (Franz et al., 2007a) and can therefore be considered unsuitable as indicator organisms. The recording of physical (pH, temperature, oxygen) and chemical (fibre content, nutrient status) manure characteristics can be used as indicators in order to make an estimation of the relative risk of the manure considered for application. Although expensive and time consuming, the most reliable strategy to determine pathogen presence or absence is classical or molecular microbiological testing of samples.

5. Water

5.1 Problem scope

Waterborne diseases caused by viral, bacterial or parasitic pathogens occur in every region of the world. The use of water containing pathogens in the farm environment may result in contamination of the crop. The risks associated with water employed in the production of leafy vegetables and herbs that are eaten raw must be identified.

5.2 Potential impact

The production of leafy vegetables is water intensive. Production requirements are met by irrigation with water drawn directly from natural sources such as streams, rivers, lakes or ponds, or by their diversion through canals or irrigation ditches; with waters collected in catchment basins, including rainwater or runoff from urban or agricultural activity; with groundwater captured in wells; with reclaimed wastewater from sewage treatment plants; or with potable water sources. Water is also used in the application of farm chemicals to crops and for the cleaning of field equipment. The microbiological quality of water and the risk of crop contamination vary with water source and agronomic practice.

5.3 Available data

5.3.1 Human pathogens in water and their transfer to leafy vegetables

A range of microbiological hazards can be transmitted to humans through contact with or the ingestion of contaminated water. Excreta-related bacterial species (including *Salmonella enterica*, *E. coli* O157:H7, *Campylobacter* spp. and *Yersinia* spp.), intestinal helminths (*Ascaris lumbricoides*, *Trichuris trichuria*), amoebae (*Entamoeba coli*) and protozoa (*Giardia intestinalis*, *Cryptosporidium parvum*, *Toxoplasma gondii*, *Cyclospora cayetanensis*) are associated with recurrent outbreaks of disease in different parts of the world (WHO, 2006). Waterborne viral epidemics have not been confirmed to date, although some species of enteric viruses have been detected in natural waters sources (Deetz et al., 1984), raw or treated waste-water (Jothikumar et al., 1993) and groundwater collected in wells (Borchardt et al., 2003).

The role of contaminated water used in the production of vegetable crops as a vector for the transmission of these pathogens to humans is less clear. However, poor irrigation water quality indicated by elevated faecal coliform counts has long been known to correlate with the incidence of human pathogens in leafy vegetable crops (Norman and Kabler, 1953). Studies carried out in both the developed and developing world have provided convincing evidence that helminthic diseases caused by *Ascaris* and *Trichuris* and bacterial diseases such as cholera are endemic in populations that consume salad vegetables irrigated with raw or untreated sewage (Gunnerson, Shuval and Arlosoroff, 1984; Shuval, Yekutiel and Fattal, 1985). Irrigation water containing pathogens has been reported to be used in the production of leafy vegetables and herbs in some countries (Thurston-Enriquez et al., 2002). Epidemiological evidence from specific outbreaks also points to the role of irrigation water in the introduction of pathogens to the production environment. For example, contamination of iceberg lettuce in a large outbreak caused by *E. coli* O157 in Sweden was linked to the use of contaminated irrigation water drawn from a small stream (Soderstrom, Lindberg and Andersson, 2005). Furthermore, experimental

evidence confirms that water used for irrigation can transfer human pathogens to a variety of growing leafy vegetables and herbs (Song et al., 2006; Melloul et al., 2001; Solomon, Yaron and Matthews, 2002; Amoah, Dreschel and Abaidoo, 2005). For example, Okafo, Umoh and Galadima (2003) reported the detection of *Salmonella* and *Vibrio* on lettuce and amaranthus when the irrigation water was contaminated. Edmonds and Hawke (2004) detected *Campylobacter* in watercress grown in and harvested from contaminated water, while Prazak et al. (2002) found 8% (4/50) cabbages positive for *L. monocytogenes* after final irrigation in the field. Okafo, Umoh and Galadima (2003) also found that the contamination rate varied with the weather and season, e.g. isolation rates for a range of pathogens were higher in the dry than the wet seasons (due to increased irrigation with contaminated stream water in the dry season). The risks associated with the contamination of leafy vegetables and herbs with enteric pathogens in irrigation water were quantified in several risk assessment exercises (Petterson et al., 2001; Stine et al., 2005; Hamilton et al., 2006). These collectively provide strong evidence that water quality is an important risk factor in the production of these foods.

5.3.2 Pathogens in agricultural waters from various sources

Potable supplies or rainwater stored in closed containment systems are considered safe for the production of leafy vegetables and herbs provided they are delivered to crops through well maintained distribution systems. In contrast, the microbiological quality of waters derived from surface or subsurface sources is highly variable. The risk of contamination with pathogens generally increases according to the following ranking (Leifert et al., 2008):

1. Potable or rain water.
2. Groundwater collected in deep wells.
3. Groundwater collected in shallow wells, due to inadequate installation or improper maintenance.
4. Surface waters, particularly in proximity to animals, human habitation and their wastes.
5. Raw or inadequately treated wastewater.

Published surveys reveal variable levels of contamination with specific pathogens, including species that may be endemic in specific regions. In one study carried out in Central America, *Cryptosporidium* oocysts and *Giardia* cysts were routinely isolated in irrigation water derived from surface sources (Chaidez et al., 2005).

5.3.3 Pathogens in groundwater

The microbiological quality of groundwater generally improves with distance below surface. Deep well water is normally of good microbiological quality, although contamination with pathogens is occasionally reported even in confined or relatively impermeable rock aquifers assumed to be protected from pollution (Borchardt et al., 2007). Evidence derived from surveys carried out in disparate regions suggest that pathogens are more commonly found in shallow aquifers and wells (Marteau et al., 1998; van der Hoek et al., 2001; Borchardt et al., 2003; Shortt et al., 2003). The transport of microorganisms through groundwater is influenced by several hydrological factors. Both the extent and rate of travel of microorganisms are enhanced in highly porous aquifers. Seepage of concentrated sources of pathogens and direct contamination of wells in shallow porous aquifers has been demonstrated experimentally (Sinton, 1986).

5.3.4 Pathogens in surface waters

Agricultural waters may be drawn from streams, rivers, lakes, ponds or manmade reservoirs designed for catchment and storage. Animal faeces are the main source of pathogens in these water sources, and a range of environmental factors exert individual or collective effects on overall microbiological quality and the presence of pathogens. For example, birds can contribute high levels of enteric bacteria to closed water reservoirs (Converse et al., 1999) and grazing cattle have been shown to affect the quality of surface waters (Howard, Johnson and Ponce, 1983). Intense livestock production correlates with the presence of pathogens in adjacent aquifers (Johnson et al., 2003). Contamination may also occur indirectly through runoff from fields or farms (Janzen, Bodine and Luszcz, 1974). Leakage from defective septic systems, the discharge of raw sewage and inadequately treated wastewater from industrial activity can contribute variable levels of pathogens to surface waters. Menon (1985) isolated *Salmonella* from the effluents of meat and poultry plants, five of seven sewage treatment plants and in water samples collected along a Canadian river system.

The microbiological quality of surface waters is often difficult to predict due to seasonal and temporal differences in microbial load and profiles (Geldereich, 2006; Miller et al., 2007). *Campylobacter* were isolated at greater frequency in the autumn and winter months than in the spring and summer from ponds, lakes and small mountain streams in Washington State, USA (Carter et al., 1987). Weather effects are known to influence the quality of surface waters. According to one analysis, about 50% of waterborne outbreaks occur as a consequence of heavy rainfall (Curriero et al., 2001). Increases in *Cryptosporidium* oocysts and *Giardia* cysts following heavy rainfall events have been documented (Atherholt et al., 1988). Severe climactic events, notably floods, also seriously affect the quality of water. The persistence of Poliovirus 1 in soil flooded with contaminated water and its transfer to leafy vegetables during cultivation has been demonstrated experimentally (Tierney, Sullivan and Larkin, 1977).

Most surveys of agricultural water quality tend to focus on the aqueous phase. There is growing evidence that infectious viral (Lewis, Austin and Loutit, 1986), bacterial (Burton, Gunnison and Lanza, 1987) or parasitic (Amoah, Dreschel and Abaidoo, 2005) species, or enteric bacteria used as indicators of faecal contamination (Sherer et al., 1992), can survive and accumulate in sediments.

5.3.5 Pathogens in reclaimed wastewater

Reclaimed wastewater water is subjected to different levels of treatment and may contain variable levels of pathogenic microorganisms. WHO recently established guidelines for the use of wastewater in agricultural production (Carr, Blumenthal and Marra, 2004; WHO, 2006). Wastewater used for unrestricted crop irrigation purposes should contain $\leq 10^3$ thermotolerant coliforms/100 ml and ≤ 1 helminth egg per litre. The guidelines specifically address irrigation of vegetables that are consumed without cooking. A review of water quality guidelines for the production of leafy vegetables and herbs is recommended, however, due to the increasing implication of these foods in outbreaks of foodborne illness.

5.3.6 Fate of human pathogens: effect of timing of irrigation

The interval between final irrigation and harvest influences the extent of contamination, as pathogens have been shown to decline with time following cessation of irrigation before harvest. Keraita et al. (2007) and Amoah, Dreschel and Abaidoo (2005) showed that helminth populations on leafy vegetables declined daily following cessation of manual irrigation. Helminths and enteroviruses appear to survive for the longest periods of time after cessation of

irrigation (Feachem et al., 1983). There is currently insufficient data to provide effective guidelines that would apply to the range of leafy vegetables and herbs grown in the various regions of the world.

5.3.7 Influence of irrigation systems

The impact of different irrigation strategies (overhead sprays, drip irrigation systems or flooding of fields through furrows) on the incidence of pathogens in leafy vegetable and herb crops is not well understood. Enteric bacteria and viruses aerosolized in spray irrigation systems have been shown to travel considerable distances from source (Tetltsch and Katzenelson, 1978). The delivery of irrigation water through overhead systems can clearly result in extensive contamination of the production environment. However, pathogens deposited on plant surfaces are subject to lethal stresses that severely restrict survival, including intense ultraviolet (solar) radiation, wide temperature fluctuations, low relative humidity and low availability of free moisture (Tetltsch and Katzenelson, 1978; Beattie and Lindow, 1995; Brandl, 2006). Soil is a comparatively less hostile environment, although competition with native microorganisms can lead to rapid declines in pathogen populations (Jiang, Morgan and Doyle, 2002).

In one study, no differences were found in levels of *E. coli* or *Salmonella* on lettuce irrigated with wastewater using either drip or furrow irrigation systems (Bastos and Mara, 1995). Contradictory conclusions were reported in another study where the transfer of *E. coli* from contaminated water to lettuce occurred at a greater rate on plants irrigated by flooding of furrows than through a drip irrigation system (Song et al., 2006). Despite the lack of corroborating scientific evidence, there is general agreement that subsurface irrigation lowers the risk of transfer to growing plants (Hamilton et al., 2006). There is evidently a need for additional scientific information to address these issues, specifically the risks associated with overhead application compared with other forms of irrigation. In the absence of new scientifically validated irrigation strategies, current WHO and country guidelines should continue to be applied.

Passage of contaminated water through irrigation equipment can contaminate the system. Persistence of bacteria and viruses in irrigation pipes has been described (Tetltsch and Katzenelson, 1978) and bacterial pathogens are capable of growth in stagnant systems (Jerman, Spencer and Duran, 2003).

5.3.8 Contamination of farm chemicals and cleaning water

There is clear evidence that human pathogens can survive and grow in pesticide solutions and that their application to the surface of leafy vegetables constitutes a risk, particularly near harvest time (Guan et al., 2001; Iyumi et al., 2008). Ng, Fleet and Heard (2004) showed that *Pseudomonas*, *Salmonella* and *Escherichia coli* could survive and grow in two of ten commercial insecticide, herbicide and fungicide formulations used in the cultivation of vegetables. The transfer of pathogens from contaminated water to inert surfaces has been examined in detail in both the food processing and home environments (Mattick et al., 2003). In contrast, little is known about the surface-associated behaviours of waterborne pathogens in the agricultural environment. In the absence of clear experimental evidence to the contrary, it is prudent to consider that the transfer of pathogens between water and agricultural equipment is likely. Surfaces routinely rinsed or cleaned with water include tools and harvesting equipment, including mechanical harvesters.

5.4 Uncertainty and data gaps

Current guidelines for the microbiological quality of water used to irrigate crops meant to be eaten raw are based on the presence of coliform bacteria and *E. coli*. It remains unclear how these indices correlate with the presence of specific human pathogens. Measurements derived from methods applied for the detection of both bacterial groups will undoubtedly continue to be used in the absence of better indicators of water quality. There is an urgent need for the development of irrigation water quality indicators that will provide more realistic assessments of the risk of contamination with human pathogens. Inexpensive field deployable methods are highly desirable.

5.5 Mitigation strategies

Protection of surface water and groundwater resources from pollution (wildlife, waste from animal production, agricultural run-off, human activity, sewage, industrial effluent) is essential for the production of safe leafy vegetables and herbs. Open water distribution systems, including ditches or irrigation canals, are similarly prone to contamination with human pathogens. Appropriate management measures (e.g. restriction of livestock feeding, watering and grazing, location of water uptake in relation to potential sources of contamination) are required to ensure the quality of irrigation water. Additional protection of water sources from seepage, by the lining of canals, for example, may be warranted where water supplies are delivered in peri-urban or mixed agricultural areas. Other options have been considered to improve microbial quality of surface waters, such as sand filtration or storage in catchments or reservoirs to achieve partial biological treatment before use (Carr, Blumenthal and Marra, 2004). The efficacy of these treatments will have to be validated for the range of pathogens likely to contaminate surface waters. Irrigation water that meets microbiological standards established for drinking water provides the best margin of safety for the production of leafy vegetables and herbs.

The use of reclaimed water is either not permitted for the production of leafy vegetables and herbs in some jurisdictions, or is subject to strict microbiological guidelines. Where use of wastewater is imperative or guidelines are lacking, health protection measures described in detail in Chapter 2 of the WHO guidelines (WHO, 2006) should be followed to reduce the risk associated with the use of wastewater for crop irrigation. Wastewater treatments in combination with other measures must meet a health-based target based on tolerable burden of disease from wastewater use. Additional measures—including crop restriction (the diversion of poor quality water to non-vegetable crops), improved irrigation methods, cessation of irrigation before harvesting, control of human exposure, and safe food preparation techniques—are needed to achieve overall pathogen reductions of 6–7 log units.

Further mitigation measures have been proposed to ensure the quality of irrigation water in the absence of reliable supplies. One issue that could be considered closely is the advantage of taking or fetching water without disturbing sediments, as human pathogens can settle and survive in large numbers in solids (Hendricks, 1971; Burton, Gunnison and Lanza, 1987). This practice has the potential to improve the quality of water used on the crop and reduce exposure of farm workers to pathogens (Ensink et al., 2006; Keraita, Dreschel and Konradsen, 2008).

Manmade distribution systems (engineered systems including pipes, pumps, etc.) and wells are subject to mechanical failure and ageing. Regular maintenance and inspection schedules are necessary to ensure the integrity of irrigation systems and wells to prevent contamination with pathogens, their accumulation or the proliferation of species capable of growth in natural

environments.

Agricultural chemicals applied to food crops need to be prepared with clean and preferably potable water. Chemical solutions requiring storage can be kept under cool conditions between applications to avoid the risk of pathogen growth. Water used to clean farm machinery, harvesting and transportation equipment, including containers and implements, should be of good microbiological quality.

Requirements for testing of irrigation waters and microbiological standards applied to the production of vegetable crops vary widely between jurisdictions, and vary for groundwater, surface water or reclaimed wastewater. Sampling strategies for testing are also inconsistent. Densities of infectious agents in natural waterways are subject to changes in climate. Seasonally adjusted testing schemes may be necessary in some regions to ensure the consistent quality of irrigation water supplies. The rate of testing could be enhanced when there is a change in the source of irrigation water or following severe climate events, such as heavy rains or flooding. Water at higher risk of change in microbiological quality due to proximity to animal production, potential sources of agricultural run-off or human habitation could also be tested more frequently (Doran and Linn, 1979).

6. Harvest, field packing and packinghouses

Leafy vegetables and herbs are harvested with the roots intact or by cutting leaves from their roots in the field. Packing with or without minor processing may occur in the field after harvest. Packing may also occur in packinghouses. This section addresses the activities that occur in the field, namely harvest and field packing (e.g. cutting from the plant, removing outer or damaged leaves, field coring, crating, maintaining hydration of product, cooling) and those that occur in packinghouses.

6.1 Problem scope

During harvesting and minor manipulation (e.g. removal of outer leaves and coring) or direct packing in the growing field or packinghouse, leafy vegetables and herbs may be at risk of the introduction of microbial contamination.

A key characteristic of these operations is that they involve considerable contact between fresh produce and workers (handler), between fresh produce and different types of tools and equipment surfaces, between fresh produce and water or ice, and between fresh produce and the field environment (soil, dust, insects, etc.).

6.2 Potential impact

Leafy vegetables and herbs may be harvested by hand or harvested mechanically. The harvested leaves or plants may be hand sorted, they may undergo some minor processing such as removal of outer wrap leaves or coring, washing or spraying, and they may be placed in bulk containers or packed ready for dispatch in boxes or covered with plastic film. These processes involve many points of contact with people, surfaces, water and the environment (soil, dust) and represent potential opportunities for contamination with foodborne pathogens.

Packing may take place in the open field or in a designated packinghouse. The location of the packing station or packinghouse (which may be no more than a shed with a roof and open or limited walls) in or very near the growing fields may result in exposure to dust and wildlife carrying food safety hazards. Other operations in the packinghouse include sorting, trimming, washing and drying, grading, packing, pre-cooling and storage. The sources of microbiological contamination in the packinghouse include the raw material, personnel, handling tools and equipment, packinghouse physical environment and water or ice. There is no physical evidence (including appearance) to indicate when fresh produce has been contaminated with pathogenic microorganisms. Scientific laboratory tests would be required to detect the presence of such potential hazards. The efficacy of testing is not clear as the probability of detecting contamination is limited by the number of samples that can reasonably be tested (ICMSF, 1986). Furthermore, tests represent additional cost to the packinghouse operation, which is often beyond the reach of many small and medium-scale operators, especially in developing countries.

6.3 Harvest

6.3.1 Available data

Harvesting is an important step that influences the safety and quality of leafy vegetables and herbs. It links field production to post-harvest life and senescence.

Mukherjee et al. *(*2004) isolated *Salmonella* from one of four organic lettuces but not from 49 conventionally grown lettuce that were sampled in the field. At the time of harvest, Johnston et al. *(*2006) did not detect *Salmonella*, *Shigella* or *E. coli* O157:H7 in 175 samples of various leafy vegetables and herbs from the USA and Mexico. Johnston et al. *(*2006) detected *L. monocytogenes* in 7% of 43 cabbages entering packinghouses, while Prazak et al. *(*2002) found 4.8% (6/125) positive on arrival at the packinghouse. The microbiological load can vary also between batches from the same producer (Johnston et al., 2006).

At the time of harvest, the presence of pathogens on produce appears to depend on the risk of exposure during growing (from soil, water, etc.). When leafy produce is harvested it may be cut from the roots (e.g. basil), or it may have the roots intact (e.g. coriander). The amount of soil contamination at harvest would be expected to be greater for the latter. Produce cultured in hydroponic or soilless systems may also have the roots intact but would be contaminated by water rather than soil. Issues surrounding contamination from soil are addressed in Section 4.0 (Soil Amendments and Fertilizers). Other risk factors are discussed in Sections 3.0 (Production environment of leafy vegetables and herbs) and 5.0 (Water).

Leafy vegetables and herbs can be harvested by hand or mechanically. The amount of handling varies with the species, e.g. cabbages versus delicate leafy herbs. The delicate nature of leafy vegetables and herbs increases their susceptibility to damage and bruising. Mechanical injuries to plant tissues can create openings to internal surfaces that are conducive to microbial contamination and growth. Attachment of bacteria and the potential for infiltration, internalization or both (hereafter referred to as internalization) has been demonstrated experimentally (Takeuchi et al., 2000; Takeuchi, Hassan and Frank, 2001; Saggers et al., 2008). This is discussed further in Section 7.0 (Processing). While much of this knowledge is from experimental studies, it indicates that damage should be minimized, especially in the field environment where exposure to soil and dust is possible.

Direct hand contact is used to trim extraneous matter or defects, sort, tie, transfer or pack product. People in the harvest area, either harvesters or visitors (e.g. children, contractors), who have contact with produce, equipment or the environment may be a source of contamination. The most common aetiological agents causing outbreaks due to leafy vegetables and herbs are enteric or systemic pathogens (Table A2.1 in Annex 2). Infected persons may transmit these pathogens via the faecal–oral route (WHO, 2008). Excretion can occur during the incubation period of infection (e.g. hepatitis A), during the infection (e.g. salmonellosis, shigellosis, viral and parasitic infections) or during convalescence (e.g. salmonellosis). Some individuals may become long-term carriers of a pathogen following convalescence and continue intermittent excretion for months or years (e.g. typhoid and non-typhoid salmonellosis). Thus field workers harvesting produce may be unaware that they are infected. If they are aware, they may not be sufficiently ill to stop working or they may be dependent on their work for income and reluctant to cease. Contamination of produce may occur while handling the produce if the worker has poor hygiene practices and/or lack of access to adequate sanitation and washing facilities to practice good hygiene.

Disease epidemiology varies in different regions of the world. The epidemiology of these foodborne pathogens will be influenced by factors such as sanitation, hygiene standards and the presence of a specific vector if required in the infection life cycle. In areas of poor sanitation and hygiene, pathogens with a predominant or essential human host are dominant e.g. shigellosis, typhoid, and certain viral and parasitic infections. Infection rates of some diseases may be higher in specific age groups, e.g. in endemic areas, hepatitis A infection rates are higher in young children who are asymptomatic (Bell, 2002), and these groups will present a

greater risk if present in the harvest environment.

Poor hygiene practices of food handlers has been suspected as the route of contamination in investigations of foodborne outbreaks associated with leafy vegetables and herbs (Harris et al., 2003; De Roever, 1998) and other fresh produce. For example, an infected food handler shredding lettuce by hand was implicated in an outbreak of hepatitis A (cited by Harris et al., 2003) and *Vibrio cholerae* contamination of sliced fruit has been linked to poor food handler hygiene practices and an asymptomatic carrier (Ackers et al., 1997). While there was no outbreaks identified confirming contamination by a field worker, epidemiological evidence has been used to suggest this occurs (Harris et al., 2003). Wachtel et al. (2003) showed that lettuce leaves could be easily contaminated via contaminated hands.

Mechanical harvesting equipment may also be a source of contamination. Contamination of machinery can occur during the harvest of low-lying produce (e.g. baby spinach) as both soil and plant material can by sucked into the machinery. Stafford et al. (2002) found in an outbreak investigation that *Salmonella* was able to persist on shredders used for the processing of fresh-cut lettuce. Lack of adequate cleaning resulted in the contamination of successive lettuce batches processed. It is therefore feasible that harvest equipment causing damage or in contact with damaged leaves may be a source of contamination if not adequately cleaned.

Similarly equipment for field trimming and coring may be a source of microbial contamination and microbial transfer among sequential units. The cut surface of the plant is especially vulnerable for pathogen contamination and growth, and pathogens attached to cut surfaces are extremely difficult to remove by subsequent sanitation procedures (see Section 7.0 Processing). Field coring involves removal of the cores and dirty or damaged wrapper leaves of lettuce during the harvesting process, followed by spraying with a solution that may contain disinfectants or anti-browning agents (Brown and Rizzo, 1999; Izumi et al., 2005; Suslow et al., 2003). The harvested lettuce is then transported to the cooling facility and vacuum cooled to below 5°C, usually within a short time post-cutting. However, cooling can be delayed due to the distance from field to cooling facility or if the load of the produce needing to be cooled exceeds the capacity of the cooling equipment. Although field coring and removing wrapper leaves may reduce the microbial populations, as outer or damaged leaves may harbour microorganisms, this field practice may potentially increase food safety risks for a number of reasons: (i) removing cores in the field creates openings in the produce, which exposes internal tissues to the field environment and renders them more vulnerable to pathogen contamination and growth (Doyle et al., 2007); (ii) field cored produce is usually processed into fresh-cut products directly without any additional in-plant sorting and inspection; (iii) pathogens that are internalized or attached to cut surfaces are extremely difficult to remove by subsequent sanitation procedures (see Section 7.4.3); (iv) the equipment and apparatus used for field coring can be contaminated, and then serve as a vehicle to contaminate produce; and (v) sanitary conditions in field environments are far more difficult to maintain compared to that of in-plant food processing environments. Although a significant data gap exists in this area, unpublished data have shown that a contaminated coring knife could transfer *E. coli* O157:H7 to up to 20 successive heads (McEvoy et al., 2008). Additional studies have shown that *E. coli* O157:H7 can grow significantly on cored areas within 4 hours at 30°C, followed by additional growth within 8 hours, but do not grow at 5°C (McEvoy et al., 2008). This underscores the importance of maintaining sanitary conditions of the harvest equipment, and cooling produce as soon as possible to minimize any potential amplification of pathogens.

Containers used for the collection of the harvested product can be contaminated by produce residues and soil particularly, if the plant roots remain attached. As bacteria are able to grow in

some products and viruses and parasites may survive (see Section 2.3.3), accumulation of vegetable matter on containers is not desirable. The level of contamination would be expected to increase if regular cleaning is not undertaken.

Cooling and cold chain maintenance for leafy vegetables and herbs begins at this point in the supply chain and the risks involved are discussed further below in Sections 6.4.1 and 8.0.

6.3.2 Uncertainty and data gaps

Field coring and trimming of lettuce is used by fresh-cut processors in some countries. As this is a recently developed technique that has the potential to increase the risk of microbial contamination, research studies are needed in the following areas:

1. The potential for pathogen internalization via the cut surfaces during harvesting and vacuum cooling.
2. The effect of field coring and trimming on pathogen contamination and its subsequent survival and growth on harvested produce (especially on the cut surface).
3. The effect of current post-harvest handling conditions—e.g. holding time, temperature, modified atmospheres and spraying of chlorinated water—on the survival and growth of pathogens.

An understanding of the impact of water sprays under various pressures on cored and trimmed produce such as lettuce under real conditions is also required.

6.3.3 Mitigation recommendations

The recommendations in the Codex Code of Hygienic Practice for Fresh Fruits and Vegetables (CAC, 2003b) incorporating the application of GAPs and GMPs, and especially its sections 3.2.3 Personnel health, hygiene and sanitary facilities, and 3.2.4 Equipment associated with growing and harvesting, for fresh fruit and vegetables are relevant. Complementary to this is the Codex Recommended International Code of Practice - General Principles of Food Hygiene (CAC, 2003a) and the need for training programmes defined in the current Code Section 10 (CAC, 2003a), taking note of the increased primary processing taking place in the field and the need for field worker awareness of food safety.

Further research is required to determine more specific and appropriate cleaning and hygiene maintenance scheduling and monitoring programmes. Particular attention should be paid to harvesting machines that may suck up soil and other field contaminants and components that may damage or cut plants.

It is recommended that non-essential persons and casual visitors are not allowed in the harvest area for leafy vegetables and herbs, particularly children, as they may present an increased risk of contamination of product or the environment.

6.4 Field packing and packinghouses

6.4.1 Available data

Field packed produce or produce entering the packinghouse has the potential to be contaminated and is a source of contamination for the packinghouse environment.

There are many surfaces during field packing and in a packinghouse that may come into contact with leafy vegetables and herbs. These include handling equipment, conveyors, sorting

tables, containers/bins/boxes, water hoses, etc. Johnston et al. (2006) conducted a study of 8 packing sheds in the USA and Mexico packing 11 different types of produce, including leafy vegetables and herbs. For the leafy vegetables (Swiss chard, turnip greens and kale) and herbs (parsley and cilantro), the mean counts of microbiological indicators on product was low. The range of total aerobic bacteria on product from pre-wash, wash and post-wash were 5.2 (±1.05), 5.2 (±1.05) and 5.5 (±0.88) log cfu/g, respectively, and *E. coli* counts were all 0.7 (±0.00) log cfu/g. The contact surfaces in the receiving bins pre-washing had significantly higher mean counts of total aerobic bacteria (3.7 (±1.10) log cfu/g) than the wash area (2.5 (±0.96) log cfu/g) and boxes (3.0 (±0.72) log cfu/g), while all had *E. coli* counts of 0.70 (±0.00) log cfu/g.

Poorly cleaned and maintained equipment can harbour microorganisms, including pathogens, and provide a reservoir of contamination (Stafford et al., 2002). Johnston et al. *(*2006) observed that the contamination with *E. coli* on cabbages increased from a mean log count of 0.7 cfu/g to 0.86 cfu/g as they moved from the conveyor belt to the final box. Prazak et al. *(*2002) similarly found *E. coli* and *L. monocytogenes* on transport bins, conveyor belts and cooler surfaces used for processing cabbages in packinghouses. As they demonstrated that common types of *L. monocytogenes* were in the packing shed environment, they concluded that contact surfaces were an important source of contamination, and highlighted the importance of hygiene and equipment sanitation. More research is needed to determine the specific contributions of different types of equipment to possible cross-contamination and pathogen amplification both inside and outside of the packinghouse.

Water is used widely in packinghouses for cleaning (produce, equipment and surfaces), transport, cooling and packing. The role of water has been referred to in Section 5. As leafy vegetables and herbs may be ready-to-eat at this stage or further processing may not remove contamination, use of potable water is required. Prazek et al. *(*2002) analysed water used to wash, spray and cool cabbages in farms and packing sheds. They detected *L. monocytogenes* in unchlorinated wash water, although no *E. coli* was detected.

A prime concern for leafy vegetables and herb growers and producers is maintaining quality and cooling as a means to extend shelf life. Pre-cooling involves rapid removal of field heat in produce prior to long-term storage. The first step to achieve good pre-cooling is to harvest the crop during cool weather (especially in the morning). Small-scale growers and handlers can also reduce the temperature of their produce by placing them outside overnight. More controlled types of pre-cooling can be achieved by icing, force-air cooling, hydro cooling, and vacuum cooling. Selection of an appropriate pre-cooling method depends on the type and volume of produce and the type and length of the supply chain. For most leafy vegetables and herbs, vacuum cooling is widely used, as the large surface areas of these produce allows for a rapid release of water vapour and thus quick cooling (Thompson et al., 1998). However, as there is a need to avoid moisture loss due to evaporation in leafy produce, water is sprayed on the product prior to vacuuming. Water is a significant input in the pre-cooling process and potable water is required. The presence of disinfectants in the water can be used to prevent cross-contamination. The use of unchlorinated municipal water that was recirculated through a hydro-cooler and used for ice in a packing shed has been implicated in an outbreak of shigellosis attributed to parsley (CDC, 1999). Because of the strong pressures involved, vacuum cooling also raises concerns of pathogen internalization. Li, Tajkarimi and Osburn *(*2008) have shown that vacuum cooling significantly increased the internalization of *E. coli* O157:H7 into lettuce tissue, after which stringent surface sterilization and quadruple washing was not able to eliminate the internalized bacteria. They suggested that the vacuum cooling changed the structure of the lettuce tissue, such as the stomata, that may have allowed the internalization. These studies emphasize the need to avoid contamination on leaves and to use potable water. Further research is required to

determine the role of these experimental findings in commercial practice. Further discussion on internalization is provided in Section 7 (Processing).

6.4.2 Uncertainty and data gaps

There is limited evidence linking outbreaks due to leafy vegetables and herbs to specific packinghouse operations.

There is uncertainty about the disinfecting levels achieved with water for cleaning produce and this issue discussed in more detail in Section 7.0 (Processing). There are restrictions on the use of disinfectants, such as chlorine, in some countries. It is not known whether low levels will reduce pathogen numbers and whether disinfectants will inhibit pathogen growth while maintaining produce quality. Information is required on the available (optimum) chlorine concentration in the water level required for effective sanitization.

The potential for cooling technologies to promote survival and persistence of pathogens through internalization of microorganisms requires further investigation to determine the significance in commercial practice and necessary control.

6.4.3 Mitigation recommendations

GAPs, GHPs and GMPs play an important role in the mitigating the risk of contamination with microbiological hazards buring harvest and packing. However, in this stage as well as during primary production the lack of information and/or knowledge regarding such microorganisms may mean that inadequate attention is given to these potential hazards. Thus training and education of workers can play and important role in risk mitigation. Specific attention to cooling processes specific to this product is also required, and control of inputs such as water used for cooling and packing.

7. Processing

7.1 Problem scope

This section specifically applies to processing operations in a factory environment (e.g. grading, removing outer or damaged leaves, coring, cutting, washing, sanitizing and packaging) where raw leafy vegetables and herbs are physically altered from their original form but remain in the fresh state and are ready-to-eat (RTE). Processing has been addressed under the headings of primary preparation (Section 7.3), further processing (including washing, sanitization, drying and chilled storage) (Section 7.4) and packaging (Section 7.5), and each may have a positive or negative impact on microbial risks.

7.2 Potential impact

During processing, leafy vegetables and herbs may be exposed to microbial contamination and microorganisms may persist and grow. However, to a large extent, the microflora of leafy vegetables and other fresh produce reflect the species present at the time of harvest (Nguyen-The and Carlin, 1994). Contamination from incoming leafy vegetables and herbs and contamination from food handlers has already been discussed in Section 6.0 (Harvest, field packing and packinghouses). Of particular concern during processing is the contact between the leafy vegetables and herbs and the multiple surfaces in the factory environment; the microbiological status of water; and the potential for tissue injury during primary preparation.

Some processes have the potential to reduce microbial risks (e.g. disinfection), control microbial growth (e.g. chilling) and protect the product from further exposure (e.g. packaging). However, current technologies or practices do not effectively eliminate any hazard acquired during post-harvest processing or packaging of fresh and fresh-cut leafy vegetables and herbs. According to industry experience, and from extrapolation of laboratory experiments, only a slight risk reduction appears possible. The main food safety aim of post-harvest handling is prevention of increasing risk.

7.3 Primary preparation

Primary preparation includes cleaning, trimming and coring of raw materials. All leafy vegetables and herbs are subject to primary preparation and are therefore exposed to food workers, multiple contact surfaces, water, processing aids and the process environment. These have already been described for produce handled in the field and packinghouses (Section 6.0).

There is evidence that foodborne bacteria adhere to plant surfaces and in particular may internalize in damaged and cut tissue (Takeuchi, Hassan and Frank, 2001; Takeuchi et al., 2000; Saggers et al., 2008; CCFRA, 2007). As well as during harvest, surface and internal contamination may also occur during primary preparation. Damage to produce during transfer (i.e. from the field to the processing facility) and during primary preparation may facilitate internalization.

The risks associated with exposure to food contact surfaces (e.g. containers, conveyors, equipment and utensils) are similar to those in packinghouses (Section 6.4.1). Surfaces are a known source of contamination in these environments and pathogens may colonize biofilms that

they form or are formed by other bacteria making them difficult to remove or inactivate (Carpentier and Cerf, 1993; Czechowski, 1991). Crates that have been used in the field or for non-RTE foods are a particular vehicle for the introduction of contaminants into the factory environment. Cross-contamination of subsequent batches of produce may occur if crates and pallets are not adequately cleaned and if forklifts or similar transport are not restricted to dirty (receipt) and clean (processing) areas respectively.

The growth of any microbial contaminants may be encouraged by exposure to unsuitable temperatures over significant periods of time.

A significant input in primary processing is washing water, which should be of potable quality (Section 5.0).

7.3.1 Mitigation recommendations

Implementation of GMP and GHP with Standard Operating Procedures, are pre-requisites for a HACCP-based food safety programme, and are required at all stages of processing.

Grading and selection prior to primary preparation, i.e. discarding or trimming damaged or decayed material (both at harvest and at the processing plant) will reduce the level of the most difficult to remove bacteria (e.g. attached and internalized viable cells).

Product flow and segregation from incoming soiled to outgoing washed product to avoid cross-contamination will be particularly important, as is exposure to chilling environments e.g. forced air cooling and cold storage (CAC, 2003b).

Areas that require particular attention in processing include knives, blades, conveyors and other food contact surfaces and utensils, particularly where biofilms may form and accumulate, together with management of the microbiological quality of water in contact with the produce.

7.4 Further processing

Fresh-cut leafy vegetables and herbs are additionally subject to size reduction, washing, sanitization, packaging and chilled storage. These steps vary according to product type and intended use.

7.4.1 Size reduction

Size reduction techniques can include cutting, slicing, shredding and chopping.

Foodborne outbreaks have been attributed epidemiologically to mishandling during the preparation of leafy vegetables in kitchens and processing facilities. Poor hygiene practices during the shredding of lettuce has been implicated in outbreaks of hepatitis A (Lowry et al., 1989) and *Shigella sonnei* (Davis et al., 1988).

The potential for cross-contamination between foods and the surfaces in which they come in contact during cutting and preparation has been demonstrated. Contaminated shredding equipment was identified as the source of contamination in an outbreak of salmonellosis attributed to shredded lettuce produced in a commercial setting (Stafford et al., 2002). In this case, the same *Salmonella* serovar was detected on the cutting blades during the investigation, indicating the contamination had persisted at least for the duration of the patients' incubation period and the preliminary case investigation. This was highlighted also in a study in a domestic kitchen, where *Campylobacter* was detected from prepared salads as well as the cleaning materials and food-contact surfaces during a series of food preparation sessions (Redmond et al.,

2004). Typing results were used to show that specific *Campylobacter* strains isolated from prepared chicken salads were the same as those isolated from raw chicken pieces, indicating microbial transfer during food preparation.

Data gaps

There are references available for several whole products, but comparisons between whole and cut products are scarce in the scientific literature, particularly for products from the same batch. Although it would appear logical that shredding and cutting increase the probability for microbial growth during storage, it has been shown that there is no significant difference in the growth of *L. monocytogenes* on cut and whole lettuce (Beuchat and Brackett, 1990). Delaquis et al. (2006) found that anti-listerial compounds could be released by cutting the tissue of iceberg lettuce. The differences in growth and survival of pathogens on whole and fresh-cut leafy vegetables is not sufficiently documented and not fully understood. This requires further investigation.

Mitigation recommendations

GMPs and GHPs with Standard Operating Procedures, as pre-requisites for a HACCP-based food safety programme, should be implemented.

7.4.2 Washing and sanitizing

Washing (primary and secondary) is the removal of soil, other gross debris, and plant tissue exudates that occur during cutting. Sanitizing is treating water with an agent that is designed to prevent cross-contamination during washing (CCFRA, 2002). Washing and sanitizing has the potential, if properly controlled, to reduce the overall microflora of leafy vegetables and herbs. However, it will not eliminate contamination; therefore, minimizing the potential for contamination in the field from the seed onward is key to assuring microbiological quality.

While there have been studies on the efficacy of water sanitizers, these are often carried out using widely differing protocols, which may not reflect natural conditions such as microbial loads, the degree of microbial attachment, the effects of leaf cultivar or species, growing or distribution conditions, or the efficacy or scale of washing systems used in commercial preparation. Suspension tests may also be erroneously used, which will give an exaggerated reduction since free organisms are known to be much more susceptible to the effects of sanitizers than those attached to surfaces (Aarnisalo et al., 2000; Best, Kennedy and Coates, 1990). Often, extremely high inocula are used in order to clearly demonstrate any reduction by use of a particular agent or process. Research methods should more closely reflect real conditions encountered in commercial processing.

Owing to practicalities, most industry trials of process water sanitizing agents use naturally colonized vegetable tissue, which is at the mature biofilm stage and therefore very resistant to the effects of washing, even in sanitized water. Commercial washing processes in relation to surfaces for fresh-cut produce are typically within a few minutes of chopping, shredding or dicing. Such washing occurs before the establishment of the protective extracellular polysaccharide, yet the washing process does not remove all of the bacteria (Liao and Cook, 2001; Zhang and Farber, 1996; Brocklehurst, 1994; Baranyi, Roberts and McLure, 1993) since it fails to address the substantial biofilm element of the attached population.

Washing in potable or sanitized water typically results in TVC/ACC reductions of 1–2 logs (CCFRA, 1999, 2002). However, the degree of risk reduction that can be expected from these current washing disinfection technologies is difficult to quantify in reality (i.e. under non-

inoculated conditions) given the sporadic nature of contamination by pathogens.

The most common sanitization agents used internationally are chlorine-based compounds, with free chlorine concentrations of 50–100 ppm frequently being used. Initial removal of debris and organic matter is a prerequisite before the decontamination step as such material will reduce the efficacy of the sanitizer. Hypochlorous acid is the active biocide and its concentration in the solution is pH dependent. At pH 7, 78% of hypochlorous acid remains in solution and for this reason citric acid is commonly used to maintain the pH at such levels. Maximum solubility of chlorine is achieved in water at about 4°C.

A summary of studies on the use of chlorine-related sanitizers and the practical aspects of their use is provided in Table A3.7 in Annex 3. These are experimental studies, and limited comparison can be made with different methodologies, strains, inocula, produce varieties and preparations between studies. While the reductions were greater with the use of sanitizers, the differences were not large in all cases. Some studies report reductions in total aerobic plate counts in lettuce pieces of ≤ 2 $\log_{10}$ cfu/g with less than 200 ppm HClO (one of the most commonly used sanitizers). For *E. coli* (including the O157 serotype) the reduction was mostly less than 1.5 $\log_{10}$ cfu/g. However, higher reductions were observed if an additional sanitizer was added. *L. monocytogenes* was reduced by 0.7 $\log_{10}$ cfu/g when washed with chlorine added at 100 ppm for 1 minute, compared with 0.5 $\log_{10}$ cfu/g reduction in water alone. The greatest reduction in salmonellae (3 $\log_{10}$ cfu/g) was achieved using 1600 ppm for 5 minutes, while reductions were less than 1.2 $\log_{10}$ cfu/g using lower concentrations and with mild heat. Other compounds provided greater reductions, but practical considerations in their use have to be considered.

Research has demonstrated that the removal of virus particles by washing in chlorinated water (100 ppm) was similar to that found with bacteria (reduction by 1–2 log) (FSA, 2004). The use of agitation marginally improved the sanitization, but increasing the wash time above two minutes had little, if any, benefit. The researchers cautioned that if contamination levels are high, it is likely that sufficient virus particles to cause infection would remain after washing. In practice, however, viral contamination of leafy vegetables is most commonly linked to post-wash handling at or near the point of consumption (Koopmans and Duizer, 2002; PHLS, 1993).

No additional available post-harvest technologies have yet been proven in practice to reduce the levels of pathogenic organisms associated with fresh and fresh-cut leafy vegetables or herbs by more than 1–2 logs while retaining satisfactory organoleptic characteristics (WHO, 1998). See Table A3.8 in Annex 3 for examples of performance and practical considerations of some other interventions.

Data Gaps

The degree of risk reduction that can be expected from current washing disinfection and packaging technologies is difficult to quantify given the sporadic nature of contamination by pathogens. Research studies should more closely reflect real conditions encountered in commercial processing.

Mitigation recommendations

GMPs and GHPs with Standard Operating Procedures, as pre-requisites for a HACCP-based food safety programme, should be implemented.

7.4.3 Microbial attachment and internalization

The location of the pathogen on the leafy vegetable or herb will affect the outcome of washing and sanitizing. There are increasing reports of experimental studies of the interaction of micro-organisms and plant tissues.

Pathogens have been shown to attach to both cut and intact surfaces of lettuce tissue (Takeuchi et al., 2000). Leaves of different plant species support different numbers of epiphytic bacteria, which may compete with human pathogenic bacteria. A significant relationship between the amount of sugar detected on leaves before colonization and the maximum bacterial population size attained on the plant species has been reported (O'Brien, and Lindow, 1989). It was hypothesized in the same report that patterns of attachment and subsequent competition of bacterial microflora for limiting resources and/or differential tolerance to environmental stresses are important factors in determining the abundance of particular bacterial species on plants. In any case, conditions on the surface of packaged leafy vegetables and herbs are favourable for pathogenic bacterial growth, albeit limited owing to poor nutrient availability and survival if temperature conditions are conducive (Bennik et al., 1996; Nguyen-The, Halna-du-Fretay and Abreu da Silva, 1996).

Microbial attachment to cut produce surfaces preferentially occurs at the plant cell-wall junction and the cell-wall components found there, including pectate, may provide a receptor site for bacterial attachment (Saggers et al., 2008). Attachment is a multi-step process (Garrood, Wilson and Brocklehurst, 2004) comprising:

- initial adhesion within less than an hour of contact;
- secretion of extracellular polysaccharide by adhered bacteria; and
- colonization of adhered bacteria.

Recent work has shown that the number of bacteria attached to cut produce surfaces after 2 minutes of contact is proportional to the inoculum concentration raised to the power of 0.79, with the degree of attachment reaching a plateau generally after one hour of exposure (Garrood, Wilson and Brocklehurst, 2004). The rate of attachment was independent of temperature.

Internalization has been found to be possible during pre-harvest under experimental conditions, but only after exposure of young plants (seedlings) to high pathogen loads (Warriner et al., 2003). Similarly, during post-harvest, internalization of microorganisms into damaged or cut surfaces of leafy vegetables has been demonstrated under experimental conditions using exposure to high inocula that would not be encountered under implementation of GAP (Kiba et al., 2006). Internalized bacteria would resist sanitization, but the microbial reduction effect of sanitization appears to be limited from the studies described in Section 7.4.2. It is not known whether microorganisms that have entered plant tissue through internalization can grow to a significant extent under real or expected storage or handling conditions.

WHO reported that internalization of microbial cells due to a negative temperature differential between the water and the fruit or vegetable would appear possible (WHO, 1998). From research carried out on internalization of bacteria into tomatoes during washing, it was concluded that the temperature of the chlorinated water should be at least 10°C (Bartz and Showalter, 1981; Zhuang, Beuchat and Angulo, 1995) higher than that of the tomato to achieve a positive differential, thereby minimizing the uptake of wash water through stem tissues and open areas in the skin or leaves (these open areas may be due to mechanical damage or may be naturally present). Since this effect is reported to be due to contraction of the tissue on exposure

to water colder than the tissue (this is common plant tissue behaviour), the recommendation to maintain a positive temperature differential is therefore applicable to other plant tissues (Brocklehurst, personal communication, 12 June 2008).

The lack of significantly effective options other than heat or irradiation, which can result in several log reductions of colonizing or internalized pathogens, requires research focusing on both fundamental attachment mechanisms and inactivation of the pathogens *in situ*.

Data Gaps

Further research is required on the precise mechanisms involved in microbial attachment to leafy vegetables and herbs in order to best direct efforts in the development of effective detachment techniques.

Internalization has been found to be possible during pre-harvest under experimental conditions, but only after exposure of young plants (seedlings) to high pathogen loads. There is no evidence indicating that internalization is significant in practice, particularly when GAP is implemented. More information is required regarding non-experimentally-induced internalization under actual GAP and GMP/GHP conditions in the field and in the factory.

Further information is required on the temperature differential between water and fresh produce (i.e. leafy vegetables and herbs) during washing to minimize the uptake of wash water through stem tissues and open areas in the skin or leaves.

Many potential washwater sanitization approaches are available. However, each will have its own limitations, such as unsuitability for particular produce types owing to damage, health and safety issues, and environmental issues, particularly on discharge. Dependent on the understanding of microbial attachment to produce surfaces, further research is required to develop truly effective sanitizers that are practicable, economically viable, acceptable to the consumer and legal. All washing approaches in commercial use must have been assessed and validated as fit for purpose under actual conditions of use. The development of common validation approaches would assist in identifying effective approaches.

The lack of significantly effective options other than heat or irradiation, which can result in several log reductions of colonizing or internalized pathogens, requires research focusing on both fundamental attachment mechanisms and inactivation of the pathogens *in situ*.

Mitigation recommendations

While the extent of the impact of microbial attachment and internilization on food safety is not yet known, the application of good hygienic practices as recommended in the Codex Code of hygienic practice for fresh fruits and vegetables (CAC, 2003b) is nevertheless important.

7.4.4 Drying

Drying will reduce the amount of free water and may reduce microbial growth.

Data Gaps

No scientific data clearly support the assumption that drying may reduce growth of foodborne pathogens.

Mitigation recommendations

While the lack of data prevents the elaboration of specific recommendations, it is nevertheless important to apply good hygienic practices, as they have been described by the Codex Alimentarius (CAC, 2003a).

7.5 Packaging

Packaging under hygienic controlled conditions immediately after drying has a role of protection in both whole and fresh-cut produce. The design of packaging systems and the selection of materials have an effect on the risk of foodborne pathogens in leafy vegetables and herbs; it is therefore important to apply sound packaging technology knowledge in order to select the correct materials and package design.

Modified atmosphere packaging (MAP) can be used with certain leafy vegetables to extend chilled shelf life (e.g. suppression of pinking in iceberg lettuce). MAP is defined as an atmosphere created by altering the normal composition of air to provide an atmosphere capable of extending shelf life (Phillips, 2006). The normal composition of the air is modified using gases such as oxygen, carbon dioxide and nitrogen. The oxygen level in packs is usually kept between 1 and 5% to reduce the respiration rate and therefore oxidative breakdown (Lee, Arul and Castaigne, 1995). The product is sealed in a wrap such as polyethylene, polypropylene, polyvinyl chlorine or edible film.

Packaging is frequently used to avoid water loss from leafy vegetables, particularly for fresh-cut products. Packaging for leafy vegetables maintains a high humidity, which is an important factor for survival and growth of pathogens (Brandl and Mandrell, 2002; Dreux et al., 2007). Brandl and Mandrell (2002) studied *Salmonella* on cilantro and Dreux et al. (2008) studied *L. monocytogenes* on parsley and found a rapid decline of both pathogens on the leaf surfaces under low humidity (60% RH or less) and growth under saturated humidity. The distribution of humidity within the package is also a factor that correlates to the bacterial counts. Valentin-Bon et al. (2008) sampled leafy vegetables from the top and bottom of bags that presented condensation on the bottom of the bags. The authors found that the samples collected from the bottom yielded a mean count of 7.65 $\log_{10}$ cfu/g, while the top-sampled bags had a mean total count of 6.96 $\log_{10}$ cfu/g. Research to assess the distribution of pathogen numbers in relation to spatial variability of temperature and humidity in bulk and packaged presentations is required.

Leafy vegetables and herbs have a high respiration rate, which is increased further by tissue damage. Depending on the permeability of the packaging materials and the length of storage, the available oxygen inside the package may decrease to levels that permit growth and toxin production by the anaerobic pathogen *Clostridium botulinum* in contaminated products. This risk is particularly high if the products undergo severe temperature abuse (e.g. more than 13°C) for more than 7 days (Petran, Sperber and Davis, 1995). Packaging conditions may also lead to an increase in CO_2 concentration, which can favour the growth of pathogens over the normally present background microflora, as shown for *L. monocytogenes* (Carlin et al., 1996)

Maintenance of the cold chain is essential to suppress pathogen growth in packaged products (Phillips, 2006). If the cold chain is not properly maintained, growth of background microflora may be reduced, creating conditions more favourable for growth of pathogens such as *Clostridium botulinum* and *E. coli* O157 (Chau et al., 2008). The cold chain is discussed in more detail in Section 8.

Notwithstanding the role of packaging, other factors, such as initial bacterial numbers and temperature, play as important a role in maintaining product quality and safety. For example, in

surveys of the prevalence of pathogens, including in both packaged and unpackaged vegetables, performed in the United Kingdom at retail level, no difference in the microbiological quality was observed between the two types of product (Sagoo, Little and Mitchell, 2003; Sagoo et al., 2003).

7.5.1 Data gaps

There is a scarcity of scientific literature comparing the safety of wrapped and unwrapped fresh leafy vegetables (either whole or cut). Most of the studies available compare shelf life, but not pathogen growth. For wrapped products there is difficulty assessing the distribution of humidity inside the package, and therefore little is known about its impact on pathogen growth. This warrants further investigation. Furthermore, investigations into the impact of humidity distribution in non-packaged presentations (e.g. differences in humidity at the surface of the bin with respect to the centre or bottom) are also required. The impact of high humidity below saturation (e.g. 70–100% RH) on bacterial pathogens on leafy vegetables is also unknown.

7.5.2 Mitigation recommendations

While general guidance with regard to packaging should be followed, i.e. packaging design and materials should provide adequate protection to minimize contamination of produce and, prevent damage, and the packaging materials or gases used must be non-toxic and not pose a threat to the safety and suitability of food under the specified conditions of storage and use (CAC, 2003a), the data gaps identified above highlight the need for additional information before more specific recommendations can be made.

7.6 Chilled Storage

Refrigeration will not eliminate contamination that occurred previously (e.g. during packing-house operations or processing). However, growth of bacteria may be slower under refrigerated conditions (Abdul-Raouf, Beuchat and Ammar, 1993). Temperature is the single most important factor contributing to bacterial growth and survival (ICMSF, 2006); therefore, temperature control and maintenance of adequate cold chain conditions are critical to food safety.

Storage facilities must be designed and constructed in such a way as to minimize damage to fresh produce, to avoid access by pests, and to reduce the opportunity for potential contamination from physical objects (glass, wood, metal, etc.).

Condensate and defrost water from evaporator-type cooling systems (e.g. cold rooms) is a potential source of microbial contamination.

The cold chain is discussed in more detail in Section 8.0.

8. Marketing and the cold chain

8.1 Problem scope

The cold chain continuum starts at the point of harvest of fresh leafy vegetables and herbs, and ends at consumption, as these products are maintained in a fresh state and consumed raw.

8.2 Potential impact

Fresh leafy vegetables and herbs may be contaminated with foodborne pathogens while growing, at harvest and at subsequent points in the processing and distribution chain. As these products are highly perishable, maintenance of the cold chain is important for both product quality and safety (maintenance of the cold chain minimizes the growth of bacteria). The survival of bacteria may be influenced by chilling temperatures over time; however, the viability of viruses and parasites is influenced very little or not at all by low temperatures.

The initial population of pathogenic bacteria on leafy vegetables and herbs is assumed to be low at harvest. An increase in the number of pathogenic bacteria at any stage post-harvest will lead to exposure of consumers to higher numbers of pathogens, and therefore prevention of contamination throughout the supply chain is essential. Temperature is the single most important factor contributing to bacterial growth and survival (ICMSF, 2006); therefore temperature control and maintenance of adequate cold chain conditions are critical to food safety.

8.3 Available data

Primary processing involves initial cooling, cleaning and segregation. Cold-chain operations at this stage include the use of ice in the field to achieve pre-cooling as close as possible to the point of harvest, forced air cooling, hydrocooling, water spray vacuum cooling or vacuum cooling (with no spraying) in the packinghouse. For leafy vegetables, vacuum cooling is the most favoured technique due to their large ratio of surface area compared to their mass. For packaged herbs, forced-air cooling is favoured (ASHRAE, 2006a).

Secondary processing of leafy vegetables and herbs involves transforming the primary products by washing, cutting and packaging. The cold-chain operations can involve the use of a temperature-controlled packing room or a second chilling after processing, followed by cold storage.

For long-distance distribution, e.g. air transport, the use of refrigerated transport or the use of other coolants (e.g. ice, dry ice, gel packs) is necessary. The ice is normally placed in plastic bags inside insulated boxes containing the product (ASHRAE, 2006b). Well planned distribution operations (either domestic or export) take into account the location and capacity of refrigerated distribution centres, the refrigerated modes (sea, air or land) and the volumes to be transported. Carrying out loading and unloading operations in refrigerated docks will avoid re-warming of the product during distribution (Estrada-Flores and Tanner, 2005).

Retailers use different levels of refrigeration: cold storage is required in supermarket distribution centres; preparation rooms (e.g. cutting of whole lettuce at the store and the use of a secondary packaging) can be air-conditioned; walk-ins and display cabinets need to be

refrigerated. A developing area is the foodservice/convenience/restaurant/take way/food stall sector. In many countries, this sector is represented by a large amount of small businesses and long food chains.

In wet markets, a typical scenario is the lack of refrigeration facilities. In these cases, an alternative cooling system is still possible e.g. spraying with potable water or alternatives that use very simple evaporative cooling systems.

Finally, consumers may store leafy vegetables and herbs in domestic refrigerators that are designed for general food storage and thus have numerous temperature zones within. For example, refrigerators in Australia were shown to have a minimum temperature of 0.6°C and a maximum temperature of 5.5°C at temperature settings of 3°C (Langley and Grant, 2004). In the USA, 20% of domestic and commercial refrigerators were found to operate at >10°C (Jol et al., 2005) and in 250 domestic refrigerators measured in Greece, a large variability was found, with almost 10% of temperature distributions having an average >10°C (Taoukis et al., 2005). The mean European domestic refrigerator temperature (according to a compilation of published surveys) is 6.64°C (Nauta et al., 2003)

It is difficult to quantify the portion of the risk of foodborne disease associated with fresh and fresh-cut leafy vegetables and herbs attributable to failures in the cold chain as no information was found where failure of refrigeration was proven to cause an outbreak. Increased use of temperature logging and recording during distribution and studies on microbial growth may lead to investigations in the future.

8.3.1 Pathogen contamination

Contamination of leafy vegetables and herbs during distribution and marketing (this includes food service) might occur through contact with contaminated surfaces during transport and storage; through airflow during cooling and storage (e.g. dirty evaporators); or through drip of contaminated condensation on cooling equipment (Evans et al., 2004). The potential for cooling, such as hydrocooling, to cause internalization has been discussed (See Sections 6.4.1 and 7.4.3). However, no reference specific to the impact of this on leafy vegetables and herbs in practice was found. Epidemiological reports more often indicated contamination occurred from poor food handler practices and hygiene of food preparation equipment (Table A2.3 in Annex 2).

8.3.2 Pathogen growth and survival during distribution

Growth and survival or decline of pathogens will depend on pathogen type, produce type, temperature, relative humidity, atmosphere and packaging (ICMSF, 2006; Harris et al., 2003). Refrigeration will not eliminate contamination that occurred previously (e.g. during packing-house operations or processing); however, growth of bacteria may be slower under refrigerated conditions (Abdul-Raouf, Beuchat and Ammar, 1993). Therefore, refrigeration during distribution and before consumption is likely to decrease the risks of bacterial infection where amplification is required to cause infection; however, pathogens with low infectious doses may remain viable and in sufficient concentration to cause illness. In the risk assessment of *L. monocytogenes* in ready-to-eat foods performed previously by FAO/WHO, the impact of an increase of contamination of ready-to-eat foods with this bacterium on the number of predicted cases has been clearly illustrated (FAO/WHO, 2004). Evidence for the risk of foodborne illness transmission is provided in the outbreaks listed in Tables A2.2 and A2.3 in Annex 2.

The growth of bacteria in leafy vegetables and herbs has been discussed in previous sections. Although low temperatures will inhibit growth and survival of most bacteria, psychrotrophs—

including *L. monocytogenes*—can survive and multiply in cold chain conditions. Growth of *L. monocytogenes* at 3–5°C in refrigerated fresh-cut packaged leafy vegetables has been demonstrated (Nguyen-The and Carlin, 2000; Beuchat and Brackett, 1990). Even so, the lag time for this bacterium is extended and the generation time is slowed under refrigerated conditions compared to growth at 37°C. Crepet et al. (2007) analysed published data on the prevalence and concentration of *L. monocytogenes* in fresh vegetables and estimated the mean contamination on fresh produce to be between 0.1 and 1 cfu/100 g. *E. coli* has also been shown to survive for several days under refrigerated conditions (Abdul-Raouf, Beuchat and Ammar, 1993).

Viruses are known to survive longer on fresh produce kept cold, and some can survive longer than the shelf life of the product (DeRoever, 1999; Beuchat, 1996; Konowalchuk and Spiers, 1974, 1975). Badawy, Gerba and Kerby (1985) showed that rotavirus could survive on lettuce, radish and carrots for 25–30 days when kept at 4°C, but only 5–25 days at ambient temperatures.

8.3.3 Relationship between shelf life and safety in leafy vegetables

Increasing times between production and consumption of the product (i.e. the shelf life) has the potential to increase food safety risks if the extended time allows survival or further growth of the pathogen. Shelf life extension may increase the risk of further contamination whenever products are not protected by packaging. In addition, longer shelf life may increase the risk of temperature abuse during storage. The National Advisory Committee on Microbiological Criteria for Foods in the USA reviewed the microbiological safety of fresh produce, and found that longer food chains could permit amplification of pathogens and thereby create a health risk for the consumer (De Roever, 1999).

Leafy vegetables and herbs kept under high humidity (e.g. sealed packaging) and short-term non-refrigerated storage can support the growth of pathogenic bacteria. For example, more than 1 log growth of *E. coli* O157 (Abdul-Raouf, Beuchat and Ammar,) and *L. monocytogenes* (data reviewed by Nguyen-The and Carlin, 2000) was observed at room temperatures (e.g. 20°C) within one day. Similarly, *Salmonella* increased by 0.7 $\log_{10}$ cfu/g in one day on cilantro leaves stored at 22°C (Brandl and Mandrell, 2002). Therefore, even a short shelf life would not prevent an amplification of bacterial pathogens on leafy vegetables stored without refrigeration.

Some studies show no differences in shelf life between whole and fresh-cut versions of leafy vegetables. ASHRAE (2006a) recommends that whole lettuce is kept less than 20 days at 0°C and 95–100% RH, while fresh-cut produce should be kept 7–14 days at the same conditions. This suggests that fresh-cut versions have half the shelf life of the whole versions, but this relationship has not been tested experimentally. Shelf life depends on the type of product and the practices followed for each.

8.3.4 Implications of failure in the cold chain

Small (but frequent) breaks may occur in the cold chain, such as when loading or unloading products during refrigerated transport, temperature variations in refrigerated equipment, short power shortages or sporadic (but short-lived) temperature abuse. During the cold chain, the likelihood of short, temperature excursions above and below the optimum temperature is high. Although they may be unavoidable, e.g. warming during loading and unloading, they need to be minimized. The impact of these situations on bacterial proliferation has not been fully assessed, particularly for leafy vegetables. Estrada-Flores and Tanner (2005) found that temperature abuse of less than 4 hours with a maximum product temperature of 9°C did not lead to significant

growth of *E. coli* on fresh produce. Product exposure to higher temperatures or for longer times is likely to lead to increased food safety risks and further research is required to look into this.

In a deficient cold chain, gross temperature variations may occur in refrigerated transport and cold stores (Tanner et al., 2005; Smale, 2004; Estrada-Flores, Eddy and Smale, 2006; Estrada-Flores and Platt, 2007). At the consumer's end, a survey of temperatures in display cabinets and domestic refrigerators in Europe and the USA was performed by EFSA (EFSA, 2007). The results showed that storage temperature at both the retail and domestic level can vary significantly between refrigerators, with some surveyed temperatures being as high as 16°C. A wider range of pathogens can grow whenever the storage temperature is over 5°C and the level attained depends on the duration. For instance, *E. coli* O157 could not grow below 5°C, although it grew at 8°C or above (Abdul-Raouf, Beuchat and Ammar, 1993; Francis and O'Beirne, 2001; Koseki and Isobe, 2005; Luo et al., 2008). For psychrotrophic pathogens, such as *L. monocytogenes*, which can grow on leafy vegetables below 5°C, higher storage temperatures might increase the risk by increasing the growth rate and thus concentration on leafy vegetables by the time of spoilage. As shown by Carlin, Nguyen-The and Abreu da Silva (1995), the log increase of the pathogen at spoilage tripled when the storage temperature was increased from 3°C to 10°C.

8.3.5 Effect of marketing practices

It is expected that different handling practices in RTE, food service, supermarket and wet markets may introduce different risks. However, no published scientific studies comparing the microbiological quality of the various marketing channels for a specific leafy vegetable or herb supply through different channels were found. There are various studies presented in Table A2.4 in Annex 2, detailing surveys of leafy vegetables and herbs at different retail points. Little et al. (1999) surveyed imported unprepared lettuce from various retail outlets in the United Kingdom and found that 18% (27/151) of samples had Enterobacteriaceae counts $\geq 10^4$ cfu/g. The temperatures of storage and the counts varied, but, in general, samples from greengrocer shops and market stalls were more likely to have higher counts than supermarkets. In a recent study (Valentin-Bon et al., 2008), a sample of 100 bags of lettuce and spinach purchased from stores in Washington DC, USA, presented a wide range of total bacterial counts (4 to 7 $\log_{10}$ cfu/g) and coliforms (1 to 4 $\log_{10}$ MPN/g). The authors of this study found large variations in counts not only among the samples, but also within same-brand products that had identical "use by" dates and were tested on the same day.

It should be noted that a certain amount of processing (e.g. slicing, dicing) and packaging can take place at retail level. Both processing and packaging have been addressed in Section 7.

8.4 Uncertainties and data gaps

There are studies that correlate shelf life with cold chain breaks; however, there are very few studies that correlate pathogen growth with cold chain breaks. In particular, the effect of cumulative temperature abuse (e.g. more than one occasion where the optimum temperature is exceeded throughout the chain) on the safety of leafy vegetables is unknown. Experimental trials are required to assess the growth of pathogens in real cold chain scenarios (i.e. with fluctuating temperatures) using both mathematical modelling and experimental assessments.

Furthermore, there is no information available on the direct impact of the cold chain for leafy vegetables or herbs on foodborne diseases. Epidemiological data needs to be collected and correlation with cold chain failures considered.

Research is needed to assess the growth and survival of foodborne pathogens in commercial conditions or in scenarios that represent real-life conditions to complement the experimental studies. The latter use inocula at high levels and organisms for which the physiological state may be different to natural settings. At least one published work shows that a high inoculum could lead to growth of a pathogen on refrigerated lettuce, whereas with a low inoculum, the pathogen declined (Abdul-Raouf, Beuchat and Ammar, 1993).

Experimental trials are required to assess growth of pathogens in real marketing scenarios, taking into account the different handling practices and commercialization times. Both mathematical modelling and experimental assessment are needed. There is uncertainty regarding the potential production of toxins by *Clostridium botulinum* in leafy vegetables under marketing conditions (e.g. normal refrigeration).

8.5 Mitigation recommendations

Prevention of contamination early in the supply chain is required as it is not eliminated by cold-chain management. The General Principles of Food Hygiene and the Code of Hygienic Practice, (CAC, 2003a) and for the Transport of Food in Bulk and Semi-Packed Food (CAC, 2001) are relevant, together with the current Code of Hygienic Practice for Fresh Fruit and Vegetables (CAC, 2003b).

Increased emphasis on training in cold-chain logistics and management is recommended, in line with advancing knowledge and technologies for both refrigeration and temperature monitoring and expanding international trade. Technologies that are available include temperature indicators, data logger temperature sensors (e.g. Tinytags™), and radio-frequency identification (RFID). Most of these measures monitor the chain up to the retailer. Measures to monitor through to consumers would be desirable, although these would need to be clear and easy to interpret, agree with the calculation of “use by” dates or the time-temperature indicator would need to supersede the “use by” date.

Given the fact that there is a large uncertainty in the effect of cold-chain breaks on food safety, it is not possible to provide recommendations to mitigate the risks of contamination, growth and survival of pathogens throughout the supply chain, other than emphasizing the importance of cold chain maintenance and good handling practices.

It is also important to have guidelines for the handling of fresh-cut and leafy vegetables in general for retailers, food services and markets.

9. Education and training

9.1 Problem scope

Training and education of growers and handlers along the entire food chain continuum should be considered as a primary preventative control measure, or risk mitigation strategy (US EPA, 1997). There is a need to create greater awareness among all workers associated with fresh produce production, packing, processing, distribution, storage, retail and catering of the risks associated with contaminated fresh produce and the need for preventative control measures along the food chain. This increased awareness is also required for consumers. The overall goal of education should be to encourage increased adoption of effective food safety behaviours. Education is said to be the least expensive, yet most effective, way to reduce foodborne illness (FAO/WHO, 2008a).

9.2 Potential issues

General requirements for training are outlined in the Codex Code of Hygienic Practice for Fresh Fruits and Vegetables (CAC, 2003b, Sections 10.1 and 10.2). In addition, Annex I to that code for ready-to-eat fresh pre-cut vegetables require further training programmes (CAC, 2003b, Section 10.2). Routine and regularly scheduled food safety awareness training is required for all persons involved in the leafy vegetables and herbs supply chain from farm to plate (International Fresh-cut Produce Association, 2006). However, training needs to address a range of specific issues and move beyond the basic training that is typically covered. Particular attention should be given to tackling high-risk food safety practices, understanding why certain behaviours occur, and the barriers to achieving behavioural change.

The human pathogens most often associated with produce (*Salmonella* and *E. coli* O157:H7) cause infection and illness by the faecal-oral route of food contamination. Therefore, food safety programmes for leafy vegetables and herbs should pay special attention to controlling, reducing and eliminating potential faecal contamination from people and animals (domestic and wild) through the most likely conduits, i.e. hands, water, manures and soil. A study of the microbiological status of morning glory harvested from around Phnom Penh, Cambodia, and collected from vendors in the marketplace showed 15% of samples were contaminated with *Shigella*, 100% with *E. coli* and 29% with *Clostridium perfringens* (WHO Regional Office for the Western Pacific, 2005).

Worker health and poor hygiene practices have been cited as contributing factors to foodborne illness. In particular, education of agricultural workers is viewed as a high priority since opportunities to eliminate microbial contamination is not possible once present on leafy vegetables and herbs. Furthermore, pathogen contamination early in the supply chain will have a much larger impact due to a magnification effect (e.g. higher incidence of foodborne illness linked to wider distribution of product).

9.3 Key considerations in education and training

Many training programmes addressing GAP, GHP and GMP are available around the world (FAO, 2007). Investment in training and education on food safety for agricultural workers through to consumers varies considerably, particularly between developed and developing

countries. Observations show the main focus for training programmes has been on capacity building and delivery of training materials to improve awareness, knowledge and skills. The extent to which training programmes have been evaluated for efficacy and impact is not known. It is important to evaluate training programmes to gain feedback on adoption and effectiveness in improving GAP, GHP and GMP.

There is a need for a systematic approach to education and training throughout the leafy vegetables supply chain from farm-to-fork. An evidence-based approach to developing, implementing and evaluating food safety education interventions need to be considered.

High-risk food safety practices and behaviours need to be targeted in education interventions. Sociological and behavioural research is needed to inform pertinent and effective food safety messages and message delivery mechanisms that will result in behavioural changes.

There are real benefits in actively engaging the target audience as a key partner in programme development, implementation and evaluation. The process assists with building trust and understanding of issues and concerns. Producers that receive targeted risk communications based on identified knowledge gaps and actively participate in the decision-making process for increasing food safety will be more receptive to adopting food safety behaviours.

Social and community support networks are important for sustainable action, especially in rural communities of developing countries (WHO Regional Office for the Western Pacific, 2007).

9.3.1 Priority target groups for training and education

Priority groups for training and education include:

- agricultural workers, especially farmers and field workers;
- food handlers and vendors in marketplaces; and
- consumers.

9.3.2 Topics for inclusion in training programmes

Topics for consideration for training programmes have been recommended in the current Codex Code of hygienic practice for fresh fruits and vegetables and are listed below (CAC, 2003b, Section 10.2).

- Good health and hygiene for personal health and food safety.
- Hand washing for food safety and proper hand washing techniques.
- Using sanitary facilities to reduce the potential for contaminating fields, produce, other workers and water supplies.
- Techniques for hygienic handling and storage of leafy vegetables and herbs by transporters, distributors, storage handlers and consumers.
- Shared responsibility among stakeholders: agricultural workers, government, NGOs and the media.

The proposed training requirement should address safe growing and handling practices, including general clean handling procedures, control of cross-contamination, and personal hygiene. Other important topics for training and education have been highlighted throughout

this report.

When designing a training programme, consideration should be given to the following:

- Longstanding entrenched worker behaviours, attitudes and social taboos.
- Transient workforce with no prior training in food safety and hygiene.
- Children and infants accompanying mothers working in the field, with the potential for transfer of pathogens with a human reservoir.
- Diverse cultural, social and traditional practices.
- Literacy and education levels.
- Language and dialect of workers.
- Need to make food safety practices realistic and easy to implement (identify enabling factors, motivators and incentives).
- Raising awareness among workers of symptoms and signs of disease, and encourage them to act upon it (take personal responsibility for health).
- Importance of food safety training when new crops are being grown for the first time.
- An additional issue for consideration in training programmes for personnel at post-harvest stages include the influence or significance of different packaging systems. Further information on packaging is available in Section 7.5 of this report.

9.4 Consumer education

The aims of consumer education are to:

- Raise awareness about microbial hazards and safety of leafy vegetables and herbs without causing alarm or damaging consumer confidence in these nutritious foods.
- Develop knowledge and skills in good hygienic practices and consumers' roles and responsibilities in protecting leafy vegetables and herbs from contamination and deterioration.
- Develop culturally appropriate food safety messages and materials targeting high-risk food safety behaviours.

Consumer awareness research conducted recently in Australia and New Zealand highlights growing consumer concern about the safety of fresh produce, including microbial hazards (FSANZ, 2008). Increased outbreaks of foodborne illness linked to fresh produce globally and intensive media coverage of events such as the *E. coli* 0157:H7 spinach outbreak in the USA in 2006 have contributed to greater public awareness of microbial risks associated with leafy vegetables and herbs.

Research conducted on consumers' and food handlers' knowledge of food safety and their actual food handling practices and behaviours shows that there can be a wide gap between what people know about safe food handling practices and their own behaviour (IFIC, 2008; Byrd-Bredbenner et al., 2007; Clayton, Griffiths and Price, 2003; Hertzman and Barrash, 2007).

Consumers (and food handlers) remain an important target group for food safety education, and there is a need for specific information on how to handle fresh and fresh-cut leafy vegetables and herbs safely. Consumers need to understand their roles and responsibilities in

protecting leafy vegetables and herbs from contamination and deterioration, and in preventing foodborne illness.

9.4.1 Important Considerations

There can be significant differences in the types of leafy vegetables and herbs consumed by people in different countries and the ways that they are eaten: raw or cooked.

Consumers' food handling practices, preparation methods and storage facilities also vary considerably, especially between developed and developing countries. Major differences include a lack of domestic refrigerators in rural communities of developing countries, and access to clean safe water.

Outside of the USA and the United Kingdom there has been little if any research conducted specifically on consumers' food safety and handling practices for leafy vegetables and herbs (Li-Cohen and Bruhn, 2002).

Consumer information should be relevant and meaningful to the target audience, and adapted according to country and specific circumstances. Food safety information must be based on sound science and must be easy to implement. An evidence-based approach to consumer information and education should be adopted, aimed at behavioural change. Consumers (target group) should be involved during development and pre-testing of food safety information and materials (Godwin et al., 2006)

Where possible, education should build upon successful intervention strategies and programmes such as the WHO "Five Keys to Safer Food" and "Fight BAC" campaign.

Clear consistent messages on handling leafy vegetables and herbs safely should be communicated to consumers by all stakeholders—industry, government, consumer organizations and the media—to avoid giving contradictory advice and causing confusion (Cuite et al., 2007).

Consumer information on handling leafy vegetables and herbs safely should cover:

- selecting produce in the marketplace (supermarkets, retailers);
- transporting to home;
- storage and refrigeration;
- washing;
- personal hygiene and kitchen sanitation; and
- use of leftover food.

Selection of fresh leafy vegetables

Many leafy vegetables, such as lettuce, are fragile and must be handled with care to avoid mechanical damage and to minimize discoloration and pathological problems.

Consumers need specific guidance on how to select safe leafy vegetables, avoiding bruised, damaged or slimy products.

Transportation

Increases in product temperatures during transportation can be considerable. Guidance is needed on keeping time as short as possible between purchase of leafy vegetables at retail or markets to the home.

Refrigeration

Where applicable, the current recommendation is to store leafy vegetables and herbs in the refrigerator. Research shows that mean temperatures of domestic refrigerators vary considerably and often operate above 5°C (Peck et al., 2006; Gilbert et al., 2007). The temperature at which a refrigerator operates is critical for the safe storage of chilled foods. A recommendation made in 1991 in the United Kingdom concerning the microbiological safety of foods advised that the maximum temperatures in domestic refrigerators should not exceed 5°C.

Consumers should be advised to regularly check the temperature of domestic refrigerators and keep them below 5°C.

Consumers also need guidance on how to store leafy vegetables and herbs safely (e.g. remove outer leaves and place in plastic bags and store in coolest part of the refrigerator and cautioned on not keeping leafy vegetables too long, even when refrigerated.

Washing

It is generally recommended in consumer food safety information materials to wash unwashed leafy vegetables and herbs before use. It is widely recognized by food microbiologists that washing cannot guarantee the removal of microbial contamination from produce. Research has shown washing will remove soil and loosely attached microorganisms but has only a minor effect on those organisms that are attached. A one to two log reduction of microbial load on leafy vegetables can be achieved through washing in the commercial setting. However, from the consumer perspective, it must be communicated that washing cannot ensure the safety of leafy vegetables and herbs.

Water quality and safety for washing leafy vegetables and herbs should be covered in consumer information materials. Water can be a major source of microbial contamination and foodborne illness. Current guidance is for consumers to use clean, potable running tap water for washing and rinsing leafy vegetables before use.

Consumer information materials should provide guidance on how to wash leafy vegetables safely in the kitchen to avoid cross-contamination (e.g. from a dirty kitchen sink).

Personal hygiene

Hands are a very common vehicle for the transfer of human pathogens to food products, including leafy vegetables. Food handlers' hands may become contaminated when they engage in activities such as touching raw meat products, using the toilet, coughing, etc. Correct hand washing methods using soap and clean water before handling leafy vegetables should continue to be promoted to consumers.

Use of leftovers

Guidance should be provided on what to do with leftover leafy vegetables, such as prepared salads that have been kept at ambient temperature for more than 2 hours.

Cross-contamination

There is potential for cross-contamination and transfer of pathogens from fresh leafy vegetables and herbs to cooked and RTE foods; also from other raw meats, poultry and fish onto leafy vegetables (Wachtel et al., 2003).

Li-Cohen and Bruhn, in 2002, published the most extensive consumer handling study of fresh produce from the time of purchase to the plate. Approximately half of all respondents did not wash their hands before handling fresh produce; 56% reported that they always wash the sink before handling fresh produce; and of those that washed the sink some 11% use water only.

The recent consumer study conducted by the International Food Information Council (IFIC, 2008) showed that while more than three-quarters of the USA population (82%) say they are confident in their ability to safely prepare food, many do not take steps to reduce the spread of bacteria in their kitchen. Less than half (48%) report using separate cutting boards for raw meat or poultry and produce.

Consumers need specific guidance on how to handle, prepare and store leafy vegetables and herbs safely to avoid cross-contamination with pathogens from various sources e.g. hands, sinks, cutting boards, raw meats.

Specific information for fresh-cut produce and RTE bagged salads

Consumers need specific and clear guidance on how to safely handle fresh-cut and pre-cut leafy vegetables that are RTE. Clear labelling is therefore important. There is anecdotal evidence to suggest that some consumers find it difficult to distinguish between produce that can be consumed without further washing and that which requires washing before consumption, particularly bagged produce such as herbs and spinach. Furthermore, advice on this matter has been conflicting. For example, since 2001, consumers in the United Kingdom were advised by the Food Standards Agency to wash RTE bagged leafy salads before consumption. However, an expert panel recently reviewed the available scientific evidence and has now determined that consumers should NOT re-wash washed RTE bagged salads (Palumbo et al., 2007; United Western Growers, 2006). The Advisory Committee on the Microbiological Safety of Food in the United Kingdom has agreed (ACMSF, 2008) that this 2001 re-washing advice be rescinded owing to its lacking a scientific basis.

Consumers need clear guidance on keeping washed RTE bagged leafy salads refrigerated (1–4°C) until used. They also need to adhere to information on the pack regarding ‘use-by’ date.

Label information needs to be clear and easy to read, with specific directions for product storage and use. Consumers need to adhere to information on the pack regarding ‘use-by’ date.

10. Data gaps

Provision of scientific advice on the microbiological hazards associated with leafy vegetables and herbs involved data collection and a review of the scientific literature applicable to all steps in the farm-to-fork continuum (i.e. pre-harvest, harvest, packing, processing, marketing and the final consumer). While the meeting addressed the questions from the Codex Committee on Food Hygiene based on the available data, in doing so the experts also identified a number of gaps in the data. The identification of data gaps should not hinder the implementation of the best risk management practices based on current knowledge, but rather serve as an indication of those areas where further research would facilitate greater understanding of some of the challenges that need to be overcome in many countries to produce microbiologically safe leafy vegetables and herbs and where more knowledge might lead to improved risk management measures in the future. These gaps, which have been raised in each section of the report, are summarized below.

10.1 Pre-harvest

There is a large amount of uncertainty associated with the contamination and colonization of leafy vegetables and herbs in the field. Although many inputs were identified that might affect the risk of contamination and colonization, the meeting identified a lack of knowledge on the threshold pathogen density needed for plant colonization, the likelihood of infection, and the subsequent level of colonization up to harvest.

No information was found on the impact of prior land use on the microbiological safety of leafy vegetables and herbs or on the recommended minimum distance between the contaminant and the growing area, or on recommended levels of pathogen or microbial contamination allowed in the proximity of the farming area. Further research is essential in this area.

Several lines of evidence exist to support the hypothesis that animal density may be an important risk factor in the contamination of crops, with increased animal density increasing the transmission of microorganisms between animals and increasing faecal contamination of the environment. Yet specific information linking animal density with risk of produce contamination is not currently available. For example, the extent to which domestic animal holding and slaughter operations, landfills, wastewater treatment facilities, urban development and human settlements contribute to the contamination of produce have not been described. Such data would facilitate modelling of the complex interactions among factors, such as wildlife populations, climate, topography, hydrology, weather and sanitary practices around industrial and residential activities to more precisely estimate the risk associated with these activities.

Although there is circumstantial evidence that climate, topology, weather, hydrology and geographical features may contribute to an increase of microbial contamination in the leafy vegetable and herb industries, there is little direct evidence and more research needs to be conducted in all of these areas. Another area of uncertainty due to lack of data is the impact of climate and weather on wildlife populations, pathogen survival in soil, and the population kinetics of insects and other vectors that are potential vehicles in the spread of pathogenic bacteria.

Current guidelines for the microbiological quality of water used to irrigate crops meant to be eaten raw are based on the presence of coliform bacteria and *E. coli*. It remains unclear how these indices correlate with the presence of specific human pathogens. There is an urgent need

for the development of irrigation water quality indicators that will provide more realistic assessments of the risk of contamination with human pathogens. Inexpensive field deployable methods are highly desirable.

Further studies on the direct impact of flooding on the microbiological safety of leafy vegetables and herbs are required.

More research is needed on the effect of the manure type added to soil and soil management systems on pathogen survival and the effect of different application methods.

No evidence in the literature of internalization from seed to harvest in the leafy vegetables and herb industry has been identified, yet research on decontamination processes for the elimination of *E. coli* from salad vegetables and herbs suggests that seeds are carriers of microbial contaminants. Just how these contaminants are retained during plant growth and carried through to harvest is not clear, and is therefore an area where further insights would be beneficial.

10.2 Harvest

While considerable data is available in the literature on the impact of harvesting on microbiological safety and quality, further information is required on the potential contamination impact of the more recently developed techniques of field coring and trimming of lettuce. Such field practices were considered to have the potential to increase food safety risks for a number of reasons (Section 6.3.1), and therefore investigation of the impact of these practices under different conditions was considered important.

10.3 Packing

Leafy vegetables and herbs may be packed directly in the field or in a packinghouse located in the field or in close proximity. Pre-cooling of fresh produce (e.g. by vacuum cooling) is an essential step in the packing procedure as it maintains product quality and extends shelf life. Vacuum cooling is one means of pre-cooling. However, further research is required into this technique as experimental studies have shown that it may promote the survival in storage of internalized bacteria. The role of these findings *in vivo* must also be examined.

10.4 Processing

Regarding sanitization, research studies should more closely reflect real conditions encountered in commercial processing.

The degree of risk reduction that can be expected from current washing disinfection and packaging technologies is difficult to quantify given the sporadic nature of contamination by pathogens.

Further research is required on the precise mechanisms involved in microbial attachment to leafy vegetables and herbs in order to best direct effort in the development of effective detachment techniques.

Internalization has been found to be possible during pre-harvest under experimental conditions, but only after exposure of young plants (seedlings) to high pathogen loads. There is no evidence indicating that internalization is significant in practice, particularly when GAP is implemented. More information is required regarding non-experimentally-induced infiltration under actual GAP and GMP/GHP conditions in the field and factory.

Further information is required on the positive temperature differential between water and fresh produce (i.e. leafy vegetables and herbs) during washing necessary to minimize the uptake of wash water through stem tissues and open areas in the skin or leaves, whether due to mechanical damage or naturally present.

The lack of significantly effective options other than heat or irradiation, which can result in several log reduction of colonizing or internalized pathogens, requires research focusing on both fundamental attachment mechanisms and inactivation of the pathogens *in situ*.

Many potential process water sanitization approaches are available. However, each will have its own limitations, e.g. unsuitability for particular produce types owing to damage, health and safety issues, and environmental issues, particularly on discharge. Further research is required on the use of sanitizers that are practical and acceptable; the impact that processes have on internalization and attachment of microorganisms (bacteria, viruses and parasites); and the relevance of these in practice.

10.5 Marketing and the cold chain

While there are studies that correlate shelf life with cold chain breaks, there are very few studies that correlate pathogen growth with cold chain breaks. In particular, the effect of cumulative temperature abuse (e.g. more than one occasion where the optimum temperature is exceeded throughout the chain) on the safety of leafy vegetables is unknown. Experimental trials are required to assess the growth of pathogens in real cold chain scenarios (i.e. with fluctuating temperatures) using both mathematical modelling and experimental assessments.

No information was available on the direct impact on foodborne diseases of the application of the cold chain for leafy vegetables or herbs. Epidemiological data needs to be collected and correlation with cold chain failures considered.

Research is needed to assess the growth and survival of foodborne pathogens in commercial conditions (*in vivo*) or in scenarios that represent real-life conditions, to complement the experimental studies (*in vitro*), which often use inocula at high levels and organisms for which the physiological state may be different to natural settings.

While there is information for several whole products, comparisons between whole and cut versions are scarce in the scientific literature, particularly using material of the same batch of produce. The differences in growth and survival of pathogens on (i) whole and fresh-cut leafy vegetables, and (ii) wrapped and unwrapped fresh leafy vegetables during storage are not sufficiently documented and not fully understood. This requires further investigation.

There is uncertainty in assessing the role of RH and microbial behaviour. It is difficult to assess the exact humidity in different parts of packages or un-wrapped (bulk) leafy vegetables. The impact of high humidity below saturation (e.g. 70–100% RH) on bacterial pathogens on leafy vegetables is also unknown.

Experimental trials are required to assess the growth of pathogens considering spatial variability in a bulk presentation (non-packaged), e.g. differences in humidity at the surface of the bin relative to the centre or bottom. Such trials are also required to assess growth of pathogens in real marketing scenarios (reflecting actual contamination levels, temperature fluctuations, etc.), taking into account different handling practices and commercialization times. Both mathematical modelling and experimental assessment are needed.

10.6 The final consumer

Further information is required on cross-contamination from culinary herbs, and subsequent foodborne illness, when used as a garnish on cooked and RTE foods. In addition, further education is required on the correct storage of leafy vegetables and herbs.

11. Response to Codex, and Recommendations

11.1 Response to Codex

As outlined in Section 1, the Expert Meeting was conducted to provide scientific advice to Codex in response to a number of specific questions related to fresh and fresh-cut leafy vegetables and herbs. While the details of how these questions were addressed and the scientific information gathered and used for that purpose have been outlined in the preceding sections of this report, the response to each of the questions is summarized below.

11.1.1 Environmental Hygiene

What is the role of wild animals, especially in high concentrations, as a potential source of contamination? (Section 3.1)

The meeting noted the large number of infectious agents that have been identified in wildlife, including those of concern in relation to the safety of leafy vegetables and herbs. Microbial pathogens may be present in the faeces of wild animals without causing outward signs of illness or disease, making it difficult, if not impossible by visual inspection, to determine if an animal is carrying a specific pathogen. In general, the most abundant species in a particular region are of the greatest concern as the risk of faecal contamination by these animals is the highest. Given the broad host range and the sporadic and unpredictable nature of carriage of foodborne pathogens by both domestic and wild animals, all animals entering leafy vegetable and herb production areas should be considered as potential hazards.

What is the relative contribution from wild animals and other environmental reservoirs as a source of human pathogens in the production environment? (Section 3.1)

Although indistinguishable pathogens have been recovered from animals and leafy produce implicated with human disease outbreaks, it was not considered possible to conclusively determine if the animals were indeed the source of the product contamination or a sentinel of broader environmental contamination that infected the animals and contaminated the crop simultaneously. While most mammalian pests of concern were considered to range fairly close to crops, birds, in contrast, have an ability to transmit pathogens over substantial distances and may present a greater challenge in terms of control. Thus, while the meeting identified wildlife and other environmental reservoirs such as landfill and wastewater and sewage treatment sites, it was not possible to determine the relative contribution of these to contamination of fresh produce. However, it was noted that the relative contribution will vary according to production area, and will also be influenced by other factors, such as topography, hydrology and climatic conditions. Therefore, site-specific assessments of the potential for wildlife and other environmental reservoirs to contaminate a production area need to be undertaken.

What are the most important types of animals and pathogens that they may carry? (Section 3.1)

Risks posed by wild animals and livestock are dependant upon the prevalence, incidence, and magnitude of pathogen carriage in the animal hosts, the degree of interaction between the animals and the growing environment, animal behaviour and ecology. The meeting considered that the most abundant species in a particular region be considered the greatest concern. Numerous studies on the prevalence of pathogens in animals have been undertaken. However, these have not been conducted in every species and every geographical region. Thus it was

considered that the prevalence of pathogens in a particular region may differ significantly from those reported in the literature and based on studies in another part of the world.

E. coli O157 has become an important cause of illness attributed to leafy vegetables and herbs, with ruminant animals among the most common reservoir species for this pathogen. In addition, *E. coli* O157 and other Shiga-toxigenic *E. coli* (STEC) are present in a large variety of domestic and wild animals. STEC have also been isolated from other wildlife, such as rodents, birds (gulls, geese, starlings and passerines), insects (houseflies, beetles) and molluscs (slugs). These may be incidental hosts due to their proximity to ruminant hosts or independent hosts. While the prevalence of transmission between ruminant hosts and associated incidental insects and pests is not certain, experimental studies have been used to demonstrate that flies, for example, are capable of transmitting microorganisms from one source to another.

Salmonella enterica serotypes were also identified as important pathogens with a herd or flock prevalence in domestic animals of between 0% and 90%, depending on the animal species and region. Prevalence in wildlife appears to be much lower, but *Salmonella* has been isolated from both wild animals and birds.

L. monocytogenes is a saprophytic organism found in the environment. Domestic livestock, especially cattle, other small ruminants, wild animals and birds play an important role in the amplification and environmental dissemination of this bacterium.

It was noted that there are a number of other organisms that are occasionally present in the manure of domestic and wild animals including *Yersinia pseudotuberculosis*, *Cryptosporidium*, *Giardia* and hepatitis E virus. While these have not been reported as causes of foodborne disease outbreaks in leafy vegetables, they have been linked with foodborne diseases transmitted by other types of fruits and vegetables. In addition, it is possible that pathogens may be present in animal species that have not been extensively studied, or that novel zoonotic pathogens may emerge. Re-emergence of pathogens is often associated with areas where people, animals and agriculture are in close proximity. This may be of concern in pre-harvest food safety, as excreta may reach crops.

Since the *Norovirus* genus comprises viruses that infect humans, pigs, cattle and mice, the possibility for zoonotic transmission of infection exists. Recent findings highlight a possible route for indirect zoonotic transmission of noroviruses through the food chain.

Is there evidence of a population density above which risk of contamination of fresh produce and subsequent consumer illness is most likely to occur? (Could we apply an Integrated Pest Management approach where "surveys" are routinely conducted for pests in a field, but no action is taken unless the population exceeds a given density for a given pest? (Sections 3.1 and 3.1.4)

Infection dynamics in animal populations depend on whether the animals are maintenance or spillover hosts. The risk of contamination was considered dependant upon the prevalence, incidence and magnitude of pathogen carriage in the animal hosts, the degree of interaction between the animals and the growing environment, animal behaviour and ecology. In general, the most abundant species in a particular region are of the greatest concern as the risk of faecal contamination by these animals is the highest.

Several lines of evidence exist to support the hypothesis that animal density may be an important risk factor in the contamination of crops, although specific information linking animal density with produce contamination is still lacking. Increase in animal density was found to increase the transmission between animals and faecal contamination of the environment, and

may increase environmental contamination with pathogens and increase the risk of environmentally acquired zoonotic infections. Understanding the status of a maintenance or spillover host is significant in order to determine whether the management of diseases in an animal host would affect the risk of microbial contamination of leafy vegetables.

Are there specific times during the production cycle when exposure of the production environment to high densities of wildlife produces the greatest risk that fresh produce will be contaminated? (Section 3.1)

Specific factors were identified that might attract livestock and wild animals, accidentally or intentionally, to crops and growing fields. These would coincide with when the growing fields provide food, water or shelter; adjacent fields provide crops or foods such as insects; cultivation encroaches on their habitat; or when animals are moving between appropriate habitats, such as buffer zones, when livestock are intentionally allowed to forage on crop waste or animals are used for work (horses, water buffalo, oxen), and when livestock and poultry are free-range. Animal behaviours were identified that may predispose increased crop contamination, including predictable and unpredictable events such as animals moving in groups, migration and dispersion patterns, and territorial marking. Control was considered especially important as the crop nears the time of harvest. However, the meeting also noted recent research indicating that leaf age influences bacterial colonization and population levels on lettuce, and that young lettuce leaves may be associated with a greater risk of contamination, thus indicating that control measures are also important when the crop is young or when baby leaves are being grown.

Are there specific mitigations (e.g. removing animal attractants and harbourage in the production environment) that can be used to minimize ingress of wild and domestic animals into growing areas while avoiding significant adverse impacts on native fauna and water shed conservation? (Section 3.1.5)

The meeting highlighted the importance of educating farmers and increasing their awareness and knowledge of, firstly, their production system and environment (e.g. knowledge of the indigenous wildlife and their behaviour in the growing area) as well as their understanding of what constitutes a hazard within that environment, and, secondly, the types of interventions that could be used to mitigate that hazard (e.g. these could range from use of distress devices to selection of a different crop). Region-specific information concerning microbiological hazards present in wildlife, appropriate pest control strategies and environmental regulations would facilitate this.

The Meeting recognized that control of wild animal populations may be difficult or restricted by specific animal protection guidelines, and that these differ between regions of the world. However, to the extent feasible, where wildlife is a concern, practices to deter or redirect wildlife to areas where crops are not destined for fresh produce markets was recommended as a consideration. When low-environmental-impact strategies and traditional low-cost deterrents are not successful, some invasive approaches, such as regulated harvest and culling, wildlife translocation or human relocation, may be necessary. It was found that aggressive depopulation activities may have negative environmental impacts, e.g. relocating animals may result in perturbations in animal behaviours causing increased pathogen dispersion. If the above methods are unsuccessful at reducing or eliminating risks, it was recommended that consideration be given to growing alternative crops in the specific location, or not growing leafy vegetables and herbs at times that animal intrusion is expected to be unavoidable (during particular migration periods, etc.). Crops that are considered less susceptible to contamination, such as vegetables that will be cooked, would have a lower risk for human health.

The meeting identified exclusion and dissuasion of livestock and wild animals from the growing fields as essential, and various approaches to achieve this were identified, as follows:

- Maintain a clean environment to limit optimal rodent habitat; restrict wildlife access to refuse; avoid attracting wild animals to human settlements; prevent wild populations being augmented and artificially sustained by human-induced food availability; manage waste handling collection and transportation; and disposal of carcasses.
- Dissuasive feeding in forests to bait animals for easier removal and to distract them from agricultural fields.
- Physical barriers, such as fencing, may reduce crop damage by wildlife and ingression into the crops; however, it was noted this method can be counterproductive since it may promote high densities and aggregation of the target species. Success depends on the behaviour of different animal species, e.g. burrowing animals breach the barrier and may permit access to other species.
- Repel the wildlife using substances or distress machines, such as those emitting noise or calls (predator calls). These are available in a large variety of formats, including sonic fences for birds and ultrasonic (very high frequency) rodent repellers.
- Restoration of previous ecological situations, such as the re-introduction of predators that formerly inhabited the area. It was noted this may generate social conflicts that can easily outbalance the advocated benefits of this management decision.

Wildlife contraception has been considered, but there is still little information on the reliability of this method under field conditions.

Several lethal methods to reduce wildlife pest populations have been identified, and these have both benefits and limitations. Increased hunting or hunting the target species can reduce wildlife numbers and their risks. Trapping pests is usually ineffectual and inefficient. Care during handling captured animals is needed in order to avoid zoonotic disease risks for personnel. Poisoning may not be very selective. Bait may not be effective when food for wildlife is readily available. Poisoning will always present a danger to livestock and humans.

Are there specific proximity and topographical features, weather events or other considerations that should be considered when assessing the potential for a production area to have a high risk of harvested produce being contaminated with foodborne pathogens? (Section 3.2)

The meeting found there was circumstantial evidence that climate, topology, weather, hydrology and geographical features may contribute to an increase of microbial contamination in leafy vegetables and herbs, although there was little direct evidence. The impact of climate and weather may be complicated through the indirect interactions with other factors such as wildlife populations, pathogen survival in soil, and insect populations. While more work in this area is needed before specific recommendations can be made, the expert meeting did recommend that, prior to farm establishment and planting or changing to the production of leafy vegetables and herbs, an assessment of the site be undertaken in terms of the potential of climate, topology, weather, hydrology and geographical features to contribute to an increased risk of microbiological contamination.

What is the relative importance of fields being in the proximity of animal production facilities, urban and suburban environments, animal refuges, etc?(Section 3.1, 3.5.4)

The likelihood of contamination of fresh leafy vegetables and herbs from animal production facilities, urban and suburban environments, animal refuges, etc., appears to be dependent on multiple factors, including density and size of human and animal populations, the geographical region, location, degree of infrastructure, conditions of sanitation and hygiene, water quality in the area, potential for direct contact between crops and people or animals, or indirect contact via waste material, and the extent of application of GAP. Thus, it is not possible to quantify the relative importance of proximity to such environments, but an assessment of the production and processing sites in the context of these factors would provide valuable information at the local level.

What are the primary vehicles and vectors for transmission of zoonotic, pathogenic micro-organisms from animal rearing facilities to produce production areas?

Livestock and their wastes are the primary source of contamination in animal rearing facilities. Sites for growing leafy vegetables and the vegetables themselves can be contaminated directly by livestock entering the growing fields, and also by indirect means, such as contaminated faecal waste, water, aerosols and dust from livestock production and feeding facilities, and also from other human activities such as landfills and wastewater treatment sites. Wildlife may play a role in dispersal of pathogens from these sources to fields used for vegetable production. The extent to which these sources contribute to the contamination of product are dependent upon other factors, such as climate, topography, hydrology and weather. It was noted also that farm workers and contaminated equipment and transport may be vehicles by which pathogens are transferred from contaminated locations to the growing field.

Are buffer zones a viable risk mitigation strategy and if so, what size zone is required? (Section 3.1.5)

The Meeting reviewed evidence for the use of buffers and clearing of natural vegetation. It was considered that concern and controversy exists about the efficacy of this approach in terms of food safety, as well as the effects on wildlife conservation. The behaviour of the species involved is important, e.g. many rodents will not travel more than 50 m from their nest and living place. However, other wildlife, such as birds and larger animals, may travel many kilometres in search of food. Thus, site-specific knowledge of the wildlife of concern and their behaviour would be required in order to assess whether a buffer zone would be a relevant mitigation strategy.

Is periodic flooding of production areas of concern and, if so, what time intervals are needed before the land is used for the production of different classes of fresh produce?

The Meeting concluded that flooding of production areas was a concern. Preparedness, in particular the establishment of response plans to deal with the adverse effects of accidental or natural flooding is essential. Consideration should be given to the whole food chain. When growing fields have been contaminated or damaged, assessments should be carried out to establish measures to reduce the risk of pathogens (e.g. delayed harvesting or further processing, such as heat treatment) or to assure disposal. Proper disposal of food stocks found to be unfit for human consumption may need to be undertaken under the supervision of appropriate authorities.

If flooding occurs, additional or alternative measures may need to be considered to ensure that (i) wells, septic systems, and water and sewage treatment systems are capable of operating safely and effectively during periods of excessive rainfall; and (ii) crop growing areas are protected from faecal contamination.

Are there specific land uses that pose a risk to subsequent production of fresh produce and what strategies can be employed to mitigate those risks?

Evidence was provided that wildlife and livestock are potential sources of foodborne hazards in growing fields. Any prior use of fields that involve the presence of these animals or their wastes will present a potential risk of contamination. Enteric microorganisms have been reported to survive for over two years in manure-amended soil. However, the likely duration of persistence of the microbial hazards will vary according to temperature, soil type, nutrient availability, the indigenous microflora, etc.

What is the significance of detection of pathogens in the environment where produce is being grown e.g. E. coli O157 in waterways, Salmonella in ponds and canals or ditches in close proximity to growing fields? (Section 3.5)

It was recognized that surface waters, in particular, may at times be contaminated with pathogens. Intervention is required to avoid contact of the contaminated water with fresh leafy vegetables and herbs during growing and processing, to seek measures to prevent contamination of the water source, and to improve the microbial quality of surface waters before use. It was noted that the efficacy of these treatments will have to be validated for the range of pathogens likely to contaminate surface waters.

11.1.2 Soil amendments and fertilizers

Under what conditions can fertilizers derived from animal or human waste be safely employed for the production of fresh and fresh-cut produce? (Section 4.5)

The Meeting found that composting, if done properly, can be a practical and efficient method to inactivate human pathogens in manure. Several countries have established regulatory requirements for composting in their risk management programmes. Further, a sufficient time interval between applying manure and planting the leafy vegetable and herb crops can result in a sufficient decline of pathogens.

However, based on the scientific literature, it was deduced that some manure types and manure handling strategies present a higher risk than others. The risks of prolonged pathogen survival are likely to be reduced in manures characterized by high fibre content, high pH and high level and diversity of background flora, and which are stored under conditions characterized by elevated temperatures, temperature fluctuations and sufficient aeration. These characteristics and conditions are more associated with solid farmyard manure as compared to liquid manure (slurry). The subsurface application of manure is likely to result in a faster pathogen decline. Although factors like ammonia gas, desiccation and microbial antagonism are suggested to contribute to pathogen decline in bio-waste, the combination of time and temperature is generally thought to be the most effective in reducing or eliminating pathogen loads.

What criteria and testing requirements should be employed to verify that fertilizers derived from animal waste are free of potential pathogens? (Section 4.5)

Composting is the most common method used for reduction of the risk of contamination in manures used as fertilizers. The criteria and testing requirements used for composting are combinations of compost processing parameters including the minimum temperature the compost should reach, the duration of holding at that temperature and a requirement for turning or mixing of the compost to ensure complete composting. These parameters vary with the composting system, and the C:N ratio may also be included.

In contrast to water and plant material, the use of generic *E. coli* as an indicator organism for pathogen presence is questionable for substrates like soil and manure, where *E. coli* has a natural niche. Human pathogenic bacteria were reported to survive longer during composting treatments compared to indicator organisms such as *E. coli*. With non-thermal inactivation, higher levels of native coliforms in manure were shown to be associated with shorter survival times of *E. coli* O157:H7. Thus, generic *E. coli* can be considered unsuitable as an indicator organism. Although expensive and time consuming, the most reliable strategy to determine pathogen presence or absence is classical or molecular microbiological testing of samples.

Does the use of "green" fertilizer (i.e. composted plant waste) represent any significant risk in relation to increasing the likelihood that pathogenic microorganisms will be present on fresh or fresh-cut produce?

It was found that the effects of these soil amendments on the inactivation, survival or growth of human pathogens has been studied to only a limited extent. The evidence available indicates that bacterial foodborne pathogens are able to survive for extended periods in some composted plant waste. This can be dependent on the composition of the compost and the composting process used.

Does the "ploughing under" of field waste represent any significant risk in relation to subsequent crops having an increased likelihood that pathogenic microorganisms will be present on fresh and fresh-cut produce? (Sections 4.3.2 and 6.3.1)

While no direct evidence was found to assess "ploughing under" of field waste, it is noted that produce at harvest may be contaminated (Section 6.3.1) and these contaminants would therefore be ploughed in with the plant material. Subsequent persistence and growth would vary with the pathogen and soil environment (Section 4.3.2).

11.1.3 Water

What are the primary hazards associated with fresh produce for which water is an important source or vehicle? (Section 5.3)

A range of microbiological hazards were identified as being transmitted to humans through contact with or the ingestion of contaminated water; however, the role of contaminated water used in the production of leafy vegetable and herb crops as a vector for the transmission of foodborne pathogens to humans was found to be less clear. Excreta-related bacterial species, including *Salmonella enterica*, *E. coli* O157:H7, *Campylobacter* spp. and *Yersinia* spp., intestinal helminths (*Ascaris lumbricoides, Trichuris trichuria*), amoebae (*Entamoeba coli*) and protozoa (*Giardia intestinalis*, *Cryptosporidium parvum*, *Toxoplasma gondii*, and *Cyclospora cayetanensis*) are associated with recurrent outbreaks of disease in different parts of the world. Waterborne viral epidemics have not been confirmed to date, although some species of enteric viruses have been detected in natural waters sources, raw or treated wastewater and groundwater collected in wells.

What is the relative risk associated with different forms of irrigation and what are the conditions under which these forms of irrigation can be safely employed? (Section 5.3.4)

The impact of different irrigation strategies (overhead sprays, drip irrigation systems or flooding of fields through furrows) on the incidence of pathogens in leafy vegetable and herb crops is not well understood, and evidence in the literature is contradictory. Despite the lack of corroborating scientific evidence, there was general agreement that subsurface irrigation lowers the risk of transfer to growing plants. There is a need for additional scientific information to

address these issues, specifically the risks associated with overhead application compared with other forms of irrigation. In the absence of new, scientifically-validated irrigation strategies, current WHO and country guidelines should continue to be applied.

What are the relative risks associated with different sources of water used for irrigation? (Section 5.3.2)

From the available evidence, the risk of contamination of leafy vegetables and herbs with irrigation water from different sources generally increases according to the following ranking:

1. Potable or rainwater.

2. Groundwater collected in deep wells.

3. Groundwater collected in shallow wells, due to inadequate installation or improper maintenance.

4. Surface waters, particularly in proximity to animals, human habitation and their wastes.

5. Raw or inadequately treated wastewater.

Does the distribution system substantially contribute to the risk of contamination? (Section 5.3.4)

Passage of contaminated water through irrigation equipment can contaminate the system. Persistence of bacteria and viruses in irrigation pipes and growth of bacterial pathogens in stagnant systems have been described.

What are the practical, cost-effective strategies that can be employed to protect water supplies and their distribution systems and to minimize the potential for agricultural water to serve as a source of contamination of fresh produce or spreading contamination in the production environment? (Section 5.5)

Protection of surface water and groundwater resources from pollution (wildlife, waste from animal production, agricultural run-off, human activity, sewage and industrial effluent) was identified as essential for the production of safe leafy vegetables and herbs.

Appropriate management measures (e.g. restriction of livestock feeding, watering and grazing; location of water uptake in relation to potential sources of contamination) are required to ensure the quality of irrigation water. Additional protection of water sources from seepage, by the lining of canals, for example, may be warranted where water supplies are delivered in peri-urban or mixed agricultural areas. Other options have been considered to improve microbial quality of surface waters, such as sand filtration or storage in catchments or reservoirs to achieve partial biological treatment before use.

Further mitigation measures have been proposed to ensure the quality of irrigation water in the absence of reliable supplies. One measure to be considered closely is the advantage of abstracting water without disturbing sediments, as human pathogens can settle and survive in large numbers in this environment

Is there evidence of a time interval between exposure of the crop to a given quality of water and harvest of fresh produce at which the risk is higher or lower? (Section 5.3.3)

The interval between final irrigation and harvest influences the extent of contamination, as pathogens have been shown to decline with time following cessation of irrigation before harvest. The rate of decline depends on the pathogen of concern. Helminths and enteroviruses appear to survive for the longest periods after cessation of irrigation. Helminth populations on leafy

vegetables declined daily following cessation of manual irrigation. There is currently insufficient data to provide effective guidelines that would apply to the range of leafy vegetables and herbs grown in various regions of the world.

What national and international microbiological criteria currently exist for different agricultural water sources and how effective are these criteria for mitigating the risks associated with their use with fresh produce? (Section 5.5)

Requirements for testing of irrigation waters and microbiological standards applied to the production of vegetable crops were found to vary widely between jurisdictions; were variable for groundwater, surface water or reclaimed wastewater; and sampling strategies for testing were also inconsistent. In terms of international guidance, WHO recently established guidelines for the use of wastewater in agricultural production, indicating that wastewater used for unrestricted crop irrigation purposes should contain $\leq 10^3$ thermotolerant coliforms/100 ml and ≤1 helminth egg per litre. The guidelines specifically address irrigation of vegetables that are consumed without cooking. Measurements of the efficacy of existing guidelines were not available.

Are there additional criteria that would be beneficial? (Section 5.4)

While the Meeting did not identify additional criteria, it noted current inefficiencies. Existing criteria for the microbiological quality of water are based on the presence of coliform bacteria and *E. coli* and it remains unclear how these indices correlate with the presence of specific human pathogens. Measurements derived from methods applied for the detection of both bacterial groups will undoubtedly continue to be used in the absence of better indicators of water quality. There is an urgent need for the development of irrigation water quality indicators that will provide more realistic assessments of the risk of contamination with human pathogens. Inexpensive field deployable testing methods are highly desirable.

Are there specific time intervals or events after which water sources should be tested? (Section 5.5)

Seasonally adjusted testing schemes may be necessary in some regions to ensure the consistent quality of irrigation water supplies. The rate of testing should be enhanced when there is a change in the source of irrigation water or following severe climate events, such as heavy rains or flooding. Water at higher risk of change in microbiological quality due to proximity to animal production, potential sources of agricultural run-off or human habitation requires testing more frequently.

What are the relative risks associated with other uses of water in the primary production environment (e.g. pesticide applications, cleaning of equipment)? (Section 5.3.5)

Evidence was found that human pathogens can survive and grow in insecticide, herbicide and fungicide solutions and that their application to the surface of leafy vegetables constitutes a risk, particularly near harvest time. The transfer of pathogens from contaminated water to inert surfaces has been examined in detail in both the food processing and home environments. In contrast, little is known about the surface-associated behaviours of waterborne pathogens in the agricultural environment. In the absence of clear experimental evidence to the contrary, it is prudent to consider that the transfer of pathogens between water and agricultural equipment is likely. Surfaces routinely rinsed or cleaned with water include tools and harvesting equipment, including mechanical harvesters.

How effective are current criteria for the use of agricultural water sources for nonirrigation uses in mitigating the risks associated with their use with fresh produce? (Section 5.5)

The Meeting was not able to access and assess other criteria; however, it was considered desirable for water that is in contact with produce through the food chain be of potable quality.

What are the relative risks associated with uses of water in the packing environment?

Leafy vegetables and herbs may be sprayed with water in the field after harvest. It is uncertain whether water spraying under certain pressures would cause pathogen internalization, especially for field processed produce (e.g. cored lettuce) and further research is required. A risk ranking of water from different sources is provided above, and potable water is required for application at harvest and post-harvest processing. Water is used widely in packinghouses for cleaning produce, equipment and surfaces, for transport of produce and cooling, and in packing ice. Foodborne pathogens have been detected in unchlorinated wash water, and unchlorinated municipal water recirculated through a hydro-cooler and used for ice in a packing shed has been implicated in an outbreak. As leafy vegetables and herbs may be ready-to-eat at this stage or further processing may not remove contamination, use of potable water is required.

How effective are current criteria for water uses in the produce packing environment? (Section 6.4.1)

There is evidence that produce may be contaminated at harvest and thus on entry to the packing-house. It is recommended that only potable water should be used in food handling and processing. However, sanitized water may also be used provided that it is treated and maintained in such a condition that no risk to the safety and suitability of the food results from its use. While the use of such water is required to prevent further introduction of contaminants, it can also reduce the total viable counts on the produce by 1–2 log cfu/g. While the reduction of pathogens can be difficult to quantify in reality, experimental data indicates small reductions do occur. However, the extent of reduction will vary according to the type and level of disinfectant used in the water, and there are restrictions on the use of disinfectants such as chlorine in some countries. It is not known whether low levels will reduce pathogen numbers and whether disinfectants will inhibit pathogen growth while maintaining produce quality.

What is the potential for water used for transport of produce in the packing environment (e.g. fluming) to serve as a means of cross-contamination? What are the conditions of use that mitigate this potential?

Evidence specifically for leafy vegetables was not found.

What are the conditions of water use that foster infiltration of pathogenic microorganisms into fresh produce and how can this be avoided? (Section 7.4.3)

From experimental evidence, infiltration of microbial cells due to a negative temperature differential between the water and vegetables would appear to be possible. The demonstration of the efficacy of a positive temperature differential (i.e. produce tissue at lower temperatures than that of the washwater) has not been reported in the literature for leafy vegetables specifically, although it has been reported for other vegetables and this approach is adopted in industrial practice.

What is the level of uptake of microorganisms that can be expected in the absence of factors contributing to infiltration? (Section 7.4.3)

Pathogens have been shown to attach to both the cut and intact surfaces of leafy vegetables. The level of uptake and growth on leaves has been shown experimentally to vary with the leaf

variety and microorganism. Important factors in determining the greatly varying abundance of particular bacterial species on plants depend on the level of epiphytic bacteria present and subsequent competition among a diverse bacterial microflora for limiting resources or differential tolerance of environmental stresses. Cut surfaces may provide additional receptors for microbial attachment.

Experimental work has shown that the number of bacteria attached to cut produce surfaces after 2 minutes of contact is proportional to the inoculum concentration raised to the power of 0.79, with the degree of attachment reaching a plateau generally after one hour of exposure. The rate of attachment was independent of temperature. Further research is required to more precisely define the extent and mechanisms of attachment.

What is the efficacy of water washes on the removal of pathogenic microorganisms from fresh produce?

The Meeting considered washing of leafy vegetables and herbs has the potential, if properly controlled, to result in some risk reduction through reduction of the overall microflora. However, it was noted this will not result in elimination of contamination; therefore minimizing the opportunity for contamination in the field from the seed onward is required.

Washing in potable or sanitized water may result in 1–2 log reductions in total bacterial populations; however, it was considered the degree of risk reduction that can be expected from current washing, disinfection and packaging technologies was difficult to quantify given the sporadic nature of contamination by pathogens.

Difficulties were encountered in assessing the efficacy of washing leafy vegetables and herbs as studies were often carried out using widely differing protocols, which may not reflect natural conditions. For example differences occur in microbial loads, the degree of microbial attachment, the effects of leaf cultivar or species, growing or distribution conditions, and the efficacy or scale of washing systems used in commercial preparation. An overview of the data available on the efficacy of a range of sanitizers is provided in Annex 3.

11.1.4 Personnel health, personnel hygiene and sanitary facilities

What is the potential for farm workers to serve as a source of contamination for fresh and fresh-cut produce? (Section 6.3.1)

Field workers, whether healthy carriers of pathogens, ill or convalescing, may be a source of contamination if poor sanitation or lack of facilities prevails. The disease agents will vary with the epidemiology of foodborne disease in the workers' community. While there was no outbreak identified confirming contamination by a field worker, epidemiological evidence has been used to suggest this occurs.

The Meeting noted that casual visitors to the field, especially children, may be an important source of contamination. Infection rates of some diseases may be higher in specific age groups, e.g. in endemic areas, hepatitis A infection rates are higher in young children who are asymptomatic, and these groups may present a greater risk if present in the harvest environment.

What is the potential for food workers in packaging, processing, distribution, and marketing facilities to serve as a source of contamination for fresh and fresh-cut produce? (Section 6.3.1)

These processes may involve multiple opportunities for exposure of product to food workers and the risks are similar to those for field workers. Poor hygiene practices of food handlers

when preparing food have been suspected as the route of contamination in investigations of foodborne outbreaks associated with leafy vegetables and herbs.

Can public health data on the incidence and prevalence of enteric or parasitic disease among farm workers and food workers and characterization of carrier status provide useful surveillance systems that need to be in place to collect such data?

The Meeting did not have evidence to support this. Disease surveillance on the incidence and prevalence of enteric or parasitic disease in the community will provide information on the potential hazards that may be introduced and that need to be managed.

What mitigation strategies (e.g. improved health status, provision of toilet and hand washing facilities, training and accountability, protective clothing) are available to reduce the risk of foodborne disease attributable to farm workers as a source of contamination, and what are the relative risk reductions that can be achieved by these mitigations?

The Meeting identified many training programmes on GAP, GMP and GHP with elements of training on personal hygiene and provision of facilities and equipment. The main focus for training programmes has been on capacity building and delivery of training materials to improve awareness, knowledge and skills. The extent to which training programmes have been evaluated for efficacy and impact on risk reduction is not known. Health screening of employees, for example via a questionnaire, was discussed as a possible mitigation strategy and one that is already used in some places. While this might be a useful tool to identify potentially high risk workers and deploy them to non-food contact activities, this also presents a number of challenges in terms of its implementation and ensuring any personal information is used appropriately.

11.1.5 Packing and post-harvest process operations

Does conducting post-harvest processes (e.g. removal of wrapper leaves, coring) in the field at the time of harvesting represent any increased risk of contamination of fresh or fresh-cut produce?

Although field coring and removing wrapper leaves may reduce contamination as outer or damaged leaves may harbour microorganisms, it was considered that field coring may potentially increase food safety risks for a number of reasons:

- field coring exposes internal tissues to the field environment and increases vulnerability to pathogen contamination and growth;
- field cored produce is processed usually into fresh-cut products directly without any additional in-plant sorting and inspection;
- pathogens that are internalized or attached to cut surfaces are extremely difficult to remove by subsequent sanitation procedures;
- field coring equipment and apparatus may be a source of cross-contamination; and
- sanitary conditions of field environments are more difficult to maintain compared with in-plant food processing environments.

Although a significant data gap exists, unpublished data have shown that a contaminated coring knife could transfer *E. coli* O157:H7 to up to 20 successive heads, and that the bacterium can grow significantly on cored areas within 4 hours at 30°C. Thus this is an area where more work is needed in order to get a better understanding of the potential risks.

Do current technologies and practices effectively eliminate any increased risk? (Section 7.2)

During processing, leafy vegetables and herbs may be exposed to further risk of microbial contamination from workers, surfaces, equipment, water and aerosols, and microorganisms may persist and grow. Processes have the potential to provide a reduction of microbial risks (e.g. washing), provide control of amplification of risks (e.g. chilling), and protect the product from further exposure (e.g. packaging). An extensive review of experimental studies of processing technologies was undertaken and current technologies or practices except for irradiation and heating were not found to effectively eliminate any increased risk incurred during packaging and post-harvest processing of fresh and fresh-cut leafy vegetables and herbs. According to industry experience, and from extrapolation of laboratory experiments, only a slight risk reduction appears possible. The main food safety aim of post-harvest handling is prevention of increasing risk.

What washing or disinfection mitigation technologies are currently available, feasible and practical for reducing the levels of pathogenic microorganisms on fresh and fresh-cut produce? (Section 7.4.2)

Chlorine-based sanitizers have been the most commonly used chemical disinfectants. Other washing technologies include, among others, the use of ozonated water, acidic electrolysed water, electrolysed/oxidizing water, and organic acids. A summary of available sanitizers is provided. Each has limitations, which may include: acceptance by regulators and by the public; quality impact on the product; cost; worker safety; and material effects on equipment. A disinfection process has to be selected on a case-by-case basis taking these limitations into account.

What degree of risk reduction can be expected from these technologies? (Section 7.4.2; Tables A3.7 and A3.8 in Annex 3)

Numerous experimental studies have been undertaken of washing fresh produce and the use of a variety of sanitizers. However, limited comparison could be made since these studies are often carried out using widely differing protocols, which may not reflect natural conditions such as microbial loads, the degree of microbial attachment, the effects of leaf cultivar or species, variations in strains and inocula, growing or distribution conditions, or the efficacy or scale of washing systems used in commercial preparation. While the reductions were greater with the use of sanitizers compared to water alone, the differences were not large in all cases. Most studies report reductions in total aerobic plate counts in lettuce pieces of ≤ 2 $\log_{10}$ cfu/g when less than 200 ppm HClO (one of the most commonly used sanitizers) is used. The reductions in *E. coli* (including the O157 serotype) were mostly less than 1.5 $\log_{10}$ cfu/g, and higher if an additional sanitizer was added. *L. monocytogenes* was reduced by 0.7 $\log_{10}$ cfu/g when washed with chlorine added at 100 ppm for 1 min compared with 0.5 log reduction in water alone. The greatest reduction in salmonellae, 3 $\log_{10}$ cfu/g, was achieved using 1600 ppm for 5 minutes, while reductions were less than 1.2 $\log_{10}$ cfu/g using lower concentrations and with mild heat. Other compounds provided greater reductions, but practical considerations in their use have to be considered.

In addition to lack of exceptional performance, barriers to commercial uptake include absence of regulatory approval; consumer acceptance (e.g. chlorine-based agents or irradiation in some countries); negative impact on organoleptic quality (e.g. allylisothiocyanate odour); cost; scale-up difficulties; health and safety risks for workers (e.g. ozone, gaseous treatments); and practical application.

Does infiltration of pathogenic microorganisms into the interior of the produce play a significant role in reducing the effectiveness of washing and disinfection treatments designed to reduce contamination? (Section 7.4.3)

Infiltrated (internalized) bacteria would resist sanitization, although the microbial reduction effect of sanitization appears to be limited from the studies described above. Based on the evidence available it is not known whether bacteria that have entered plant tissue through infiltration can grow to a significant extent under real or expected storage or handling conditions compared with the experimental conditions used.

What additional technologies are available for reducing the levels of pathogenic micro-organisms on fresh and fresh-cut produce? What degree of risk reduction can be expected from these technologies? Are there any barriers to their application?

Other approaches identified for pathogen reduction on vegetables include the use of bacteriophages, physical barriers in packaging, radiation, ultrasound, ultraviolet light and heat. Heat or irradiation, which can result in several log reductions, may eliminate colonizing or internalized pathogens. The level of reduction is related to the dose applied, which has to be balanced against the effect on quality. Irradiation is not legally accepted in all countries and may have poor public acceptance. The other technologies were reported to provide not more than 1.5 log reductions, and are often applied in combination with another hurdle. However, these technologies vary in terms of both their efficacy and application in different plant varieties, and may affect quality attributes.

11.1.6 Maintenance of the cold chain

What portion of the risk of foodborne disease associated with fresh and fresh-cut produce is attributable to failure to maintain the cold chain (Section 8.0)?

There was no information available on the direct impact of the cold chain for leafy vegetables or herbs as a cause of foodborne diseases. Epidemiological data needs to be collected, and correlation with cold chain failures considered. Data describing commercial conditions and naturally occurring pathogens is limited.

Are there any practical technologies that are available that can be used by industry, competent authorities or consumers to verify that fresh and fresh-cut produce have been maintained under continual refrigeration (Section 8.5)?

Technologies that are available include temperature indicators, data logger temperature sensors (e.g. Tinytags™), and radio-frequency identification (RFID). Most of these technologies are used to monitor the chain up to the retailer. While expanding the use of such technologies to monitor through to consumers would be desirable, the indicators used would need to be clear, and easy to interpret and understand, in order to avoid confusion and to ensure a clear message about shelf life. Other issues to be taken into consideration include their validation for use post-retail under a diverse set of conditions, and liability in the case of malfunction leading to foodborne illness.

Is there increased risk of foodborne disease associated with further extending the shelf life of fresh and fresh-cut produce (Section 8.3.3)?

Leafy vegetables and herbs kept under high humidity (e.g. sealed packaging) and short-term non-refrigerated storage can support the growth of pathogenic bacteria with up to 1 log increases reported within a day. Therefore, even a short shelf life would not prevent an amplification of bacterial pathogens on leafy vegetables stored without refrigeration.

Any increase in shelf life would need to be determined on the basis of the time that the food remains safe while still meeting the quality parameters. Thus, increasing the shelf life was considered to have the potential to increase food safety risks if the extended time allows further growth or survival of the pathogen. Shelf life extension may increase the risk of further contamination whenever products are not protected by packaging. In addition, longer shelf life may increase the risk of temperature abuse during storage. Evidence for differences between the shelf life of fresh and fresh-cut product was variable and depends on the temperature and relative humidity.

11.2 Conclusions and recommendations

Following the review and analysis of the current information and data on fresh and fresh-cut leafy vegetables and fresh herbs, and addressing the questions posed by Codex in the areas of environmental hygiene, including wild animals, fertilizers, water use, personnel health and hygiene, sanitary facilities, packing and process operations, as well as the issue of the maintenance of the cold chain and education, the meeting reached the following conclusions, and made the recommendations below based on these.

Firstly, it was noted that extensive guidance is already available in terms of improving the safety of leafy vegetables and herbs and therefore the meeting recommended that countries and the industry should

- Implement the recommendations of the Codex Code of Hygienic Practice for Fresh Fruits and Vegetables (CAC, 2003b) and the Codex Recommended International Code of Practice – General Principles of Food Hygiene (CAC, 2003a).

Foodborne pathogens may be present in the faeces of asymptomatic domestic and wild animals. Therefore, all animals entering leafy vegetable and herbs production areas should be considered a hazard. Wild animals have been linked to contaminated leafy vegetables responsible for large outbreaks. However, it has not been possible to conclusively determine if they were the source or if there was a common environmental source of contamination. While controlling wild animal populations may be difficult, mitigation strategies to dissuade or exclude wildlife from growing fields could be considered. In terms of the growing environment, local climatic and geographical characteristics could affect the microbiological contamination of leafy vegetables and herbs but too little are known about the impact of these factors at this stage. Contamination of leafy vegetables can also be due to livestock wastes and their use on fields. Similarly, farm workers and their equipment can also be a source of contamination. Leafy vegetables and herbs may be fertilized with manures from animals, humans and bio-wastes provided they are properly composted and applied. This is a practical and efficient means for disposal of this waste. In light of these issues, the meeting recommended that:

- Before planting, consideration should be given to the prior use of the land, with a risk assessment prior to the cultivation of new or alternative crops.
- A risk assessment on the impact of climate, topology, weather, hydrology and geographical features on the microbiological contamination of leafy vegetables and herbs during the growing phase should be undertaken prior to planting.
- Practices to deter or redirect wildlife to areas where crops are not destined for fresh produce market be considered.
- Efforts be made to avoid cross-contamination of farm land with potentially contaminated close-by environmental sources.

- Areas prone to flooding during the growing season should be avoided.
- When growing fields have been contaminated or damaged (e.g. by flooding), an assessment of the risk be undertaken to establish measures to reduce the risk of pathogens (e.g. delayed harvesting, heat treatment of produce) or to ensure disposal.
- Consideration be given to additional or alternative measures to ensure (i) wells, septic systems and water and sewage treatment systems are capable of operating safely and effectively during periods of excessive rainfall; and (ii) crop growing areas are protected from faecal contamination.

The impact of different irrigation systems in the growing fields of leafy vegetables is not well understood. However, subsurface irrigation that protects to some extent the leaves of vegetables from being sprayed most probably lowers the risk of contamination of leafy vegetables. Because leafy vegetables and herbs can be considered ready-to eat, it is important to use water of potable quality during harvest and subsequent packing and processing. Thus the meeting recommended that:

- Surface water and groundwater resources be protected from pollution or decontaminated prior to use, and the efficacy of these measures ad treatments be validated for the range of pathogens likely to be a source of contamination.
- In the absence of reliable water supplies, consideration should be given to abstracting water without disturbing sediments, as human pathogens can settle and survive in large numbers in this environment. This practice has the potential to improve the quality of water used on the crop and reduce exposure of farm workers to pathogens.
- Consideration be given to new indicators of irrigation water quality that will provide more realistic assessments of the risk of contamination with human pathogens. Inexpensive field deployable methods are highly desirable.

Post-harvest operations cannot effectively eliminate foodborne pathogens from leafy vegetables and herbs. They should aim at preventing any increase in the risk for consumers. Temperature is the single most important factor contributing to bacterial growth and survival. Therefore, temperature control and maintenance of adequate cold chain conditions are critical to food safety. In light of this the meeting recommended that:

- Increased emphasis should be placed on education and training concerning the role of cold-chain maintenance during distribution, along with advancing knowledge and technologies for both refrigeration and temperature monitoring.

Field workers, food workers and visitors to the field or processing areas can be an important source of contamination, particularly if poor hygiene practices exist. Therefore the meeting recommended that:

- Harvest, processing and packing areas be restricted to essential personnel only.
- Measures are taken to create greater awareness among all workers (from growers to food handlers and consumers) associated with fresh produce production, packing, processing, distribution, storage, retail and catering of the risks associated with contaminated fresh produce and the need for preventative control measures along the food chain.

Education on food safety is important for all participants in the food chain for leafy vegetables and herbs from the farmer to the consumer. Thus the meeting recommended:

- Investment in training and education on food safety for agricultural workers through to consumers, and evaluation of training programmes to gain feedback on adoption and effectiveness in improving GAP, GHP and GMP.
- A systematic approach to education and training throughout the leafy vegetables supply chain from farm to fork. An evidence-based approach to developing, implementing and evaluating food safety education interventions need to be considered.
- High-risk food safety practices and behaviours be targeted in education interventions.

Finally, although there was a substantial amount of information available, the meeting identified a number of data gaps and noted that while immediate actions can be taken to reduce the food safety risks associated with leafy vegetables and herbs, addressing these data gaps will enable the refinement of existing mitigation strategies, as well as the identification of new strategies. Therefore the meeting recommended that:

- Further research be undertaken that can be extrapolated to current pre-harvest and post-harvest practices for leafy vegetables and herbs.

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ANNEX 1

Botanical and common names for some leafy vegetables and herbs

Botanical name	Common name(s)
	Leafy Vegetables
Lactuca sativa	lettuce (cos, iceberg, romaine, baby, red and green butter, red and green leaf, oak)
Cichorium endivia	endive, Belgian endive, witlof (Australia), witloof (USA), French endive
C. endivia var. *crispa*	endive
C. endivia var. *latifolium*	escarole
C. pumilum and *C. intybus*	common wild chicory, radicchio, puntarelle
Spinacia oleracea	spinach, baby spinach, English spinach, Chinese spinach
Brassica oleracea (Capitata Group)	cabbage
Brassica oleracea (Acephala Group)	kale, borecole
Eruca sativa (syn. *E. vesicaria* subsp. *sativa* (Miller) Thell.; *Brassica eruca* L.)	arugula, rocket
Brassica juncea	Indian mustard, brown mustard, mustard greens, red mustard (Thailand)
Beta vulgaris	Beet[root], mangold
Beta vulgaris subsp. *cicla*	chard, Swiss chard, silverbeet, perpetual spinach
	Herbs
Ocimum basilicum	basil
Mentha ~ 25 species	mints
Coriandrum sativum	coriander, Chinese parsley, cilantro
Petroselinum crispum	parsley (curly)
P. crispum var. *neapolitanum*	parsley (flat leaf, Italian)

ANNEX 2

Table A2.1 Microbial hazards associated with leafy vegetables and herbs.

Microbial hazard	Produce
Bacteria	
Campylobacter jejuni	lettuce
Clostridium botulinum	cabbage
Shiga-toxigenic *Escherichia coli* (including O157)	(coleslaw source?), lettuce, spinach, parsley?
Listeria monocytogenes	cabbage (coleslaw), lettuce
Salmonella enterica	lettuce, cabbage (coleslaw), fresh spices, coriander
Shigella sonnei	lettuce, parsley
Staphylococcus aureus	leafy vegetables
Vibrio cholerae	cabbage
Yersinia pseudotuberculosis	lettuce
Parasites	
Cyclospora cayetanensis	lettuce, basil
Giardia lamblia	lettuce
Fasciola hepatica	watercress
Viruses	
Calicivirus	leafy vegetables
Hepatitis A	lettuce, watercress
Norovirus, SRSV	cabbage (coleslaw?), watercress
Rotovirus	leafy vegetables

SOURCES: Compiled from: Harris *et al.,* 2003; EU Risk Profile, 2002; CDC, 2006; and country reports

Table A2.2 Country reports of outbreaks attributed to leafy vegetables and herbs and total number of outbreaks attributed to fresh produce from 1996-2006 (unless specified)

Country	Leafy vegetables and herbs [a] [b]	Herbs [b]	Total fresh vegetable outbreaks or suspected outbreaks
Australia	16 complex (64)		25
Brazil	18 (75) (+17 salads)		24
Canada	3 (12)	5 (20)	25
CSPI [d]	191 (70)		272
Denmark	0 (0)		1
Finland	13 (33)		40
France	1 (50)		2
Japan	0 (0)		1
Netherlands			76
New Zealand	3 (23)	2 (15)	13
Poland [c]	Not specified		33
Sweden	5 (22)	1 (4)	23
USA	24 (24)	6 (6)	98

NOTES: (a) Includes complex dishes with leafy vegetables and other ingredients. (b) Numbers in brackets are percent of total fresh vegetable outbreaks or suspected outbreaks. (c) No data for 2006; may include fresh and preserved produce. (d) Dta from Center for Science in the Public Interest (CSPI) 1998–2005.

Table A2.3 Examples of fresh leafy vegetables and herbs reported to be associated with foodborne illness outbreaks (1970 – 2007).
NOTE: Where information on number of cases, deaths, country, etc., was not cited, those fields have been left blank.

Year	Product	Aetiological agent	Contributing factors	Cases (deaths)	No. of out-breaks	Country	Reference
Fresh leafy vegetables							
	Coleslaw, onions	*Salmonella* Agona					Clark et al., 1973
1970	Watercress, wild	*Fasciola hepatica*	Intermediate snail host, *Lymnaea truncatula*, found in growing waters contaminated by infected cattle and sheep	44	1	UK	Hardman et al., 1970
1979	Lettuce, celery, tomatoes	*L. monocytogenes*	Lettuce contamination only possible., hospital patients	23	1	USA	Ho et al., 1986
1981	Cabbage salad	*L. monocytogenes*	Sheep faeces contaminating field	66 (18)	1	Canada, USA	Schlech et al., 1983
1986	Lettuce, shredded	*Shigella sonnei*	Food handler possible source. Laboratory experiments showed that *Shigella* multiplies on shredded lettuce at room temperature.	347	1	USA	Bean et al.,1990; Davis et al., 1998
1986	Lettuce, salad	Hepatitis A	Infected food handler with poor hygiene (lettuce was shredded by hand)	103	1	USA	Lowry et al., 1989
1987	Cabbage (MAP, shredded)	*Clostridium. botulinum*			1	USA	Solomon et al.,1990
1988	Lettuce, iceberg	Hepatitis A	Contamination suspected to have occurred prior to distribution (possible source Mexico)	202 (3 restaurants)	1	USA	Rosemblum et al., 1990
1993	Garden salad (carrots, iceberg, romaine lettuces, endive)	Enterotoxigenic *E. coli* O6:NM		>128	3	Mexico; USA	CDC, 1994; Nguyen-The and Carlin, 1994
1993	Sliced raw vegetables	*Giardia intestinalis*	Office setting	19 lab +8	1	USA	Mintz et al., 1993
1994	Lettuce	*Shigella sonnei*	Imported from Spain. Isolated *Salmonella* and *E. coli* from lettuce, *Shigella* ND	110 culture confirmed	1	Norway, (Sweden)	Kapperud et al., 1995; Frost et al., 1995;
1994	Lettuce, iceberg	*Shigella sonnei*	Imported from Spain.	218	1	UK	Little & Gillespie, 2007; HPS, 1994

Year	Product	Aetiological agent	Contributing factors	Cases (deaths)	No. of out-breaks	Country	Reference
1995	Lettuce leaf	*E. coli* O157:H7	Irrigated with surface water. Poor handling at grocery store	29	1	USA (Montana)	Cited by De Roever, 1998
1995	Lettuce iceberg	*E. coli* O157:H7	Possible cross-contamination from meat	30	1	USA (Maine)	Cited by De Roever, 1998
1996	Lettuce iceberg	*E. coli* O157:H7	Isolates from both outbreaks were indistinguishable; PFGE possibly same grower; Numerous GMP violations were observed in the on-farm processing facility; potential bovine and avian faecal contamination	≤26 20	1 1	USA (Illinois) USA (Connecticut)	Cited by De Roever, 1998
1996	Lettuce	*Campylobacter jejuni*	Suspected cross-contamination with chicken	14 (0)	1	USA	CDC, 1998
1997	Lettuce, iceberg	*E. coli* O157:H7				Canada	CCDR, 1997
1997	Lettuce, mesclun (mixed)	*C. cayetanensis*	Unidentified		2	USA	CDC, 1997a; Cited by De Roever, 1998
1998	Lettuce, iceberg	*Y. pseudotuberculosis* O:3	Likely from contaminated irrigation water or animal faeces (wild deer)	47 (1)	1	Finland	Nuorti *et al.,* 2004
1999	Lettuce	*E. coli* O157	Not reported. Lettuce from central Europe	37	1	Sweden	Welinder-Olsson et al., 2004
1999	Fresh salad vegetables	Norovirus	No physical separation raw, non-disinfected foods and prepared salads., poor personal hygiene of ill food handler	159	1	Israel	Grotto et al., 2004
2000	Lettuce	*Salmonella* Typhimurium DT104	Could not be confirmed; problem with traceability	361		UK	Horby et al., 2003
2000	Lettuce, iceberg	*Salmonella* Typhimurium DT204b	Imported lettuce	392	2	England, Wales; Scotland; Iceland; Germany the Netherlands	Crook et al., 2003
2000	Salad (lettuce and fresh green leafy herbs)	*C. cayetanensis*	Imported lettuce. Most probably contaminated through human waste	34	1	Germany	Doller et al., 2002

Year	Product	Aetiological agent	Contributing factors	Cases (deaths)	No. of out-breaks	Country	Reference
2001	Lettuce (in salad mix)	*Salmonella* Bovismorbificans	Deficiencies in equipment cleaning; *Salmonella* found in food residue on lettuce shredder	41	1	Australia (Qld)	Stafford et al., 2002
2001	Salad pre-pack RTE	*Salmonella* Newport		9	1	UK	O'Brien and Fisher, 2001
2002	Watercress, commercial	*Fasciola hepatica*	Snail host present in growing beds, cattle grazing nearby, no flood protection, poor control	18	1	France	Mailles et al., 2006
2003	Salad vegetables	Norovirus (3 genotypes), sapovirus, rotavirus	Fresh produce from Middle East most probable source	37	1	At sea (British Navy ship) Northern Arabian Gulf	Gallimore et al., 2005
2003	Lettuce	*Salmonella* Braenderup	Imported from Spain	40+	1	UK	Little & Gillespie, 2007
2003	Lettuce	*E. coli* O157:H7		57	1	USA	
2004	Lettuce	*Salmonella* Newport	Imported from Spain	375	1	UK	Little & Gillespie, 2007
2004	Rucola/rocket	*Salmonella* Thompson	Imported from Italy	20	1	Norway (possible cases also in Sweden, England & Wales)	Nygård et al., 2004
2005	Lettuce, iceberg	*E. coli* O157	Lettuce produced locally, crop irrigated from a stream	120	1	Sweden	Soderstrom et al., 2005
2005	Lettuce (various mixes of romaine lettuce, red cabbage, carrots)	*E. coli* O157:H7		>18	1	USA	FSnet, 2005a
2005	Lettuce, iceberg	*Salmonella* Typhumurium var Copenhagen DT104	Imported from Spain	60	1	Finland	Takkinen et al., 2005
2005	Lettuce	*Salmonella* Typhumurium DT104	Imported from Spain	96	1	UK	Little & Gillespie, 2008
2006	Lettuce, iceberg	*E. coli* O121:H19	Lettuce suspected at fast food outlet	4	1	USA	FSnet, 2006
2006	Spinach	*E. coli* O157:H7	Contamination by fields by feral swine faeces	205 (3)	1 (26 States)	USA	CDC, 2006; Jay et al., 2007
2007	Lettuce, iceberg	*E. coli* O157:H7		35			

Year	Product	Aetiological agent	Contributing factors	Cases (deaths)	No. of out-breaks	Country	Reference
Fresh leafy herbs							
1997	Basil likely; basil pesto used in a variety of food dishes	*Cyclospora* spp.	One distributor of basil pesto common among cases	185 in 20 clusters		USA	CDC, 1997b
1998	Parsley	*Shigella sonnei*	Parsley grown in Mexico. Unchlorinated town water used for ice in packing shed	437	8	USA	Cited by Harris et al., 2003
1999	Basil	*Cyclospora* spp.	Contaminated basil (from either USA or Mexico) in chicken pasta salad and tomato basil salad. 2 different events. *Cyclospora* detected in leftover salad.	62		USA	Lopez et al., 2001
2001	Basil	*C. cayetanensis*	Case control study linked disease to imported Thai basil	17		Canada	Hoang et al., 2005
2005	Parsley	*E. coli* O157:H7		>12	1	USA	FSnet, 2005b
2007	Basil	*Salmonella* Senftenberg	Imported from Israel, same PFGe type	UK (33), Netherlands (2), Denmark (3), USA (?)		UK, Netherlands, Denmark, USA	Pezzoli et al., 2007

Table A2.4 Microbial load on fresh leafy vegetables and herbs.

Product	Country of origin	Food chain point and samples	Microorganisms(s)	Data	Reference
		Summary of pathogens in raw vegetables, to 1998	*Salmonella* *Campylobacter*	Most 4–8% Most <3%	WHO, 1998
Pre-harvest or on farm					
Various	USA 2001/2. Study biased by farmers recruited by personal invitation	Sampled variety of vegetables in field: organic farms (O) n=476 from 32 farms; conventional farms (C) n=129 from 8 farms. Leafy vegetables included kale, spinach, amaranth, Swiss chard, lettuce, cabbage, bok choi and basil. lettuce=55 C=49 O=4 leafy vegetables=84 O=84 C=4 cabbage=54 O=39 C=15	Coliform count overall leafy vegetables O>C lettuce cabbage bok choi *Escherichia coli* Leafy vegetables Lettuce Cabbage Bok choi *Salmonella* *E. coli* O157:H7	92% +ve., aver.=2.9 log MPN/g. O=2.9±1.8 C=2.9±1.8 mean log MPN/g O=3.3±1.8 C=2.0±0.1 mean log MPN/g O=4.0±2.3 C=3.5±2.1 mean log MPN/g O=2.6±1.8 C=1.6±1.6 mean log MPN/g O=3.0±1.1 C=5.3±2.6 mean log MPN/g total O=10.7% (9/84) C=25.0% (1/4) Cert O=0% (0/19) Non-cert O=13.8% (9/65) total O=22.4%(12/49) Conv=16.7%(1/6) CertO=0% (0/10) NonCertO=30.8% (12/39) total O=10.2% (4/39) Conv=0%(0/15) CertO=0% (0/9) NonCert O=13.3%(4/30) total O=13.3% (2/15) Conv=0% (0/3) CertO=0% (0/3) NonCertO=16.7% (2/12) Organic samples from farms that used manure or compost aged <12 months had a prevalence of *E. coli* 19 times greater than that of farms that used older materials. one organic lettuce (-ve for *E. coli*) ND	Mukherjee et al., 2004
Watercress	New Zealand, 2000	Samples collected from 11 waterways, together with growing water	*E. coli* *Campylobacter* spp.	46% watercress >10^2 cfu/g 11% +ve watercress and 80% waters	Edmonds and Hawke, 2004

Product	Country of origin	Food chain point and samples	Microorganisms(s)	Data	Reference
Lettuce, amaranthus	Nigeria, 2002	collected from farm and irrigation water; sampled in wet and dry seasons amaranthus: dry n=72, wet n=33 lettuce: dry n=44, wet n=11	amaranthus: *Salmonella* *Vibrio* *E. coli* lettuce: *Salmonella* *Vibrio* *E. coli*	number +ve dry=4 wet= 0 dry=0 wet=0 dry=5 wet=2 dry=0 wet= 0 dry=7 wet=0 dry=3 wet=2	Okafo, Umoh and Galadima, 2003
	USA, 2003–4	14 organic certified farms, n=473 samples 30 semi-organic farms, n=911 19 conventional farms, n=645 Total n=2029 Included leafy vegetables as well as other vegetables. Leafy vegetables included: lettuce, spinach, cabbages, kale, Swiss chard, collard, and small numbers of bok choi	*E. coli*	Prevalence data and not counts The use of animal wastes for fertilization of produce plants increased the risk of *E. coli* contamination in organic (OR=13.2, 95% CI=2.2–61.2, P-valueb0.0001) and semi-organic (OR=12.9, 95% CI=2.9–56.3, P-valueb0.0001) produce significantly.	Mukherjee, Speh and Diez-Gonzalez, 2007
Post-harvest					
Lettuce and fennel	Spain,1973–5	5 retail outlets lettuce n=120 fennel n= 89	APC Total coliforms Faecal coliforms Faecal streptococci APC Total coliforms Faecal coliforms Faecal streptococci *Salmonella* 1 or > serovars includes *S.* Typhi	average 2y., count/100 g (fresh weight) lettuce: 6.59×10^7 5.95×10^4 6.13×10^3 2.24×10^3 fennel: 2.32×10^6 7.82×10^4 7.78×10^3 3.15×10^3 68.3% lettuce +ve 71.9% fennel +ve	Ercolani, 1976
cabbage, lettuce	USA, 1987–8	supermarket 25 g samples cabbage n=92 lettuce n=92	*Listeria* spp. lettuce cabbage *L. monocytogenes* *L. seeligeri* *L. innocua*	 1+ve (1.1%., 95%CL 0.0-3.2) 2+ve (2.2%., 95%CL 0.0-5.2) Isolated from:1 cabbage 1 cabbage 1 lettuce	Heisick et al.,1989

Product	Country of origin	Food chain point and samples	Microorganisms(s)	Data	Reference
Spinach, cabbage, lettuce and parsley	1978-90	Summary of four studies. Leafy vegetables and herbs, including spinach, cabbage, lettuce and parsley.	Total aerobic bacteria field samples retail samples	 10^5 to 10^7 cfu/g 10^4 to 10^6 cfu/g	Garg, Churey and Splittoesser, 1990; King et al., 1991; Ruiz, Vargas and Garcia-Villanov, 1987; Stewart et al., 1978
Spinach, lettuce, parsley	Canada, 1992	n=1564 fresh vegetables; 10 types; two different retail levels n= 533 farmers' outdoor markets n= 1031 samples supermarkets	thermotolerant Campylobacters: *Campylobacter jejuni* predominant species (88%), with the remainder being *C. lari* (8%) and *C. coli* (4%). supermarkets	outdoor markets: spinach, 3.3% lettuce, 3.1% parsley, 2.4% washed with chlorinated water 0% all were negative for *Campylobacter*s	Park and Sanders, 1992
Cilantro, lettuce, cabbage	Cost Rica, <1995		*Cryptosporidium* sp. oocysts). *Giardia intestinalis* *Entamoeba histolytica* cysts *Listeria monocytogenes* Hepatitis A virus and Rotavirus	5.2% (4/80) of cilantro leaves 8.7% (7/80) of cilantro roots 2.5% of lettuce Cabbage –ve 5.2% (4/80) of cilantro leaves 2.5% (2/80) of cilantro roots. 6.2% (5/80) of cilantro leaves, 2.5% (2/80) cilantro roots 3.8% (3/80) lettuce 20% (10/50) cabbage salad three of the lettuce pools	Monge and Chinchilla, 1995; Monge and Arias, 1996
	USA 1995	Salad bars & three grocery-store deli. operations, lettuce	total plate count coliform count	5.7 $\log_{10}$ cfu/g 5.3 $\log_{10}$ cfu/g temp 8.7–18.89 °C, av 12.8±2.4°C	Albrecht et al., 1995
Lettuce	Costa Rica, 1996	Farmer markets from San José, Costa Rica, during months of low (April–June) and high (December–January) incidence of diarrhoea associated with rotavirus.	Rotavirus Hepatitis A virus	Three sample pools, collected during the period of high prevalence of diarrhoea positive for rotavirus (ELISA) and in one of them rotavirions were visualized by electron microscopy. Two samples pools collected during the same period were positive for Hepatitis A virus (PCR). In almost all the pools faecal coliform bacteria were detected by cultivation and bacteriophages were visualized by electron microscopy.	Hernandez et al., 1997

Product	Country of origin	Food chain point and samples	Microorganisms(s)	Data	Reference
	Peru, ~1997	Several small markets in a peri-urban slum. Samples were collected in low-incidence season, beginning of high-incidence season, and end of high-incidence season.	*C. parvum* oocysts *Cyclospora cayetanensis* oocysts Suggests that washing vegetables does not completely remove oocysts.	14.5% 1.8%	Ortega et al., 1997
Ready-to-eat salads in sealed bags		Five nationally and regionally distributed brands from major supermarket chains. Tested at retail and at expiry date (14–16 days after packaging) At time of purchase, product temperature was 4 to 7°C	mean mesophilic microbial count mean headspace O_2 and CO_2 concentrations ethanol content	retail 1.0×10^7 cfu/g expiration 6×10^7 cfu/g 1.2 and 12%, not different at retail and expiry date. retail 700 ppm expiry 1500 ppm	Hagenmeaier and Baker, 1998
Cilantro, culantro, loose-leaf lettuce, parsley	USA, 1999	Imported produce n=1000 From Mexico, Canada, Costa Rica, Guatemala, the Netherlands, Honduras, Belgium, Italy, Israel, Chile, Peru, Columbia, Trinidad & Tobago, New Zealand, Nicaragua, the Dominican Republic, France, Argentina, Ecuador, Haiti and Korea	*Salmonella*, and *E. coli* O157 cilantro n= 177 culantro n=12 lettuce n=116 parsley n=84 cilantro n=30 culantro n=12	44/1003 (4%) +ve 96% ND 16 (9.0%) *Salmonella* +ve 6 (50%) *Salmonella* +ve 1 (0.9%) *Salmonella* +ve 1 (0.9%) *Shigella* +ve 1 (1.0%) *Salmonella* +ve 1 (1.0%) *Shigella* +ve 1 (3%) *Salmonella* + ve ND	FDA, 2001
lettuces	UK, 1999	Imported unprepared whole lettuce sampled from supermarkets, greengrocers, shops and market stalls	Enterobacteriaceae levels all acceptable *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *E. coli* O157:H7, *V. cholerae*, *L. monocytogenes*	27/151 (18%) $\geq 10^4$ cfu/g varied with type of retail premises and the temperature at which the lettuces were displayed. Samples from greengrocers, shops, and market stalls were more likely to contain levels $>10^4$ cfu/g than those from supermarkets. ND	Little et al., 1999

Product	Country of origin	Food chain point and samples	Microorganisms(s)	Data	Reference
Lettuce, dill	Norway, August 1999–Jan. 2001	n=475 No association with import or domestic source	*Cryptosporidium* oocysts or *Ascaris*/other eggs *Cryptosporidium* oocysts and *Giardia* cysts +ve *Cryptosporidium* oocysts+ve *Giardia* cysts +ve	0/475 Total 29/475 (6%) Concentration mean 3 oocysts/100g produce 19/475 (26%) +ve 5 (26%) in lettuces 10/475 2 (20%) in dill 2 (20%) in lettuce	Robertson and Gjerde, 2001
Cabbage	USA, 1999–2000	Prevalence during production and post-harvest processing of cabbage in farms and packing sheds in south Texas. n=855 Cabbage n=425 from 4 farms	*L. monocytogenes* water environmental cabbage (common types cabbage and conveyor belts)	Total +ve 26/855 (3%) 3/205 water 2 from farms and 1 packing shed 3 packing shed surfaces 20/425 +ve 7 isolates from farms 13 from packing sheds	Prazak *et al.,* 2002
Lettuce, cilantro, parsley	USA, 2001/2 Primarily packing-houses also re-packers and w/sale	Domestic and imported produce surveys indicated an extremely low prevalence of pathogen contamination. Domestic n=1028, each product ×10 subsamples fruits and vegetables Outer leaves roots removed - cilantro, loose-leaf lettuce, parsley	*Shigella* only parsley *Salmonella* all samples *E. coli* O157 all samples Individual varieties Cilantro n=85 Lettuce n=142 Parsley n=90	5 /1028 +ve 6/1028 +ve ND *Salmonella*=1+ ve *Salmonella*=1+ ve *Shigella*=1+ve	FDA, 2003
Lettuce, coriander, dill, parsley	Norway, 2000	Norwegian markets lettuce n=200 (includes Chinese leaves) pre-cut salads n= 100 growing herbs n=130 parsley and dill n=100 For all product groups, TCB were isolated from a small proportion of samples	thermotolerant coliform bacteria (TCB) *E. coli* *E. coli* O157, Salmonella spp., *Staphylococcus* spp. *L. monocytogenes*, *Y. enterocolitica*	lettuce 10 cfu/g +ve 5 lettuce, 1 growing herb (coriander), 1dill and 2 parsley all -ve lettuce 1 (0.5%) +ve lettuce 6 (0.3%) +ve (PCR –ve culture)	Johannessen, Loncarevic and Krusel, 2002

Product	Country of origin	Food chain point and samples	Microorganisms(s)	Data	Reference
Leafy vegetables and herbs	USA Mexico 11/2002–12/2003	Packing, processing: bin, merry-go-round, box., Wash dip or spray with chlorine 5–250 mg/L 8 packing sheds 11 types produce Total leafy vegetables n=175 USA n=109 (swiss chard, 9;turnip greens, 33; collards, 15; cabbage, 43; kale, 9) Mexico leafy vegetables n=66; (Cabbage, 66.) Total herbs n=222 USA n=165 (Cilantro n=93; Parsley n=63; Dill n=9) Mexico n=57 (Cilantro, 48; parsley, 9)	Indicators Environmental swabs – *E. coli* APC Total coliforms Total Enterococcus *E. coli* Note: increase *E. coli* in cabbage from conveyor to box	Generally low and no significant change during processing Low and no significant difference between sheds <1.0 $\log_{10}$ cfu/g 4.0–7.9 $\log_{10}$ cfu/g <1.0–4.5 $\log_{10}$ cfu/g <1.0–5.4 $\log_{10}$ cfu/g <1.0–4.0 $\log_{10}$ cfu/g	Johnston et al., 2006
			Salmonella, Shigella, *E. coli* O157:H7	0/466 25g samples	
			Listeria 25 g samples	3/466 (1%) total cabbages +ve 3/43 (7%) domestic cabbages +ve	

vegetables	UK.
	Most imported (70%).
	Spain 31%,
	Netherlands 15%,
		Retail RTE vegetables grown near ground: (broccoli n=209
		cabbage n= 159; carrot n= 478;
		cauliflower n= 70 ; celeriac n= 11; celery n= 193; cress n= 12 ; lettuce n= 415;
		mushrooms n= 425; radish n= 17;
		spring onions n= 87; watercress n= 65)
		Other (cucumber n= 221; pepper n= 184; tomato n=428)
		Organic
		n=3200 organic
		n=3852 conventional
			E. coli
			
			Listeria spp. not *L. monocytogenes*
			
			Salmonella,
			Campylobacter,
			E. coli O157
			
			PHLS guidelines:
			98.5% (3146) satisfactory
			1% (39) acceptable
			0.5% (15) unsatisfactory
				1.5% 48/3200 +ve
				0.3% ≥ 10^2 cfu/g
				0.2% 6/3200 +ve
				0.1% 4/3200 ≥ 10^2 cfu/g
				Watercress *L. innocua* >10^3 cfu/g
				ND
				ND
				ND
					Sagoo, Little and Mitchell, 2001.

RTE salad veg.	UK, 2001	Retail food service areas and customer self-service bars Prepared unwrapped and handled on site by staff and customers	*E. coli* *L. monocytogenes* *E. coli* O157, *Campylobacter* spp., *Salmonella*	3% (87) 10^2 to 10^5 cfu/g (<1%) (1) 840 cfu/g ND	Sagoo, Little and Mitchell, 2003
Lettuce, parsley, cilantro	Cost Rica Late 2001–early 2002	Markets n=50 (25 dry and 25 wet season) 5 markets	*Cyclospora* sp., *Cryptosporidium* sp., *Microsporidia* Faecal coliform	All products evaluated +ve at least once Lettuce +ve dry season Wet > dry	Calvo et al., 2004

Product	Country of origin	Food chain point and samples	Microorganisms(s)	Data	Reference
Lettuce RTE	Spain	16 university restaurants n=140	APC Total coliform count	3.01 to 7.81 log_{10} cfu/g <0.47 to 3.38 log_{10} MPN/g	Soriano et al., 2000
Lettuce head	Norway, 2005	organically grown lettuce were collected from 12 producers n=179 head lettuce	*E. coli* *E. coli* O157, *Salmonella* *L. monocytogenes* serogroups 1 and 4	16/179 (8.9%) +ve 12/16 <100 cfu/g 4/16 >100 cfu/g (i.e. 100, 120, 1700 and 5000 cfu/g, respectively) The two samples with the highest number of *E. coli* came from the same producer, who had three positive samples. ND 2/16 +ve	
Lettuce, watercress	Brazil	Leafy salads n= 133 n= 181 for *L. monocytogenes*	APC psychrotrophic Enterobacteriaceae Faecal coliform *Salmonella* *L. monocytogenes* Other *Listeria* spp.	51% > 6.0 log_{10} cfu/g 42% - 10^5 and 10^6 cfu/g 97 (73%) - 10^2 cfu/g (Brazilian standard) 4 (3%) detected Two of the *Salmonella*-positive samples had <10^2 cfu/g faecal coliforms 1 (0.6%) of 181 samples *L. welshimeri* (one curly lettuce) *L. innocua* (2 samples of watercress).	Fröder et al., 2007
Lettuce, cabbage	Ghana, 2006	Fresh vegetables produced in intensive urban and peri-urban smallholder agriculture with informal wastewater irrigation. n= 180 lettuce, cabbage, and spring onion 9 major markets and 12 specialized selling points in 3 major cities	faecal coliforms helminth egg counts (*Ascaris lumbricoides, Ancylostoma duodenale, Schistosoma heamatobium,* and *Trichuris trichiura*.)	geometric mean values ranging from 4.0×10^3 to 9.3×10^8/g wet weight lettuce av. 1.1 helminth eggs/g cabbage av. 0.4 helminth eggs/g	Amoah et al., 2006
Lettuce, parsley, cilantro	Canada, 2007	retail distribution centres and markets n= 1183 samples, leaf lettuce n=263 organic leaf lettuce n=112 head lettuce n=155 parsley n=127 cilantro n=61	*Salmonella* *Shigella* and VTEC generic *E. coli*, most <1 log cfu/g	organic leaf lettuce 1+ve 0 +ve parsley 13.4%+ve., <5–16 000 cfu/g organic leaf lettuce 11.6% +ve., <5–290 cfu/g cilantro 4.9% +ve., <5-7600 cfu/g head lettuce 0%., <5 cfu/g	Arthur et al., 2007

Product	Country of origin	Food chain point and samples	Microorganisms(s)	Data	Reference
Lettuce, arugula, parsley, dill	Poland, 2006	commercial groceries, supermarkets, street vendors, and food stall markets n=15 heads of lettuce (5 each of iceberg, curly, and arugula), n=5 bunches parsley leaves, and dill.	*E. cuniculi* *E. bieneusi*	Parsley leaves, grocery 1.2×10^2 spores Curly lettuce, market stall 1.9×10^2 spores	Jedrzejewski et al., 2007
Lettuce, arugula, endive, spinach	Spain, 2005–6	Fresh and minimally-processed vegetables at 4 retail supermarkets. Fresh cut: Arugula n=5 Endive n=21 Lettuce n=29 (iceberg, batavia and romaine) Spinach n=10 Whole fresh vegetables n=28 iceberg lettuce n=3 oakleaf, romaine and trocadero lettuces, endive and lettuce hearts) each n=5	presumptive *E. coli* +ve% *L. monocytogenes* *Salmonella* *E. coli* O157:H7 *Y. enterocolitica* thermotolerant *Campylobacter*	fresh-cut: arugula 40 lettuce 3.4 spinach 20 whole trocedero 20 endive 20 fresh-cut lettuce 3.4 fresh-cut lettuce 3.4 spinach 10 ND	Abadias et al., 2008

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ANNEX 3

This annex presents an overview of available data on the impact of disinfectants/sanitizers and other interventions on microbiological hazards on fresh leafy vegetables and herbs.

Table A3.1 Effect of chlorine-related sanitizers on fresh leafy vegetables and herbs.

Agent (concentration and duration)	Produce type	Treat-ment	Reduction (cfu/g)	Reference	Practical comments
NaClO (200 ppm, 10 min)	Lettuce leaves	Dipping	Aerobic bacteria, moulds and yeasts: 2.5–3 log (Water wash: 0.8–0.9 log) Total coliforms: <2 log (Water wash: 0.8 log)	Nascimento et al., 2003	NaClO is one of the most commonly used sanitizers in practice. Health and safety dangers in handling. Sanitizing efficacy is lost rapidly in the presence of organic compounds.
NaClO (100 ppm, 2 and 5 min)	Lettuce leaves	Dipping	*E. coli*: 2.6–2.9 log (Water wash: 0.8 log), *L. monocytogenes*: 1.5–1.7 log (Water wash: 0.7 log)	Akbas and Olmez , 2007	
NaClO (20 ppm, 30 sec)	Lettuce leaves	Submerge	*L. monocytogenes*: 1–1.2 log	Li et al., 2002	
NaClO (300 and 600 ppm, 3 min)	Lettuce and spinach (internalized in leaves) pieces	Agitation	*E. coli* O157:H7: 0.5 log in each vegetable	Niemira, 2007	
NaClO (150 ppm, 10 min)	Lettuce pieces	Soak	Aerobic bacteria: 2 log	Koseki et al., 2001	
NaClO (50–200 ppm, 30 sec)	Lettuce pieces Diced tomato	Agitation	Aerobic bacteria: <0–1.3 log Mould and yeasts: <0–0.9 log Aerobic bacteria: <0 Mould and yeasts: <0.1 log	Simmons, Ryu and Beuchat, 2006	
NaClO (25–200 ppm, 10 min, 4°C) NaClO (25–200 ppm, 10 min, 22°C)	Lettuce pieces	Stirring	*L. monocytogenes*: 0.2–1.3 log *L. monocytogenes*: 0.6–1.7 log	Zhang and Farber, 1996	
NaClO (200 ppm, 10 min) NaClO (200 ppm, 1 min, 50°C)	Lettuce pieces	Immersion	*E. coli* O157:H7: 1.2 log, *Salmonella*: 1.2 log, *S. aureus*: 1.4 log, aerobic bacteria: 0.9 log *E. coli* O157:H7: 1.2 log, *Salmonella*: 1.2 log, *S. aureus*: 1.5 log, aerobic bacteria: 1.5 log	Kondo, Murata and Isshiki, 2006	
NaClO (100 and 300 ppm, 10 min) NaClO (100 ppm) + Tergitol (0.1%) (10 min) NaClO (100 ppm) + lactic acid (0.5%) (10 min)	Lettuce pieces	Stirring	*Y. enterocolitica*: 2.36–3.15 log *Y. enterocolitica*: 1.03–2.73 log *Y. enterocolitica*: >6 log	Escudero et al., 1999	
NaClO (75 ppm, 5 min)	Lettuce pieces	Submerge	Aerobic bacteria: 2 log	Lu et al., 2007	
NaClO (200 ppm, 5 min)	Lettuce pieces	Submerge	Aerobic bacteria: 1.2–4 log	Koseki and Isobe, 2006	

Agent (concentration and duration)	Produce type	Treat-ment	Reduction (cfu/g)	Reference	Practical comments
NaClO (200 ppm, 1 min)	Lettuce pieces	Agitation	*E. coli* O157:H7: 0.86–0.88 log (Water wash: 0.58–0.59 log), *Salmonella*: 0.96–1.04 log (Water wash: 0.53–0.67 log)	Koseki et al., 2003	
NaClO (20 ppm, 1 min, 50°C) NaClO (20 ppm, 1 min, 20°C)	Lettuce pieces	Agitation	*E. coli* O157:H7: 1.1 log (Water wash: 0.9 log) *E. coli* O157:H7: 1.0 log (Water wash: 0.7 log)	Li et al., 2001	
NaClO (100 ppm, 10 min)	Cabbage Lettuce Parsley	Dipping	*E. coli*: 1.41 log (Water wash: 0.65 log) *E. coli*: 0.72 log (Water wash: 0.38 log) *E. coli*: 1.56 log (Water wash: 0.85 log)	Seymour et al., 2002	
Chlorine (10 ppm, 5 min)	Shredded lettuce	Agitation	*L. innocua:* 1–1.5 log	Francis and O'Beirne, 2002	
NaClO (100 ppm, 1 min)	Shredded lettuce	Wash	*L. monocytogenes*: 0.7 log (Water wash: 0.5 log)	Hellstrom et al., 2006	
NaClO (100 ppm, 5 min) NaClO (100 ppm) + Citric acid (0.1%) (5 min)	Chinese cabbage pieces	Mixing	*E. coli* O157:H7: 2.0–2.7 log *E. coli* O157:H7:1.7–2.2 log (Water wash: 0.7–1.0 log)	Inatsu et al., 2005a	
NaClO (100–1600 ppm, 5 min)	Parsley bunches	Submerge	*Salmonella*: 1.7–3.0 log	Lapidot, Romling and Yaron, 2006	
NaClO (5–150 ppm, 5 min)	Parsley bunches	Agitation	*Shigella*: 1.2–6 log	Wu et al., 2000	
Aqueous ClO_2 (20 ppm, 10 min) Aqueous ClO_2 (20 ppm, 10 min) + Ultrasonication (170 Hz)	Lettuce leaves	Stirring	*E. coli* O157:H7: 2.3 log (Water wash: 1.3 log), *Salmonella*: 2.2 log (Water wash: 1.5 log) *E. coli* O157:H7: 2 log, *Salmonella*: 3.2 log	Huang et al., 2006	Unstable, must be generated on site, can be explosive when concentrated, as with other chlorine compounds. Not legally approved in all countries.
Aqueous ClO_2 (2-5 ppm, 10 min, 4°C) Aqueous ClO_2 (2-5 ppm, 10 min, 22°C)	Lettuce pieces	Stirring	*L. monocytogenes*: 0.4–1.1 log *L. monocytogenes*: 0.4–0.8 log	Zhang and Farber, 1996	
Aqueous ClO_2 (300 ppm, 10 min) Aqueous ClO_2 (3000 ppm, 10 min)	Green pepper pieces	Shake	*L. monocytogenes*: 1.87 log/5 g (uninjured surfaces), 1.53 log/5 g (injured surfaces) *L. monocytogenes*: 3.67 log/5 g (uninjured surfaces), 1.35 log/5 g (injured surfaces)	Han et al., 2001	

Agent (concentration and duration)	Produce type	Treat-ment	Reduction (cfu/g)	Reference	Practical comments
Gaseous ClO_2 (4.7 mg, 30 min) Gaseous ClO_2 (6.7 mg, 1 h) Gaseous ClO_2 (8.7 mg, 3 h)	Lettuce leaves		*E. coli* O157:H7: 3.4 log, *L. monocytogenes*: 5.0 log, *Salmonella*: 4.3 log *E. coli* O157:H7: 4.4 log, *L. monocytogenes*: 5.3 log, *Salmonella*: 5.2 log *E. coli* O157:H7: 4.4 log, *L. monocytogenes*: 5.4 log, *Salmonella*: 5.4 log	Lee, Costello and Kang, 2004	Experimental approach only. Health and safety dangers in handling. How to ensure sufficient contact with leaf in practice with commercial throughput requirements.
Gaseous ClO_2 (4.1 mg/L, 30.8 min)	Cabbage pieces Lettuce pieces Peach		*E. coli* O157:H7: 3.13 log, *L. monocytogenes*: 3.60 log, *Salmonella*: 4.42 log *E. coli* O157:H7: 1.57 log, *L. monocytogenes*: 1.53 log, *Salmonella*: 1.58 log	Sy et al., 2005	
Gaseous ClO_2 (1.74 mg/L, peak 10 min) Gaseous ClO_2 (1.29 mg/L, peak 10 min)	Lettuce pieces shredded cabbage		Aerobic bacteria: 0.84 log (Psychrotrophic count: 0.88 log), Yeast: 0.64 log Aerobic bacteria: 0.25 log (Psychrotrophic count: 0.27 log), Yeast: 0.46 log	Gomez-Lopez et al., 2008	
Gaseous ClO_2 (0.5 mg/L, 2 min) Gaseous ClO_2 (0.5 mg/L, 10 min) Gaseous ClO_2 (5.0 mg/L, 2 min) Gaseous ClO_2 (5.0 mg/L, peak 10 min) Gaseous ClO_2 (3.0 mg/L, 10 min)	Lettuce pieces		*E. coli* O157:H7: 0.5 log/piece *Salmonella*: 0.7 log/piece *E. coli* O157:H7: 1.6 log/piece *Salmonella*: 1.9 log/piece *E. coli* O157:H7: 3.0 log/piece *Salmonella*: 1.5 log/piece *E. coli* O157:H7: 3.9 log/piece *Salmonella*: 2.8 log/piece *E. coli* O157:H7: 3.3 log/piece *Salmonella*: 2.5 log/piece	Mahmoud and Linton, 2008	
Gaseous ClO_2 (0.3 mg/L, 30 min) Gaseous ClO_2 (3 mg/L, 10 min) Gaseous ClO_2 (0.6 mg/L, 30 min)	Green pepper pieces		*E. coli* O157:H7: 3.05 log/5 g (uninjured surfaces), 1.88 log/5 g (injured surfaces) *E. coli* O157:H7: 7.39 log/5 g (uninjured surfaces), 3.60 log/5 g (injured surfaces) *E. coli* O157:H7: >6 log/5 g (uninjured surfaces), 3.36 log/5 g (injured surfaces)	Han et al., 2001	
$NaClO_2$ (0.5 g/L) + citric acid (1 g/L) (15 min) $NaClO_2$ (0.5 g/L) + citric acid (10 g/L) $NaClO_2$ (0.5 g/L) + citric acid (1 g/L) +heat (50°C) $NaClO_2$ (0.5 g/L) + citric acid (1 g/L) + sonication	Chinese cabbage pieces	Mixing	*E. coli* O157:H7: 2.2–2.9 log (Water wash: 0.7–1.4 log) *E. coli* O157:H7: 3.7–3.8 log (Water wash: 0.4–0.5 log) *E. coli* O157:H7: 3.7–3.9 log (Water wash: 1.3–1.5 log) *E. coli* O157:H7: 2.8 log (Water wash: 1.1–1.3 log)	Inatsu et al., 2005a	Active principle is ClO_2. Its use as a sanitizer of fresh produce is not allowed in some countries.

Agent (concentration and duration)	Produce type	Treat-ment	Reduction (cfu/g)	Reference	Practical comments
$NaClO_2$ (0.5 g/L) + citric acid (1 g/L) (15 min)	Chinese cabbage pieces	Agitation	*E. coli* O157:H7: 2.0 log (Water wash: <1.0 log), *L. monocytogenes*: 2.2 log (Water wash: <1.0 log), *S. aureus*: 2.4 log (Water wash: <1.0 log) , *Salmonella*: 2.0 log (Water wash: <1.0 log), aerobic bacteria: 2.0 log (Water wash: 0.6 log)	Inatsu et al., 2005b	

Table A3.2 Examples of performance and practical considerations of some other interventions.

Measure	Concentration	Produce type	Treat-ment	Control	Reduction (cfu/g)				Reference
					E. coli O157:H7	*L. mono-cytogenes*	Aerobic bacteria	Moulds and yeasts	
Ozonated water AcEW[(1)] (containing 30 ppm of residual chlorine)	5 ppm (10 min) (10 min)	Lettuce pieces	Soak	Untreated			1.5 log 2.0 log		Koseki et al., 2001
AcEW (containing 30–35 ppm of residual chlorine)	(60 s) (90 s)	Leafy greens (Organic baby lettuce (red and green romaine, red and green oak leaf, lollo rosa, tango), organic red and green chard, organic mizuna, organic arugula, organic frisee, and organic radicchio)	Agitation	Untreated	2.4 log (Water 1.0 log) 2.5 log (Water 1.0 log)	2.0 log (Water 1.0 log) 2.5 log (Water 1.0 log)	2.3 log (Water 1.0 log) 2.5 log (Water 1.0 log)		
Ozonated water	10 mg/L (1 min)	Lettuce pieces	Shake	Untreated	1.21 log (Water 0.88 log)				Singh et al., 2002
Ozonated water	1.3 mM (3 min)	Lettuce pieces	Stirring	Untreated			1.2 log (Methophilic) 1.8 log (Psychro-trophic)		Kim, Yousef and Chism, 1999
Ozonated water Acetic acid Citric acid Lactic acid Ozone+citric acid	5 ppm (5 min) 1% (1 min) Ozone (3 ppm) +1% citric acid (1 min)	Lettuce pieces	Submerge	Untreated Untreated	1.09 log 0.17 log 0.84 log 1.07 log 2.31 log (Water 0.57 log)	No effect 0.59 log 1.03 log 0.93 log 1.80 log (Water 0.53 log)			Yuk et al., 2006

Measure	Concentration	Produce type	Treat-ment	Control	Reduction (cfu/g)				Reference
					E. coli O157:H7	*L. mono-cytogenes*	Aerobic bacteria	Moulds and yeasts	
EO[2] water (containing 45 ppm of residual chlorine)	(1 min) (3 min)	Lettuce leaves	Shake	Untreated	4.16 cfu/mL (Water 1.38 log) 4.17 cfu/mL (Water 1.76 log)	3.94 cfu/mL (Water 1.56 log) 4.40 cfu/mL (Water 1.75 log)			Park et al., 2001
Nisin Nisin+0.02M EDTA Nisin+0.02% phytic acid Pediocin Peduicun+0.02M EDTA Pediocin+0.02% phytic- acid Nisin Nisin+0.02M EDTA Nisin+0.02% phytic acid Pediocin Pediocin+0.02M EDTA Pediocin+0.02% phytic- acid	50 mg/L (1 min) 100 AU/mL[3] 50 mg/L 100 AU/mL	Cabbage pieces Broccoli pieces	Agitation	Untreated		2.77 log 2.94 log 4.35 log 1.94 log 2.44 log 2.50 log (Water 0.86 log) 2.55 log 2.47 log 4.18 log 1.11 log 1.90 log 1.70 log (Water 0.48 log)			Bari et al., 2005
Nisin Z Coagulin Nisin Z+coagulin	200 AU/mL (2 min) 400 AU/mL 200 AU/mL	Lettuce pieces	Wash	Untreated		3.2–3.5 log cfu/cm^2 3.2–3.5 log cfu/cm^2 3.2–3.5 log cfu/cm^2 (Water 0.6 cfu/cm^2)	2.4 log cfu/cm^2 2.4 log cfu/cm^2 2.4 log cfu/cm^2 (Water 1.9 cfu/cm^2)		Allende et al., 2007

Measure	Concentration	Produce type	Treat-ment	Control	Reduction (cfu/g)				Reference
					E. coli O157:H7	*L. mono-cytogenes*	Aerobic bacteria	Moulds and yeasts	
Citric acid	1 g/L (15 min)	Chinese cabbage pieces	Mix	Untreated	1.0-1.4 log (Water 0.9 log)				Inatsu et al., 2005a
Gamma-ray	1.0 kGy 1.5 kGy	Lettuce pieces	Irradiation	Untreated			2.35 log 3.1 log		Zhang, Lu and Wang, 2006
Gamma-ray	1.05 kGy	Cilantro bunches	Irradiation	Untreated	>6.6 log (Water 1 log)		2.41 log (Water 0.46 log)	1.91 log (Water 0.55 log)	Foley et al., 2004
Ultrasound					2.5 log improvement when combined with other hurdles and sanitizers e.g. chlorine, acetic acid, warm temperatures. This has been observed for a range of bacterial pathogens.				Seymour et al., 2002; Ajlouni et al., 2006' Delaquis et al., 1999
UV		Lettuce			Log reduction dependent on H2O2 concentration, temperature and treatment time. At optimal conditions surface reduction 4.12 ± 0.45 and internal counts by 2.84 ± 0.34 log cfu. Reductions applicable to salmonella on and within lettuce.				Hadjok, Mittal and Warriner, 2008

NOTES: 1. AcEW = Acidic electrolyzed water. 2. EO = Electrolyzed oxidizing water. 3. AU/mL = arbitrary units per millilitre

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Annex 4

Optimum cold chain conditions for leafy vegetables and fresh-cut salad

Product	Temp. (°C)	RH (%)	Controlled atmospheres	Recommended shelf life	References
Corn salad, lamb's lettuce, field salad, mâche (*Valerianella locusta,* syn. *V. olitoria*). Dandelion (*Taraxacum officinale* Wiggers.). French or round sorrel (*Rumex scutatus*) Garden sorrel (*R. acetosa* L.) Miner's lettuce, Winter purslane., (*Montia perfoliata*, syn. *Claytonia perfoliata*). Mizuna (*Brassica rapa* L. subsp. *japonica* (Group Japonica)). Purslane (*Portulaca oleracea* L.) Rocket salad, roquette, arugula, rucola, rugula (*Eruca vesicaria* subsp. *sativa*.	0–2 Top icing can be used.	95–100	CA is generally not beneficial. MAP is mostly beneficial for controlling RH. However, lamb's lettuce retains acceptable quality after 28 days in sealed plastic bags with reduced O_2 and elevated CO_2 at <4°C (39°F). MAP of sorrel reduces yellowing and decay.	Rocket: 7–10 days. Others: 10–14 days	Aharoni *et al.,* 1993; Cantwell, 2001; Cantwell and Reid, 1993; de Leiris, 1987; Péron and Rees, 1998; USDA ARS, 2002
Watercress (*Nasturtium officinale*)	0	95	The rate of yellowing can be reduced by storing in atmospheres >7% CO_2 with not less than 5% O_2	2–3 weeks	Aharoni, Reuveni and Dvir, 1989; Hruschka and Wang, 1979
Bean sprouts	0	95–100	The shelf-life of mung bean sprouts can be increased by storage under modified atmosphere in which O_2 is reduced and CO_2 is increased For instance, they can be held for 4 to 5 days at 8°C (46°F) in packages containing 5% O_2 + 15% CO_2. Darkening of sprouts is reduced and development of sliminess is delayed.	5–10 days	Brackett, 1999; Cantwell, 2001; DeEll *et al.,* 2000; Lipton, Asai and Fouse, 1981; Varoquaux *et al.,* 1996

Product	Temp. (°C)	RH (%)	Controlled atmospheres	Recommended shelf life	References
Basil (*Ocimum basilicum*) Chervil, salad chervil (*Anthriscus cerefolium*) Coriander, cilantro (couriander), Chinese parsley (*Coriandrum sativum*) Dill (*Anethum graveolens*) Savory (Summer savory – *Satureja hortensis*; winter savory – *Satureja montana*)	Chervil, coriander, dill and savory should be stored at 0 °C. Basil should be stored °C	95–100	A 5 to 10% O2 + 4 to 6% CO2 CA is only moderately beneficial for fresh herbs	1–2 weeks	Aharoni *et al.,* 1993; Aharoni, Reuveni and Dvir, 1989; Cantwell, 2001; Cantwell and Reid, 1993; Gorini, 1981; Lange and Cameron, 1994, 1998; Loaiza and Cantwell, 1997; Saltveit, 1997
Lettuce (*Lactuca sativa*) (butterhead, crisphead, green leaf, iceberg, romaine) (chopped, shredded, whole leaf)	1–3	95–100	Browning of the cut edges of butterhead lettuce is reduced by modified atmospheres rapidly created by flushing with N_2 to get 1 to 3% residual O_2, with CO_2 levels of 5 to 10%. A 0.5 to 3% O_2 + 5 to 10% CO_2 CA reduced cut edge browning of green leaf lettuce. Fresh-cut iceberg lettuce packages commonly have <1% O_2 to effectively slow browning and >10% CO_2 to inhibit microbial growth	6 (packaged in air)–12 days (packaged with MAP)	USDA ARS 2002 Suslow and Cantwell, no date.
Spinach (*Spinacia oleracea*) (whole leaves, cut leaves)	0–3	95–98	Storage in 0.8 to 3% O2 + 8 to 10% CO2 is beneficial.	6 (packaged in air)–12 days (packaged with MAP)	USDA ARS, 2002

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