1. PUBLISHABLE SUMMARY

- **A summary description of the project context and objectives**

Vegetables and fruits are important components of a healthy, balanced diet. Fresh produce is perceived overall as safe, and increased consumption is encouraged. Still, there are some concerns expressed related both to chemical and microbial contaminants on fresh produce. Globalization of food chains and a shift from a spot market system to 'supermarketization' resulted in increasing requirements to comply with private and regulatory standards. Foodborne outbreaks from fruits and vegetables and border rejections due to non-compliance testing contribute to the burden of disease, generate economic loss and food waste, and lead to a loss of trust and confidence in the safety of fresh produce. A commodity often grown in open fields with intensive use of water, fruit and vegetables are subject to the impact of extreme weather events that can in turn affect occurrence of contaminants, perhaps increasingly in the future as climate continues to change. Veg-i-Trade was an EU-funded large collaborative project launched in 2010 to assess the impact of international trade and climate change on the safety of fresh produce. Veg-i-Trade brought together a multidisciplinary team of 22 international partners. Its research integrated several approaches, including sampling and testing, field studies on pre- and post-harvest practices, assessment of current food safety management systems, risk assessment modelling and risk communication. The objectives of the project were to characterize emerging risks, formulate adaptation scenarios, identify bottlenecks in implementation of quality assurance recommendations and suggest control measures of managerial or technological nature to take the safety of fresh produce, both for domestic markets and export, to the next level.

- **Description of the work performed since the beginning of the project and the main results achieved so far**

The global food market

The organizational structure, governance and relationships between actors in the pear and mango export supply chain from South African orchards to retailers in Europe have been investigated. The primary theme in the pear value chain is to attain economies of scale and scope to interact directly with retail groups to achieve the best possible returns for growers. Conversely, the mango chain was characterised by a global sourcing theme. The dominant role player in the mango chain is the category manager, bridging the gap between producers and retailers and controlling the sourcing of fruit globally to offer a diversified range of year-round fresh-cut fruits to overseas customers. In another part of the Veg-i-Trade project, interviewing 118 producers located in both EU and non-EU countries, this type of highly integrated chains or big companies dealing with stringent standards as a result of market self-regulation were noted to have elaborated advanced Food Safety Management Systems (FSMS) with good information about their systems' output. Producers or processors operating in a fragmented market or in a less favourable context demonstrated less mature FSMS based on 'best available' knowledge and their own experience. The 'supracontext' including an operational national food safety legal framework, access to good quality of inputs, adequate storage and lab facilities, and agricultural extension services are driving forces to implement FSMS that will enhance quality and safety of fresh produce. A favorable enabling environment, training and capacity building to transfer the "best available knowledge" is a key issue for local and global development in the field of fresh produce safety.

Safe food for all

In a Veg-i-Trade survey of over 100 fresh produce supply chain experts from both the global North and the South, concerns were raised about costly control measures and third party certification required for private standards and the EU market, even though advantages of these requirements in terms of farmer health, spill-over of knowledge to non-certified activities and environmental sustainability were acknowledged. Standards and certification were seen in their dual role both as a catalyst for upgrading 'best practices' and indirect food safety on the one hand and as a non-tariff barrier to trade on the other hand. The need for science-based prerequisites was highlighted. As, on the global scale, the amount of fresh fruit and vegetables traded still represents a limited fraction of what is produced, respectively ca. 3.5% and 9.5% of world vegetable and fruit production, the concept of safe food for all (i.e. domestic market and export market) needs attention. For example, a survey in Cameroon highlighted poor pesticide use by smallholder farmers producing goods for local markets, resulting in human health risks for the workers and local consumers. Also, in a limited sampling study in Egypt, *Salmonella* was found

on fresh produce at the local markets: the lowest prevalence of *Salmonella* still found in lettuce and strawberries in hypermarkets, followed by an intermediate prevalence if sampled in shops, and the highest prevalence was observed in products sold in open markets. From a survey in Tamil Nadu, India, it was noted that households with different income categories invariably preferred to buy from weekly farmers' markets in rural areas and even in urban areas. The majority of the urban respondents felt that food safety and quality certifications, grading, packaging and labeling of fruits and vegetables were important, whereas the rural respondents were still indecisive due to lack of understanding about these systems. In a Veg-i-Trade survey about fresh produce handling practices in Belgium and Spain, quite a high proportion of the participants (17-19%) was not aware of the importance to maintain the cold chain in storage of fresh fruits and vegetables. This illustrates that awareness is essential for improving food safety for local consumers, an issue which still needs attention in many countries.

Sampling, testing and assessment of food safety management systems

Three self-assessment tools were developed within Veg-i-Trade and shown to be useful as an internal audit system to track the current status of "best practices" or maturity of the "management systems" implemented at primary production, processing or trade. They consist of 64 to 69 indicators and address core control and quality assurance activities in the prevention and reduction of microbiological, mycotoxin and pesticide residue contamination. Each indicator uses grids with supporting information for self-assigning to situation 1 (basic level), 2 (average level) or 3 (advanced level). Such selected indicators and grids provide a 'bird's eye view' of the performance level of the current systems in place, the risk level of the context wherein the actor has to operate, and an indication of the system's output.

Interviews using this self-assessment questionnaire were combined with observations of the local situation and with sampling and testing of crops and the production environment (soil, water, contact surfaces). This identified bottlenecks in current food safety management systems used in various Veg-i-Trade partner countries. Thanks to voluntary collaboration by many producers, information from the self-assessment tool was obtained from ca. 260 companies and ca. 7000 results of microbial analysis were collected in a central Veg-i-Trade database. The surveys undertaken by Veg-i-Trade partners showed that systems in place provided a high level of safety of fresh produce. However, increased vigilance, in particular related to the selection of water sources and use of water treatment, training about personal hygiene, commitment of the work force, documentation and record keeping, and conditions of storage and transport, may help to take safety of fresh produce to the next level. Often, assurance activities, namely validation and verification, need further attention.

Sampling has been noted to be a recurring and complex issue, in particular from a global perspective. A methodology for risk-based pesticide residue monitoring in supplier control of fresh fruits and vegetables was validated. The criteria for prioritisation of analysis includes supplier conformity guarantees (historical data, report of analysis), country of origin, a product risk factor, and takes into account potential impacts of processing and cross-contamination in either reducing or dispersion of pesticide residues within the business unit. Risk level scores are attributed per commodity or batch and used to optimize resources for sampling and allocate in percentages assigned sample sizes to the incoming batches of fruits and vegetables within the processing or trade company.But whereas pesticide residue monitoring is often already well elaborated, sampling schemes for monitoring fecal indicator organisms and enteric pathogens in either produce or water were often restricted. Ambiguity in selection of microbial parameters and interpretation of test results to assess sanitary quality and safety was highlighted. This was particularly true in the case of emerging pathogens, such as verotoxin-producing pathogenic *E. coli* (VTEC) and Norovirus (NoV), for which detection is based upon molecular techniques, and also in the case of *Cryptosporidium* or *Giardia*, using immunomagnetic separation and fluorescent microscopy for detection. Such methods have been shown to be technically complex, and can only be carried out in specialised and well-equipped laboratories. In addition, information is lacking on the relationship between the detection of the VTEC virulence genes or NoV genomic copies by these molecular techniques and the actual presence of infectious agents and risk to public health.

The microbiological sampling and testing performed within the framework of Veg-i-Trade in various partner countries demonstrated that (for the samples analyzed) no VTEC or *Salmonella* were isolated from produce in EU countries (although some *Campylobacter* positive samples occurred) and only occasionally was *E. coli* present in low numbers. Nonetheless, hazards are present in the production environment (e.g. irrigation water, water at harvest, soil). Control of the treatment process of organic fertilizers and the quality of irrigation water appeared to be among the most crucial factors during primary production of leafy greens in a number of non-EU countries,

in particular when sampling small farms. For both EU and non-EU countries, the importance of water quality for the rinse step of leafy greens at harvest and also the washing step in the production of fresh-cut produce was identified as a potential pathway for dispersion of spoilage bacteria, *E. coli* or introduction of pathogens via cross-contamination. This was observed, in particular, if no sanitizing agents were used to keep the water clean in the washing tank. Still, if sanitizers were used, their effectiveness was on some occasions not monitored. From the overall Veg-i-Trade results database, it is notable that the presence of elevated levels of *E. coli* (independent whether in water, soil or produce) increased the likelihood for detecting the pathogens, but only had a low to moderate predictive value on the actual presence of pathogens. At the local level, setting *E. coli* indicator threshold values can thus be useful for alerting producers about situations of increased risk for pathogens' occurrence, and trigger initiation of corrective measures.

Pre- and postharvest practices and microbial ecology studies

Leafy greens remain one of the most relevant crops, with an increasing production of bagged salads. Soilless production systems in open fields increased the content of bioactive compounds. However, to ensure the post-cutting life, the combination of adequate production systems and suitable cultivars is considered essential. Appropriate irrigation management practices are needed to guarantee the sustainability of the environment and the quality of the leafy greens. The application of deficit irrigation preserved quality and shelf life of fresh-cut lettuce while excess irrigation accelerated cut-edge browning and microbiological growth. The safety risks associated with flooding were evaluated. Contamination with foodborne pathogens was observed after flooding, but climatic conditions (high solar radiation) caused a quick decline (within 3 weeks) of pathogens in lettuce.

Veg-i-Trade studies revealed that the microbial ecology of plants is influenced by a web of complex interactions between climate, environment, biological, technical and cultural factors, in which a shift in one of the factors may lead to changes in the whole web to potentially impact safety of fresh produce. The intrinsic microbial ecology of the host plant may impact the persistence of pathogens in leafy greens. The epiphytic bacterial community on baby leaves in the field and of cut basil leaves was investigated using culture-independent techniques, including denaturing gradient gel electrophoresis and next-generation sequencing. The diversity and abundance of the epiphytic community of baby lettuce were affected by climatic conditions. The harvest week and the variations in radiation, rainfall and relative humidity explained most of the differences. Given the diverse functions of phyllospheric microbes, understanding the variations of specific communities such as *Pseudomonas* spp. could help explain the different susceptibilities of crops to spoilage or pathogenic bacteria.

After harvest, the control of relative humidity during storage is critical as it affects microbial survival and the visual quality of the crop. Postharvest internalization of *Salmonella* into baby spinach leaves was demonstrated during washing, but a reduction in the potential internalization was observed at medium and low hydration levels. The effect of storage conditions on the survival, attachment and internalisation of enteric pathogens was investigated. Plate count data showed that both *E. coli* O157:H7 and *Salmonella* occurred in higher numbers at the cut edge than at the surface. Confocal microscopy confirmed the internalization of both pathogens at the cut edges. However internalization from the surface through the stomata was not observed in the dark or under simulated supermarket light conditions. Washing (with or without sanitizers) did not substantially reduce pathogen abundance from the cut edges. Neither packaging condition nor storage time had any quantifiable effect on attachment or internalization.

Logistic simulation models integrated with behavior of pathogens or spoilage organisms under modified atmosphere packaging suggested that advanced inventory control policies and selection of transport mode and time may offer opportunities to increase food safety while keeping product waste costs, expenditure costs and customer satisfaction under control.

Water treatment technologies

An important lesson learnt is that effectiveness of water disinfection in the fresh produce supply chain needs to be validated case by case, and its appropriate use monitored. The conditions for use of chlorine and a range of alternatives such as chlorine dioxide, peroxyacetic and lactic acids, hydrogen peroxide, electrolyzed water, UV-light, and ultrasound in wash water disinfection to prevent cross-contamination or for external water reconditioning were studied within Veg-i-Trade, both in static and dynamic lab systems. Data generated were used for modelling, in order to understand which variables (including pH, temperature, Chemical Oxygen Demand (COD), turbidity, and product to water ratios) govern water disinfectant stability and *E. coli* O157 inactivation.

Both water reconditioning (i.e. disinfection outside the processing line before reuse in the washing tank, enabling prolonged contact times) and wash water disinfection (i.e. instant inactivation of micro-organisms in the washing tank itself by keeping a disinfectant residual) were investigated. It was established that chlorine decay and *E. coli* O157 inactivation depend mainly on the COD and the residual chlorine concentration. In case of reconditioning, an option is to include a prior treatment step using gallotanins or chitosan as flocculants in combination with sand filtration to reduce COD load of the water to be treated. Ultrasound and advanced oxidation processes, including UV or ozone, with or without enhancing agents, were tested as alternatives to chlorination in reconditioning, each having particular requirements to provide effective disinfection. For wash water disinfection, chlorination and peracetic acid (combined with lactic acid) (PAA/LA) were extensively studied. Both microbial inactivation rate and disinfectant decay rate were considerably higher for chlorine than for PAA/LA. As such, a higher chlorine demand and continuous dosing is needed to keep a (low) chlorine residual, but on the other hand a higher PAA+LA concentration and residual is necessary for equally rapid *E. coli* O157 inactivation. The developed model for assessing efficacy of wash water disinfection also showed that the strategy of reconditioning and frequent refreshing of water in the washing tank dilutes only to a minor extent the microbial load and cannot guarantee microbial inactivation to prevent cross-contamination in process water. It was also noted that appropriate use of water sanitizers (either chlorine or PAA/LA) did not substantially affect the microbial load of the washed produce nor extend its sensorial shelf life and thus cannot function as a fresh produce decontamination treatment.
Overall, for assessing efficacy of water treatments, given physicochemical parameters and the product water ratio needs to be taken into account, but also operational costs, maintenance, worker's health and acceptance by competent authorities will impact technology selection by end-users.

Risk ranking of enteric pathogens, mycotoxins and pesticide residues in fresh produce

Risk ranking is a useful tool to set priorities for risk management decisions and was also pursued during the Veg-i-Trade project .

Enteric pathogens: A comparative exposure assessment model was elaborated starting from the Veg-i-Trade prevalence data for both *Salmonella* and VTEC in strawberries, basil and butterhead lettuce crops at the time of purchase and included storage (temperature and time), washing (rinsing or submersion) and consumption data of the Veg-i-Trade consumer survey collected in Belgium and Spain. Overall, although less consumed, basil posed the highest risk, followed by butterhead lettuce and then strawberries. In case of basil, higher numbers of *Salmonella* contaminated portions were calculated than for VTEC. Using the model, the number of pathogen-contaminated portions of butterhead lettuce consumed was estimated to be higher in Spain versus Belgium, and vice versa for strawberries. If present, pathogen concentrations were calculated to be higher in butterhead lettuce compared to strawberries, due to a gradual die-off in the strawberries upon storage.
Apart from pathogen/commodity risk ranking also a quantitative exposure model for generic *Escherichia coli* was developed to estimate the occurrence and levels of *E. coli* under defined circumstances of agricultural production. It was noted that apart from the regional variable distribution of *E. coli* numbers in the irrigation water or the soil, the cultivation season and interlinked with this the length of the crop cycle, rainfall and extent of solar radiation also influence the *E. coli* levels substantially in the case of open field production. Climatic conditions and water availability during the growing season and humidity during storage also significantly affected both microbial load and visual quality of the derived fresh-cut leafy greens.

Mycotoxins: Risk ranking of mycotoxins considered the relative benefit to consumers expected from legal enforcement to comply to the EU maximal allowable levels for the exposure to i) aflatoxin B1 (AFB1) via consumption of nuts and figs, and ii) ochratoxin A (OTA) via consumption of dried fruits. Mycotoxin contamination data were collected from a Veg-i-Trade database. In scenario 1, all collected data were considered, assuming no legal EU limits or border rejections, whereas in scenario 2 data with contamination levels above the legal EU limit were excluded from the calculation. Scenario 1 demonstrated that AFB1 and OTA concentrations in the considered foods can be a potential health risk, whereas in scenario 2 the estimated exposure of OTA in dried fruits and AFB1 in nuts using the current legal threshold limit resulted in appropriate consumer protection. However, in the case of AFB1 in figs there may be some public health concerns, so reconsideration of the current EU limit is recommended.
Furthermore as part of the Veg-i-Trade project, the screening of moulds and mycotoxins in tomatoes, bell peppers, onions, soft red fruits and derived tomato products triggered a risk assessment study of *Alternaria*

species and their associated mycotoxins, alternariol and alternariol monomethyl ether, in tomato derived products. The mycotoxin patulin was occasionally present in moulded soft red fruits and may need further attention in juicing.

Pesticides: Veg-i-Trade investigated the risk of exposure for the Belgian population to additive effects of multiple pesticides with a common mode of action, using as an example the triazole pestides, a chemical family of fungicides. The used MCRA 8.0 model converts pesticide residue data from the raw commodity to food-as-eaten e.g. cooked, peeled, washed through certain processing factors, some of which were established a priori within the Veg-i-Trade project. It was shown that the Belgian consumer is not at high risk of toxicity due to triazole exposure. Both acute and chronic toxicity assessments were significantly lower than the toxicological norms. Bananas, pineapples, rice and table grapes are the foods that result in most exposure to triazoles. Three of these four foods are exclusively imported, whereas table grapes are both locally produced and imported. Foods that are locally produced exclusively had far less of an impact on triazole exposure.

Climate change and impact of climatic conditions on quality/safety of fresh produce

Impact of climate change depends upon the local balance between positive and negative effects. Findings from a Delphi study set-up within Veg-i-Trade about how to respond to climate change in terms of food safety of fresh produce highlighted the need to strengthen activities such as water control, irrigation methods, training in personal hygiene, integrated pesticide management and (cold) storage control, in addition to investing in protective and segregation structures to avoid the spread of contamination. Long term adaptation includes weather forecasting, monitoring and warning systems and mapping of risk areas. Experts assessed the pressure of extreme weather events as higher for the countries from the global south and indicated higher adaptive and coping capacity and existing response strategies in the global north.

Furthermore, specific studies focused on the impact of climate change on the use of pesticides, occurrence of mycotoxins and behavior of micro-organisms on fresh produce

Pesticides: After application of crop protection products, the residual of the initial deposit will be influenced by climatic conditions. Three fungicides, including systemic and contact fungicides and using various product formulations were applied to apples in lab experiments with a rainfall simulator and in field studies in orchards to assess the factors impacting rain fastness. Drying time after application of pesticides is a key parameter in assessing rain fastness. From lab trials there were some indications that rain fastness of systemic pesticides in leaves was lower than in apples, whereas in the field trial, the leaves exhibited a better rain fastness than the apples. It is notable that experimental set-ups for studying the impact of rainfall are challenging and warrant further research.

Enteric pathogens: As few pathogens were detected within the Veg-i-Trade project, the study of the impact of climate and climate change on microbial contamination of leafy greens focused on *E. coli* as the indicator organism. The central Veg-i-Trade dataset was used as input for a statistical model. The *E. coli* concentration is positively correlated with the mean daily temperature, whereas precipitation was not shown to be significant. Daily average temperatures, projected by selected climate change scenario models, quantified the expected changes in *E. coli* concentration, ranging from a 27-40% increase in Belgium to 19-30% in Spain. However, management of farming systems (irrigation source and method, manure application) also differs regionally and interacts with climatic conditions to affect *E. coli* concentration. In case of a flooding event, high *E. coli* levels and pathogens were noted, but a holding time of 21 days combined with solar radiation reduced *E. coli* and *Salmonella* on the leaves to non-detectable levels.

Mycotoxins: *Alternaria* molds and their mycotoxins alternariol (AOH) and alternariol monomethyl ether (AME) were established as a pre-harvest problem. The risk of AOH and AME contamination in tomato- derived products could be reduced by sorting and removing any field harvested tomatoes with visible mold growth. Temperature influenced growth and mycotoxin production. Thus, as field temperatures increase as an effect of climate change, mold growth and mycotoxin production is expected to increase.

Dissemination, training and risk communication.

Veg-i-Trade was a challenging project in management and for the collaboration between various cultures and disciplines, but provided a platform to enhance dialogue. Multiple interactions and exchange of staff and students resulted in a network of skilled professionals with global experience in safety of fresh produce, quality assurance and risk assessment.

Veg-i-Trade partners visited many production and processing sites in their respective countries and thanks to this voluntary collaboration by many producers, huge amounts of information were obtained and analysed. Since these findings from Veg-i-Trade also partially stem from studying actual practices of growers and traders themselves, and relate to concerns stakeholders already have, Veg-i-Trade conclusions may serve as guidelines to take safety of fresh produce to the next level. It is clear that climate and climate change should not be ignored, and will in particular affect the introduction of contaminants at the pre-harvest stage.

Veg-i-Trade investigated risk communication for understandable messages, also to the lay public, to address the consumers' right- to-know about potential hazards, and to increase awareness for making informed decisions. In a cross-cultural study in Europe, it was shown that the emotional reactions and risk perception about safety of fresh produce by consumers differed between countries. This can be explained by the finding that trust in the government, subjective knowledge about the topic, and behavioral intentions differ as well. It was shown that the credibility of a risk message can be increased when the main argument (the hazard in fresh produce) is made vivid (e.g. with a picture) and combined with a two-sided message communicating both risks and benefits.

Due to globalization, fresh produce supply chains naturally evolved to obtain effective food safety management which is also embedded in the effort to bring sufficient and nutritious high quality fresh produce to the consumer. The fresh produce chains are increasingly challenged these days to also respond to demands for environmental sustainability and social accountability, also discussed within the Veg-i-Trade's framework of food sovereignty. In particular, because on the worldwide scale, the quantities of fresh fruit and vegetables traded represent just a limited fraction of what is produced, the concept of safe food for all is a key issue.

Some of the Veg-i-trade outcomes are available as e-learning modules for local and global training programs to take safety of fresh produce to the next level. Major achievements can also been seen in the website: www.Veg-i-Trade.org.