EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on the risk posed by Shiga toxinproducing Escherichia coli (STEC) and other pathogenic bacteria in seeds and sprouted seeds

EFSA publication; Hald, Tine

Link to article, DOI:
10.2903/j.efsa.2011.2424

Publication date:
2012

Document Version
Publisher's PDF, also known as Version of record

SCIENTIFIC OPINION

Scientific Opinion on the risk posed by Shiga toxin-producing *Escherichia coli* (STEC) and other pathogenic bacteria in seeds and sprouted seeds [1]

EFSA Panel on Biological Hazards (BIOHAZ)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 6 March 2012, replaces the earlier version published on 15 November 2011[*]

ABSTRACT

Sprouted seeds are young seedlings obtained from the germination of seeds. They are ready-to-eat foods which have caused large outbreaks. The bacterial pathogens most frequently associated with illness due to contaminated sprouted seeds are *Salmonella* and to a lesser extent STEC. *Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus* and *Yersinia enterocolitica* have also been transmitted by sprouted seeds, albeit very rarely. Dry seed contaminated with bacterial pathogens has been identified as the most likely initial source of sprout-associated outbreaks; although other routes of contamination (e.g. during production due to poor practices) may also occur. In some outbreaks, contamination of seeds with as low as 4 *Salmonella* per kg was sufficient for the sprouts to cause disease. Seeds purchased by sprouts producers are usually not grown specifically for this purpose. They may be contaminated during production, harvest, storage and transport, and there may be difficulties in traceability of seeds from production to sprouting. Bacterial pathogens on seeds may survive for long periods during seed storage. There is so far no guarantee of a bactericidal step which is able to control contamination of seeds with bacterial foodborne pathogens acquired prior to germination. Due to the high humidity and the favourable temperature during sprouting, bacterial pathogens present on dry seeds can multiply on the sprouts. Contamination with pathogenic bacteria must be minimized by identification of seed crops intended for sprouted seeds production before planting, and application of GAP, GHP, GMP, HACCP principles at all steps of the production chain. The relevance of decontamination treatments of seeds and of microbiological criteria is also discussed.

KEY WORDS

Decontamination techniques, foodborne pathogenic bacteria, *Salmonella,* seeds, Shiga toxin-producing *E. coli* (STEC), sprouted seeds, microbiological criteria, mitigation options.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on MRA in sprouts: Ana Allende**,** Gunnar Andersson, Paul Cook, Marie-Agnès Jacques, James McLauchlin, Kirsten Mooijman**,** Stefano Morabito, Christophe Nguyen-The for the preparatory work on this scientific opinion and, the hearing experts: Cynthia Andersson and Jelle Kuijper and EFSA staff: Maria Teresa da Silva Felício, Marta Hugas and Ernesto Liebana Criado for the support provided to this scientific opinion.

[*] Editorial amendment on page 46 (line 10) "Two additional three class microbiological criteria …" was replaced with "Two additional microbiological criteria …".

Suggested citation: EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on the risk posed by Shiga toxin-producing *Escherichia coli* (STEC) and other pathogenic bacteria in seeds and sprouted seeds. EFSA Journal 2011;9(11):2424. [101 pp.] doi:10.2903/j.efsa.2011.2424. Available online: www.efsa.europa.eu/efsajournal

SUMMARY

The European Commission asked the Panel on Biological Hazards to issue a scientific Opinion on the public health risk of Shiga toxin-producing *E. coli* (STEC) and other pathogenic bacteria that may contaminate seeds and sprouted seeds and in particular the Panel was asked: (i) to assess the public health risk caused by STEC and other pathogenic bacteria that may contaminate both seeds and sprouted seeds intended for direct human consumption; (ii) to the extent possible, to identify risk factors contributing to the development of STEC and other pathogenic bacteria that may contaminate these seeds and sprouted seeds; (iii) to recommend possible specific mitigation options, and to assess their effectiveness and efficiency to reduce the risk throughout the food chain (from the seed production until final consumption); and lastly, (iv) to recommend, if considered relevant, microbiological criteria for seeds and sprouted seeds, water, and other material that may contaminate the seeds and sprouts throughout the production chain.

On the assessment of the public health risk, the BIOHAZ Panel concluded that sprouted seeds are ready-to-eat foods with microbial food safety concern due to the potential for certain pathogenic bacteria to contaminate the raw materials (seeds) and to grow during germination and sprouting, and to their consumption patterns (raw or minimally processed). The various types of sprouted seeds (sprouts, shoots, cress) may not represent the same risk for contamination with, and growth of, pathogenic bacteria. There is mostly data on risks from sprouts, and a scarcity of data for shoots and cress: for these final two categories the production is more comparable to that of fresh-cut leafy vegetables. Alfalfa and mung bean sprouts have been the most commonly consumed and most frequently implicated products in outbreaks. *Salmonella* and pathogenic *Escherichia coli* (including STEC) are the most commonly reported bacterial pathogens causing outbreaks associated with the consumption of contaminated sprouts. As found for *Salmonella*, very low contamination levels of dry seeds (e.g. 4 MPN/kg) can cause sprout associated-outbreaks. Other bacterial pathogens (e.g. *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Yersinia enterocolitica*) have also been implicated with sprout-associated outbreaks, although these have been reported very rarely. Epidemiological data shows that a single contaminated seed lot may be used by several sprouting plants, even in different countries, causing widespread related outbreaks. Due to limitations in available data, it has not been possible to carry out a quantitative microbiological risk assessment and estimate the proportion of foodborne infections attributable to the consumption of contaminated sprouted seeds. Large outbreaks involving these products (e.g. the outbreak in Germany 2011) illustrate the potential to cause major public health emergencies affecting previously healthy people and not limited to those considered particularly vulnerable to infections. Reliable methods for decontaminating all types of seeds or sprouted seeds are not currently available.

On the identification of risk factors, the BIOHAZ Panel concluded that pathogenic bacteria can be carried and transmitted by animals, humans and the environment, and they may contaminate seeds in the field and throughout the sprouted seed production chain. Seeds may be contaminated via diverse routes. The most relevant risk factors are associated with the effect of agricultural practices on seed production, storage and distribution: contaminated irrigation water and/or manure, presence of birds and rodents in storage facilities, dust and soil particles are potential sources of contamination. Processing conditions (e.g. temperature, humidity) prevailing during germination and sprouting of contaminated seeds favour the growth and dissemination of pathogenic bacteria and should be considered as major risk factors. The widespread distribution of seed lots is a risk factor that may increase the size and geographical spread of outbreaks. Poor traceability of the seed lots may delay the action taken by health authorities to control outbreaks. Poor traceability will also present difficulties for the sprouted seed producers to assess the hygienic quality of lots of seeds and consequently increases the risk of using contaminated seeds in the production process. Microbiological methods to detect emerging pathogens in seeds and sprouted seeds may not yet exist or be applied, posing an additional challenge in the identification of the source of outbreaks. There is limited information on risk factors during the production of shoots and cress compared to sprouts.

Considering that sprouted seeds are ready-to-eat foods, contamination by asymptomatic carriers of pathogenic bacteria should not be excluded as a potential risk factor. Considering the above mentioned risk factors, it has been difficult to date to control the hygiene of the production process of sprouted seeds.

Regarding possible specific mitigation options, the BIOHAZ Panel indicated that food safety management based on HACCP principles should be the objective of operators producing sprouted seeds including GAP, GHP and GMP along the whole chain from seed production to the final sprouted product. The hazard analysis should include risk classification of commodities, regions of origin, and operators and suppliers of seeds. In seed and to some extent sprout production, it can be difficult to define critical control points (CCPs) including hazard control measures, critical limits and monitoring. Preventing seed contamination is particularly important because of the long survival of bacterial pathogens on seeds and their multiplication during sprouted seed production. This concerns both seeds intended for industrial and home sprouting. GAP and GHP concerning primary production, harvest and storage should be applied with a high level of stringency, similar to that applied for the primary production of fresh-produce, to minimize the risk of contamination with pathogenic bacteria. These include but are not limited to: (i) identifying seed crops intended for sprout production before planting; (ii) safe use of fertilizers and irrigation water; (iii) minimizing contamination of seeds with soil during harvest and preventing mechanical damage of seeds; (iv) ensuring that workers harvesting and handling seeds follow hygiene and health requirements; (v) ensuring that seeds are transported, processed and stored under conditions which will minimize the potential for microbial contamination; (vi) removing damaged seeds, from which it may be more difficult to remove pathogenic bacteria, or avoiding lots with too many damaged seeds; (vii) improving traceability of seed lots and minimizing, as far as possible, mixing of seed lots. Washing of seeds to remove dirt before sprouting is recommended. Measures to prevent introduction of pathogens in sprouted seeds production remain of the foremost importance. During sprouting, GMP, GHP and HACCP principles should be applied as for other ready-to-eat foods. Use of potable water is necessary during sprouting. Decontamination of seeds prior to sprouting, is currently practiced in some EU Member States as an additional risk mitigation measure as part of a combined intervention strategy. To date, no method of decontamination is available to ensure elimination of pathogens in all types of seeds without affecting seed germination or sprout yield. Decontamination of seeds would need to be optimised for each type of seed. The safety and efficacy of different seed decontamination treatments (e.g. chemical, heat treatment, irradiation alone or in combination) should be evaluated in a harmonised way at EU level. The consequence of any decontamination treatment on the background microflora and its potential impact on the pathogenic bacteria during sprouting should be taken into account. A chill chain for sprouts and shoots from end of production to consumption is necessary to limit growth of bacterial pathogens. Stakeholders, including consumers and also those practicing home-sprouting, at all parts of the production chain, should be informed of the food safety risk posed by sprouted seeds.

Regarding microbiological criteria, the BIOHAZ Panel indicated that as sprouted seeds are ready-to-eat foods, finding pathogenic bacteria in seeds used for sprouting or in sprouted seeds indicates a public health risk. Microbiological testing alone may convey a false sense of security due to the statistical limitation of sampling plans. A negative sample result does not ensure the absence of the pathogen in the tested lot, particularly where it is present at low or heterogeneous prevalence. It is currently not possible to evaluate the extent of public health protection provided by specific microbiological criteria for seeds and sprouted seeds. This highlights the need for data collection to conduct quantitative risk assessment. Microbiological criteria including the design of sampling plans for pathogenic bacteria should be considered as one of the components of the food safety management system for the sprouted seed production chain. Existing food safety criteria and process hygiene criteria in Regulation (EC) 2073/2005 relevant to sprouted seeds or seeds are: a *Salmonella* food safety criterion, *L. monocytogenes* food safety criterion, and a total *E. coli* process hygiene criterion. Consideration should be given to the development of new or revision of the existing microbiological criteria for pathogens most frequently associated with outbreaks involving sprouts (*Salmonella* spp.

and pathogenic *E. coli).* Currently, there are no criteria for pathogenic *E. coli.* If such criteria were to be proposed serotypes of concern and associated with severe human disease should be considered. Microbiological criteria for *Salmonella,* pathogenic *E.coli* and *L. monocytogenes* could be considered for seeds before the start of the production process, during sprouting and in the final product, to this respect: (i) detection and mitigation of a contamination problem earlier in the sprouted seed production chain (seeds) may have advantages as it avoids contamination being amplified during the full sprouting process; (ii) testing seeds alone does not permit to detect contamination which may come at a later stage in the production process. Therefore microbiological criteria could be useful during the sprouting process and/or for the final product; (iii) when considering a microbiological criterion for the final sprouted seeds, the time required for the detection methods for pathogenic bacteria combined with the short shelf-life may not allow to withdraw the product in the event of a non-compliance, and (iv) an additional value of testing would be to build up knowledge about the hygienic performance of seed and sprouted seeds producers. The Panel also indicated that when targets for seeds are considered it should be taken into account that low levels of *Salmonella* (4 MPN/kg) have been sufficient to cause sprouted seed associated-outbreaks. A 2-class sampling plan "absence in 25g", n=5; c=0, as specified in EC Regulation 2073/2005 for sprouted seeds, will not give sufficient confidence to demonstrate absence of a target pathogen at these low levels in seeds. In order to increase probability of rejection of a positive lot it would be necessary to analyze kilogram quantities of the sample. In order to reduce the number of analytical samples when testing seeds, pooling strategies can be applied. Different approaches have been proposed; any pooling strategy needs to be validated and standardized taking into account the low level of contamination expected in the seeds. Seeds intended for home sprouting should be subjected to a sampling protocol for pathogens having at least the same level of stringency as for seeds intended for commercial sprout production. Ideally the inspection lot on which the sampling plan is applied should represent only one seed production lot. Testing seeds will be especially important if operators use new seed commodities or source seeds from new suppliers where a previous history of microbiological testing may be limited or lacking. Dust and debris from seed storage areas may also be regularly tested for *Salmonella* and STEC. Total *E.coli* counts may be included to provide evidence of faecal contamination. During the industrial sprouting process testing spent irrigation water for pathogenic bacteria has been proposed as an alternative strategy to the analysis of a large number of sprout samples. However, there are some uncertainties regarding the sensitivity of this strategy. Sampling could be conducted on sprouted seed production environments. It could be applied for pathogenic bacteria such as *L. monocytogenes* as well as indicator bacteria. There are currently no indicator organisms that can effectively substitute for the testing of pathogens in seeds, sprouted seeds or irrigation water. Testing for *E. coli*, *Enterobacteriaceae* and *Listeria* spp. can inform process hygiene control. Further work may be required to assess the value of tests for these indicator organisms. It is important to use standard methods for testing, preferably EN/ISO methods especially developed for analyzing microorganisms in seeds, spent water and sprouts. Due to the short shelf life of sprouted seeds rapid (e.g. molecular) methods for detection and/or typing of pathogenic bacteria are important to obtain timely information on the microbiological status of sprouted seeds. Alternative (rapid) methods should be validated according to EN/ISO methods (e.g. EN/ISO 16140).

TABLE OF CONTENTS

Abstract 1
Summary 2
Table of contents 5
Background as provided by the European Commission 7
Terms of reference as provided by the European Commission 8
Assessment 9
1. Introduction 9
2. Sprouted seeds production 10
2.1. Description of EU sprouted seeds sector 10
2.2. Seed production process (including on-farm production and seed processing) 12
2.2.1. On-farm seed production 12
2.2.2. Seed processing 13
2.3. Sprouted seeds production 14
3. Bacteriological hazard identification and characterisation 16
3.1. Sprouted seed-associated outbreaks 16
3.2. Rapid Alert System on Food and Feed (RASFF) notifications on sprouted seeds 19
3.3. *Salmonella* 20
3.4. Pathogenic *Escherichia coli* 20
3.5. *Yersinia enterocolitica* 22
3.6. *Listeria monocytogenes* 22
3.7. *Bacillus cereus* 23
3.8. *Staphylococcus aureus* 23
3.9. Other pathogenic bacteria 23
3.10. Resistance of foodborne pathogenic bacteria to antimicrobial agents 23
3.11. Conclusions 24
4. Exposure assessment 24
4.1. EU monitoring data on occurrence of foodborne pathogenic bacteria in seeds and in sprouted seeds 24
4.2. Survival and growth of foodborne pathogenic bacteria on seeds and sprouted seeds 25
4.2.1. General growth and survival characteristics of foodborne pathogenic bacteria 25
4.2.2. Survival on seeds 26
4.2.2.1. Attachment and localisation of pathogenic bacteria on seeds 26
4.2.2.2. Fate of pathogenic bacteria on seeds 26
4.2.3. Growth during sprouting 28
4.2.3.1. Sprouted seeds 28
4.2.3.2. Shoots and cress 29
4.2.4. Survival and growth on sprouted seeds 29
4.3. EU consumption data for sprouted seeds 30
5. Control and reduction/elimination of bacterial hazards in seeds and sprouted seeds 30
5.1. Human exposure routes 31
5.2. Critical points in seed production where bacterial contamination is likely to occur 31
5.3. Critical points during the sprouting process where bacterial contamination is likely to occur33
5.4. GAPs and GHP during seed production and storage 33
5.5. GMP, GHP and HACCP principles during sprouting 34
5.6. Decontamination techniques for seeds and sprouts, shoots and cress derived from seeds .. 36
5.6.1. Chemical decontamination of seeds, dry and during sprouting 37
5.6.2. Alternative decontamination treatments of seeds and sprouted seeds 38
5.6.3. Decontamination treatments of sprouts, shoots and cress 40
5.6.4. Conclusions on decontamination methods 40
6. Microbiological criteria for seeds and sprouted seeds 45
6.1. Introduction to microbiological criteria 45
6.2. Specific criteria/limits for seeds and sprouted seeds 45

6.2.1. Existing microbiological criteria/limits in EU legislation 45
6.2.2. Microbiological criteria/limits in guidelines 46
6.3. The value of existing criteria and the possible establishment of new ones 46
7. Sampling and analytical methods for the detection and enumeration of foodborne pathogenic bacteria in seeds and sprouted seeds 49
7.1. Introduction 49
7.2. Sampling and sample size 49
7.2.1. Heterogenous distribution of pathogens 49
7.2.2. Pooling of samples 50
7.2.3. Testing for indicator bacteria 51
7.2.4. Seeds 52
7.2.5. Spent irrigation water 55
7.2.6. Sprouted seeds 56
7.2.7. Environmental and process control samples (dust, swabs) 57
7.3. Standardisation of methods for detection and enumeration of bacterial foodborne pathogens in seeds and sprouted seeds 57
7.3.1. General considerations 57
7.3.2. Standard methods 58
7.3.3. Molecular methods 58
7.3.4. Rapid methods. 59
7.4. Conclusions and recommendations 60
7.4.1. Conclusions 60
8. Considerations on the assessment of the contribution of seeds and sprouted seeds as a source of foodborne infection in humans 61
Conclusions and Recommendations 63
References 68
Appendices 84
A. Data reported in the zoonoses database on occurrence of foodborne outbreaks where implicated foodstuffs were sprouted seeds (2004-2010) 84
B. RASFF notifications 85
C. Data reported in the zoonoses database on occurrence of zoonotic agents in sprouted seeds (2004-2010) 88
D. Food consumption data on sprouted seeds 90
E. Review legislation 98
Glossary 100

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

In May 2011 a major outbreak of STEC O104 occurred in Germany. More than 3,000 people were reported ill with symptoms of bloody diarrhoea from this unusual strain, which was not considered of public health concern so far. Many of them were suffering from a complication that causes kidney failure (Haemolytic Uremic Syndrome, HUS). The outbreak resulted in the death of more than 40 people. Other countries reported a certain number of people becoming ill by the same strain, most of whom had recently visited the region of northern Germany where the outbreak occurred.

At the end of June, there was a second outbreak in Bordeaux, France, which was caused by the same *E. coli* strain as the one found in Germany. In both cases, investigations pointed to the direction of sprouted seeds.

The Commission mandated EFSA to carry out a tracing back exercise, using data from both outbreaks to identify the initial source. Based on the initial results of the tracing back exercise, fenugreek seeds from Egypt were considered to be a possible initial source.

Regulation (EC) 852/2004[4] on the hygiene of foodstuffs lays down general hygiene requirements to be respected by food businesses at all stages of the food chain. All food business operators have to comply with requirements for good hygiene practice in accordance with this Regulation, thus preventing the contamination of food of animal and of plant origin. Establishments other than primary producers and associated activities must implement procedures based on the HACCP principles to monitor effectively the risks.

In addition to the general hygiene rules, the following microbiological criteria have been laid down in Regulation (EC) No 2073/2005[5]:

- *Listeria monocytogenes* food safety criterion: 100 cfu/g, if the manufacturer is able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit 100 cfu/g throughout the shelf-life. In other cases absence in 25 g applies;
- *Salmonella* food safety criterion: absence in 25 g of sprouted seeds;
- *E. coli* process hygiene criteria in pre-cut fruit and vegetables (ready-to-eat): n=5, c=2, m=100 cfu/g, M=1000 cfu/g.

On 9th June EFSA issued a Technical Report on information of STEC in food and animals, in support of the needs for information following the outbreak in Germany. It provides a summary of the historic data available on STEC in food and animals, and data on the food-borne outbreaks caused by STEC. It contains a table, with STEC in fruit and vegetables and products thereof, including sprouted seeds.

Furthermore, EFSA scientific Opinion on Monitoring of verotoxigenic *Escherichia coli* (VTEC) and identification of human pathogenic VTEC types[6], identified other *E. coli* serotypes most frequently associated with severe human infections (including HUS) in the EU.

In addition, between 2004 and 2010, 11 food-borne outbreaks caused by sprouts have been reported in the EFSA/ECDC annual EU summary report on trends and sources of zoonoses and zoonotic agents and food-borne outbreaks[7]: 10 *Salmonella* outbreaks (DK 1, FI 2, SE2, EE1, UK 3, NO 1) and 1 *Staphylococcus aureus* outbreak (DK). Between 2004 and 2009, microbiological testing demonstrated that 1.8% and 0.35% of about 2000 samples were positive for *Salmonella* and *Listeria* respectively.

[4] OJ L 139, 30.4.2004, p.1-54
[5] OJ L 338, 22.12.2005, p.1-26
[6] EFSA Journal (2007) 579, 1-61
[7] 2009 Report: EFSA Journal 2011; 9(3): 2090

On 9th June EFSA issued an urgent advice on the public health of STEC in fresh vegetables providing an assessment of consumers' exposure to STEC through consumption of raw vegetables and suggesting possible pre-harvest and post-harvest mitigation options.

Furthermore, on 29th June, EFSA and the European Centre for Disease Prevention and Control (ECDC) have jointly issued a rapid risk assessment of the cluster of HUS in Bordeaux, France, strongly recommending to consumers not to grow sprouts for their own consumption and not to eat sprouts or sprouted seeds unless they have been cooked thoroughly.

Even if a low level of bacterial contamination may be present in some seeds, the specific production conditions for sprouts, characterised by high temperature and high humidity, can provide ideal conditions for microbial growth.

In view of the above, there is a need to:

- Assess the public health risk posed by Shiga toxin-producing *Escherichia coli* (STEC) and other pathogenic bacteria that may contaminate seeds and sprouted seeds intended for direct human consumption (without heat treatment).
- Given the identified risk, to recommend risk mitigation options.
- Assess the need to establish specific microbiological criteria.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is asked to issue a scientific Opinion on the public health risk of STEC and other pathogenic bacteria that may contaminate seeds and sprouted seeds and, in particular:

1. To assess the public health risk caused by STEC and other pathogenic bacteria that may contaminate both seeds and sprouted seeds intended for direct human consumption;
2. To the extent possible, to identify risk factors contributing to the development of STEC and other pathogenic bacteria that may contaminate these seeds and sprouted seeds;
3. To recommend possible specific mitigation options and to assess their effectiveness and efficiency to reduce the risk posed by STEC and other pathogenic bacteria that may contaminate these seeds and sprouted seeds throughout the food chain. The mitigating options should cover all parts of the food chain from the seed production until final consumption;
4. To recommend, if considered relevant, microbiological criteria for seeds and sprouted seeds, water, and other material that may contaminate the seeds and sprouts throughout the production chain. This may include process hygiene criteria.

ASSESSMENT

1. INTRODUCTION

Sprouted Seeds comprise different types of products obtained from seeds, according to the part of the plant which is collected and consumed, and in particular whether the seed is still present or is removed. For the purpose of this Opinion, these products obtained from seeds are all included under the generic term "sprouted seeds" and comprise:

- "Sprouts", obtained from the germination of true seeds and their development in water, collected before the development of leaves. The final product still contains the seed.

- "Shoots" which are obtained from the germination and the development of the seeds (true seeds usually, although tubers or bulbs may also be used) in water, to produce a green shoot with very young leaves and/or cotyledons. The shoots and the leaves are harvested at the end of the production process and the final product does not include the seed teguments and the roots.

- "Cress" is obtained from the germination and development of true seeds in soil or in hydroponic substrate, to produce a green shoot with very young leaves and/or cotyledons. Cress is usually sold as the entire plant in its substrate or soil and the aerial part is harvested by consumers. In the present Opinion "cress" refers to this particular mode of production and not to some botanical species.

These three categories of sprouted seeds are considered in the Opinion, although the majority of information available on the risk posed by pathogenic bacteria concerns "sprouts" as defined above.

Seeds intended for direct human consumption without sprouting are not considered in this Opinion.

The scope of this Opinion is to assess the risk, for public health, due to the presence of Shiga toxin-producing *Escherichia coli* (STEC)[8] and other pathogenic bacteria that may contaminate seeds and sprouted seeds intended for direct human consumption, and to evaluate the most important options to control the risk, including the setting of microbiological criteria for seeds used for sprouting and sprouted seeds, water, and other material that may contaminate the seeds and sprouts throughout the production chain.

In accordance with the terms of reference, this Opinion does not consider hazards such as parasites and viruses, and contamination by fungi and mycotoxins. Furthermore, due to the lack of scientific information, and the limited time available for this assessment, it was not possible to quantify the contribution of seeds and sprouted seeds contaminated by Shiga toxin-producing *Escherichia coli* and other pathogenic bacteria to the prevalence of these bacteria in food, and to the incidence of foodborne disease in humans. The BIOHAZ Panel therefore proposes a qualitative risk assessment which is the feasible option within the time frame and resources and knowledge available.

During the 1990s, sprouted seeds became a more common food component. During the 90s there were increasing reports in the scientific literature of bacterial food borne illness associated with sprout consumption which have raised concerns from public health agencies and consumers about the safety of these products. The microbial pathogens most commonly associated with consumption of sprouted seeds were *Salmonella* spp., and pathogenic *Escherichia coli* (NACMCF, 1999).

[8] Also known as Verocytotoxin-producing *Escherichia coli* (VTEC).

Sprouted seeds exhibit a unique hazard potential, since: (i) the germination stage breaches the inhibitory barrier of the seed coat, allowing bacterial pathogens which may be present to grow on nutrients from the sprouted seed (EC, 2002), and (ii) are usually consumed raw, being ready-to-eat products. Contamination of seeds intended for sprouting, or the sprouts themselves can occur at any point in the production chain from the farm, during transport and storage, and up to the point of final consumption. This Opinion will focus on two areas of concern, i.e., 1) seed contamination, from the production in the field to the seed conditioning facility, and 2) sprouted seed production. Seed appears to be the primary source of contamination in sprout-associated outbreaks (CAC, 2003). Further, the conditions during the sprouting process may permit growth of pathogenic bacteria if they are present on or in the seed. After sprouting (e.g., during packaging, distribution, retail sale, and preparation for consumption), microbial hazards and risk factors are similar to those previously identified for fresh produce (NACMCF, 1999).

In the EU, sprouted seed producers usually consider themselves as primary producers and not as food business operators, although this may depend on the Member State and on the processing operations done on the final products within the production environment.

Sprouted seeds are mostly consumed raw, although some may receive a rapid cooking process (especially mung bean sprouts which can be stir-fried in Asian cooking). Therefore, it is the opinion of the Panel that all sprouts, shoots and cress should be considered as ready-to-eat foods.

2. SPROUTED SEEDS PRODUCTION

2.1. Description of EU sprouted seeds sector

An organisation of producers (Freshfel Europe, http://www.freshfel.org/asp/index.asp) was invited to nominate representatives of the sprouted seed production sector to provide information to the WG members on current production practices in the EU and to participate in a technical hearing. Information provided by this professional association has been used in this section of the present Opinion (www.efsa.europa.eu/en/supporting/pub/203e.htm).

Sprouted seeds are commonly produced from the following seeds: adzuki beans, alfalfa, broccoli and other *Brassica* spp., buckwheat, cabbage, chickpeas (garbanzo), clover, cress, leek, lentils, linseed, mung beans, mustard, garlic, grass pea, green and yellow peas, onion, quinoa, radish, red beet, rice, rye, sesame, snow pea, soy, sunflower, triticale and wheat among others (Beales, 2004; EC, 2002; HC, 2001; Schrader, 2002) and fenugreek . In addition to sprouts, in the EU this sector also produces other types of sprouted seeds, known as shoots and cress. Sprouts and shoots differ in their maturity stage, as sprouts are just harvested after germination whereas shoots are very young plants. Sprouts and shoots are germinated and grown from seeds without using any substrate and only water is used. In contrast, cress is a plant with the same maturity stage than shoots but it is germinated and grown using different types of substrates such as soil or other synthetic substrates.

All these products are eaten singly or in salad mixes, as garnishes and also following mild cooking (stir frying) in some Asian dishes. Sprouts grown from different plant species vary in texture and taste: some are spicy (e.g. radish), some are used in Asian foods (e.g. mung bean), and others are delicate (e.g. alfafa) and are used in salads and sandwiches to add texture. Currently, there is a trend towards supplying mixtures of sprouted seeds. Thus, it is not uncommon to find products with 2 or more sprouted seeds mixed together. Table 1 shows which varieties of sprouted seeds are most commonly available on the EU market. However, this is a very dynamic sector, and new varieties are introduced every year to increase the assortment available to consumers.

Table 1: Top five types of seeds used for sprouting in the EU (source: Freshfel Europe).

Top five types of seeds used for sprouting		Scientific name
1.	Mung Bean sprouts	*Vigna radiata* (L.) R.Wilczek
2.	Alfalfa sprouts	*Medicago sativa* L.
3.	Radish sprouts	*Raphanus sativus* L.
4.	Peas shoots/cress	*Pisum sativum* L.
5.	Sunflower shoots/cress	*Helianthus annuus* L.

Among the seeds intended for sprout production (Table 2), there are very different types of seeds, which show differences in the nature of the seed surface, the seed size and water content. The effect these differences have for the sprout physiology and food safety is not understood.

Table 2: Seeds used for production of sprouts, shoots and cress in EU (source: Freshfel Europe).

Seeds have and can be used for sprout production [(a)]		
1.	Adzuki	*Vigna angularis* (Willd) Ohwi & Ohashi
2.	Alfalfa	*Medicago sativa* L.
3.	Basil cress	*Ocimum basilicum* L.
4.	Borage cress	*Borago officinalis* L.
5.	Broccoli	*Brassica oleracea* L.
6.	Chick peas	*Cicer arietinum* L.
7.	Coriander	*Coriandrum sativum* L.
8.	Fennel	*Foeniculum vulgare* Hill
9.	Garden cress	*Lepidium sativum* Cambess
10.	Garlic	*Allium schoenoprasum* Regel & Tiling
11.	Leek	*Allium porrum* L.
12.	Lentil	*Lens culinaris* Medik
13.	Limon cress	*Ocimum americanum* Auct. ex Benth
14.	Mung Bean	*Vigna radiata* (L.) R.Wilczek
15.	Onion	*Allium cepa* L.
16.	Peas	*Pisum sativum* L.
17.	Radish	*Raphanus sativus* L.
18.	Root beet	*Beta vulgaris* L.
19.	Shiso	*Perilla frutescens* L. ex B.D. Jacks
20.	Sunflower	*Helianthus annuus* L.
21.	Wheat	*Triticum aestivum* L.

(a): The list is ordered alphabetically and not intended to be exhaustive because of the constant change in consumer preference, manufacturers' innovation and food preparation. Source for the plant names: The International Plant Names Index (http://www.ipni.org/ipni/plantnamesearchpage.do)

The sprout sector in Europe has an estimated turnover of about 150 – 200 m € for farms with a market value of 500 m € at the consumer level. There are approximately 100 companies active in producing sprouts, shoots and cress all over Europe and most of them are small companies with only few large companies in the market (source: Freshfel Europe). The top producing countries in terms of turnover are Netherlands (50 m €), UK (40 m €), Denmark (30 m €) and France (15 m €) (source: Freshfel Europe).

In addition to the industry sprout sector, there are also home-sprouted seeds. There are no data available about the market share and sprouting conditions.

The seed sprout production chain can be described as being divided in three parts: the production of seeds, the processing of seeds and the production of sprouted seeds.

2.2. Seed production process (including on-farm production and seed processing)

2.2.1. On-farm seed production

The seed production involves pre-harvest and post-harvest activities such as field preparation, planting, growth (including flowering and seed setting), irrigation, fertilization, pollination, swathing, field drying, seed harvest, storage and transport. Seed producers are involved in all these parts of the chain (FSANZ, 2010).

Plants for seed production are grown in typical agricultural environments and seeds are generally treated as raw agricultural products. There is a wide range of seeds that can be used for sprouting and thus a diverse range of agricultural practices may be associated with seed production. On farm seed production consists broadly of the steps described in Figure 1. Some growers may modify some of these practices depending on many factors, such as the needs of the crop, resources of the operation, and requirements, if any, imposed by the buyer or distributor (FSANZ, 2010; NACMCF, 1999).

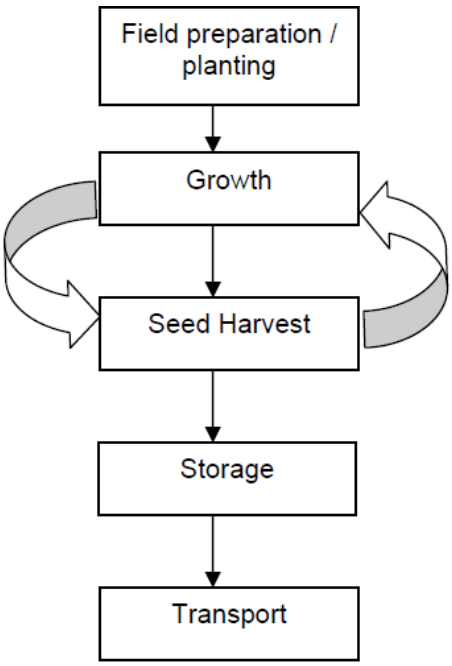

Figure 1: Typical seed production process (adapted from:(FSANZ, 2009)).

To minimize damage to seeds during harvest, the plant material may be allowed to dry for a number of days until the moisture content falls to the desired percentage (i.e. 14-16 %) or a chemical desiccant/defoliant is sprayed over the crop. Although it is mainly avoided, during harvest, extraneous material from the ground, including soil and other potential contaminants, can be also included in the final seed preparation. The plant material is then threshed inside the harvester to separate the seed from the other material (FSANZ, 2010).

One of the main concerns is that seeds are produced for several end-uses (e.g. edible seeds, animal feeds, oil production, horticulture etc) and not specifically for sprout production. In addition sprouted

seeds producers frequently purchase seeds from distributors (EFSA, 2011e) and may not know the origin and primary purpose of the seeds they use (source: Freshfel Europe).

2.2.2. Seed processing

Seed processing involves the receipt of harvested seeds from seed producers through to the supply of seed to sprout producers. In general, the seed can be purchased directly by sprout producers for sprouting, or is purchased by seed distributors. It is assumed that, seed distributors receive cleaned/graded seeds from seed processors, match customer requirements, and sell seeds to customers including sprout producers (FSANZ, 2010). Figure 2 shows the main steps that are generally involved in seed processing.

The seed processing mainly consists in the elimination of extraneous material such as soil, weed seeds and other debris. The cleaning usually consists in passing the seeds through a series of sieves and then further cleaned via use of a gravity table, where seeds are separated by their weight. The cleaning processes may reduce, but is unlikely to eliminate, pathogenic microorganisms. If performed incorrectly, these steps may serve as a source of contamination or cross contamination (NACMCF, 1999). For instance, the use of scarification, a process whereby the seed coat is broken or scratched to increase the germination rate, may increase contamination of pathogens during subsequent processing steps, and make decontamination procedures, more difficult. Once cleaned, seeds are generally packed into bags for the bulk seed market.

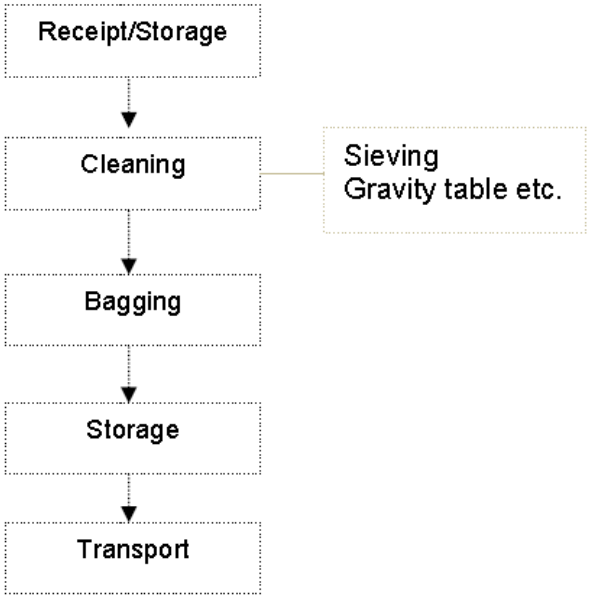

Figure 2: Typical seed processing chain (from: (FSANZ, 2010)).

As previously mentioned, for most seed crops, only a small proportion of harvested seed goes to sprout manufacturers. Further, the decision whether to direct seed to agricultural uses or to sprouting is often not made until after harvest. For example, alfalfa seeds do not start as food products but may be produced as seeds to be grown as crops, with only a small percentage set aside for sprouted seeds (Beales, 2004). Thus, the seed grower does not necessarily know whether seed will be sold for food use as seeds or sprouts and, therefore, may have little incentive for following GAPs. Finally, seed processing, shipping and selling practices often involve mixing multiple lots of seeds of different origins, which can be accompanied by a varietal mixing, complicating traceback and providing an opportunity for cross contamination (NACMCF, 1999).

Many countries have Codes of Practices and Guidelines that provide information on preventing field contamination. However, although these guidelines are applicable in the relevant countries, imported seeds may come from sources where the guidelines do not apply (Beales, 2004).

2.3. Sprouted seeds production

A brief summary of all the steps during sprouted seeds production is shown in Figure 3.

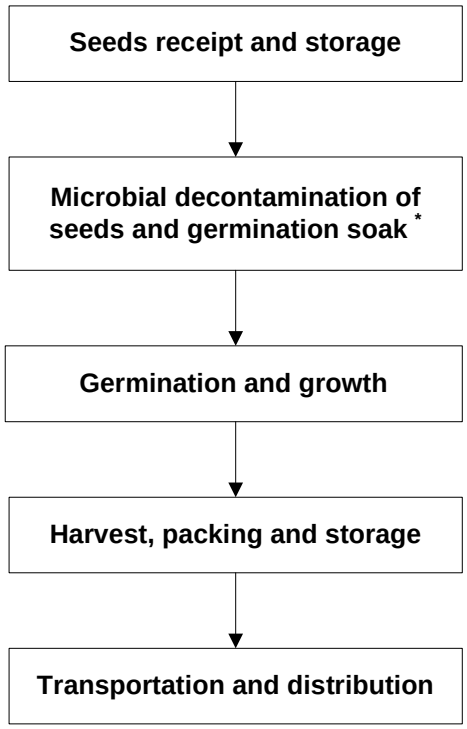

Figure 3: Generic Process Flow Diagram for sprouted seed production (adapted from: (FSANZ, 2010)). In EU the process may include other steps, depending on the type of seed, type of sprouted seed and the sprouted seed production plant.

* Seed decontamination and germination soak are not used in all sprouted seeds plants in EU.

Sprouted seeds production consists broadly of the steps depicted in Figure 3. Sprouted seeds production in the EU is very diverse. A wide range of seeds are used, which may be used to produce sprouts, shoots or cress. The size of production environments varies as well as the type of equipment used. Some sprouting facilities specialize in a few types of seeds whereas others produce a wide range of sprouted seeds (source: Freshfel Europe).

The batch of seeds received at the sprouted seeds plant, and used for a sprouted seeds production lot is frequently a mixture of different lots of seeds from different origins. The seeds may be stored for some time before sprouting, either in the plant (e.g. up to two years for seeds representing a small production volume) or at some steps of the seeds distribution (e.g. fenugreek seeds from 2009 sprouted in 2011, (EFSA, 2011e).

Soaking causes seeds to swell and softens seed coats so that a sprout will grow out of the seed. Rinsing removes residues from soaking. When applied, seed decontamination may involve chemical sanitizers (e.g. chlorine or hydrogen peroxide) or heat, or a combination of both (see chapter 5).

Germination and growth require large amounts of water and involves frequent watering of the sprouts (NACMCF, 1999). Water is always renewed and does not accumulate in the sprouting equipment. As germination and sprouting release heat, watering also permits cooling the bulk of sprouting seeds to maintain an adequate temperature. The frequency and duration of watering depends on the type of sprouted seeds and the equipment used (*e.g.* from 15 min to several hours intervals; (NACMCF, 1999)). Temperature during sprouting is frequently maintained around 21-26°C, and sprouting lasts for a few days (NACMCF, 1999). This varies with the type of product and with the production plant practices. Some plants may use lower temperatures and extend sprouting duration. Germination and growth conditions depend on the type of sprouted seeds and broadly comprise:

- To produce sprouts, seeds are germinated in beds, bins, or rotating drums. It occurs in a very humid environment and is frequently done in the dark.

- To produce shoots, seeds are placed in trays to permit growth of an aerial green shoot over the roots. No substrate is added, the shoots grow from the seeds with water only, regularly provided. Light is provided to permit accumulation of chlorophyll in the shoot.

- To produce cress, seeds are placed in trays in soil or hydroponic substrate. Light is provided to permit the development of a very young, green seedling. Conditions are very similar to the production of fresh produce in green houses (e.g. baby leaves or young herbs plants sold in pots).

For harvest, the whole sprouts are collected entirely, with in some cases an additional step to remove the seed coats. Shoots are cut to keep only the green, aerial part of the young plant. Cress is sold in small trays with their roots and substrate.

Sprouted seeds can be packaged at the production plant or transported in bulk to secondary packagers for supply to both commercial and retail customers where they may be incorporated into other products. At the retail level, sprouts may be sold packaged both as ready to eat and ready to cook products, as well as loose ingredients in salads (both alone or with other products), or incorporated into other foods including sandwiches. Packages may contain one type of sprouted seeds or mixtures of different types. The shelf life of sprouts usually comprises between 3 and 10 days depending on the type of product. Storage conditions vary depending on the type of sprouted seeds, sprouts and shoots being stored at refrigeration temperatures, whereas cress, as a whole plant, are stored at ambient temperature but sometimes also refrigerated. Sprouted seeds are packaged in polymeric films maintaining a high humidity.

The results of a Californian survey indicated there were significant gaps in sprout manufacturers' understanding and knowledge of food safety, Good Manufacturing Practices (GMPs), and US regulatory requirements. In addition, most sprout producers were not registered as food processing establishments as required by California regulations, and thus had not been previously inspected for compliance with GMPs. The survey found sprouts being produced in buildings, sheds, greenhouses, modified buses, fields, or a combination of these. The 45 firms covered by the inspection survey produced 24 different types of sprouts, the most frequently observed products were mung bean, alfalfa, clover, and radish sprouts. Only 25 firms reported producing over 5000 pounds (2,268 kg) of sprouts per day (NACMCF, 1999). Such information is not publically available in EU. Some features are similar to those outlined in the Californian survey: mung bean and alfalfa are the main seeds used for sprouts, the firms producing sprouted seeds have very diverse sizes and practices and sprouted seeds firms are usually not considered as food business operators but as primary producers.

Sprouted seeds may be produced at home by consumers, using commercial kits and/or domestic equipment. Sprouting conditions may be variable but in any case, very high humidity at favourable temperatures would be necessary to permit seeds sprouting.

3. BACTERIOLOGICAL HAZARD IDENTIFICATION AND CHARACTERISATION

The following sections of this chapter provide a brief summary on:

- the available data on sprout-associated outbreaks described in the scientific literature as well as those reported in the scope of the annual reporting of investigated EU foodborne outbreaks,
- the Rapid Alert System on Food and Feed (RASFF) notifications regarding seeds intended for sprouting or sprouted seeds,
- the main bacterial foodborne pathogens which have been implicated with sprout-associated outbreaks and/or have been isolated from samples of sprouted seeds in the scope of the EU annual reporting on occurrence of zoonotic agents in foodstuffs and
- other bacterial foodborne pathogens which have been described in fresh produce and/or fruits.

3.1. Sprouted seed-associated outbreaks

Several different bacterial pathogens have been implicated in sprouted seed-associated outbreaks or have been identified as a potential hazard. However the majority of incidents identified are associated with *Salmonella* and pathogenic *E.coli,* as already identified in 2002 by the Scientific Committee on Food (EC, 2002). Other pathogens implicated in outbreaks and incidents, include *Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes* and *Yersinia enterocolitica* (EC, 2002). Alfalfa and mung bean sprouts have been the most commonly reported sprouted seeds implicated in outbreaks of food-borne illness (FSANZ, 2010) with *Salmonella* spp. and *E. coli* O157:H7 being the most common agents (Breuer et al., 2001; Fett, 2006; Mahon et al., 1997; Mohle-Boetani et al., 2001; NACMCF, 1999; Puohiniemi et al., 1997; Taormina et al., 1999; Van Beneden et al., 1999). Table 3 shows reported outbreaks of food poisoning associated with sprouted seeds in various countries.

Table 3: Outbreaks associated with consumption of contaminated sprouted seeds (adapted from: (Beales, 2004; FSANZ, 2010; Taormina et al., 1999).

Year	Bacterial Pathogen (*Salmonella* species unless indicated otherwise)	No. of cases	Location	Type of sprouted seed	Likely source of contamination	Reference
1973	*Bacillus cereus*	4	USA.	Soy, cress, mustard	Seed	(Portnoy et al., 1976)
1982	*Yersinia enterocolitica*	16	USA	Bean sprouts	Bean sprouts immersed in contaminated well water	(Cover and Aber, 1989)
1988	*S.* Saintpaul	143	UK	Mung beans	Seed	(O'Mahony et al., 1990)
1988	*S.* Virchow	7	UK	Mung beans	Seed	(O'Mahony et al., 1990)
1988	*S.* Saintpaul, S.Havana, S. Munchen	148	Sweden	Mung beans		(Nguyen-The and Carlin, 1994)
1989	*S.*Goldcoast	31	UK	Cress	Seed and sprouter	(Joce et al., 1990)
1989	*Listeria monocytogenes*	1	Canada	Alfalfa	Growth during manufacture and prior to drying and encapsulation. Seeds originally for animal feed	(Farber et al., 1990)
1990	*S.*Anatum	15	USA	Alfalfa		(CDC, 1990)
1992	*S. enterica* 4,5,12,b:-	272	Finland	Mung beans		(Mattila et al., 1994)
1994	*S.* Bovismorbificans	492	Finland, Sweden	Alfalfa	Seed	(Ponka et al., 1995; Puohiniemi et al., 1997)
1995	*S.* Stanley	242	Finland and USA	Alfalfa	Seed	(Mahon et al., 1997)
1995-6	*S.* Newport	>133	USA, Canada, Denmark	Alfalfa	Seed	(Van Beneden et al., 1999)
1996	*S.* Stanley	30	USA	Alfalfa	Seed	(Barrett and Chaos, 1996; CDC, 1996)
1996	*E.coli* O157	>10,000	Japan	Radish	Seed	(Watanabe et al., 1999)
1996	*S.* Montevideo and *S,* Meleagridis	500	USA	Alfalfa	Seed	(Mohle-Boetani et al., 2001; NACMCF, 1999; Taormina et al., 1999)
1997	*S.* Anatum, *S.* Infantis	109	USA	Alfalfa	Seed	(Taormina et al., 1999)
1997	*E.coli* O157	79	USA	Alfalfa	Seed	(Breuer et al., 2001)
1996	*S.* Meleagridis	78	Canada	Alfalfa	Seed	(Sewell and Farber, 2001)
1997-8	*S.* Seftenberg	60	USA	Alfalfa	Seed and sprouting drum	(Mohle-Boetani et al., 2001)
1998	*S.* Havana,, *S.* Cuba	40	USA	Alfalfa	Seed	(CDC, 1998; Mohle-Boetani et al., 2001)
1998	*E.coli* O157	8	USA	Alfalfa and Clover	Seed and /or sprouter	(CDC, 1998; Mohle-Boetani et al., 2001)
1999	*S.* Mbandaka	83	USA	Alfalfa	Seed	(CDC, 1999)
1999	*S.* Muenchen	157	USA	Alfalfa	Seed	(Proctor et al., 2001a)
1999	*S.*Paratyphi var Java	51	Canada	Alfalfa	Seed	(Stratton et al., 2001)
1999	*S.* Saintpaul	36	USA	Clover		(CDC, 1999)
1999	*Salmonella* spp.	34	USA	Alfalfa		(CDC, 1999)

Year	Bacterial Pathogen (*Salmonella* species unless indicated otherwise)	No. of cases	Location	Type of sprouted seed	Likely source of contamination	Reference
1999	*S.* Typhimurium	120	USA	Alfalfa	Seed	(Winthrop et al., 2003)
2000	*S.* Enteritidis	27	The Netherlands	Mung beans	seed	(van Duynhoven et al., 2002)
2000	*S.* Enteritidis	75	USA	Mung beans		(CDC, 2000)
2000	*S.* Enteritidis	8	Canada	Alfalfa		(Harris et al., 2003)
2000	*S.* Enteritidis	84	Canada	Mung Beans	Seed	(Honish and Nguyen, 2001)
2001	*S.* Kottbus	31	USA	Alfalfa	Seed	(Mohle-Boetani et al., 2001)
2001	*S.* Enteritidis	26	USA (Hawaii)	Mung beans	Seed and/or sprouter	(CDC, 2003b)
2002	*S.* Abony	13	Finland	Mung beans		(MAF, 2003)
2003	*S.* Saintpaul	16	USA	Alfalfa		(CDC, 2003a)
2003	*E.coli* O157	7	USA	Alfalfa		(CDC, 2003a)
2003	*E.coli* O157	13	USA	Alfalfa		(CDC, 2003a)
2003	*S.* Chester	26	USA	Alfalfa		(CDC, 2003a)
2004	*E.coli* O157	2	USA	Alfalfa		(CDC, 2004)
2004	*S.* Bovismorbificans	35	USA	Alfalfa		(CDC, 2004)
2005-6	*S.* Oranienburg	126	Australia	Alfalfa	Seed	(OzFoodNet, 2006)
2006	*S.* Oranienburg	15	Australia	Alfalfa	Seed	(OzFoodNet, 2007)
2006	*S.* Braenderup	4	USA	Beans		(CDC, 2006)
2006	*S.* Bareilly and *S.*Virchow	115	Sweden	Mung beans	ND	(de Jong et al., 2007)
2007	*S.* Weltevrenden	45	Norway, Denmark and Finland	Alfalfa	Seed	(Emberland et al., 2007)
2009	*S.* Bovismorbificans	42	Finland	Alfalfa	seeds	(Rimhanen-Finne et al., 2011)
2010	*S.* Bareilly	231	UK	Mung beans	ND	(Cleary et al., 2010)
2011	*E.coli* O104	>3,700	Germany	Fenugreek	ND	(WHO, 2011a)

ND. Not determined

The majority of sprouted seed-associated outbreaks have been reported in the United States, however, outbreaks have also occurred in Canada, Germany, Sweden, Finland, Denmark, Germany, France, United Kingdom, Japan and Australia (FSANZ, 2010). The largest reported outbreak associated with sprouted seeds, with over 10,000 notified cases, was in Japan in 1996 and was attributed to consumption of radish sprouts contaminated with *E. coli* O157:H7 (Michino et al., 1999; Watanabe et al., 1999). The sprouted seed associated outbreak of *E.coli* O104:H4 in Germany in 2011 resulted in over 3,700 cases of illness (EFSA, 2011e).

The national differences in reporting of outbreak associated with sprouted seeds consumption may be due in part to the differences in amounts and types of sprouted seeds consumed in each country as well as to surveillance systems. In most of the outbreaks included in Table 3, the seed was suspected to be the origin of contamination, although poor production practices have also been implicated. It is also apparent from epidemiological data that seeds from a single producer or supplier may be very widely distributed (Beales, 2004; EFSA, 2011e). For example, investigations of the *S.* Stanley outbreak in 1995 indicated that contaminated alfalfa sprouts were grown by at least nine different producers. These producers obtained alfalfa seed from a single seed supplier in the USA who purchased the seeds from a seed shipper in the Netherlands. This same Dutch shipper also supplied the seed for sprouting and consumption to Finland where a further outbreak occurred. The seed was not grown in the Netherlands but, due to mixing of seed batches, it was not possible to determine the ultimate source of contamination, although evidence for contamination from rodents and birds was observed within the seed warehouse (Mahon et al., 1997).

There have been reports of sprouted seed-associated outbreaks where seed sanitising as recommended by the FDA (20,000 ppm calcium hypochlorite) has been undertaken ((Brooks et al., 2001; Proctor et al., 2001b). For example a multi-state outbreak of *E. coli* O157:NM associated with alfalfa sprouts occurred in Minnesota and Colorado in 2003, where seeds were treated as recommended (Ferguson et al., 2005). These outbreaks illustrate that using seed sanitisation in isolation may not reliably prevent cases of foodborne illness from occurring (see also chapter 5 of the present Opinion).

The Zoonoses database was searched for reported data for the period 2004 to 2010[9] on reports of foodborne outbreaks where implicated foodstuffs were sprouted seeds (Appendix A, Table 7). Nine *Salmonella* outbreaks were reported in the following countries: Denmark (one in 2007), Estonia (one in 2009), Finland (one in 2007 and another in 2009), Norway (one in 2007), Sweden (one in 2006 and another in 2007) and United Kingdom (two in 2010). In addition one *Staphylococcus aureus* outbreak has been reported by Denmark in 2008.

On May 2011, Germany reported an ongoing outbreak due to Shiga toxin-producing *Escherichia coli* (STEC), serotype O104:H4 (Frank et al., 2011a) (with some cases elsewhere in Europe and North America), with >3,200 cases, 850 cases of HUS and 50 deaths. In June 2011, France reported a cluster of patients with bloody diarrhoea and eight cases with HUS in South West France. Infection with STEC O104:H4 has been confirmed for four patients with HUS. Trace back investigations of both outbreaks support the hypothesis that the outbreaks in Germany and France were linked to fenugreek seeds used for sprouting, which became contaminated with STEC O104:H4 at some point prior to leaving the importer (EFSA, 2011e).

3.2. Rapid Alert System on Food and Feed (RASFF) notifications on sprouted seeds

Under Regulation EC N 178/2002[10] (art. 50) Member States of the European Union are obliged to notify to the EC any event and measure taken regarding consignments of food and feed (e.g. withholding, recalling, seizure or rejections of imported consignments not complying with food legislation) where a potential risk to human health has been identified. The Rapid Alert System on

[9] Preliminary data for 2010.
[10] OJ L 31, 1.2.2002, p. 1–24

Food and Feed (RASFF) team at the EC maintains daily updates of the database. The RASFF-Database includes detailed information on the number of reports, their origin and reason for notification, the countries and the products involved, and the identified hazards. Systematic analyses of notifications from the RASFF, over time and geographical areas, facilitate the identification of potentially relevant trends of (re-)emerging threats and a timely reporting (EFSA, 2010).

The RASFF database was searched for notifications during the period from 2001 to 2011: 11 food safety events were notified by the members of the RASFF-network regarding seeds used for sprouting or sprouted seeds during this period. Four out of these eleven notifications were related to seeds as follows: Germany (2008), Finland (2009) and Sweden (2007) submitted one notification each and France two in 2011 (one in the scope of the *E. coli* O104 outbreak). The remaining notifications regarded sprouted seeds and were distributed as follows: two from Denmark on alfalfa (2007) and beetroot (2010) sprouts, one from the United Kingdom on mixed sprouts (2004) and three from Germany on sprouts mixture and beetroot sprouts in the scope of the 2011 *E. coli* O104 outbreak.

Although RASFF reports are relatively infrequent, the use of this reporting system (including trend analysis) combined with more sensitive analytical testing will contribute to better control of this food type with respect to contamination with bacteriological hazards. Detailed information regarding these notifications is provided in Appendix B, Table 8.

3.3. *Salmonella*

Salmonella is an important cause of gastrointestinal illness in humans and is the most frequently reported agent associated with sprouted seed-associated outbreaks worldwide, some of which involved considerable numbers of cases (Mahon et al., 1997; NACMCF, 1999; O'Mahony et al., 1990; Puohiniemi et al., 1997). Information from testing seeds during outbreak investigation suggest an initial low level contamination ((levels of the *Salmonella* at <1 CFU/ g of seeds were reported (Inami et al., 2001; Stewart et al., 2001)), can be followed by growth during sprouting (Mahon et al., 1997; Splittstoesser et al., 1983). *Salmonellae* have been shown to grow during the sprouting process (NACMCF, 1999; Splittstoesser et al., 1983).

Although there are interserovars differences in virulence of *Salmonella enterica* (EFSA, 2011a), all members of this species are considered as potentially pathogenic with respect to contamination of ready-to-eat foods. *Salmonella* can generally grow over a temperature range 5-47ºC, and requires foods with an a_w of at least 0.93 to permit growth. The pH range for growth is between 4 and 9 and contamination occurs via the faeces of a wide range of animals (rodents, birds, cattle, pigs, humans etc) (D'Aoust and Maurer, 2007).

3.4. Pathogenic *Escherichia coli*

Pathogenic *E. coli* are capable of causing disease in humans following colonization of different organism and can cause both diarrhoeal as well as extraintestinal disease. Amongst *E. coli* capable of causing intestinal disease are the Shiga toxin-producing *E.coli* (STEC) (also known as verocytotoxin producing *E. coli* or VTEC) which are strongly associated with the most severe forms of the infection including haemorrhagic colitis (bloody diarrhoea, HC), and the HUS. STEC of the O157 serogroup have been responsible for large sprouted seed-associated (radish and alfalfa) outbreaks (CDC, 1997; Watanabe et al., 1999). The Scientific Veterinary Committee for Veterinary Measures Relating to Public Health issued an Opinion on STEC and identified among others fresh produce, in particular sprouts and unpasteurised fruit and vegetable juices as of particular public health concern (EC, 2003). These food categories have also been recently reported in the EFSA STEC-related Scientific Opinion as an important mode of transmission within the food-borne route (EFSA, 2007b). In the case of sprouted seeds, epidemiological and/or microbiological evidence suggest the seeds to be the most probable source of the pathogen (Itoh et al., 1998). *E. coli* O157 can grow rapidly to large populations

during sprout production (NACMCF, 1999). Contamination can occur via the faeces of ruminant animals (particularly cattle).

A second group of pathogenic *E.coli* are the Enteroaggregative *E. coli* (EAEC), have been implicated as a cause of persistent diarrhoea in children, mainly in developing countries, as well as acute diarrhoea in travellers coming from those regions (Nataro et al., 1998). EAEC have rarely been identified in animals, suggesting that they are not zoonotic, but exclusive to humans as a pathogen (Cassar et al., 2004).

The recent outbreak in Germany (May and July 2011), epidemiologically-linked to sprouted seeds, was associated with an EAEC which has acquired the ability to produce Shiga toxin via horizontal gene transfer and has been designated as STEC O104:H4 (Rasko et al., 2011). Such strains are extremely rare and have only sporadically been identified in the last ten years in the EU, none of which were the same strain as causing the 2011 outbreak (EFSA, 2011f). Before the German outbreak, STEC O104 was isolated from only four sporadic cases of infection in EU, two of which had a travel history in developing countries (Scavia et al., 2011). The same strain that caused the outbreak in Germany, was implicated in June 2011 in a smaller outbreak in France. In this outbreak descriptive epidemiological investigations established a connection with the consumption of sprouted seeds (Gault et al., 2011; Rasko et al., 2011).

In the 2007 EFSA Scientific Opinion on STEC (EFSA, 2007b) it was stated that: over 200 O:H serotypes of VTEC (STEC) have been identified from all sources (Scheutz and Strockbine, 2005), although many lack the full complement of known virulence factors found in strains that cause serious disease; however over 100 have been associated with disease in humans. The role of some putative virulence factors is still uncertain and they may be detected as markers of particular strains rather than contributing to the disease process. There are substantial gaps in knowledge about the interaction between VTEC and their hosts; some VTEC, including O157, may be carried asymptomatically by both adults and children. The definition of pathogenic strains has been based on phenotypic properties and the linkage of certain serotypes to serious illness. Simple methods for identification of VTEC O157 strains and improved techniques for O26, O103, O111 and O145 may have led to a degree of overestimation of the prevalence and importance of these serotypes. Although serotype may conveniently be a surrogate marker for virulence potential, the availability of molecular techniques enables simple direct detection and subtyping of virulence-associated genes. These tests can be incorporated in detection methods. The presence of LEE (Caprioli et al., 2005) and of genes for VT2, particularly subtypes *vtx*2 and *vtx*2c have been identified as markers that are more closely indicative of strains associated with HUS than the serotype alone (Friedrich et al., 2002; Persson et al., 2007). This genetic approach can be extended to include genes such as *vtx*2d, the mucus-activatable variant associated with serious human illness in some LEE negative strains, markers on other pathogenicity islands, and the *sfp*A gene of HUS-associated sorbitol fermenting VTEC O157 strains. An approach that incorporates virulence gene tests independent of serotype as predictors for risk may enable newly emerging VTEC threats to be identified. All VTEC O157 possess the intimin genes on the chromosomally located LEE region. The presence of *vtx*2, either alone or with *vtx*2c, is associated with increased risk of serious disease (Friedrich et al., 2002). There is a link between infection with some phage types (PT) of VTEC O157 with the development of HUS. A study in the UK and Ireland showed that PT 21/28 and PT2 that possessed *vtx*2 and *vtx*2c genes were isolated from HUS cases (Lynn et al., 2005).

The concept of seropathotype that has emerged classifies VTEC into five groups based on the incidence of serotypes in human disease, associations with outbreaks versus sporadic infection, their capacity to cause HUS or HC, and the presence of virulence markers (Karmali, 2003; Wickham et al., 2006). This approach attempts to combine these inputs to understand better the apparent differences in virulence of VTEC. Seropathotype A strains (VTEC O157) have a high relative incidence, commonly caused outbreaks and are associated with HUS. O26:H11, O103:H2/NM, O111:NM and O145:NM together with O121:H19 fall into seropathotype B, as they have a moderate incidence and

are uncommon in outbreaks but were associated with HUS. A further seropathotype C included O91, O104 and O113 strains all of H-type 21 and associated with HUS, but these strains were of low incidence and rarely caused outbreaks. Groups A and B possess LEE and genes of O-island 122 but group C strains may be LEE-negative and have only some of the O122 virulence genes. seropathotypes D and E are not HUS-associated and are uncommon in man or found only in non-human sources. Surveys targeting isolation of VTEC (but not specifically O157) and from non-human sources generally produce isolates from groups C and D.

It is not possible at the present time to fully define human pathogenic VTEC. The concept of seropathotype has evolved which classifies VTEC into groups based on empirical knowledge about the typical clinical outcome of VTEC infections combined with knowledge about serotype, *vtx* subtypes and presence of additional virulence factors. This concept is likely to be further refined and will provide a valuable tool in the future for the assessment of the human pathogenic potential of different VTEC serotypes.

STEC can generally grow over a temperature range 8-42ºC, can be acid tolerant (can survive for 8 weeks at pH 4), is able to grow between pH 4.5 and 9, and require foods with an a_w of at least 0.93 to permit growth (Jay, 2003).

3.5. *Yersinia enterocolitica*

Yersinia enterocolitica is an enteric pathogen can be found in diverse foods of animal origin including pork, beef, poultry, and dairy products, and is commonly isolated from different environments such as lakes, rivers, wells, and soil (Kapperud, 1991). An outbreak of yersiniosis was associated with eating non-commercially produced bean sprouts that were grown using pond water (Cover and Aber, 1989). As a psychrotroph, it is possible that *Y. enterocolitica* can grow on sprouted seeds during refrigerated storage (Abadias et al., 2008; Chao et al., 1988) just as it can with other food products. The bacterium is likely to be capable of growth during sprout production, but no specific data are available (NACMCF, 1999). Not all strains of *Y. enterocolitica* are pathogenic (EFSA, 2007a).

3.6. *Listeria monocytogenes*

Listeria monocytogenes is an environmental organism capable of causing severe human systemic infection, particularly amongst vulnerable populations. The foods associated with transmission of listeriosis are mostly ready-to-eat foods that support growth of *L. monocytogenes* (EFSA, 2007c). Because of the ubiquitous distribution, there are multiple opportunities for *L. monocytogenes* to contaminate either seeds or sprouts from the environment including from sites where sprouting seeds are produced. *L. monocytogenes* can grow at refrigeration temperatures on a variety of produce, including sprouts (Lovett, 1989; NACMCF, 1999; Schoeller et al., 2002). This pathogen has been isolated from commercially produced sprouted seeds, and a single human case has been described where associated with alfalfa tablets (Farber et al., 1990). In this instance, the bacterium is likely to have grown on this plant material which was subsequently dried and encapsulated into tablet form.

The incubation period between consumption of contaminated foods and onset of symptoms varies between 1-90 days (Linnan et al., 1988), there are difficulties in the identification of specific food vehicles associated with disease transmission. Consequently the lack of data on transmission of listeriosis sprouts does not exclude these products as a potential vehicle for infection. Because of the likely growth of the bacterium in this food type, the persistence of this organism in factory environments, and existing technology for testing and microbiological criteria, this hazard has been included in subsequent sections of this Opinion.

3.7. *Bacillus cereus*

B. cereus is a ubiquitous spore-forming bacterium commonly found in soil and on plants (Kramer and Gilbert, 1989) which can cause either emesis or diarrhoea after consumption of foods where the bacterium has been allowed to proliferate. Because of the ubiquitous distribution in the environment and the robust nature of the spores, plant materials (including seeds) will commonly be contaminated by this bacterium (Pao et al., 2005). In 1973, four cases in an outbreak were associated with the consumption of home-grown raw sprouted seeds (a mixture of soy, cress, and mustard seeds packaged in a seed sprouting kit) contaminated with *B. cereus* (Portnoy et al., 1976). The sprouted seeds contained up to 10^8 *B.cereus*/g with the soy seeds more heavily contaminated than either the cress or mustard. As with other pathogens associated with outbreaks from contaminated sprouts, the likely source was seed. *B. cereus* is capable of growth under seed sprouting conditions (Harmon et al., 1987).

3.8. *Staphylococcus aureus*

Staphylococcus aureus is a common commensal (normal bacterial flora) of skin and nasopharynx of humans as well as other mammals and a cause of food poisoning due to the production of heat resistant enterotoxins following growth of the bacterium (ICMSF, 1996). Because of the distribution of this bacterium, contamination is likely to occur via food handlers (Saroj et al., 2006a). An outbreak of staphylococcal food poisoning affecting 42 persons was reported in Denmark following consumption of bean spouts (Appendix A, Table 7).

3.9. Other pathogenic bacteria

Other intestinal bacterial pathogens potentially linked to contamination of fresh produce (including sprouted seeds) where contamination occurs via the faeces of animals (including water) include *Campylobacter* (Buswell et al., 1998; Chynoweth et al., 1998; Koenraad et al., 1997; Mason et al., 1999), *Shigella* (NACMCF, 1999; Rafii et al., 1995) and *Vibrio cholerae* serogroups O1 and O139 (Faruque et al., 1998; Wachsmuth et al., 1994). Bacterial toxins have not been reported on sprouted seeds, however the single report of an instance of staphylococcal food poisoning highlights this hazard as a possible means of exposure (see section 3.8).

3.10. Resistance of foodborne pathogenic bacteria to antimicrobial agents

Organisms such as *Salmonella* spp., and pathogenic *Escherichia coli*, including STEC O14:H4 that are, or have been associated with sprouts, shoots and cress derived from seeds may be exposed to antibiotics during the course of their history, depending on their source, primary reservoir, or method of contamination. For example, if their primary source or reservoir of such organisms is, or was, human faecal material, then they will have been exposed to antibiotics used in human medicine and to other bacteria which maybe carrying antimicrobial resistance genes. Likewise, if the primary source is waste from food production animals, then they will have been exposed to antibiotics used in relevant food production systems and to other bacteria which may be carrying antimicrobial resistance genes.

The causative strain of *E. coli* responsible for the 2011 outbreak of STEC O104: H4, centred in northern Germany exhibited resistance to first-generation β-lactam antibiotics (e.g., ampicillin) as a result of possession of a TEM-1 β- lactamase, and also to extended spectrum β-lactamases (ESBLs) as a result of production of an ESBL enzyme (CTX-M-15). The strain was also resistant to nalidixic acid, with concomitant decreased susceptibility to fluoroquinolones but was sensitive to carbapenems and to ciprofloxacin at therapeutic levels (Frank et al., 2011b).

Most ESBL- and AmpC-producing strains carry additional resistances such as to sulphonamides and other commonly-used drugs, including quinolones. Therefore, generic antimicrobial use in both humans and animals is a risk factor for the development and transmission of ESBL/AmpC and quinolone resistance, and it is not restricted specifically to the use of β-lactamase antibiotics and quinolones (EFSA, 2011c).

Antibiotics such as penicillins, cephalosporins and quinolones are used extensively in both human and veterinary medicine in developed and developing countries, often without prescription (WHO, 2011b). European data on antibiotic usage in human medicines has been collated by the European Surveillance of Antimicrobial Consumption (ESAC) group (Coenen et al., 2009). Likewise information on antimicrobial consumption in food production animals in nine European countries between 2005 and 2009 has recently been compiled by the European Medicines Agency (EMA), as part of the European Surveillance of Antimicrobial Consumption (ESVAC) project[11]. In contrast information on antibiotic usage is not available for many countries outwith the EU. Because of the widespread and generic usage of antimicrobials in the both the human and veterinary sectors and the overall lack of usage data globally, it is very difficult, if not impossible, to link antimicrobial resistance in organisms associated with sprouts, shoots and cress derived from seeds with their possible origin or reservoir.

3.11. Conclusions

In summary, *Salmonella* and *E.coli*: are the most commonly reported bacterial pathogens causing outbreaks associated with the consumption of contaminated sprouted seeds. Alfalfa and mung bean sprouts have been the seed most commonly implicated in outbreaks. Other bacterial pathogens (e.g. *Bacillus cereus, Staphylococcus aureus, L. monocytogenes* and *Y. enterocolitica*) have also been implicated in outbreaks, although these have been reported very rarely. It is not possible to estimate the proportion of all gastrointestinal infection attributed to contaminated sprouted seeds, however the large outbreaks (including the >3,700 cases in Germany in 2011) illustrate the potential to cause major public health emergencies.

In the recent outbreak of STEC O104, although the bacteria exhibited resistance to a range of therapeutic antimicrobials, resistance cannot be attributed to the specific use of such antimicrobials in either the human or veterinary settings.

4. EXPOSURE ASSESSMENT

Investigations traced back the origin of several outbreaks caused by sprouts consumption to the contamination of the dry seeds (see section 3.1 of the present Opinion, (Breuer et al., 2001; Brooks et al., 2001; EFSA, 2011e; Ferguson et al., 2005; Gill et al., 2003; Inami and Moler, 1999; Mahon et al., 1997; Winthrop et al., 2003). From this step, human exposure to foodborne pathogens through sprouts consumption depends on the prevalence and survival of the pathogens on the seeds, its growth or survival during the sprouting process and its growth or survival on the sprouts until consumption.

4.1. EU monitoring data on occurrence of foodborne pathogenic bacteria in seeds and in sprouted seeds

Data on occurrence of zoonotic agents in sprouts reported during the period from 2004 to 2010[12] were extracted from the zoonoses database (Appendix C, Table 9). Some data on the occurrence of STEC, *Salmonella* and *L. monocytogenes* in samples of sprouts are available. No STEC-contaminated sprouts samples were reported. For *L. monocytogenes* only 4 out of 14 investigations resulted in the detection of *L.monocytogenes*-contaminated sprouts, namely 2/29, 2/45, 4/1722 and 1/88 positive units/units tested. A total of 43 investigations were reported for *Salmonella* detection in sprouts and only in 7 investigations *Salmonella*-contaminated sprouts were found, with respectively 1/22, 12/20, 3/135, 9/581, 12/229, 1/174 and 1/65 positive units/units tested. Numbers of *Salmonella* in the positive units were not determined. It should be noted, however, that overall the number of investigations in sprouts is very small and each of these comprises usually a low number of units tested. In addition, small sample sizes have been analysed, namely 2.5, 10 or 25 g. In the nineties, in France *L. monocytogenes*

[11] www.ema.europa.eu/docs/en_GB/document_library/Report/2011/09/WC500112309.pdf
[12] Prelimanry data for 2010.

was found in numbers below 100/g at production and at retail in respectively 1 of 31 and 19 of 102 samples of bean sprouts (Michard et al., 1993; Pierre and Veit, 1996). In 2004 in the UK, out of 808 samples of sprouts, 28 were positive for *L. monocytogenes* with one sample containing more than 10^2 *L. monocytogenes* per g (Little and Mitchell, 2004).

No information was found on the occurrence of foodborne pathogenic bacteria on dry seeds used for sprouting, outside of outbreak investigation. In other types of dry seeds intended for human consumption, presumably not used for sprouting, *Salmonella* was detected in 0.6% of samples analysed in the UK (Willis et al., 2009).

In conclusion, *L. monocytogenes* and *Salmonella* have been found, albeit at low frequencies, in several surveys on sprouted seeds.

4.2. Survival and growth of foodborne pathogenic bacteria on seeds and sprouted seeds

4.2.1. General growth and survival characteristics of foodborne pathogenic bacteria

The survival and growth of bacteria in foods are dependent on the interaction of various intrinsic factors such as water activity and pH and extrinsic factors such as temperature and gaseous atmosphere. *E. coli* O157:H7 can survive for many weeks when desiccated, particularly at refrigeration temperature (Bagi and Buchanan, 1993). On the surface of dry seeds, bacteria are exposed to low water activities, which will not permit growth of any foodborne pathogenic bacteria. Sprouting occurs during a few days, at temperatures of between 20 – 25°C and high humidity, with pH of sprouts usually close to neutral, conditions theoretically allowing growth of foodborne pathogenic bacteria to high populations (e.g. *Salmonella*, VTEC, *L. monocytogenes*, *B. cereus*) or allowing toxin production (e.g. by *S. aureus* or *B. cereus*). However, the diversity of pH on the surface of the sprouts obtained from the very wide range of seeds used is not documented.

During the shelf life of sprouted seeds, the products are usually packaged in conditions maintaining high relative humidity, and bacterial growth will depend mostly on storage temperature. Refrigerated storage will inhibit growth of *E. coli* O157:H7 for instance, which have a minimum growth temperature around 8-10°C under optimal conditions (Buchanan and Bagi, 1994), but not of *L. monocytogenes*. In case of temperature abuse a wider range of pathogens may be able to grow. Whenever the product is packaged in polymeric films, respiration of the sprouted seeds may deplete oxygen to levels sufficiently low to potentially permit development of strict anaerobic bacteria such as *C. botulinum*, as described for temperature abused packaged fresh cut vegetables (Nguyen-The and Carlin, 2000).

Dry seeds contain between 10^3 and 10^5 non pathogenic bacteria per g, which rapidly multiply during sprouts production, and the sprouted seeds may contain between 10^8 and 10^9 non pathogenic bacteria per g until consumption (NACMCF, 1999). This very high bacterial population may limit the growth of pathogenic bacteria through competition, during sprouting and subsequent storage of the sprouts. The non pathogenic bacterial population of shoots and cress has not been characterised, but this may be similar to fresh produce where competition has been shown for packaged fresh-cut vegetables during storage (Carlin et al., 1996).

During sprouting, bacterial growth is presumably supported by nutrients leaking from the germinated seeds and from the sprout or shoot tissues. Some plants contain compounds with antimicrobial properties, and considering the very broad range of species used for sprouted seeds productions, it is possible that bacteria including pathogens may be inhibited by compounds leaking from seeds or sprouts or shoots. This has not been documented for sprouted seeds but was shown for some fresh cut leafy greens (Carlin and Nguyen-The, 1994).

Location of the bacteria on the seeds/sprouts surface, their potential internalisation, their ability to adhere on the seeds/sprouts tissues, are also important factors which will influence the efficacy of intervention strategies such as washing and decontamination.

In conclusion, the physico-chemical environment of sprouts production is favourable to growth of pathogenic bacteria. Refrigeration is important to limit the growth of pathogenic bacteria during storage of sprouted seeds. Other factors, such as competition from the very abundant background microflora of sprouts, may however limit growth of bacterial pathogens.

4.2.2. Survival on seeds

4.2.2.1. Attachment and localisation of pathogenic bacteria on seeds

Localisation and attachment of foodborne pathogens on seeds is an important factor which may influence their survival and resistance to mitigation strategies such as seed surface decontamination.

Plant pathogenic bacteria may be able to enter seeds by a variety of routes such as the vascular system of the contaminated plant, pollen germ tube and the dorsal suture of the silique (seed pod) or hilum of the mature seed, and may be present inside the seed (Harman, 1983; Mundt and Hinkle, 1976). Whether the same can occur for foodborne human pathogens is not known. *Salmonella* and *E. coli* O157 have been found internalised inside plant tissues under laboratory conditions, but whether this is possible in natural conditions is unclear (EFSA, 2011f). In addition, it has not been demonstrated if human foodborne pathogens internalised in plant tissues can be transmitted internally to the seed via the vascular system. Using *Arabidopsis thaliana* as a model plant system, Cooley *et al*. (2003) found that following inoculation of the roots and shoots with *S.* Newport and *E. coli* O157:H7, these pathogens were recovered from the flowers and seeds of the mature plants. However, the authors also demonstrated that pathogens can become associated with seed via direct contact with contaminated material such as chaff.

Cracks or openings in the seed coat increases the opportunity for bacterial attachment and ingress, and may enhance the potential for penetration into the seed (Charkowski et al., 2001; Wade et al., 2003). The prevalence of seeds with visible cracks or other imperfections is highly variable, with rates of 3-85% being reported in alfalfa seeds (Wade et al., 2003).

Salmonella and *E. coli* O157:H7 artificially inoculated on seeds (Jaquette et al., 1996) (see also section 5.2), or spontaneously transferred from the inoculated mother plants to seeds (Cooley et al., 2003), can tolerate high concentrations of disinfectants, but can nevertheless be reduced by several $\log_{10}$ units (Bang et al., 2011; Bari et al., 2011; Beuchat, 1997; Fett, 2002a; Gandhi and Matthews, 2003; Saroj et al., 2006b). This suggests that these bacteria are located, at least in part, in protected areas on the seeds, but that most are presumably exposed on the surface of the seeds.

4.2.2.2. Fate of pathogenic bacteria on seeds

Once present on or in seeds, pathogenic bacteria are likely to survive for extended periods of time. In many outbreaks, the same batch of contaminated seeds was used by several sprout production sites, causing a wide dissemination of the pathogenic bacteria and increasing the number of cases. Dry seeds can be stored for several weeks or months before sprouting. Extended periods of survival of pathogens on seed is likely to increase the number of sprout production plants using contaminated seeds.

The survivability of *E. coli* O157:H7 on alfalfa seeds stored for prolonged periods at three temperatures was investigated by Taormina and Beuchat, (1999). Seeds were inoculated with about 3 $\log_{10}$ cfu/g of *E. coli* O157:H7. A significant decrease in the number of *E. coli* O157:H7 on dry seeds

was observed within 1 week of storage at 25°C (1.1 $\log_{10}$ reduction) and 37 ° C (2.74 $\log_{10}$ reduction), but not at 5 °C. Between 1 and 38 weeks, populations on seeds stored at 5 °C remained relatively constant at levels above 2 $\log_{10}$ counts, at 54 weeks (when the experiment came to an end) *E. coli* O157:H7 still survived, although at counts below 0.30 $\log_{10}$ cfu/g. However, longer survivability cannot be excluded. The pathogen was recovered from alfalfa seeds after storage at 25 °C or 37 °C for 38 weeks but not 54 weeks. With a lower initial inoculum (2 $\log_{10}$ cfu/g) *E. coli* O157:H7 was detected after 8 weeks but not after 13 weeks on the dry seeds and on the sprouted seeds (Wu et al., 2001).

In a later study by the same group (Beuchat and Scouten, 2002), the survivability of *E. coli* O157:H7 and *Salmonella* inoculated on dry alfalfa seeds (respectively 4.8 and 3-3.2 $\log_{10}$ cfu/g) as a function of temperature (5 °C, 25 °C, 37 °C) and also a_w (0.15, 0.36, 0.54) over a 52-weeks storage was studied. For both bacteria, survival was enhanced by low temperatures and low water activity, but *Salmonella* survived better on seeds than *E. coli* O157:H7. After 52 weeks at 5°C *Salmonella* declined by 0.2-0.3 $\log_{10}$ compared to 0.6-1 $\log_{10}$ for *E. coli* O157:H7. After 25-26 weeks at 25°C, declines were 0.7-2 $\log_{10}$ for *Salmonella* and 2-3 $\log_{10}$ for *E. coli* O157:H7. After 4 weeks at 37°C, *Salmonella* declined by 0.5-2 $\log_{10}$ compared to 2.5-3 $\log_{10}$ for *E. coli* O157:H7. Globally, for both pathogens, at 37 °C and 14 days of storage, all samples were found negative after enrichment. At 25 °C and after 25 days of storage, all samples were still found contaminated, although reductions over 2 $\log_{10}$ units were found at an a_w-value of 0.15 and almost 3 $\log_{10}$ units reductions were found at an a_w-value of 0.36-0.54. At 5 °C and up to 52 weeks of storage, $\log_{10}$-reductions were below 1 at all a_w-values studied. Loss of germination followed the same pattern as loss of viability but more than 72% of seeds germinated after all storage conditions tested.

In conclusion from these studies, *Salmonella* and *E. coli* O157:H7 declined over time on dry seeds; but for storage at ambient temperatures and for high initial inoculum (e.g. over 100 cfu/g), they persisted for at least several weeks.

An epidemiological traceback investigation of the recent 2011 *E. coli* O104 outbreak in Germany and France suggested that imported seeds in 2009 which became contaminated at some point prior to leaving the importer were the most likely cause of the outbreak (EFSA, 2011e). The survival of *Salmonella* on dry seeds described in the above studies seems shorter than that previously reported in other dry environments (e.g. flour, milk powder) (Mitscherlich and Marth, 1984).

Information available for survival of human pathogenic bacteria on seeds is only available for high numbers of bacteria grown under laboratory conditions and inoculated onto seeds. The fate of foodborne pathogens naturally occurring on seeds may be different and is not documented. Naturally occurring, non pathogenic bacteria can persist for long periods on seeds. For instance, the natural microflora of rice seed decreased by less than 0.5 $\log_{10}$ after 260 days of refrigerated storage and approximately 2 logs after 277 days under ambient storage conditions (Piernas and Guiraud, 1997). *Erwinia herbicola*, a common saprophyte of alfalfa seed, could be isolated for at least 3 years after treatment of seeds with 1% sodium hypochlorite (NACMCF, 1999). In addition, methods for recovery and enumeration of foodborne pathogen may underestimate the number of bacteria that have survived on dry seeds. In other foods, it was shown that selective agents of the media, or the composition of diluent used for serial dilutions, can influence the apparent number of survivors (Jordan et al., 1999) and may hinder recovery of stressed bacteria (Blackburn and McCarthy, 2000; McCarthy et al., 1998). An additional enrichment step has been found necessary to recover *Salmonella* surviving on dry seeds, presumably because of sublethal injury (Liao and Fett, 2003). In contrast, Wu et al. (2001) found a good correlation between detection of *E. coli* O157:H7 during survival on dry seeds using conventional selective media, and its presence on the sprouted seeds.

4.2.3. Growth during sprouting

4.2.3.1. Sprouted seeds

The key aspect of sprouts that increases the risk of foodborne disease compared to other fresh produce, is the growth of bacteria during sprouting. In addition, the regular circulation of water during sprouting may disseminate the pathogens throughout the batch of seeds, even if initially only a few seeds are contaminated.

NACMCF (1999) reviewed the growth of foodborne pathogenic bacteria, artificially inoculated onto seeds, during sprouting in laboratory scale equipment. *B. cereus*, *L. monocytogenes*, various serovars of *Salmonella* multiplied by 3 to 5 $\log_{10}$ units during the sprouting process and reached a maximal population of 6 to 8 $\log_{10}$ cfu/g of sprouts at the end of sprouting. Similar multiplication was found for *E. coli* O157 (Barak et al., 2002; Castro-Rosas and Escartin, 2000; Hara-Kudo et al., 1999). In contrast, *Vibrio cholera* grew only during the first days of sprouting and then declined (Castro-Rosas and Escartin, 2000). Published studies have mostly examined growth of bacteria on mung bean and alfalfa sprouts and to a lesser extent radish sprouts.

In the above studies, high initial inoculum (2 to 4 $\log_{10}$ cfu/g) of pathogenic bacteria were artificially applied on the seed at the start of sprouting. In contrast, levels of *Salmonella* founds in dry seeds implicated in sprouts outbreaks were much lower: 13 and 16 *Salmonella* MPN/kg of seeds in two different outbreaks (Fu et al., 2008), 4 cfu/kg of seeds in one outbreak (NACMCF, 1999), 20-100 MPN/kg of seeds for two outbreaks (Stewart et al., 2001).

Salmonella naturally present on alfalfa seeds multiplied during the first 2 days of sprouting from -1 to 2.5-3 $\log_{10}$ MPN/g (Stewart et al., 2001). The bacteria did not exceed this level during the 3rd days, although it was 3 $\log_{10}$ units lower than the population reached at the end of sprouting in studies where *Salmonella* was artificially inoculated. Similarly, Charkowski et al. (2002) reported that inoculation 1 cfu *Salmonella* per ml of sprouting water may result in very low growth during sprouting (e.g. 1.3 $\log_{10}$ units). Therefore, artificial inoculation of seeds with high numbers of pathogens may largely overestimate the numbers of pathogens which are present at the end of sprouting with naturally contaminated seeds.

Under laboratory conditions, seeds are usually sprouted under static conditions. However, in industrial settings seeds may be regularly rinsed with clean water during sprouting (see chapter 2). This may wash out some pathogenic bacteria and reduce the number present on the sprouts at the end of the process although the extent of any removal will, depend on the adhesion of pathogens to the seeds and sprouts. Frequent rinsing during sprouting reduced the final population of *E. coli* O157:H7 by 10 to 100 fold compared to static sprouting conditions (Charkowski et al., 2002). Rinsing had no effect on *Salmonella*, presumably because of a stronger adhesion to sprouts tissues (Barak et al., 2002).

The growth of *Salmonella* naturally present on seeds (13 to 16 MPN/kg) during sprouting in jars (as usually done in laboratory studies) was compared with sprouting in a minidrum to more closely simulate industrial conditions (Fu et al., 2008). No growth of *Salmonella* was observed during sprouting in the minidrum, whereas growth was observed during sprouting in the jars. In this latter situation, growth did not exceed 3-4 $\log_{10}$ units, which ended in sprouts containing between 2 and 3 $\log_{10}$ *Salmonella* per g of sprouts. This study confirms that most laboratory experiments presumably overestimated the growth of *Salmonella* during sprouting.

The impact of sprouting conditions (temperatures and watering frequencies) on growth of *Salmonella* during sprouting (3 days) was investigated by Fu et al. (2008). No growth was observed at 20°C with normal watering frequencies but growth (2 $\log_{10}$ units) occurred at 30°C, or at 20°C with reduced frequency of watering. This shows that under some sprouting conditions, the growth of *Salmonella*

may be limited, but this should be confirmed by further studies. The results of this study cannot be explained by a direct effect of temperature, because 20°C for 3 days under high humidity and neutral pH should in theory permit substantial growth of *Salmonella*.

Sprouting water may transport pathogens from contaminated seeds to originally pathogen free seeds in the same sprouting batch, or contaminate the sprouting equipment, thereby increasing the total amount of pathogens in the production facility. In laboratory experiments with artificial inoculation of seeds (Charkowski et al., 2002; Liu and Schaffner, 2007b), and with naturally contaminated seeds (Stewart et al., 2001), the number of pathogens in the sprouting water was similar to that on the sprouts, after some times of sprouting. In contrast, (Hora et al., 2005) showed that *Salmonella* artificially inoculated in a few seeds placed in the seedbed before sprouting did not always result in *Salmonella* being found in the water irrigating the seeds. In addition, inoculation of seeds during the sprouting process with *Salmonella* or *L. monocytogenes*, instead of inoculation at the start of sprouting, resulted in no multiplication of these pathogenic bacteria (Castro-Rosas and Escartin, 2000; Schoeller et al., 2002). The rapid multiplication of the background microflora of seeds during the first day of sprouting might exert a barrier and/or competitive effect against pathogens. Therefore, dissemination of pathogenic bacteria during sprouting may sometime be limited and secondary contamination may not always permit pathogen growth.

In conclusion, the sprouting process permits multiplication, and dissemination of pathogenic bacteria in a batch of sprouting seeds. Outbreak investigations have shown that the presence of low numbers of *Salmonella* in the dry seeds can potentially lead to infections in humans from consuming contaminated sprouts. However, understanding the basis of pathogen growth in sprouted seeds has mostly been quantified in laboratory experiments using high levels of artificially inoculated pathogens, which probably overestimates the extent of multiplication and dissemination resulting from "natural" contamination. Competition with the non pathogenic microflora, ability of the pathogenic bacteria to adhere to the sprout tissues or to be released from the sprout tissues, are presumably important factors, yet currently difficult to predict and quantify.

4.2.3.2. Shoots and cress

No information is available concerning the fate of foodborne pathogenic bacteria during production of shoots and cress although cress has been associated with an outbreak in the past (Table 3).

Pathogens present on seeds may grow during production of shoots due to the frequent watering, similarly to that occuring during sprout production. However, as the shoots represent only the aerial part of the plant, not directly in water, which is not known to what extent they will be contaminated at harvest.

Cress is grown in soil or hydroponic substrates. Whether pathogenic bacteria present on seeds could multiply in the soil and substrates used for cress production is not known. Compared to sprouts and shoots, the substrates used may represent an additional source of contamination, but this is not documented.

4.2.4. Survival and growth on sprouted seeds

Sprouts for the retail market are usually packaged and stored at refrigeration temperatures for 3 to 10 days. Under these conditions, *Salmonella* and *L. monocytogenes* which had multiplied to a high level on the sprouts during sprouting, remain at a constant level or slightly declined (Jaquette et al., 1996; Schoeller et al., 2002). However, in these studies, the level of pathogenic bacteria at the end of sprouting was presumably higher than what would happen with naturally contaminated seeds, as discussed in the previous section. The fate of lower populations of pathogens during sprouts storage is not known.

The fate of pathogenic bacteria on sprouts during storage at abuse temperature is not documented. This may be relevant for practical conditions, because sprouts may continue to grow during storage, would generate heat, and may hinder rapid and homogeneous refrigeration throughout the package.

The fate of pathogenic bacteria during storage of packaged shoots and cress is not known. It is presumably similar to that of packaged fresh produces (e.g. baby leaves) which permit survival or growth of bacterial pathogens depending on temperature.

Some sprouts may be lightly heat treated during preparation before consumption (e.g. stir-fried). The impact of these heat treatments of sprouts on bacterial pathogens is not documented.

4.3. EU consumption data for sprouted seeds

Available data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011g; Merten, 2011) on consumption of sprouted seeds in the EU are provided in Appendix D for all original food descriptors listed in Table 10 (Appendix D). All statistics are reported according to age class, country, dietary survey and gender. Average and 95th percentile consumption are reported in grams/day and grams/day per kg body weight, for total population and consumers only (Appendix D, Table 11).

From the data presented it is evident that there is a large variation in consumption patterns across different Member States, age group and gender both in the percentage of the consumers sampled that declared consumption of the specific sprouted seed and in the average daily amount of product consumed. This would make the task of an EU risk assessment very complex, as the consumption figures are very different depending on the Member State.

As an example the % of consumers who indicated consumption of sprouted seeds ranges from 0 (in many population groups and countries) to 14.0% (in UK female adults) and the average daily amount of product consumed ranges from 0.3 to 83.8 grams/day.

As sprouted seeds are mainly consumed within composite dishes without being the main ingredient, we can expect that the consumption figures in this database are an underestimation. This assumption is confirmed by an outbreak investigation which showed that some consumers were not aware that they had consumed sprouts in their food (Proctor et al. 2001).

The mode of consumption of sprouts may vary, and with respect to exposure to bacterial pathogens, the proportion of sprouts eaten raw or after a heat treatment is not known.

5. CONTROL AND REDUCTION/ELIMINATION OF BACTERIAL HAZARDS IN SEEDS AND SPROUTED SEEDS

Outbreaks associated with sprouted seeds have led to the development of different guidelines for producers and processors, covering Good Agricultural Practices (GAPs), Good Manufacturing Practices (GMPs) and Good Hygiene Practices (GHPs), in different parts of the world. These include the recommended Code of Practice by the National Advisory Committee on Microbiological Criteria for Foods in the US (NACMCF, 1999), Guidance for sprouts manufacturers in Canada (CFIA, 2008), Production and processing standards in Australia and New Zealand (FSANZ, 2010), FDA recommendation in the US (FDA, 1999a) and the Code of Hygiene Practice for Fresh Fruits and Vegetables by the Codex Alimentarius Commission (CAC, 2003). The guidelines include specific advices on hygienic production and decontamination of seeds and sprouted seeds to control and reduce/eliminate the presence of pathogens at all stages of the food chain, from agricultural production of seeds on the farm to production and preparation of sprouted seeds.

Recommendations concerning sprouted seed production are not homogeneous neither around the world nor within the EU (source: Freshfel Europe). However, most of the published guidelines agree on the importance of identifying specific points where contamination is more likely to occur such as growing, storage, of seeds, germination of seeds during sprout production and harvest, packaging and storage of sprouts, where cross contamination might occur. They are mainly focussed on using seeds with the lowest risk of carrying foodborne pathogenic bacteria, seed decontamination and the adequate hygienic conditions of process water and all the equipment used during the sprouting process (CDC, 2009). These steps during seed and sprouted seeds production, where control and/or reduction of microbial hazards in seeds and sprouts, shoots and cress derived from seeds could be possible, might permit implementation of HACCP principle for hazard prevention and control. Thus, based on the HACCP, control of critical points is recommended. However, it should also be taken into account that HACCP as such, is not applicable to primary production because no interventions currently exist to reduce pathogens to an acceptable level or to completely eliminate them (EFSA, 2011f; Gil et al., 2011). Nevertheless, HACCP could be implemented in all the steps related to sprout production. Mitigation strategies to reduce pathogen numbers such as the introduction of a decontamination step can be applied, and their efficacy to control or reduce microbial risks will also be discussed in this section.

5.1. Human exposure routes

The four routes through which humans can become exposed and potentially infected by the most important foodborne pathogens which have been linked to sprouted seeds-associated outbreaks, namely *Salmonella* and pathogenic *E. coli*, are shown in Figure 4. These have been reviewed by EFSA in a previous Scientific Opinion (EFSA, 2007b).

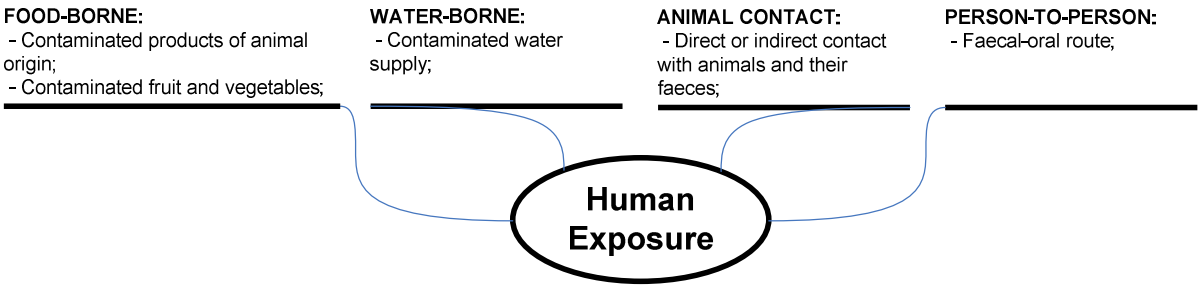

Figure 4: Human exposure routes to foodborne pathogens.

Outbreaks may have more than one exposure route involved. For example, primary human infection may originate from consumption of contaminated food or direct contact with an animal carrying pathogenic *E. coli*, while secondary infection may occur by the faecal-oral route, after contamination of food through handling by an infected person shedding the bacteria. As a result, multiple exposure routes are likely especially during the later stages of an outbreak.

5.2. Critical points in seed production where bacterial contamination is likely to occur

Epidemiological investigations suggest that seed are the likely source in most, if not all, sprout-associated illness outbreaks (Mahon et al., 1997; NACMCF, 1999; Puohiniemi et al., 1997). The mechanism by which crop seeds and seeds intended for germination become contaminated is not fully understood, although literature on herbs and spices and for feed and oil seeds may be useful. Since seeds are raw agricultural products, they could be contaminated by a variety of potential sources of faecal contamination, including contaminated agricultural water, contaminated soil, use of inadequately treated manure as a fertilizer, location of fields near animal rearing facilities, access by

feral animals, soil, dust and plant debris, inadequate agricultural worker hygiene, distribution, sorting, grading, packing or mishandling by the consumer (NACMCF, 1999).

The investigation of some past outbreaks linked to the consumption of sprouted seeds were reviewed by NACMCF (1999) who identified some of the possible factors responsible for contamination of the dry seeds: use of chicken manure to fertilize the field, irrigation of the field with water from a canal, contamination of the field with manure, presence of livestock close to the field, transport in dirty vehicles, presence of rodents and birds in the seed conditioning premises. However, the implication of these various risk factors has not been confirmed by microbiological evidence (e.g. presence of the outbreak strain in the putative source of contamination).

These risk factors are very similar to those identified for fresh fruit and vegetables (EC, 2002; FAO/WHO, 2008), but as production of dry seeds differs in several respect from that of fruit and vegetables, the respective contribution of the risk factors may also differ. For instance, contamination of dry seeds via dust during harvest and conditioning, via rodents and birds during storage, may be more important than for fresh fruits and vegetables which are stored for much shorter periods. Conversely irrigation of fresh produce may occur much closer to harvest than in the case of dry seed production. As pathogenic bacteria decline over time in the soil and on aerial plant surfaces, it is possible that the risk of transfer to the harvested crop from contaminated irrigation water may be lower for dry seeds than for fresh produce. In addition many seeds are protected from external contamination until harvest by various types of "envelops" (e.g. legumes seeds enclosed within pods). However, transfer of contamination from the external envelops to the seeds is presumably possible during harvest.

Few studies have investigated whether pathogens may be able to enter seeds by a systemic route such as the vascular system, pollen germ tube and the dorsal suture of the silique or hilum of the mature seed (Delaquis et al., 1999; Harman, 1983; Mundt and Hinkle, 1976). However, it has been proven that cracks and openings in the seed coat increases the opportunity for bacterial attachment and may enhance the potential for penetration into the seed (Fett, 2002a). Mechanical damage of the seeds may therefore contribute to the risk of contamination with bacterial pathogens.

The critical points in fresh fruits and vegetables and sprout production have been summarized by the Codex Alimentarius Comission (CAC, 2003) and the National Advisory Committee on Microbiological Criteria for Food (NACMCF, 1999).The most relevant points related to seeds and sprouts, shoots and cress derived from seeds are summarized as follows.

- Insects, animals and in particular birds, have the potential to cause contamination during seed production in the agricultural environment. In addition, domestic animals may be allowed to graze (e.g. on alfalfa fields). While such contact is not likely to be a significant problem for the primary use of seed, i.e., seed for forage production, even low level, sporadic contamination of seed for food use may result in significant public health concerns because the sprouting process amplifies pathogen levels (NACMCF, 1999).

- Contaminated agricultural water is a potential source of microbial contamination.

- People working with seeds may transfer microorganisms of significant public health concern. Their safety and hygiene practices, from land preparation, planting, weeding, and pruning, to harvest, could influence whether produce becomes contaminated (James, 2006).

- Equipment and tools used during growing, harvesting, handling and storage could also be a source of contamination.

- Harvesting procedures expose the seed to a substantial amount of dirt and debris and likely spread localized contamination throughout the harvested seeds (NACMCF, 1999).

- Seeds can be contaminated during storage as they are kept in warehouses in open containers where they can be exposed to rodent, birds, faeces of farmyard animals and insect pests which are potential vectors of contamination. Equipment used to remove debris from seeds is not routinely disinfected (Mohle-Boetani et al., 2001). Certain organisms, e.g. *Salmonella*, can survive for months under dried conditions and therefore could persist on seeds and in seed storage environments.

5.3. Critical points during the sprouting process where bacterial contamination is likely to occur

Although seeds are likely to be the primary source of contamination in sprout-associated foodborne illness outbreaks, practices at the sprouting establishment may increase or decrease the extent of the microbial hazards. In one outbreak, unhygienic conditions in the sprout production environment suggested that contamination with foodborne pathogenic bacteria may have occurred directly at the sprouting stage (NACMCF, 1999). In the outbreak linked to sprouts which occurred in 2011 in Germany, personnel from the sprouting production site were found carrying the STEC in their faeces, but since they had not become ill prior to the outbreak, it was concluded that they were not the source of the food contamination (EFSA, 2011d). It is not known if this may have contributed to the dissemination of the outbreak strain in the production environment. Since there is evidence of asymptomatic carriers in humans for certain pathogen, this is a potential risk factor for sprouted seed contamination during production. Unhygienic seed storage facilities (whenever done in the sprouting plant), poor sanitation of equipment and poor personal hygiene can be sources of contamination with pathogenic bacteria.

Water used during sprouting can be a source of initial contamination or a vehicle for subsequent cross contamination. For instance, when only the roots of fully developed radish sprouts were immersed in water containing *E. coli* O157:H7, the pathogen was found throughout the edible portion (Hara-Kudo et al., 1997).

5.4. GAPs and GHP during seed production and storage

During seed production and harvesting, the application of Good Agricultural Practices (GAPs) and Good Hygienic Practices (GHPs) are recommended, aiming at preventing contamination of seeds with pathogens (CAC, 2003). Compared to fresh fruits and vegetables, seed production presents additional risks because seeds can be produced for several end-uses and not only for sprout production (e.g. edible seeds, animal feed, oil production, horticulture, etc). Foodborne pathogenic bacteria may survive for long periods on seeds (see section 4.2 of the current Opinion), and pathogens present on seeds can multiply during sprouting (see section 4.2 of the current Opinion). Therefore the risk for consumers from the contamination of seeds used for sprouting may be higher than in the case of seeds intended for other uses. The level of stringency of GAP in the case of seeds intended for sprouting should be particularly high, similar to that for fruits and vegetables eaten raw. Recent EFSA advice on the public health risk of Shiga toxin-producing *Escherichia coli* in fresh vegetables, which addressed mitigation options for vegetables, does remain valid in this context (EFSA, 2011f). Thus, primary production of seeds for sprouting should not be carried out in growing fields where the presence of pathogens would lead to an unacceptable level of such microorganisms in the final seed crop, and efforts should aim at avoiding introduction of pathogens in the field and in the crop. According to existing guidelines available in particular, but not exclusively in (CAC, 2003), of special importance would be:

- Avoid access of wild or domestic animals to the immediate environment. For instance, some guidelines propose a delay in planting of the seed crop by 12 months if the land has been grazed by cattle and 6 months if grazed by other livestock (Beales, 2004). The NACMCF (1999) also recommends that wild animal populations should be assessed and, where possible,

deterred possibly by physical deterrents or by redirecting the animals to fields where ready-to-eat crops are not produced.

- Use irrigation and agricultural water which are of adequate microbiological quality. In this case, the use of clean water, defined as water that does not compromise food safety in the circumstances of its use, and/or potable water, defined as water which meets the quality standards of drinking water are recommended. Growers should identify the sources of water used on the farm (municipality, re-used irrigation water, well, open canal, reservoir, rivers, lakes, farm ponds etc.). They should assess its microbial and chemical quality, and its suitability for intended use, and identify corrective actions to prevent or minimize contamination (e.g. from livestock, sewage treatment, human habitation). Special care should be taken when reclaimed or run-off water is used for agricultural purposes (FDA/CFSAN, 2001).

- Control of sourcing, handling and treatment of manure and slurry that are to be used for fertilising fields. To minimize microbial contamination growers should adopt appropriate treatment procedures that are designed to reduce or eliminate pathogens in manure (EFSA, 2005), biosolids and other natural fertilizers. Manure, biosolids and other natural fertilizers which are untreated or partially treated may be used only if appropriate corrective actions are being adopted to reduce microbial contaminants such as maximizing the time between application and harvest of seeds.

- People working with seeds should be advised to follow hygiene and health requirements as preventive measures. Additionally, hygienic and sanitary facilities should be available to ensure that an appropriate degree of personal hygiene can be maintained.

- During the harvesting phase, special attention should be made in order to reduce the intake of soil and other environmental contamination with the seeds, and seed damage (FSANZ, 2010). Thus, equipment and tools used during growing and harvesting should be maintained in good condition. Growers and harvesters must always follow the technical specifications recommended by manufacturers for the proper usage and maintenance of equipment. Harvesting equipment should be adjusted to minimize soil intake and seed damage and should be cleaned from any debris or soil. Diseased or damaged seeds, which may be more susceptible to microbial contamination, should not be used for the production of sprouts for human consumption.

- Seeds should be stored and transported under conditions which will minimize the potential for microbial contamination. Seeds grown for the production of sprouts for human consumption should be segregated from products intended for other uses and clearly labelled and stored in clean areas. They should be off the floor and away from walls to avoid contamination due to rodents (Beales, 2004).

5.5. GMP, GHP and HACCP principles during sprouting

Sprouts, shoots and cress derived from seeds can be sold as ready-to-eat (RTE) food, and consequently they should be produced under conditions of hygiene at least as strict as those used for RTE foods supporting growth of bacterial pathogens.

Sprouted seeds have unique attributes and microbiological issues that increase the risk of foodborne diseases due to the potential for pathogenic bacteria to grow during the germination and sprouting of the seeds (see section 4.2 of the present Opinion).

In addition, sprout production involves contact with food handlers, with different types of equipment and water. Therefore, GMP, GHP and HACCP principles have been proposed to reduce contamination of seeds and to prevent cross contamination during production (CAC, 2003):

- Sprout producers should know the origin of the seeds as well as the agricultural practices used for the seed production. Traceability of seeds intended for human consumption is of great importance in establishing its microbial quality and for the implementation of food safety management measures. This may include testing of seed lots before entering production (see chapter 7).

- Hygiene practices and health screening of workers continues to be an important measure in order to prevent direct or indirect contact between diseased or healthy carriers with seeds, sprouts or the production environment.

- Initial rinses should be done thoroughly to remove dirt of seeds and increase the efficiency of subsequent microbial decontamination (CAC, 2003) whenever such decontamination is applied.

- CAC (2003) considered that "due to the difficulty of obtaining seeds which can be guaranteed as pathogen free, it is recommended that seeds be treated prior to the sprouting process". However CAC (2003) does not recommend specific decontamination treatment. In the US, treatments that may achieve 5 $\log_{10}$ units reduction of *Salmonella* on seeds are recommended (NACMCF, 1999). Microorganisms are more accessible to sanitizers when present in seeds than on sprouted seeds (Caetano-Anolles et al., 1990), although it is recognised that most of the decontamination treatments cannot ensure complete elimination of pathogens. This will be discussed in section 5.6.

- Wherever chemical decontamination in applied, post-treatment rinsing of seeds should be done with potable water.

- Pre-germinating soaking, whenever applied, may use antimicrobial agents to avoid cross contamination, according to (CAC, 2003). This is a critical step as seeds are commonly soaked in water for 3 to 10 hours at ambient temperature, allowing the proliferation of microorganisms. The quality of the water should be regularly monitored to ensure the avoidance of cross-contamination. In accordance with this, the use of running water is recommended (FAO/WHO, 2002).

- Use of potable water for sprout production. Where water is recycled or re-used, as it could happen in washing tanks, interventions should be taken to treat and maintain the quality of the water to reduce the build-up of microorganisms. The treatment process should be effectively monitored and controlled; it should be compatible with a safe disposal of the used water, considering its environmental impact.

- During germination potable water should be used. Whenever soils or other substrates are used (*e.g.* for cress) CAC (2003) recommends that they are adequately treated, including heat treatment. Consequence of soil pasteurisation on the risk for cress production in case of seed contamination is not documented.

- Final rinse of sprouted seeds is frequently needed to remove hulls and cool down the product to slow down microbial growth. As previously, the water should be of potable quality and renewed sufficiently or treated with decontamination agents to maintain its quality and prevent cross-contamination.

- Sprout production, harvesting, draining and storage of sprouted seeds should be done with cleaned and disinfected tools dedicated for this use, which should be maintained in good conditions.

- The final product should be kept in a way to permit rapid cooling if additional cooling is needed (e.g. small containers). Maintaining a chill chain for sprouts and shoots from end of production to consumption is necessary to limit growth of bacterial pathogens.

- CAC (2003) also recommends microbiological testing at several steps (seeds, spent irrigation water during sprouting, the final sprouted seeds). This will be discussed in section 7.

5.6. Decontamination techniques for seeds and sprouts, shoots and cress derived from seeds

The primary step to prevent contamination from occurring is to respect the preventive measures included in the GAPs, GMPs, GHPs and Sanitation Standard Operating Procedures (SSOPs) in primary production, postharvest handling and processing. However, most of these guidelines deal with generic preventive measures and interventions, which are not always easy to implement by individual farmers or to translate to a farm/company specific situation (Gil et al., 2011), Therefore interventions aimed at controlling and/or reducing microbiological contamination of seeds were designed because the seed quality is difficult to control and it is not until late in the production process that it is determined whether seed will be used for agriculture or food production (NACMCF, 1999).

Based on the majority of published data, seeds should be rinsed in large volumes of potable water as many times as necessary to remove dirt and increase the efficiency of the chemical decontamination treatment. There have been extensive investigations into the efficacy of various chemical sanitising agents and other disinfection treatments in reducing levels of pathogenic micro-organisms in contaminated seeds (Bang et al., 2011; Bari et al., 2011; Beuchat, 1997; Fett, 2002a; Gandhi and Matthews, 2003; Jianxiong et al., 2010; Saroj et al., 2006b). These are summarized in Table 4. Most of the scientific literature indicates that sanitising reduces, but does not necessarily eliminate, pathogens from contaminated seed. As mentioned in previous sections (see section 5.6 of the present Opinion), sanitizing seeds intended for sprouting presents a unique challenge in the arena of produce safety in that even a low residual pathogen population remaining on contaminated seed after treatments appears capable of growing to very high levels during sprouting, which means that reduction on the seeds is not equivalent to reduction in the final product. Chemicals sanitizers, "when used appropriately with adequate water quality, may also help to minimize the potential microbial contamination of processing water and subsequent cross contamination of the product" (FDA/CFSAN, 2008). This latter usage of sanitizers has also been investigated during sprouting of seeds.

A review of the different sanitation methods available for seeds as well as for fresh vegetables, which can be also applied to sprouts, shoots and cress derived from seeds, was compiled by the National Advisory Committee on Microbiological Criteria for Food (NACMCF, 1999), in the Food Safety Guidelines for the Fresh-cut Produce Industry (Gorny, 2001) and throughout the Forum on Washing and Decontamination of Fresh Produce (CCFRA, 2002-2008). Additionally, the efficiency of numerous chemical and physical methods for assuring the microbiological safety of seeds and fresh produce has been covered in several reviews (Allende et al., 2006; Ariefdjohan et al., 2004; Beuchat, 1997; Gómez-López et al., 2009; Ölmez and Kretzschmar, 2009; Parish et al., 2003; Rico et al., 2007; Sapers et al., 2006; Weissinger and Beuchat, 2000). However, as far as we know, specific scientific literature focusing on decontamination treatments for shoots and cress is currently not available.

5.6.1. Chemical decontamination of seeds, dry and during sprouting

In the US, the FDA has recommended soaking seeds in 20,000 ppm of calcium hypochlorite before sprouting to reduce pathogens (Montville and Schaffner, 2004). Alternatively, sprout producers may use one or more than one sanitizing method to achieve a 5-$\log_{10}$ CFU reduction of pathogens on seeds (NACMCF, 1999). Decontamination of sprouts is also being done using relatively high levels of chlorine, within the range 50-200 ppm (NACMCF, 1999). Most investigations concern the decontamination of seeds (Table 4). Surface decontamination of sprouts might be limited by the inaccessibility of the bacteria. *E. coli* O157:H7 was found both on the outer surfaces and the inner tissue of radish sprouts grown from artificially inoculated seeds (Itoh et al., 1998). In addition, seeds withstand much higher concentrations of sanitizers than sprouts (see Table 4 for the impact of decontamination methods on the germination of the treated seeds).

Although chlorine washing is commonly used for seed decontamination, its efficacy seems to be very variable. Chlorine washing of dry seeds at 200 and 20,000 ppm was shown to result in a reduction of pathogens by 3 $\log_{10}$ CFU/g or less suggesting that other alternative treatments such as gaseous acetic acid could be more effective than chlorine washing in controlling pathogenic bacteria on seeds (Nei et al., 2011). Gandhi and Matthews (2003) reported that combined treatment of seeds with 20,000 ppm $Ca(OCl)_2$ followed by 100 ppm chlorine or calcinated calcium during germination and sprout growth did not eliminate *Salmonella* from alfalfa seeds artificially inoculated with 10^6-10^7 cfu/g. However, previous studies found that the addition of 0.4% calcinated calcium into *E. coli* O157:H7 contaminated radish sprouting medium completely inhibited growth of the organism (Bari et al., 1999). Results from various works using 10,000 to 20,000 ppm chlorine on dry seeds, summarized in Table 4, showed between 2 to 5 $\log_{10}$ reduction of *Salmonella* or *E. coli* O157 inoculated on dry seeds (Fett, 2002a, 2002b; Taormina and Beuchat, 1999).

The above results were obtained with seeds artificially inoculated with pathogenic bacteria. Two studies undertaken using naturally contaminated seeds gave different results. Treatment of an alfalfa seed lot naturally contaminated with around 1MPN per 100g *Salmonella* Mbandaka with unbuffered 2,000 ppm or buffered and unbuffered 20,000 ppm free chlorine from $Ca(OCl)_2$ for 10-15 min or 30min, all eliminated the pathogen (Suslow et al., 2002). In contrast, treatment of alfalfa seeds naturally contaminated with 1 MPN *Salmonella* per 10 g for 10 min with 20,000 ppm of calcium hypochlorite caused a reduction, but not elimination, of *Salmonella* contamination in the finished sprouts (Stewart et al., 2001).

Montville and Schaffner (2004) conducted a statistical analysis of published seed sanitisation studies focussing on reduction in levels of *Salmonella* and EHEC and this revealed a high degree of variability in the results. Evaluation of the disinfection technologies is greatly affected by several factors such as physicochemical properties of process wash water and methodology used for applying the treatment to the produce (Fett, 2006; Gil et al., 2009; Pirovani et al., 2004; Ukuku and Fett, 2004) along with several other factors such as the inoculation procedure, drying times prior to washing, the physiological status of the test microorganism (fresh laboratory cultures versus environmentally stressed micro-organisms), type and condition of the seed, sprout, shoot and cress (broken, cracked or wrinkled seed coats) and the method of detection with special emphasis on the detection limit (Beuchat et al., 2004; Beuchat et al., 2001; Burnett et al., 2004; Charkowski et al., 2001; Lang et al., 2004; Singh et al., 2002).

The use of naturally contaminated seeds, sprouts, shoots and cress rather than artificially inoculated ones may give a more accurate prediction of the efficacy of decontamination treatments for eliminating bacterial human pathogens in commercial practice. This may be due to differences in bacterial populations per gram of product (normally much lower on naturally contaminated product than on artificially contaminated product used for laboratory studies), possible differences in the location and physiological status of the pathogens and the potential presence of pathogens in biofilms (Fett, 2006). However, seeds naturally contaminated with *Salmonella* or pathogenic *E. coli* have been

very rarely used (Stewart et al., 2001; Suslow et al., 2002). The lack of a standardized methodology and validation procedure makes difficult to select the most appropriate sanitizing strategies for the disinfection of seeds, sprouts, shoots and cress. Therefore, the evaluation of efficacy of different seed decontamination treatments should be harmonized to facilitate comparison among different studies.

Highly efficacious disinfection strategies are supported by laboratory studies where various sanitizing agents and methods are used (Fonseca, 2006). These laboratory studies may overestimate the efficacy of sanitizers because experiments are carried out under laboratory conditions without taking into account very important parameters such as the presence of organic matter in the process wash water (Allende et al., 2007; Sapers et al., 2006; Stopforth et al., 2008). It should be taken into account that the water quality in a washing tank deteriorates rapidly and it usually contains high organic loads including soil, leaves, and other debris as well as microorganisms associated with the produce, which might reduce the efficacy of specific sanitizing treatments (Allende et al., 2008). One possibility to reduce the high amount of organic matter present in the washing tank could be to introduce a pre-washing step. In some cases, the decontamination solution is used for only one batch of seeds. In this case, large amounts of water are needed for the washing step, thereby reducing the risk of cross-contamination between different production batches.

Alternative chemical decontamination treatments which have been suggested to reduce pathogenic microorganisms on seeds include the use of hydrogen peroxide and peroxyacetic acid among others (Table 4). In the case of seeds, Weissinger and Beuchat, (2000) reported that immersing inoculated seeds in solutions containing 8% H_2O_2 for 10 min resulted in reductions of about 2.0 to 3.2 $\log_{10}$ *Salmonella* CFU/g. Treating alfalfa seeds with 1 and 3% peroxyacetic acid using an air-mixing wash basin resulted in similar reductions in *Salmonella* Stanley of 1.77 and 1.34 $\log_{10}$, respectively (Rajkowski and Ashurst, 2009).

Some authors have attributed a detrimental effect to decontamination treatments due to the reduction of native microbial load in addition to the reduction of pathogenic bacteria, which might favour the growth of any pathogen remaining in the seeds, or contaminating the seeds after decontamination. However, one study showed that the population of *Salmonella* spp. inoculated on sanitized alfalfa seeds was less than 1 log higher after 3 days of sprouting than on non-sanitized seeds(Liao, 2008). Therefore, the reduction in *Salmonella* levels by decontamination treatments (several log units) presumably outweighs these potential negative effects on the native microbial flora.

In the US, the impact of the 20,000 ppm hypochlorite treatment of seeds has been assessed in the course of two *Salmonella* outbreaks, where the same lot of contaminated seeds had been used by different sprout producers, some using, and some not using seed decontamination. In one report, sprouts from the producers using seed decontamination were not linked to *Salmonella* cases (Gill et al., 2003). In the second report, sprouts from a producer using seed decontamination resulted in fewer cases of illness (Brooks et al., 2001).

Because there may be constraints on the use of chemical disinfection in some sectors (***e.g.*** in organic producers of sprouts, shoots and cress) alternative decontamination technique, such as physical decontamination treatments (e.g. heat treatments, UV light) should be also considered.

5.6.2. Alternative decontamination treatments of seeds and sprouted seeds

There are numerous examples of alternative sanitizing methods, including combined decontamination treatments, which can be currently found in the literature on treating seeds and sprouts, although only few of them have been implemented by the industry. Some of these studies are summarized in Table 4. In the case of shoots and cress, there is no available literature about the effect of alternative decontamination treatments on the safety of this type of product.

The efficacy of dry heat alone or in combination with chemical and physical treatments to reduce pathogens in seed intended for human consumption has been investigated. Neetoo and Chen (2011) showed that alfalfa seeds subjected to dry heat treatments at 55 and 60ºC achieved ≤1.6 and 2.2 $\log_{10}$ CFU/g reduction in the population of *Salmonella* spp. after a 10-d treatment, respectively, while higher temperatures of 65ºC for 10 days or 70ºC for 24 h eliminated a 5 log population of *Salmonella* and *E. coli* O157:H7. They also reported that even one of the most aggressive treatments (65ºC for 10d) did not affect seed germination but reduced the sprout yield by 21%. The application of dry heating followed by high hydrostatic pressure (HHP) can substantially reduce the dry heating exposure time while achieving equivalent decontamination (Neetoo and Chen, 2011). Dry heating at 55, 60, 65 and 70ºC for 96, 24, 12 and 6 h, respectively, followed by a pressure treatment of 600 MPa for 2 min at 35ºC was able to eliminate a 5 $\log_{10}$ CFU/g initial population of *Salmonella* and *E. coli* O157:H7 in alfalfa seeds intended for sprouting without affecting the germination percentage of seeds. Simultaneous combinations of different HHP and temperatures have been also reported to efficiently eliminate pathogens, such as 550 MPa for 2 min at 40°C, 300 MPa for 2 min at 50°C, and 400 MPa for 5 min at 45°C (Neetoo et al., 2009). The sequential application of dry heat (50ºC) for 17 h followed by the application of aqueous chemicals (1% oxalic acid, 0.03% phytic acid, 50% ethanol, electrolyzed acidic water, and electrolyzed alkaline water) were effective in greatly reducing *E. coli* O157:H7 populations on radish, broccoli, and alfalfa seeds, without compromising the quality of the sprouts (Bari et al., 2009). Bari et al., (2009) also determined that sequential application of dry heat treatment (50ºC) for 17 h of dry heat followed by a 1.0-kGy dose of irradiation completely eliminated *E. coli* O157:H7 from radish and mung bean seeds whereas only a minimum radiation dose of 0.25 kGy was required to completely eliminate the pathogen from broccoli and alfalfa seeds.

Wet heat treatment of seeds intended for human consumption is a decontamination method currently applied in several countries including Japan. Heat treatment consisting of a hot water treatment at 85ºC for 10 s has been reported to be more effective for disinfecting inoculated *E. coli* O157:H7, *Salmonella*, and nonpathogenic *E. coli* on mung bean seeds than the calcium hypochlorite treatment (20,000 ppm for 20 min) recommended by the U.S. Food and Drug Administration (Bari et al., 2010). This study also showed that hot water treatment at 85ºC for 40 s followed by dipping in cold water for 30 s and soaking in chlorine water (2,000 ppm) for 2 h reduced the pathogens to undetectable levels (initial inoculum of 5.8 $\log_{10}$ cfu/g), and no viable pathogens were found in a 25-g enrichment culture and during the sprouting process. In other studies, treatment of mung bean seeds inoculated with *Salmonella* at 55ºC for 20 minutes, 60ºC for 10 minutes, or 70ºC for 5 minutes led to an approximate 5 $\log_{10}$ reduction (Weiss and Hammes, 2003). Treating seed at 80ºC for 2 minutes was even more effective resulting in a >6 $\log_{10}$ reduction. None of these temperature/time treatments led to a decrease in germination of the treated seeds (Fett, 2006). In another study treatment of seeds at 23 or 55°C with 1% $Ca(OH)_2$ was most effective in reducing populations compared to the use of 20,000 ppm of chlorine, without compromising seed viability (Scouten and Beuchat, 2002). Therefore, wet heat treatments or a combination of wet heat followed by chlorine treatments could be a viable alternative to decontaminate seeds intended for sprout production. However, it should be taken into account that germination of some seed varieties might be affected. Thus, more research is needed to determine the optimum wet heat treatment to avoid yield losses for different types of seeds. Treating with aqueous chemicals at elevated temperatures can lead to greater reductions of pathogen populations on seeds, than temperature or chemicals alone, although this may be detrimental to seed germination.

Treatment with 7.8% (v/v) acetic acid at 55ºC for 2–3 h reduced the population of *E. coli* O157:H7 and *Salmonella* inoculated on alfalfa (*Medicago sativa* L.) and radish seeds (*Raphanus sativus* L.) by more than 5.0 $\log_{10}$ CFU/g (Nei et al., 2011). *E. coli* O157:H7 populations were reduced to an undetectable level with a gaseous acetic acid treatment for 48 h, without significantly affecting the germination rates of treated seeds (germination rates greater than 95%). However, these treatments were unable to eliminate *Salmonella* in both seed types (Nei et al., 2011; Weissinger et al., 2001).

Other combinations have also been tested. Addition of high levels of the surfactant Tween 80 (1%, w/v) to 1% $Ca(OH)_2$ led to only an additional 1 $\log_{10}$ reduction or less in the population of *Salmonella*

on alfalfa seeds (Holliday et al., 2001; Weissinger and Beuchat, 2000). Sonication of seed during treatment with aqueous antimicrobial compounds also did not have a significant effect, only slightly increasing the $\log_{10}$ kill obtained (Bari et al., 2003; Rajkowski et al., 2003).

On dry seeds irradiated to reduce pathogenic bacteria before production of sprouts, D_{10} values for *Salmonella* strains were between 0.74 and 1.1 kGy (Rajkowski et al., 2003), higher than those reported in most foods, presumably because of the low humidity present on the surface of seeds (EFSA, 2011b). The US Food and Drug Administration (FDA) has approved irradiation treatment of seeds intended for sprout production at doses up to 8 kGy (FDA, 2011). Exposure of inoculated alfalfa seeds to a 2 kGy dose of gamma irradiation led to a 3.3. and 2.0 $\log_{10}$ reduction in *E. coli* O157:H7 and *Salmonella* populations, respectively, while still maintaining commercially acceptable yields as well as nutritive values of sprouts grown from the treated seeds (Bari et al., 2004; Fan et al., 2004; Fett, 2006; Rajkowski and Thayer, 2000, 2001; Thayer et al., 2003). However, higher dosages led to unacceptable reductions in yields (Fett, 2006).

Jianxiong et al. (2010) reported that the application of a combination of antagonistic bacteria and lytic bacteriophages were effective at controlling the growth of *Salmonella* on sprouting mung beans and alfalfa seeds under a range of sprouting temperatures (20 to 30°C). Therefore, the combination of antagonistic bacteria and bacteriophages could represent a chemical-free alternative for controlling the growth of *Salmonella* on sprouting seeds.

5.6.3. Decontamination treatments of sprouts, shoots and cress

As previously mentioned there is limited information on the efficacy of decontamination treatments of sprouts, shoots and cress derived from seeds. The work done on the decontamination of fresh-produce (Parish et al., 2003) may apply to some extent to sprouts, shoot and cress, although this should be specifically investigated. In the case of irradiation, the US Food and Drug Administration (FDA) has approved irradiation treatment of fresh produce, including sprouts, at a maximum dose of 1 kGy (FDA, 2011).

5.6.4. Conclusions on decontamination methods

Despite considerable efforts, to date, no chemical method of disinfection has been able to ensure pathogen-free for all seed types. There are very few disinfection treatments that consistently achieve a substantial (i.e. > 5-$\log_{10}$) reduction in pathogen numbers. Even the US recommended 20,000-ppm chlorine ($CaOCl_2$) treatment has most often only a median reduction of 2.5 $\log_{10}$ CFU/g and produces equal to or greater than a 5-$\log_{10}$ reduction only approximately 9% of the time (Montville and Schaffner, 2005).

Measures to prevent introduction of pathogens in sprouted seed production (including primary production of seeds) remains of the foremost importance.

Alternative treatments such as heating seeds, alone or in combination with other treatments, could greatly improve the efficacy of seed decontamination. However, the temperature/time parameters should be optimized for each type of seed variety as not all of them will be suitable for the same treatment. Decontamination treatments should not kill seed or reduce the germination rate.

Table 4: Physical and chemical treatments for the inactivation of pathogens on inoculated sprouting seeds (adapted from: (Beales, 2004; Fett, 2006; FSANZ, 2010).

Treatment	Conditions	Time [(a)]	Seed Type	Bacterium	Logarithmic Reduction (cfu/g)	Seed Germination	Reference
Acetic acid, vapour	242 µl/L air, 45°C	12 h	Mung bean	*Salmonella*	> 5, no survivors	No effect	(Delaquis et al., 1999)
Acetic acid, vapour	242 µl/L air, 45°C	12 h	Mung bean	*E. coli* O157:H7	> 6, no survivors	No effect	(Delaquis et al., 1999)
Acetic acid, vapour	242 µl/L air, 45°C	12 h	Mung bean	*L. monocytogenes*	4	No effect	(Delaquis et al., 1999)
Acetic acid, vapour	300 mg/L air, 50°C	24 h	Alfalfa	*Salmonella*	0.8	No effect	(Weissinger et al., 2001)
Acetic acid, water	7.8% (v/v)	2-3 h	Alfalfa and radish	*E. coli* O157:H7	> 5	No effect	(Nei et al., 2011)
Acidic EO water	1,081 mV, 84 ppm chlorine	10 min	Alfalfa	*Salmonella*	1.5	No effect	(Kim et al., 2003)
Acidic EO water	1150 mV, 50 ppm chlorine	64 min	Alfalfa	*E. coli* O157:H7	1.6	Significant reduction	(Sharma and Demirci, 2003b)
Acidic EO water	1,079 mV, 70 ppm chlorine	15 min	Alfalfa	*Salmonella*	2	No effect	(Stan and Daeschel, 2003)
Allyl isothiocyanate	50 µl/950-cc jar, 47°C	24 h	Alfalfa	*E. coli* O157:H7	>2.0, survivors present	Slight reduction	(Park et al., 2000)
Ammonia, gas	300 mg/L	22 h	Alfalfa	*Salmonella*	2	No effect	(Himathongkham et al., 2001)
Ammonia, gas	300 mg/L	22 h	Mung bean	*Salmonella*	5	No effect	(Himathongkham et al., 2001)
Ammonia, gas	300 mg/L	22 h	Alfalfa	*E. coli* O157:H7	3	No effect	(Himathongkham et al., 2001)
Ammonia, gas	300 mg/L	22 h	Mung bean	*E. coli* O157:H7	6	No effect	(Himathongkham et al., 2001)
Biocontrol	*Enterobacter asburiae* + Lytic bacteriophages	4 days	Mung bean	*Salmonella*	5.7 to 6.4	No effect	(Jianxiong et al., 2010)
$Ca(OH)_2$ (Calcium Hydroxide)	1%	10 min	Alfalfa	*E. coli* O157:H7	3.2		(Holliday et al., 2001)
$Ca(OH)_2$	1%	10 min	Alfalfa	*Salmonella*	2.8 - 3.8	No effect	(Holliday et al., 2001); (Weissinger and Beuchat, 2000)
$Ca(OCl)_2$ (Calcium Hypochlorite)	20,000 ppm	3 min	Alfalfa	*E. coli* O157:H7	> 2.3, survivors present	Reduced rate	(Taormina and Beuchat, 1999)
$Ca(OCl)_2$	20,000 ppm	10 min	Alfalfa	*Salmonella*	2	Slight reduction	(Weissinger and Beuchat, 2000)
$Ca(OCl)_2$	18,000 ppm	10 min	Alfalfa	*Salmonella*	3.9	No effect	(Fett, 2002a)
$Ca(OCl)_2$	18,000 ppm	10 min	Alfalfa	*E. coli* O157:H7	4.5	No effect	(Fett, 2002a)
$Ca(OCl)_2$	16,000 ppm	10 min	Mung bean	*Salmonella*	5	No effect	(Fett, 2002b)
$Ca(OCl)_2$	16,000 ppm	10 min	Mung bean	*E. coli* O157:H7	3.9	No effect	(Fett, 2002b)
$Ca(OCl)_2$	2,000 and 20,000 ppm	10 min	Alfalfa	*Salmonella*	Undetected levels of naturally inoculated		(Suslow et al., 2002)

Treatment	Conditions	Time [a]	Seed Type	Bacterium	Logarithmic Reduction (cfu/g)	Seed Germination	Reference
$Ca(OCl)_2$	20,000 ppm	20 min	Mung bean	*E. coli* O157:H7 and *Salmonella*	2.5, 2.7, respectively	No effect	(Bari et al., 2010)
Chlorine dioxide, acidified	500 ppm	10 min	Alfalfa	*E. coli* O157:H7	>2.4, survivors present	Significant reduction	(Taormina and Beuchat, 1999)
Chlorine dioxide + air drying + dry heat	500 ppm + 25 º C + 55 ºC	5 min + 2 h + 36 h	Radish	*E. coli* O157:H7	5	Slight reduction	(Scouten and Beuchat, 2002)
Citrex™	20,000 ppm	10 min	Alfalfa	*Salmonella*	3.6	No effect	(Fett and Cooke, 2003)
Citrex™	20,000 ppm	10 min	Alfalfa	*E. coli* O157:H7	3.4	No effect	(Fett and Cooke, 2003)
Dry heat	50°C	60 min	Alfalfa	*E. coli* O157:H7	1.7	No effect	(Bari et al., 2003)
Dry heat	70°C	3h	Alfalfa	*Salmonella*	3	Slight reduction	(Weissinger and Beuchat, 2000)
Dry heat	50 °C	24 h	Mung bean	*E. coli* O157:H7	3	No effect	(Bari et al., 2009)
Dry heat	50 °C	24 h	Radish	*E. coli* O157:H7	5	No effect	(Bari et al., 2009)
Dry heat	50 °C	17 h	Alfalfa and broccoli	*E. coli* O157:H7	5, survivors present	No effect	(Bari et al., 2009)
Dry heat	55 °C	10d	Alfalfa	*Salmonella*	1.6	No effect	(Neetoo and Chen, 2011)
Dry heat	60 °C	10 d	Alfalfa	*Salmonella*	2.2	No effect	(Neetoo and Chen, 2011)
Dry heat	65 and 70 °C	10 d and 24 h, respectively	Alfalfa	*Salmonella and E. coli* O157:H7	5	No effect	(Neetoo and Chen, 2011)
Dry heat	50º C	17 h	Mung bean	*E. coli* O157:H7	2	No effect	(Bari et al., 2009)
Dry heat + high hydrostatic pressure	600 mPa 55, 60, 65 and 70 ºC	96, 24, 12 and 6 h, respectively	Alfalfa	*Salmonella and E. coli* O157:H7	5	No effect	(Neetoo and Chen, 2011)
Dry heat + high hydrostatic pressure	600 mPa, 40 ºC	2 min	Alfalfa	*E. coli* O157:H7	5	No effect	(Neetoo et al., 2009)
Dry heat + high hydrostatic pressure	550 mPa, 40ºC	2 min	Alfalfa	*E. coli* O157:H7	5	No effect	(Neetoo et al., 2009)
Dry heat + high hydrostatic pressure	300 mPa, 50ºC	2 min	Alfalfa	*E. coli* O157:H7	5	Slight reduction	(Neetoo et al., 2009)
Dry heat + high hydrostatic pressure	400 mPa, 45ºC	5 min	Alfalfa	*E. coli* O157:H7	5	Slight reduction	(Neetoo et al., 2009)
Dry heat + ethanol soaking	50 °C + 50 %	17 h	Mung bean, radish, alfalfa and broccoli	*E. coli* O157:H7	5, survivors present	No effect	(Bari et al., 2009)
Dry heat + ethanol soaking	50 °C + 50 %	17 h	Mung bean, radish, alfalfa and broccoli	*E. coli* O157:H7	5, survivors present	No effect	(Bari et al., 2009)

Treatment	Conditions	Time [a]	Seed Type	Bacterium	Logarithmic Reduction (cfu/g)	Seed Germination	Reference
Dry heat + soaking in aqueous sanitizers (1% oxalic acid, 0.03% phytic acid, 50% ethanol, electrolyzed acidic water, and electrolyzed alkaline water)	50 °C + 1%, 0.03%, 50%	17 h + 20 s	Alfalfa and broccoli	*E. coli* O157:H7	5, survivors present	No effect	(Bari et al., 2009)
Dry heat + irradiation	50 °C +1 kGy	17 h	Mung bean and radish	*E. coli* O157:H7	5, no survivors present	No effect	(Bari et al., 2009)
Dry heat + irradiation	50 °C +0.25 kGy	17 h	Alfalfa and broccoli	*E. coli* O157:H7	5, no survivors present	No effect	(Bari et al., 2009)
Fit ™	According to label	15 min	Alfalfa	*Salmonella*	2	No effect	(Beuchat et al., 2001)
Fit ™	According to label	15 min	Alfalfa	*E. coli* O157:H7	>5.4	No effect	(Beuchat et al., 2001)
H_20_2	8%	3 min	Alfalfa	*E. coli* O157:H7	>2.9, survivors present	No effect	(Taormina and Beuchat, 1999)
H_20_2	8%	10 min	Alfalfa	*Salmonella*	3.2	No effect	(Weissinger and Beuchat, 2000)
Hydrostatic pressure	300 mPa	15 min	Garden cress	*Salmonella*	5.8	Reduced rate	(Wuytack et al., 2003)
Hydrostatic pressure	300 mPa	15 min	Garden cress	*Shigella flexneri*	4.5	Reduced rate	(Wuytack et al., 2003)
Lactic acid	5%, 42°C	10 min	Alfalfa	*E. coli* O157:H7	3	No effect	(Lang et al., 2000)
Radiation, gamma	Various	NA	Alfalfa	*Salmonella*	D-value of 0.97 kGy	Dosage dependent	(Thayer et al., 2003)
Radiation, gamma	Various	NA	Alfalfa	*E. coli* O157:H7	D-value of 0.60 kGy	Dosage dependent	(Thayer et al., 2003)
Radiation, gamma	Various	NA	Broccoli	*Salmonella*	D- value of 1.10 kGy	Dosage dependent	(Rajkowski et al., 2003)
Radiation, gamma	Various	NA	Broccoli	*E. coli* O157:H7	D- value 0f 1.11 kGy	Dosage dependent	(Rajkowski et al., 2003)
Radiation, gamma	1.5 kGy, 2.0 kGy	NA	Mung bean and radish, respectively	*E. coli* O157:H7 and *Salmonella*	Undetected levels	No effect	(Bari et al., 2004)
Sodium chlorite, acidified	1,200 ppm, 55°C	3 min	Alfalfa	*E. coli* O157:H7	>1.9, survivors present	Slight reduction	(Taormina and Beuchat, 1999)
Sulphuric acid	2N	20 min	Alfalfa	*E. coli* O157:H7	5	No effect	(Pandrangi et al., 2003)
Ozone, aqueous	21 ppm, w/sparging	64 min	Alfalfa	*E. coli* O157:H7	2.2	No effect	(Sharma et al., 2002)
Ozone, aqueous	21.3 ppm, w/sparging	20 min	Alfalfa	*L. monocytogenes*	1.5	No effect	(Wade et al., 2003)
Peroxyacetic acid	1%	3 min	Alfalfa	*E. coli* O157:H7 and *Salmonella*	> 1		(Rajkowski and Ashurst, 2009)
Pulsed UV light	5.6 J/cm^2 , 270 pulses	90 sec	Alfalfa	*E. coli* O157:H7	4.9	Significant reduction	(Sharma and Demirci, 2003a)
Dielectric heating, radio frequency	39 MHz, 1.6 kV/cm	26 sec	Alfalfa	*Salmonella*	1.7	No effect	(Nelson et al., 2002)
Water, hot	3-stage: 25 to 50 to 85°C	30 min, 9 sec, 9 sec	Alfalfa	*E. coli*, generic	>4, no survivors	No effect	(Enomoto et al., 2002)

Treatment	Conditions	Time [(a)]	Seed Type	Bacterium	Logarithmic Reduction (cfu/g)	Seed Germination	Reference
Water, hot	54°C	5 min	Alfalfa	*Salmonella*	2.5	No effect	(Jaquette et al., 1996)
Water, hot	80°C	2 min	Mung bean	*Salmonella*	>6	No effect	(Weiss and Hammes, 2003)
Water, hot	85 °C	10 s	Mung bean	*E. coli* O157:H7 and *Salmonella*	2.8, 3.2 respectively	No effect	(Bari et al., 2010)
Water, hot	85 °C	20 s	Mung bean	*E. coli* O157:H7 and *Salmonella*	4.3, >5.84, respectively	No effect	(Bari et al., 2010)
Water, hot	85 °C	30 s	Mung bean	*E. coli* O157:H7 and *Salmonella*	>5.69, >5.84 respectively	No effect	(Bari et al., 2010)
Water, hot + chlorine	85 ºC + 2,000 ppm	20 s + 30 s	Mung bean	*E. coli* O157:H7 and *Salmonella*	>5.69, >5.84 respectively	No effect	(Bari et al., 2010)

(a) NA: Not applicable

6. MICROBIOLOGICAL CRITERIA FOR SEEDS AND SPROUTED SEEDS

6.1. Introduction to microbiological criteria

Microbiological testing against microbiological criteria is only one of several control options in food safety control and should not be considered in isolation from other aspects of EU food legislation, in particular HACCP principles and official controls to audit food business operators' compliance. Microbiological criteria can help in verifying that a food safety management system is operating as intended (CAC, 2007).

The establishment of a microbiological criterion should be scientifically based and preferably informed by risk assessment (CAC, 1997, 2007; EC, 1997). A microbiological criterion consists of specific elements i.e. the analytical method for detection and/or quantification, the sampling plan, microbiological limit(s), the number and size of analytical units that should be taken (sampling plan) and tested and the number that should conform to the limit(s). A microbiological criterion should also indicate the food or ingredient and the point(s) in the food chain where the criterion applies together with any actions to be taken when the criterion is not met.

Microbiological criteria are useful for validation and verification of HACCP-based processes and procedures, and other hygiene control measures (CAC, 1997). In addition, microbiological criteria are used to assess the acceptability of a batch or lot of food or food ingredients, including in circumstances where there is insufficient knowledge of production conditions. This may be particularly relevant with respect to seeds and their intended purpose. The application of microbiological criteria does not mean that all food batches/lots have to be tested, but it does clarify how the test results should be interpreted, and the anticipated actions in the event of a failure. In EU legislation, microbiological criteria are also used as a way to communicate the level of hazard control that is expected to be achieved by a food business operator.

6.2. Specific criteria/limits for seeds and sprouted seeds

6.2.1. Existing microbiological criteria/limits in EU legislation

Regulation (EC) No 2073/2005[13] on microbiological criteria for foodstuffs introduced two different types of criteria; Food Safety Criteria and Process Hygiene Criteria. A Food Safety Criterion is one which defines the acceptability of a product or a batch of foodstuff applicable to products placed on the market. If the food safety criterion is not met then the product / batch must not be placed on the market and if it already has then appropriate remedial or corrective action should be taken (e.g. withdrawal, recall etc). It is generally not possible to evaluate the extent of public health protection provided by a specific food safety criterion. Microbiological testing alone may convey a false sense of security due to the statistical limitation of sampling plans, particularly in the cases where the hazard presents an unacceptable risk at low concentrations and/or low and variable prevalence.

A Process hygiene criterion indicates the acceptable functioning of a production process through setting of contamination levels above which corrective actions are required in order to maintain the hygiene of the process. These criteria define the expected final outcome of the processes, but they neither characterize nor differentiate between the processes themselves.

Regulation (EC) 2073/2005[13] establishes several microbiological criteria relevant to the production of ready-to-eat sprouted seeds. There are no EU legislative microbiological criteria for this food if it is not considered as ready-to-eat.

[13] OJ L 338, 22.12.2005, p.1-26

The food safety criteria and process hygiene criteria in Regulation (EC) 2073/2005[14] which are relevant to sprouted seeds are a two class *Salmonella* food safety criterion, *Listeria monocytogenes* food safety criterion and a *E. coli* process hygiene criterion which comprises:

- a two class *Salmonella* food safety criterion for ready-to-eat sprouted seeds placed on the market during their shelf-life. The number of units comprising the samples should be 5 (n) and *Salmonella* should be absent in 25 g (using the EN/ISO 6579[15] detection method). Indications for preliminary testing of the batch of seeds before starting the sprouting process or the sampling to be carried out at the stage where the highest probability of *Salmonella* is expected are also included.

Two additional microbiological criteria have been laid down in Regulation (EC) No 2073/2005[14]:

- *Listeria monocytogenes* food safety criterion for ready-to-eat foods able to support the growth of *L. monocytogenes* (other than those intended for infants and for special medical purposes) placed on the market during their shelf-life: 100 cfu/g (using the EN/ISO 11290-2[16]), if the manufacturer is able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 cfu/g throughout the shelf-life. In other cases absence in 25 g applies (n=5). Application of this criterion will depend on whether or not sprouted seeds are being considered as a ready to eat food.

- *E. coli* process hygiene criterion in ready-to-eat pre-cut fruit and vegetables at the end of the manufacturing process: n=5, c=2, m=100 cfu/g, M=1000 cfu/g (using the EN/ISO 16649-1[17] or 2[18]). In the case of unsatisfactory results, actions leading to the improvement in the production hygiene and selection of raw materials should be taken. Although this criterion may apply to harvested cress and shoots there is no cutting step in sprouted seed production so the criterion does not apply under this legislation.

6.2.2. Microbiological criteria/limits in guidelines

There are various national and international guidelines on sprouted seed production and some of these include information on which pathogenic bacteria should be tested for as well as the point of application. *Salmonella* and *E.coli* O157 are the pathogens usually highlighted as target pathogens in a number of these guidelines (CAC, 2003; CFIA, 2007; FDA, 2004; FSAI, 2011; NACMCF, 1999). Whilst these guidelines do acknowledge the presence of other bacteria in sprouted seed production little specific information is provided on monitoring of hygiene indicators in the seed, sprouts, irrigation water or the process environment.

6.3. The value of existing criteria and the possible establishment of new ones

The listing of outbreaks (Tables 3, 7, 8) and microbiological data (Table 9) in this report emphasises the importance of the final product being free of pathogens or in the case of *L.monocytogenes*, levels that would not represent a risk to vulnerable consumers. Microbiological criteria should be used as an

[14] OJ L 338, 22.12.2005, p.1-26

[15] EN/ISO 6579:2002. Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp. International Organization for Standardization.

[16] EN/ISO 11290-2: 1998. Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 2: Enumeration method. International Organization for Standardization.

[17] EN/ISO 16649-1:2001. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* - Part 1: Colony-count technique at 44 degrees C using membranes and 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. International Organization for Standardization.

[18] EN/ISO 16649-2:2001. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* - Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. International Organization for Standardization.

integral part of a HACCP-based control system (see chapter 5) together with sampling and testing at those points in the production chain that will yield information to help manage microbiological hazards and risks and protect the consumer.

Due to the statistical limitations of sampling plans, negative results from microbiological testing of seeds and sprouted seeds for pathogens occurring at a low prevalence, may give a false indication of safety particularly if an insufficient number of samples are tested over time. In the EU most food safety criteria are based on two class sampling plans with 5 or 10 units tested per sample, except for powdered infant formulae where 30 units are required to be tested (Regulation (EC) 2073/2005[19]). Therefore, for pathogens present in food lots at a low frequency such as seeds, the risk of not detecting contamination is high unless very large numbers of samples are taken (see Chapter 7 and (ICMSF, 1986, 2002; Jarvis, 2008). When pathogens are present at low frequency such as on seeds the efficiency of applying food safety criteria to enhance consumer protection will be low but this can be improved to some extent by pooling and sprouting to enable the target organism to multiply prior to testing (chapter 7).

Table 5 provides an outline of the possible points of application of pathogen (food safety) and indicator (hygiene) criteria to inform food safety management in the sprouted seed production chain for ready to eat foods. It should be emphasised that these are potential points of application and it may not be necessary or informative to monitor every step. However, linkage of the information at each step illustrates the importance of working towards a shared responsibility throughout the chain from seed producers, distributors and sprout producers to ensure that consumers are not exposed to microbiological hazards from these products.

The pathogens of primary concern are *Salmonella* spp. and pathogenic *E.coli* as these have been the most frequently associated pathogens with outbreaks. Although listeriosis is rarely linked to these products, the possible growth of *L.monocytogenes* during sprout production and subsequent storage indicates a potential risk to vulnerable consumers. Testing against microbiological criteria for *L.monocytogenes* is currently included in EC Regulation 2073/2005[19] and will include some types of sprouted seeds considered ready to eat.

Salmonella, pathogenic *E.coli* and *L.monocytogenes* should be absent from seed which is intended for sprouting, whether in commercial operations or in the home. Microbiological criteria for seeds used for sprouting at home or by small caterers are particularly important because there is unlikely to be any subsequent microbiological testing of the sprouted seeds or production environment prior to consumption.

Currently, there are no criteria for pathogenic *E. coli* on seeds or sprouted seeds. Based on epidemiological and other data, consideration should be given to establishing microbiological criteria for the following STEC serotypes: O157, O26, O103, O111, O145 and O104, associated with severe human disease.

Detection and mitigation of a contamination problem earlier in the sprouted seed production chain (e.g. pooling and sprouting of seeds) may have advantages as it avoids contamination being amplified during the full sprouting process. If contamination is missed at this later point then contaminated product might be placed on the market leading to recalls and consumer warnings if contamination is subsequently detected. However, testing seeds alone does not permit to detect contamination which may come at a later stage in the production process.

There may be merit in establishing seed testing controls since such contamination, at least for *Salmonella*, has been shown to impact on subsequent contamination of sprouts leading to outbreaks of illness. It also provides a focus for monitoring of seed and the seed environment (e.g. dust) earlier in

[19] OJ L 338, 22.12.2005, p.1-26

the supply chain which may be important in assessing as well as reducing the likelihood of pathogen contamination arriving on seed at the sprouting plant. Evidence from sprout associated outbreaks of salmonellosis indicates that low levels of *Salmonella* in seeds used for sprouting (e.g. 4 MPN/kg) are sufficient to cause an outbreak. This should be considered to establish the sampling plan if some criteria are to be proposed for dry seeds used in sprouting.

Further work will be required to assess its wider applicability and in choosing a suitable sampling and testing regime to achieve sufficient sensitivity for microbiological criteria applied at this point.

Table 5: Possible points of application (x) of criteria (bacterial pathogens and indicator organisms) in sprouted seed production for ready to eat foods [(a)].

Point of application	Pathogen Criteria			Indicator Criteria	
	Salmonella **detection**	**Pathogenic *E. coli* detection**	***Listeria monocytogenes*** [(b)] **detection /enumeration**	**Total *E.coli* enumeration**	***Enterobacteriaceae*** **enumeration**
Seed storage environment - dust	x	x	-	x	-
Seeds	x	x	x	x	x
Sprouts	x	x	x	x	x
Production water [(c)]	-	-	-	-	-
Spent Irrigation water	x	x	x	x	x
Sprout process environment-swabs	-	-	x	x	x
Final product	x	x	x	x	x

[(a)] Could also apply to monitoring of cress and shoots with respect to seeds and final product. Testing of the growth medium (cellulose matting, soil etc) may be additional sampling points for cress and shoots.
[(b)] Would include testing for *Listeria* spp.
[(c)] Water used should be of potable quality in all cases. Consider periodic checks for *E.coli* if verification of water quality is needed (e.g. water treatment in the plant)

As discussed further in chapter 7, testing of dust and debris in silos, containers and sacks or at selected control points of the processing line (e.g. conveyers, aspiration filters) could provide useful information on the likelihood of contamination of seed by *Salmonella* and pathogenic *E. coli*. It will not account for all routes of contamination of seed but will provide additional information instead of sole reliance on testing of seed where contamination levels are expected to be at a low level.

There are currently no indicator organisms that can effectively substitute for the testing of pathogens in seeds, sprouts or irrigation water. However, assuming that pathogens are likely to be infrequent and at low levels there remains a need to consider other microbiological parameters that will enable the hygiene status to be assessed on a regular basis. Testing for total *E.coli*, *Enterobacteriaceae* and *Listeria* spp. in sprouts and irrigation water as well as the production environment (equipment, utensils and other surfaces that may come into contact with seeds, sprouts, spent irrigation water etc) can inform process hygiene control. Environmental sampling of the process environment provides useful information that the hygienic manufacturing conditions are being achieved and maintained. Testing for *Listeria* spp. is also important since niches in the manufacturing environment are known to potentially act as a focus for *L. monocytogenes* contamination in manufacturing sites for ready to eat food production. Further work may be required to assess the value of tests for these organisms in seeds prior to sprouting and for *E. coli* in dust samples.

Sprouts are perishable products and food business operators producing ready to eat sprouts are expected to conduct shelf-life assessment for their products and should take into account the potential for contamination and growth of *L. monocytogenes* and other microbiological hazards during sprout production and through chill storage up to the point of consumption.

As a ready-to-eat food, contamination by asymptomatic carriers of pathogenic bacteria should not be ignored. Specific criteria are not needed for those employed in the production of sprouted seed other than meeting requirements for fitness to work as a food handler.

7. SAMPLING AND ANALYTICAL METHODS FOR THE DETECTION AND ENUMERATION OF FOODBORNE PATHOGENIC BACTERIA IN SEEDS AND SPROUTED SEEDS

7.1. Introduction

In this chapter information will be given on sampling and on the analytical methods for the detection and enumeration of bacteria sampled in the possible points of application of criteria proposed in Table 5: (i) seeds, (ii) sprouted seeds (mung bean sprouts and other sprouts), (iii) spent water from seed sprouting, (iv) seed storage and processing environment (dust and swab samples). Sampling for testing of the sprouted seeds production environment is not documented and will not be discussed in this chapter. The collection and testing of such samples can provide information to asses the nature and extent of bacteriological hazards associated with production. Testing seeds and the seed storage environment could apply to all types of sprouted seeds (sprouts, shoots and cress). Testing spent irrigation water and testing the final product has been documented only for sprouts.

Because of the sporadic nature of seed contamination, effective testing of each seed production lot is recommended. Analyses can be performed on the seeds before production, during the germination process (e.g., spent irrigation water or sprouted seeds) and/or on finished product after harvest (CAC, 2003). It has been recommended that testing of production lots of seed for the absence of bacterial pathogens, including sampling and test sprouting, should be done by the seed supplier rather than the sprout producer unless the latter can carry out the screening procedure in a separate area from the main sprout production using dedicated equipment. If sampling and test sprouting is done in the normal production area then there is an increased risk for contamination of storage areas and the production environments (FSAI, 2011). In addition, testing performed by the seed supplier could also be applied to seeds sold for home sprouting.

In general, sample collection should be done on site by trained personnel, who assures aseptic sampling procedures and avoids cross contamination between samples and products tested.

Although the primary focus of microbiological testing of seeds and sprouted seeds is to detect and, where appropriate, enumerate bacterial foodborne pathogens, these will be isolated less frequently than other components of the microflora such as hygiene indicators *E. coli*, and *Enterobacteriaceae*. Microbiological sampling and analysis should consider such hygiene indicator organisms as well as pathogens. Indicator organisms can provide useful information to assess batch to batch variation in seeds and sprouts, consistency of process hygiene control and in assessing the shelf-life.

Based on the hazards considered in previous sections of this Opinion, this chapter will focus on pathogenic *E. coli*, *Salmonella*, *L. monocytogenes* and hygiene indicator bacteria.

7.2. Sampling and sample size

7.2.1. Heterogenous distribution of pathogens

Irrespectively of whether the sample is taken from seeds, sprouts or irrigation water it is essential that the sample is representative of the inspection lot as otherwise there is a risk of missing contamination localized in a part of the lot (stratification). In particular in the production of sprouts a consignment of seeds might be a mixture of production lots, which is a cause of stratification. The effect of stratification on the sampling variance has been investigated by simulation for GM seeds (Paoletti et

al., 2003). These results indicated that even at moderate levels of stratification, the uncertainty increases dramatically, even when the number of sampling points exceeded 100.

Generally information on the statistical distribution of pathogens in seeds and sprouts is lacking. There is no simple formula to calculate the number of sampling points needed whenever the distribution of the pathogens does not follow a random (Poisson) distribution. This is the case if the pathogenic bacteria are aggregated on some seeds and not homogeneously distributed among seed, for instance in the case of localised contamination occurring during storage. The heterogeneity is further increased when there is a mixture of production lots (CAC, 2004).

Evidence for heterogenous distributions of bacterial pathogens have been observed for: seeds (FDA, 1999c; ISS, 2011; Van Beneden et al., 1999); irrigation water (Liu and Schaffner, 2007a; McEgan et al., 2008); sprouts and spent irrigation water (Hora et al., 2005; Liu and Schaffner, 2007a; McEgan et al., 2008).

In EU, during the laboratory analyses that followed the tracing back investigations on the seed lots potentially involved in the German outbreak in 2011 (ISS, 2011), the EU- VTEC Reference Laboratory analysed ten lots of beet seeds and found a VTEC strain from two lots. These two lots and two negative lots were used to organize an inter-laboratory study on naturally contaminated seeds involving eight National Reference Laboratories (NRLs) from Member States. All the laboratories returned results that did not match those expected (samples expected negative were found positive and reciprocally), which could reflect the non-homogeneous contamination of the lots from which the test samples were taken, with very low amount of STEC.

7.2.2. Pooling of samples

Due to the fact that pathogens are (in general) not homogeneously distributed throughout a batch of seeds or sprouted seeds and that they are likely to be present at low concentrations, it might be necessary to analyse a large number of samples. To reduce the number of analyses to manageable proportions it may be advantageous to pool the samples to reduce heterogeneity. The practice of sprouting a sample of seeds and analysing for pathogens in the resulting sprouts and/or irrigation water, discussed in later sections, is actually a special case of pooling of seeds where the sprouting serves as a first pre-enrichment step.

It should be recognized that pooling may have an effect on the sensitivity of the analytical method and therefore any protocol for pooling must be validated.

Two ways of pooling can be distinguished: dry pooling and wet pooling. For dry pooling, the sample units are pooled before analyses, for example 10 samples each of 25 g are analysed as one (pooled) sample of 250 g. For wet pooling, individual samples (of e.g. 25 g) are pre-enriched (e.g. for *Salmonella* in 225 ml buffered peptone water) and after overnight incubation, small amounts of the pre-enriched cultures are mixed and further analysed as one pre-enriched (pooled) sample. (Price et al., 1972) reported an increased detection rate for *Salmonella* when performing wet pooling on dry products (dry milk, egg albumin, cocoa and wheat flour), than with dry pooling of the same products. The differences between dry pooling and wet pooling may be larger when the level of the target organism (pathogen) is low and the amount of background flora is high, as will be found in seeds and particularly sprouted seeds. This is explained by the fact that if the pathogen is not homogeneously distributed over the samples (which is often the case), it can happen that only one sample of 25 g contains 1 cfu of the pathogen. If this sample is mixed for example with a further 9 x 25 g samples which does not contain the pathogen, but does contain the pathogen, but do contain high amounts of background flora, the pathogen is not only 'diluted' 10-fold, but potentially has to overcome a higher level of background microflora. Both effects may result in the fact that the pathogen is not able to grow sufficiently to become detected, particularly if sublethally injured and therefore requires a

longer period of resuscitation. In other words the background flora masks the pathogen. This may be a reason why it can be very difficult to find low levels of a target pathogen in large amounts of sample. Wet pooling may therefore be an alternative. With this form of pooling, the samples are still small so that the pathogen has a better chance to grow to a detectable level in the pre-enrichment broth. When the pre-enriched cultures are subsequently pooled, the pathogen may already have grown to a sufficiently high level to become detected in the pre-enriched pool as well. Additionally, wet pooling has the advantage that individually pre-enriched samples can be stored in the refrigerator and still be available for further testing in the event that the pathogen is detected in the pooled enrichment broth.

Detection methods typically involve pre-enrichment, which in theory would allow detection of a single bacterium, provided that it is present in the sample (except when inhibition/injury/competition occurs, see below). Consequently, at low levels of contamination the probability of detection will depend on the number of sample units (e.g. 25g) that are analysed. Figure 5 depicts probability of acceptance for a defective (positive) lot as a function of the contamination level (expressed as proportion of defective 25 g samples) when 5, 25 or 80 25g samples are tested for presence/absence of a pathogen and assuming random (Poisson) distribution of the pathogen in the test material.

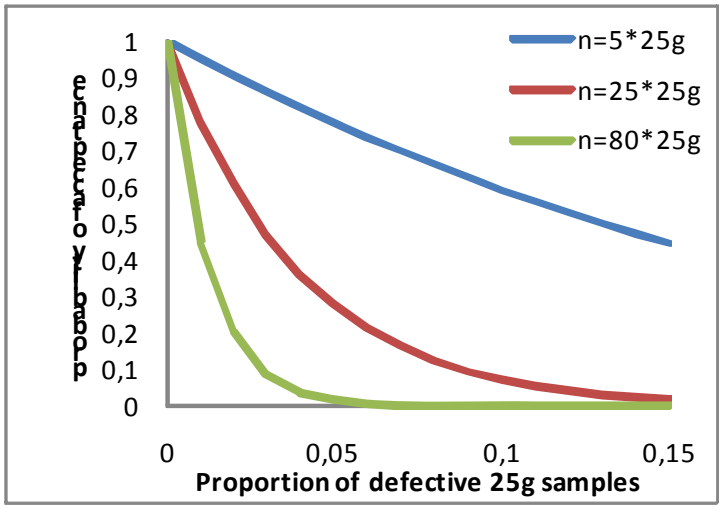

Figure 5: Relationship between contamination level (expressed as proportion of defective 25g samples) and probability of accepting a defective (positive) lot when 5, 25 or 80 25g samples are tested for presence/absence of a pathogen.

This assumption is only valid if the 25g or 50g samples are taken from a well mixed composite sample formed by representative sampling of the production lot. A contamination level of 4 cfu/kg seed as found in the investigation of an outbreak of *Salmonella* Havana would correspond to (at most) 10% contaminated 25g samples. From the blue and red curves it is apparent that a sampling plan based on 5 or 25 25g samples (125 or 625g respectively) would be associated with a large probability of accepting the seed-lot as *Salmonella* free (~60% and ~7% respectively) when it is in fact *Salmonella* positive. This is based on a standard ICMSF 2-class sampling plan assuming a 100% probability of detection when the pathogen is present in the sample assuming homogenous distribution of bacteria in the seeds which clearly is not the case.

7.2.3. Testing for indicator bacteria

As part of a HACCP program in food production, it is often recommended to sample for indicator bacteria such as coliforms, Enterobacteriaceae or *E.coli* rather than solely testing for pathogens (Brown et al., 2000). However, reliable indicator bacteria are not always available. For example coliforms as an indicator for *Salmonella* is not regarded as suitable in some systems including feed

(EFSA, 2008) and fresh produce production (Nguyen-The and Carlin, 2000). This could be due to the fact that coliforms and *Salmonella* differ in their ability to survive and multiply outside their hosts resulting in a poor correlation between the presence of coliforms and the risk of *Salmonella* contamination**.**

Similar problems may be anticipated in sprout production where a high background flora may be present and where there may also be decontamination steps which vary in their efficacy against pathogens and the background microflora. If manure is the source of contamination for seeds, any correlation between total coliforms and the presence of STEC contamination is likely to exist shortly after harvest but this may not hold if seeds are contaminated at another stage of production, e.g. during storage. *Salmonella* has been shown to multiply in storage areas and production environments if water is available from rain or condensation (Binter et al., 2011). This may result in a lack of correlation between the presences of a pathogen such as *Salmonella* and indicator organisms not exposed to situations where they are able to multiply. The risk of seed contamination is also dependent on whether or not the pathogen is present in the ecosystem where the seeds are produced.

In food manufacturing environments *Listeria* spp. other than *L. monocytogenes* are generally indicative of the likelihood of *L. monocytogenes* also being present. Total *E. coli* counts are used as a general indicator of faecal contamination and the likelihood of pathogens being present. *Enterobacteriaceae* are also used but are less reliable as an indicator of faecal contamination and, unlike total *E. coli,* are commonly found on plant materials. The presence of elevated levels of indicator bacteria (*Listeria* spp., total *E. coli*) may be indicative of elevated risk with respect to incoming seed, sprouts, irrigation water and the processing environment. However, they are not a replacement for pathogen testing particularly in situations where there are few pathogen reduction steps in producing a ready-to-eat food.

7.2.4. Seeds

The levels of pathogens in seeds are typically low and thus enumeration will have to be based on methods involving pre-enrichment, by applying standard methods for detection of bacterial pathogens such as a most probable number (MPN) approach. Even contamination levels far below 1 defective seed/25g could still result in growth of the pathogen to hazardous levels during sprouting and contamination of most or all of the sprouts in the batch. A defective seed in this context is a seed contaminated with at least one cfu of the target pathogen. Information about the distribution of bacteria on seeds is not available and thus it may sometimes be more relevant to discuss contamination in terms of defective (contaminated) seeds per sample unit (e.g. 25g) rather than cfu/sample. The seed species used for sprouting differ in size and surface texture, and thus the surface area of a given amount of seeds may vary considerably. As contamination is likely to be present on the seed surface, this may have an impact on sampling performance but it is not clear how this should be accounted for. A seed sample may also contain contaminated particles of dust and debris which contribute to the total surface area of the sample, and consequently a pathogen contaminated particle in a sample may not necessarily be a seed.

Evidence is available supporting the sporadic nature of seed contamination occurring with very low levels of pathogens (NACMCF, 1999). According to an assessment report from the Australian Food Standards, concentrations of 1-100 MPN per kg of contaminated seed are reported in the literature (FSANZ, 2009). In the 1995–1996 *S.* Newport outbreak, analysis of the implicated seed lot by MPN yielded 0.1–0.6 CFU of *S*. Newport/25 g of seed (4–24 cells /kg seed) (Van Beneden et al., 1999). Analysis of the seed implicated in the 1998 *S*. Havana outbreak revealed *S*. Tennessee, *S*. Cubana and *S*. Havana at levels of approximately 4 CFU/ kg (NACMCF, 1999). In order to produce safe food the sampling procedure should be designed to detect pathogens at these levels. In relation to the number of individually contaminated seeds, a *Salmonella* level of 4cfu/kg seed could maximally correspond to 4 contaminated seeds/kg but in the case of a heterogeneous distribution as discussed in section 7.2.1.

(more than 1 bacteria aggregated on a small number of seeds) the number of contaminated seeds may be even smaller (e.g. 1/kg).

A common strategy for analysing seed intended for sprouting is to form a representative aggregate sample (e.g. 2kg-20kg[20] (Beales, 2004), sprout the entire sample and subsequently analyse a subsample of the spent irrigation water and a portion of the crushed sprouts for the target pathogen (e.g. *Salmonella*, STEC and *L. monocytogenes*). Since the bacteria are assumed to multiply by up to 5 logs during the first 48h of sprouting, it is likely that almost any sub-sample from the irrigation water or sprouts would contain detectable levels of the target pathogen provided that the water was well mixed and the sprouts crushed prior to sub sampling (NACMCF, 1999). If the multiplication rate of the pathogen during sprouting is sufficiently high, sprouting a sample of 2kg of seeds and analysing water and crushed sprouts would result in almost the same probability of detection as if the 2kg of seed were analysed as 80 individual 25g samples. However, conflicting results regarding growth of pathogens during sprouting exists as discussed in section 4.2.3.1. Alternatively one large pre-enrichment broth may be used if it can be shown that pooling does not impair detection.

The success of this strategy may depend on the commodity being tested since pathogens may multiply at different rates on different types of sprouted seeds as well as the presence of competing microflora. In the UK follow-up of a large outbreak of *Salmonella* Bareilly and bean sprouts, *Salmonella* could only be detected in 100g samples of bean sprouts and at MPN levels of 1-7 cfu/100g (HPA, 2011). This may indicate that either multiplication during sprouting in this system was as low as 1 $\log_{10}$, or that competitive microflora was inhibitory, or that inhibitors in the beans or spent irrigation water interfered with the sensitivity of the detection method. For this reason, detection protocols based on sprouting may have to be validated for each seed commodity (see also section 7.3 of this Opinion).

The above mentioned strategy has been used in a study involving 13 growers (either producers or co-packers) of sprouts (Fahey et al., 2006). All the participants followed the FDA guidelines (seeds were treated with 20,000 ppm calcium hypochlorite) and used drums to grow sprouts. One litre of spent irrigation water was collected after 48 hours sprouting from single drums (57% of samples) or as composite samples from more than one drum (2-4; 5-7, 8-19 drums). A total of 3,191 samples were collected and analysed for the presence of *Salmonella* and *E. coli* O157. Presumptive presence of *Salmonella* or *E. coli* O157 occurred in 24 samples. Most of the presumptive samples were re-analysed and in two cases, the presence of *Salmonella* was confirmed. In a single case, the presumptive presence of *E. coli* O157 was considered as conclusive by the grower and the sprouts were destroyed without re-testing. The low frequency of confirmation of contamination may indicate that the protocols involving test-sprouting are less sensitive than expected based on theoretical considerations thus emphasizing the need to verify the sensitivity of this strategy for each seed type/commodity tested.

The size of the sample that must be sprouted in order to ensure that at least one defective seed is present in the sample depends on the average contamination level and the desired degree of confidence. It may be necessary to increase the sensitivity of the sampling plan for lots with an elevated risk of being contaminated with pathogenic bacteria. Thus when testing of seeds and sprouts for the presence of pathogenic bacteria the application of different inspection levels based on previous history or successive lots (CAC, 2004) or other forms of risk classification of the lot should be considered.

For example, at a contamination level of 1 defective seed per kg, at least 3kg of seeds must be sprouted in order to ensure, with 95% probability, that the sample would contain the pathogen given that it is present in the lot (Table 6). The probability of at least one defect seed in the sample ($p_{>0}$) given the average number of defective seeds per sample unit (λ) is calculated as 1-Poisson(0,λ) = 1-e^{-}

[20] www.sproutnet.com/sprouting_seed_safety.htm

$^{\lambda}$) (Råde and Westergren, 1990) being equivalent to ICMSF sampling plan where λ=npm where n =number of samples, p is proportion defective, m=mass of each samples.

Table 6: Probability of at least one defective (contaminated) unit in a sprouted seed sample at 0.5-5 defective seeds per kg.

Defective seeds per kg	sample mass (kg)	Defective seeds per sample (λ)	Probability of defect seed in sample (p)
0.5	1	0.5	0.39
0.5	2	1	0.63
0.5	3	1.5	0.78
0.5	6	3	0.95
0.5	10	5	0.99
1	1	1	0.63
1	2	2	0.86
1	3	3	0.95
1	6	6	1
1	10	10	1
5	1	5	0.99
5	2	10	1
5	3	15	1
5	6	30	1
5	10	50	1

The detection probability will not increase with the size of the sprouted seed sampled for all sample sizes, since when the volume of irrigation water/crushed sprouts is too large, the concentration of bacteria may be diluted to low levels despite multiplication. As the expected number of bacteria in a water sample (e.g. 100ml) goes down to levels near the LOD, the probability of a false negative increases. This effect would (naturally) be more pronounced if the bacteria multiply slower during sprouting, e.g. 2 to 3 $\log_{10}$ as reported elsewhere (Prokopowich and Blank, 1991). In this situation, the detection limit of the analytical methods will also have a significant effect on the probability of detection (Montville and Schaffner, 2005).

Sprouting as a means of detecting microbial contamination is time consuming and labour intensive but is closer to the point of product release and consumption. An alternative to sprouting is shredding seeds to a coarse powder prior to pre-enrichment and this is less labour intensive and requires fewer laboratory supplies (Inami et al., 2001). Inami et al. (2001) compared the sprouting and shredding method on 100g samples and obtained similar results. However, one key advantage of sprouting methods is that it allows pre-enrichment of a large aggregate sample (2-20kg) and thus detection of a target pathogen with a very small proportion of defective seeds. It is not clear if the shredding method would be practical at that scale or introduce interference to the isolation/detection process.

It is generally recommended to sample for pathogens after 48h sprouting. However, if the seeds are pre-soaked (e.g., soaked in water for a short time prior to being transferred to growing units for sprouting) then pre-soak time should be included. Early results will allow the sprout manufacturer to take corrective actions sooner, thus minimizing the potential for cross-contamination between batches of sprouts or to equipment and surfaces within the production environment (CFIA, 2008).

Another alternative to sprouting is the maceration of seeds in buffer or water under agitation to allow for the diffusion of the bacteria. For legumes, a maceration of 12-18h at 4°C in a ratio 2.5:1 (water:seed mass) may be used and for small dry seeds such as cruciferous types a maceration for 2-2.5h at room temperature can be used. 100ml of buffer per 10,000 seeds is used. These conditions are

part of the international testing methods published for the detection of plant pathogenic bacteria by the International Seed Testing Association (ISTA)[21].

7.2.5. Spent irrigation water

Health Canada recommend that sprout manufacturers regularly test spent irrigation water, because water that has flowed over and through the sprouts is likely to be a good indicator of the types of microorganisms in the sprouts themselves, including microbial pathogens of concern (e.g *Salmonella* and STEC). They recommend that sprouts should not be tested in place of spent irrigation water unless the production methods make it impossible to test the spent irrigation water. However, the recommendation to test spent irrigation water does not preclude additional testing of sprouts (either collected during production or as finished product). Current EU Microcriteria regulations (EC Regulation 2073/2005[22]) require testing of the food (sprouts) for *Salmonella* and *L. monocytogenes* so for these pathogens sprouts would need to be tested in addition to any testing of irrigation water. For mung-beans, even a single sample of spent irrigation water gives a higher detection probability than testing of the sprouts directly (Hora et al., 2005). However despite being more sensitive than testing sprouts directly, a single sample of spent irrigation water has been shown not to give reliable information on the status of a sprouting mung bean bed (Hora et al., 2005) and thus composite samples must be formed from multiple sampling points from each production lot and analysed for microbial pathogens of concern for this approach to be effective. Even so it can still not be assured with absolute certainty that the pathogens (*Salmonella,* STEC) will be detected (Hora et al., 2005). However, the distribution of contaminating enteropathogens in spent irrigation water is considered to be more homogenous than in seeds and therefore fewer samples are needed to form a representative sample. This testing strategy will depend on the technology used to grow sprouts and also the volume of water used, for example, to cool mung bean sprouts which may affect detection through dilution. Forming a representative sample is more challenging when seeds are sprouted in trays compared with rotating drums and the seed type or scale of sprouting (deep vs shallow trays) may impact detection probability (McEgan et al., 2009). The batch of sprouts will typically be contaminated by a few contaminated seeds and if water is collected at distance from the initial contaminated seeds (e.g. 20cm (Liu and Schaffner, 2007a; McEgan et al., 2008) then there is a significant risk that a sample will be negative. Consequently the number of sampling points and subsamples needed to detect pathogens in irrigation water depends on the initial number of contaminated seeds and the design and throughput of the irrigation system.

The concentration of pathogenic bacteria after 48h sprouting is often reported to be up to 1 $\log_{10}$ lower in the spent irrigation water than on the sprouts themselves (Beales, 2004; Montville and Schaffner, 2005); introducing a risk that the pathogenic bacterias in the irrigation water are diluted to a level where detection probability is reduced. In this case, it may be necessary to test large volumes of spent irrigation water and methods for concentrating the water sample will be advantageous. A tangential flow filtration system for concentrating *Salmonella* in spent irrigation water from mung beans was evaluated by (McEgan et al., 2009).

Samples of spent irrigation water can be collected as early as 48 hours after the start of sprouting.

An example of a sampling plan for pathogens in spent irrigation water plan can be found in appendix A of the Food safety practices guidance for sprout manufacturers published by the Canadian Food Inspection agency (CFIA, 2008).

One litre of water should be aseptically collected as the water leaves a drum or tray(s) during the irrigation cycle. Spent irrigation water samples should be collected directly into clean, sterile, pre-labelled containers. When ten or fewer trays make up a production lot, approximately equal volumes

[21] www.seedtest.org/en/download-ista-seed-health-testing-methods-_content---1--1132--241.html
[22] OJ L 338, 22.12.2005, p.1-26

of spent irrigation water should be collected from each of the 10 trays to make a total sample volume of one litre. When there are ten or more trays, collect ten spent irrigation water samples throughout the entire production lot (FSPGSM, 2008).

Detailed instructions for sampling in case of sprouting in drums or trays are also given by FDA (1999b).

Testing spent irrigation water for pathogenic bacteria has been proposed as an alternative strategy to the analysis of a large number of sprout samples. However, there are some uncertainties regarding the sensitivity of this strategy, which needs further evaluation.

7.2.6. Sprouted seeds

Sampling for microbiological testing of sprouted seeds has been investigated only for sprouts. The distribution of pathogenic microorganisms on shoots and cress may be similar to that found on other leafy vegetables.

Numerous studies have demonstrated that bacteria multiply to high levels during the sprouting process and therefore a sample of sprouts positive for a target pathogen is often assumed to contain $>10^3$ bacteria. If this assumption is true then when several samples are pooled into a composite sample, a 25g subsample withdrawn from the composite sample has a high probability of containing the pathogen. The validity of this assumption is critical for the choice of sampling strategy and it should be recognised that some studies report lower multiplication as discussed in 4.2.3.

Pooling samples from different sprout lots is sometimes applied to reduce the number of laboratory analyses to be performed. With this strategy, if a presumptive positive is found, all sprouts lots represented by the pooled sample are suspect. The suspect sprout lots should either be discarded or each sprout lot analysed separately to determine which lot(s) is (are) contaminated. However, allowing sale of suspected positive lots after re-testing will result in a lower level of protection since the detection probability of pathogens is unlikely to be 100%. Thus, if release of lots after re-testing is permitted, it is essential that the sampling and testing reflects the significantly higher risk of contamination of a suspect lot compared with a randomly selected lot for example by applying rules described in CAC (2004).

Different sampling plans have been proposed, for example the Canadian Food Inspection Agency recommends five sample units of 200g of sprouts whereas US FDA recommends 32 x 50g units from different locations in drums or trays. Both recommend keeping the samples separate during transport to the laboratory to facilitate the subsequent formation of a representative aggregate sample (CFIA, 2008).

An example of a sampling plan for sprouts can be found in can be found in appendix A of the Food safety practices guidance for sprout manufacturers published by the Canadian Food Inspection agency (CFIA, 2008). Five sample units of approximately of 200g each should be aseptically collected from different locations in the drum or growing trays, to ensure that the sample collected is representative of the lot. The sample units should be collected throughout the entire production lot (e.g., from top to bottom, side to side, and front to back of the drum or trays). Each 200g sample unit should be placed directly into individual clean, sterile, pre-labelled containers.

Another example of a sampling plan comes from the US FDA where collection of 32 samples of each 50g of units from different locations in drums or trays is recommended (FDA, 1999b).

Considering that the pathogen may be heterogeneously distributed in a sprouting lot, especially when sprouting in trays, the latter sampling plans may increase the probability of detection.

7.2.7. Environmental and process control samples (dust, swabs)

A complement to product sampling and testing, especially when bacterial pathogens are present at low concentrations, is the sampling of dust from the product rather than the product itself. This strategy is sometimes used in, for example, production of animal feed (Andersson and Häggblom, 2009). For seed sampling, samples of dust could be taken from a silo containing seeds to be sprouted or from equipment used in sorting, weighting or cleaning, as well as in storage areas or the bottom of sacks and other storage containers.

An indication that sampling of dust may be effective comes from monitoring of soybeans for soybean meal productions where *Salmonella* was isolated from approximately 30% (12-68) of samples of dust from all lots of soybean intended for feed imported to Norway during 1994-2007 (EFSA, 2008). Furthermore, as some pathogens, like *Salmonella*, can survive well in dust, these types of sample can also indicate persistent contamination (Davies and Wray, 1996).

Experience from source tracking of *Salmonella* in Swedish pig production indicates that the outbreak associated strain can sometimes be detected in environmental samples (dust/swab) from a production environment (feedmill) whereas is often proved difficult to find the strain in the product samples (Wierup, 2006). This suggests that environmental samples may be valuable for source tracking using based on molecular typing. Environmental samples from production and storage environments should be considered also for source tracking of sprout associated outbreaks.

Few studies have investigated the use of dust samples for detection of pathogenic *E. coli*. However, Miller et al (2008) studied the impact of exposure to dust in the cattle load-out area in feed yards on pathogen contamination of cattle hides. The cattle hides were sampled to determine total numbers of pathogens on leaving their home pen (before loading) and on cattle hides after exposure to the dust in the loading area. Air and dirt samples from the home pens and the cattle load-out area were also collected. The total numbers of pathogens increased after dust exposure; *Salmonella* counts increased from 1.09 $\log_{10}$ most probable number (MPN)/cm^2 to 1.74 $\log_{10}$ MPN/cm^{2}after exposure, and *E. coli* O157 counts increased from 0.80 to 2.35 $\log_{10}$ MPN/cm^{2}after sampling. *E. coli* O157 and *Salmonella* were recovered from the air samples during dust generation at 6.66 and 11.1%, respectively. This indicates that dust samples may be relevant also for detection of STEC (Miller et al., 2008).

7.3. Standardisation of methods for detection and enumeration of bacterial foodborne pathogens in seeds and sprouted seeds

7.3.1. General considerations

All bacteriological testing should be conducted in a laboratory that meets the following criteria:

- The laboratory should be physically separated from the food production facility to prevent cross-contamination.
- The laboratory should be staffed by personnel with training and experience in analytical microbiology techniques to ensure that tests are performed correctly and that all appropriate safety precautions, including appropriate waste disposal, are followed.
- Testing should be carried out by laboratories running quality assurance systems or, better, accredited under the EN/ISO/IEC 17025[23].

[23] EN/ISO/IEC 17025:2005. General requirements for the competence of testing and calibration laboratories. International Organization for Standardization.

If microbial analyses are done by the sprout manufacturer, the laboratory facilities, personnel, and quality management system should meet the above mentioned criteria and ensures that testing provides reliable information for food safety management and does not create food safety hazards.

7.3.2. Standard methods

When analysing seeds, sprouted seeds, water, dust and the environment in the scope of the verification of compliance with the currently established microbiological criteria for *Salmonella* spp., *L. monocytogenes* and *E. coli* (chapter 6), EN/ISO standard methods 6579[24], 11290-2[25], 16649-1[26] or 2[27] respectively are prescribed in Regulation 2073/2005[28].

Due to the short shelf life of this food type, rapid methods for detection may be an important complement to standard methods. Time for sprouting is 3-10 days depending on commodity. EN/ISO method for *Salmonella* takes approximately 5 days for results whereas the standard method for *E. coli* O157, EN/ISO 16654[29], takes 48 hours before results are available. As samples from sprouts and irrigation water are typically collected at 48h after initiation of the sprouting process this time should be added to the total time to achieve a test result.

7.3.3. Molecular methods

Cultural methods specifically for the detection of STEC are not available because this group of pathogenic *E. coli*, lack the metabolic and growth characteristics that allow the use of selective or differential culture media. The only international standard available, EN/ISO 16654[29], concerns the identification of *E. coli* O157 based on its capability to grow on solid media containing the supplements cefixime and potassium tellurite and on its inability to ferment sorbitol that makes *E. coli* O157 colonies colourless on media containing this sugar. This phenotype is in contrast to the majority of other *E. coli* strains, which yield red colonies. The isolated colonies, however, have to be confirmed as STEC by demonstrating presence of the Shiga toxin-genes, or by the production of Shiga toxin(s) or by confirmation by a specialist (reference) laboratory. Unfortunately, the peculiar metabolic features of STEC *E. coli* O157 are not shared with the other non-O157 STEC, which are indistinguishable from commensal *E. coli*. Therefore the EN/ISO 16654[29] method cannot be used to detect non-O157 STEC. Since STEC pathogenic to humans are partially identifiable by an array of virulence genes a number of approaches for the detection of these genes have been developed. One of the proposed strategies has been discussed and endorsed at European level in the framework of the activities of CEN TC 275 WG6. The methodology is based on the assumption that STEC are characterized by the presence, in addition to the Shiga toxins-coding genes (*stx*), of the intimin-coding *eae* gene. Moreover, although STEC may belong to a large number of serogroups, those that have been most frequently associated with severe human disease, in particular Haemolytic Uraemic Syndrome (HUS), belong to a restricted group of them, namely O157, O26, O111, O103, and O145. A horizontal method has been proposed, which is based on the screening by Real Time PCR of enriched food samples for the detection of the STEC major virulence genes and the genes associated with these

[24] EN/ISO 6579:2002. Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp. International Organization for Standardization.

[25] EN/ISO 11290-2: 1998. Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 2: Enumeration method. International Organization for Standardization.

[26] EN/ISO 16649-1:2001. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* - Part 1: Colony-count technique at 44 degrees C using membranes and 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. International Organization for Standardization.

[27] EN/ISO 16649-2:2001. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* - Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. International Organization for Standardization.

[28] OJ L 338, 22.12.2005, p.1-26

[29] EN/ISO 16654:2001. Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Escherichia coli* O157. International Organization for Standardization.

serogoups. When the presence of these genes is detected, the isolation of the strain is attempted, to confirm their presence in the same isolated bacterial colony.

A laboratory procedure based on this approach has been drafted by an ad hoc group of experts, appointed by the CEN TC 275 WG6 and co-ordinated by the EU RL VTEC, in the form of an international standard and it will be published as a Technical Specification (CEN ISO TS 13136).

The use of a molecular screening step has the advantage of a rapid identification of samples where the target has not been detected. This is particularly useful for those commodities that have a short shelf life. The ISO method for the detection of STEC in food has the following timeline:

i) 24-27 hours to perform the screening step. The presence of any STEC in the sample can excluded at this stage (release of samples negative to the screening step). In case of a target detected in the screening step, the sample is suspected to contain a STEC (presumptive positivity) and further confirmatory testing should be initiated.

ii) 48-57 hours to achieve isolation and confirmation of the STEC from the sample detected by screening above.

The approach described in the CEN ISO TS 13136 although technically feasible is not directly applicable for the detection of the STEC O104:H4 associated with the 2011 outbreak which does not contain the *eae* gene and does not belong to the typical serogroups of STEC associated with HUS. Therefore, in order to develop a detection method for this pathogen, the EU VTEC Reference Laboratory adapted the CEN ISO TS 13136 procedure by including the reagents for the detection of the genes associated with the O104 serogroup and to the H4 flagellar antigen. The laboratory procedure also recommend the use of selective media based on the ability of this strain to grow in the presence of antimicrobials as well as including a procedure for isolate confirmation based on the detection of the rare virulence genes characterizing this unusual STEC strain[30].

7.3.4. Rapid methods.

Rapid testing procedures have been described that can be used to test for *E. coli* O157:H7, *L.monocytogenes* and *Salmonella* spp. (CFIA, 2008). The EC regulations on food safety indicates the possibility of using alternative (rapid) methods to those indicated as reference methods provided they are validated according to EN/ISO 16140[31] (article 5) standard. Several rapid (e.g. molecular) methods have been validated according to this EN/ISO protocol for the detection of *Salmonella, E. coli* O157 and for the enumeration of *L. monocytogenes* in food and animal feed samples ('horizontal methods') and also certified by MicroVal or Afnor-validation. An overview of the validated methods is available at the relevant websites of MicroVal[32] and Afnor[33]. In validation studies published by MicroVal and Afnor information is given on the limit of detection (LOD_{50}) of the validated rapid methods for the different food samples tested as well as for the reference method (EN/ISO 6579[34]).

In Regulation 2073/2005[35], no method is indicated for the analyses of water. However, specific ISO methods exist for the detection and enumeration of specific organisms in water (e.g. ISO 19250[36], for the detection of *Salmonella* in water).

[30] www.iss.it/vtec/work/cont.php?id=152&lang=2&tipo=3
[31] EN/ISO 16140:2003. Microbiology of food and animal feeding stuffs - Protocol for the validation of alternative methods. International Organization for Standardization.
[32] www.microval.org/validated-methods.html
[33] www.afnor-validation.com/afnor-validation-validated-methods/validated-methods.html
[34] EN/ISO 6579:2002. Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp. International Organization for Standardization.
[35] OJ L 338, 22.12.2005, p.1-26

7.4. Conclusions and recommendations

7.4.1. Conclusions

The presence of elevated levels of indicator bacteria (*Listeria* spp., total *E. coli*) may be indicative of elevated risk with respect to incoming seed, sprouts, irrigation water and the processing environment. A survey among sprouted seed producers may be useful to determine which levels of hygiene indicator bacteria are to be expected during the production cycles.

Pathogenic microorganisms may be heterogeneously distributed and the number of samples from seeds, sprouts and irrigation water to be taken in order to be representative of a given lot/batch depends on many factors including production system.

When sampling consignments of seeds the inspection lot should represent a single production lot to avoid sampling uncertainty from stratification.

Sampling procedures for *Salmonella* and STEC in seeds used for production of sprouts for direct consumption (mung-bean sprouts or speciality sprouts) should take into account that levels of pathogens as low as a few bacteria per kilogram can be hazardous. The sampling plan "absence in 25g", n=5; c=0 will not demonstrate absence of a target pathogen at these levels. Rather it is necessary to analyze kilogram quantities of the seeds as exemplified in Table 5.

In order to reduce the number of the analytical samples needed when testing seeds, various pooling strategies may be adopted. One popular approach is to sprout a composite sample and test sprouts and spent irrigation water after 48h. However any pooling strategy needs to be validated and standardized considering the low level of contamination expected in the seed.

Available data on growth of microorganisms during sprouting indicate that the levels of bacteria (including *Salmonella* and STEC O157) will be relatively constant after 24-48h and is independent of initial levels of bacteria on the seed. Thus testing samples of sprouts and spent irrigation water collected 48 hours after initiating sprouting may give a good indication on the microbiological status of a batch of sprouted seeds.

Dust and swab samples taken from different stages in the seed (conveyers, dust filters, sacks etc) and sprout production (drums/trays, working areas etc) could potentially be an efficient way of monitoring and tracing pathogenic microorganisms and, in the case of sprout production, hygiene indicators.

The rational for test sprouting of seeds and testing of spent irrigation water is the assumption that growth of pathogenic *E. coli* and *Salmonella* spp. during sprouting result in a multiplication of 3-5 $\log_{10}$ (cfu). However some studies reported significantly lower levels of multiplication, depending on commodity and/or strain characteristics, challenging the LOD of the detection methods.

It is important to use standard methods for testing, preferably an EN/ISO method especially developed for analyzing microorganisms in seeds, spent water and sprouts. Due to the short shelf life of sprouted seeds, rapid (e.g. molecular) methods for detection and/or typing of the pathogens are important to obtain timely information on the microbiological status of sprouted seeds, provided that these methods are validated according to relevant international standards (e.g. EN/ISO 16140[37]).

[36] ISO 19250:2010. Water quality - Detection of *Salmonella* spp. International Organization for Standardization.
[37] EN/ISO 16140:2003. Microbiology of food and animal feeding stuffs - Protocol for the validation of alternative methods. International Organization for Standardization.

The distribution of pathogenic microorganisms on shoots and cress may be similar to that found on other leafy vegetables and sampling plans for these commodities should be harmonized with sampling plans for other ready-to eat vegetables being consumed raw e.g. baby leaves covered by EC Regulation 2073/2005[38].

8. CONSIDERATIONS ON THE ASSESSMENT OF THE CONTRIBUTION OF SEEDS AND SPROUTED SEEDS AS A SOURCE OF FOODBORNE INFECTION IN HUMANS

Sprouted seeds are a very diverse food sector and it has not been possible to estimate the proportion of all gastrointestinal infections which can be attributed to contaminated sprouted seeds. However, the large outbreaks (including the >3,700 cases in Germany in 2011) illustrate the potential to cause major public health emergencies. Information is predominantly available for sprouts only although the risk of infection is presumably different for other sprouted seeds (shoots and cress) which are intermediate between sprouts and fresh-produce (baby leaves). Sprouted shoots and cress probably represent a similar risk to other fresh produce and should consequently receive a similar level of food control. However, sprouted seeds are ready-to-eat foods with a higher microbial food safety concerns since:

- This food can be produced industrially or at home by consumers: in both settings, food safety has been compromised.
- Sprouted seeds producers may consider themselves as primary producers.
- Sprouted seeds are ready-to-eat foods based on the way they are consumed (raw or minimally processed).
- Based on outbreak data the bacterial pathogens most frequently associated with illness due to contaminated sprouted seeds are *Salmonella* and to a lesser extent STEC. *L. monocytogenes, S. aureus* and *B. cereus* have also been transmitted by sprouted seeds, albeit very rarely. Some outbreaks involved considerable numbers of cases.
- Based on outbreak data, dry seed contaminated with bacterial pathogens has been identified as the most likely initial source of sprout-associated outbreaks; although other routes of contamination (e.g. during production due to poor practices) may also occur.
- In the EU, the seeds purchased by sprouts producers are usually not grown specifically for this purpose. The seeds may be contaminated during production, harvest, storage and transport, and there may be difficulties in traceability of seeds from production to sprouting.
- The prevalence of bacterial pathogens on dry seeds used for sprout production is not known. However investigation of some outbreaks showed that contamination of seeds with as low as 4 *Salmonella* cfu/kg was sufficient for the sprouts to cause disease.
- Bacterial pathogens on seeds may survive for long periods during seed storage, although experimental studies predict significantly shorter survival duration than is suggested from outbreak investigations.
- There is so far no guarantee of a bactericidal step which is able to control contamination of seeds with bacterial foodborne pathogens acquired prior to germination.

[38] OJ L 338, 22.12.2005, p.1-26

- Due to the high humidity and the favourable temperature during sprouting, bacterial pathogens present on dry seeds can multiply on the sprouts, and can disseminate, from the few initially contaminated seeds to other parts of the production batch.

- The extent of multiplication during sprouting is difficult to quantify. It is presumably overestimated by experiments done under laboratory conditions and is likely to be influenced by factors difficult to control such as competition with the background microflora, adhesion of the pathogens to the sprouts. Pathogenic bacteria (*Salmonella* and *L. monocytogenes*) have been detected in sprouts in the course of food surveillance surveys.

- Sprouts are usually packaged, to maintain a high humidity, and stored for a few days at refrigeration temperature. Under these conditions pathogens are able to survive with no or limited decline. Whether they can multiply under some conditions (e.g. abuse temperatures) is not known.

- Sprouts can be consumed directly, without receiving any bactericidal treatment. For most pathogens the number of pathogenic bacteria on the product at the end of sprouting presumably represents the amount to which the consumer is exposed.

- There may be difficulties in traceability of sprouts from point of seed production to the consumer.

- Contaminated sprouts can act as a source of cross-contamination for other foods in commercial or domestic kitchens.

- There is limited information for consumers to make an informed choice on the hazards of eating this product. This is further complicated since it is not always clear from the packaging information whether the sprouts are considered as a ready-to-eat or a ready to cook product.

CONCLUSIONS AND RECOMMENDATIONS

On ToR1: To assess the public health risk caused by STEC and other pathogenic bacteria that may contaminate both seeds and sprouted seeds intended for direct human consumption.

- Sprouted seeds are ready-to-eat foods with microbial food safety concern due to the potential for certain pathogenic bacteria to contaminate the raw materials (seeds) and to grow during germination and sprouting, and to their consumption patterns (raw or minimally processed).

- The various types of sprouted seeds (sprouts, shoots, cress) may not represent the same risk for contamination with, and growth of, pathogenic bacteria. There is mostly data on risks from sprouts and a scarcity of data for shoots and cress: for these final two categories the production is more comparable to that of fresh-cut leafy vegetables.

- Alfalfa and mung bean sprouts have been the most commonly consumed and most frequently implicated products in outbreaks.

- *Salmonella* and pathogenic *E. coli* (including STEC) are the most commonly reported bacterial pathogens causing outbreaks associated with the consumption of contaminated sprouts.

- As found for *Salmonella* very low contamination levels of dry seeds (e.g. 4 MPN/kg) can cause sprout associated-outbreaks.

- Other bacterial pathogens (e.g. *Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes* and *Yersinia enterocolitica*) have also been implicated with sprout-associated outbreaks, although these have been reported very rarely.

- Epidemiological data shows that a single contaminated seed lot may be used by several sprouting plants, even in different countries, causing widespread related outbreaks.

- Due to limitations in available data, it has not been possible to carry out a quantitative microbiological risk assessment and estimate the proportion of foodborne infections attributable to the consumption of contaminated sprouted seeds.

- Large outbreaks involving these products (e.g. the outbreak in Germany 2011) illustrate the potential to cause major public health emergencies affecting previously healthy people and not limited to those considered particularly vulnerable to infections.

- Reliable methods for decontaminating all types of seeds or sprouted seeds are not currently available.

On ToR2: To the extent possible, to identify risk factors contributing to the development of STEC and other pathogenic bacteria that may contaminate these seeds and sprouted seeds.

- As pathogenic bacteria can be carried and transmitted by animals, humans and the environment, they may contaminate seeds in the field and throughout the sprouted seed production chain.

- Seeds may be contaminated via diverse routes. The most relevant risk factors are associated with the effect of agricultural practices on seed production, storage and distribution: contaminated irrigation water and/or manure, presence of birds and rodents in storage facilities, dust and soil particles are potential sources of contamination.

- Processing conditions (e.g. temperature, humidity) prevailing during germination and sprouting of contaminated seeds favour the growth and dissemination of pathogenic bacteria and should be considered as major risk factors.

- The widespread distribution of seed lots is a risk factor that may increase the size and geographical spread of outbreaks.

- Poor traceability of the seed lots may delay the action taken by health authorities to control outbreaks.

- Poor traceability will present difficulties for the sprouted seed producers to assess the hygienic quality of lots of seeds and consequently increases the risk of using contaminated seeds in the production process.

- Microbiological methods to detect emerging pathogens in seeds and sprouted seeds may not yet exist or be applied, posing an additional challenge in the identification of the source of outbreaks.

- There is limited information on risk factors during the production of shoots and cress compared to sprouts.

- Considering that sprouted seeds are ready-to-eat foods, contamination by asymptomatic carriers of pathogenic bacteria should not be excluded as a potential risk factor.

- Considering the above mentioned risk factors, it has been difficult to date to control the hygiene of the production process of sprouted seeds.

On ToR3: To recommend possible specific mitigation options and to assess their effectiveness and efficiency to reduce the risk posed by STEC and other pathogenic bacteria that may contaminate these seeds and sprouted seeds throughout the food chain. The mitigating options should cover all parts of the food chain from the seed production until final consumption.

- Food safety management based on HACCP principles should be the objective of operators producing sprouted seeds including GAP, GHP and GMP along the whole chain from seed production to the final sprouted product. The hazard analysis should include risk classification of commodities, regions of origin, and operators and suppliers of seeds.

- In seed and to some extent sprout production, it can be difficult to define critical control points (CCPs) including hazard control measures, critical limits and monitoring.

- Preventing seed contamination is particularly important because of the long survival of bacterial pathogens on seeds and their multiplication during sprouted seed production. This concerns both seeds intended for industrial and home sprouting. GAP and GHP concerning primary production, harvest and storage should be applied with a high level of stringency, similar to that applied for the primary production of fresh-produce, to minimize the risk of contamination with pathogenic bacteria. These include but are not limited to:

    - identifying seed crops intended for sprout production before planting;

    - safe use of fertilizers and irrigation water;

    - minimizing contamination of seeds with soil during harvest and preventing mechanical damage of seeds;

  - ensuring that workers harvesting and handling seeds follow hygiene and health requirements;
  - ensuring that seeds are transported, processed and stored under conditions which will minimize the potential for microbial contamination;
  - removing damaged seeds, from which it may be more difficult to remove pathogenic bacteria, or avoiding lots with too many damaged seeds;
  - improving traceability of seed lots and minimizing, as far as possible, mixing of seed lots.

- Washing of seeds to remove dirt before sprouting is recommended.
- Measures to prevent introduction of pathogens in sprouted seeds production remains of the foremost importance.
- During sprouting, GMP, GHP and HACCP principles should be applied as for other ready-to-eat foods. Use of potable water is necessary during sprouting.
- Decontamination of seeds prior to sprouting, is currently practiced in some EU Member States as an additional risk mitigation measure as part of a combined intervention strategy. To date, no method of decontamination is available to ensure elimination of pathogens in all types of seeds without affecting seed germination or sprout yield.
- Decontamination of seeds would need to be optimised for each type of seed. The safety and efficacy of different seed decontamination treatments (e.g. chemical, heat treatment, irradiation alone or in combination) should be evaluated in a harmonised way at EU level.
- The consequence of any decontamination treatment on the background microflora and its potential impact on the pathogenic bacteria during sprouting should be taken into account.
- A chill chain for sprouts and shoots from end of production to consumption is necessary to limit growth of bacterial pathogens.
- Stakeholders, including consumers and also those practicing home-sprouting, at all parts of the production chain, should be informed of the food safety risk posed by sprouted seeds.

On ToR4: To recommend, if considered relevant, microbiological criteria for seeds and sprouted seeds, water, and other material that may contaminate the seeds and sprouts throughout the production chain. This may include process hygiene criteria.

- As sprouted seeds are ready-to-eat foods, finding pathogenic bacteria in seeds used for sprouting or in sprouted seeds indicates a public health risk. Microbiological testing alone may convey a false sense of security due to the statistical limitation of sampling plans. A negative sample result does not ensure the absence of the pathogen in the tested lot, particularly where it is present at low or heterogeneous prevalence.
- It is currently not possible to evaluate the extent of public health protection provided by specific microbiological criteria for seeds and sprouted seeds. This highlights the need for data collection to conduct quantitative risk assessment.
- Microbiological criteria including the design of sampling plans for pathogenic bacteria should be considered as one of the components of the food safety management system for the

sprouted seed production chain.

- Existing food safety criteria and process hygiene criteria in Regulation (EC) 2073/2005[39] relevant to sprouted seeds or seeds are: a *Salmonella* food saf*ety criter*ion, *L. monocytogenes* food safety criterion, and a total *E. coli* process hygiene criterion.

- Consideration should be given to the development of new or revision of the existing microbiological criteria for pathogens most frequently associated with outbreaks involving sprouts - *Salmonella* spp. and pathogenic *E. coli.* Currently, there are no criteria for pathogenic *E. coli.* If such criteria were to be proposed serotypes of concern and associated with severe human disease should be considered.

- Microbiological criteria for *Salmonella,* pathogenic *E.coli* and *L. monocytogenes* could be considered for seeds before the start of the production process, during sprouting and in the final product.

    - Detection and mitigation of a contamination problem earlier in the sprouted seed production chain (seeds) may have advantages as it avoids contamination being amplified during the full sprouting process.

    - Testing seeds alone does not permit to detect contamination which may come at a later stage in the production process. Therefore microbiological criteria could be useful during the sprouting process and/or for the final product.

    - When considering a microbiological criterion for the final sprouted seeds, the time required for the detection methods for pathogenic bacteria combined with the short shelf-life may not allow to withdraw the product in the event of a non-compliance.

    - An additional value of testing would be to build up knowledge about the hygienic performance of seed and sprouted seeds producers.

- When targets for seeds are considered it should be taken into account that low levels of *Salmonella* (4 MPN/kg) have been sufficient to cause sprouted seed associated-outbreaks.

- A 2-class sampling plan "absence in 25g", n=5; c=0, as specified in EC Regulation 2073/2005[39] for sprouted seeds, will not give sufficient confidence to demonstrate absence of a target pathogen at these low levels in seeds. In order to increase probability of rejection of a positive lot it would be necessary to analyze kilogram quantities of the sample.

- In order to reduce the number of analytical samples when testing seeds, pooling strategies can be applied. Different approaches have been proposed; any pooling strategy needs to be validated and standardized taking into account the low level of contamination expected in the seeds.

- Seeds intended for home sprouting should be subjected to a sampling protocol for pathogens having at least the same level of stringency as for seeds intended for commercial sprout production.

- Ideally the inspection lot on which the sampling plan is applied should represent only one seed production lot.

[39] OJ L 338, 22.12.2005, p.1-26

- Testing seeds will be especially important if operators use new seed commodities or source seeds from new suppliers where a previous history of microbiological testing may be limited or lacking.

- Dust and debris from seed storage areas may also be regularly tested for *Salmonella* and STEC. Total *E. coli* counts may be included to provide evidence of faecal contamination.

- During the industrial sprouting process testing spent irrigation water for pathogenic bacteria has been proposed as an alternative strategy to the analysis of a large number of sprout samples. However, there are some uncertainties regarding the sensitivity of this strategy.

- Sampling could be conducted on sprouted seed production environments. It could be applied for pathogenic bacteria such as *L. monocytogenes* as well as indicator bacteria. There are currently no indicator organisms that can effectively substitute for the testing of pathogens in seeds, sprouted seeds or irrigation water. Testing for *E. coli*, *Enterobacteriaceae* and *Listeria* spp. can inform process hygiene control. Further work may be required to assess the value of tests for these indicator organisms.

- It is important to use standard methods for testing, preferably EN/ISO methods especially developed for analyzing microorganisms in seeds, spent water and sprouts. Due to the short shelf life of sprouted seeds rapid (e.g. molecular) methods for detection and/or typing of pathogenic bacteria are important to obtain timely information on the microbiological status of sprouted seeds. Alternative (rapid) methods should be validated according to EN/ISO methods (e.g. EN/ISO 16140[40]).

[40] EN/ISO 16140:2003. Microbiology of food and animal feeding stuffs - Protocol for the validation of alternative methods. International Organization for Standardization.

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APPENDICES

A. DATA REPORTED IN THE ZOONOSES DATABASE ON OCCURRENCE OF FOODBORNE OUTBREAKS WHERE IMPLICATED FOODSTUFFS WERE SPROUTED SEEDS (2004-2010)

Table 7: Reported sprouted seeds-associated outbreaks in the EU in accordance with Directive 2033/99/EC[41], 2004-2010[42]

Year	Country	Causative agent	Foodstuff implicated	Type of evidence	Human cases	Deaths [(a)]	Hospitalisations [(a)]
2006	Sweden	*S.* Virchow *S.* Bareilly	Sprouts	Epidemiological evidence	130	0	NR
2007	Denmark	*S.* Weltevreden	Bean sprouts	Microbiological evidence	19	0	0
2007	Finland	*S.* Weltevreden	Alfafa sprouts (seeds)	Microbiological evidence Epidemiological evidence	8	0	NR
2007	Norway	*S.* Weltevreden	Alfafa sprouts	Microbiological evidence	27	0	3
2007	Sweden	*S.* Stanley	Alfafa sprouts	Epidemiological evidence	51	NR	NR
2008	Denmark	*Staph. aureus*	Bean sprouts	Microbiological evidence	42	NR	NR
2009	Estonia	*S.* Bovismorbificans	Sprouts	Microbiological evidence Epidemiological evidence	6	0	3
2009	Finland	*S.* Bovismorbificans	Alfafa sprouts	Microbiological evidence Epidemiological evidence	28	0	0
2010	United Kingdom	*S.* Bareilly	Bean sprouts	Epidemiological evidence	241	1	32
2010	United Kingdom	*S.* Kottbus	Bean sprouts	Epidemiological evidence	4	0	0

[(a)] NR: not reported

[41] OJ L 325, 12.12.2003, p. 31–40
[42] Preliminary data for 2010

B. RASFF NOTIFICATIONS

Table 8: Summary of the RASFF notifications regarding seeds and/or sprouted seeds (from 2000 to 2010).

Date	Reference	Notifying Country	Reason for notification	Microbiological hazard	Product	Origin	Distribution	Reported cases[43] (a)
Jul 2004	2004.BPJ	United Kingdom	food information -official control on the market	*Samonella* spp.	alfafa, broccoli and radish sprouts	United Kingdom	United Kingdom	NR
Aug 2007	2007.0605	Sweden	food alert – official control on the market	*Salmonella* Mbandaka	organic alfafa seeds for sprouting[44]	Italy via Denmark	Sweden	Approximately 50 cases
Oct 2007	2007.0760	Denmark	food alert – official control on the market	*Salmonella* Weltevreden	alfafa sprouts	Pakistan via Italy and Netherlands	Denmark, Norway, Belgium, Germany, the Netherlands	The investigation was due to human cases of *S.* Weltevreden in Denmark; the number of cases was not reported. However, 17 persons were confirmed ill due to *Salmonella* Weltevreden in Norway. Isolates from these patients all showed the same DNA- profile as for the *Salmonella* Weltevreden isolates found in alfalfa sprouts in Denmark
Mar 2008	2008.0295	Germany	food information -official control on the market	*Salmonella* Mbandaka	organic alfafa seeds[45]	Italy	Germany	NR

[43] As reported to the RASFF database.

[44] The Italian firm declared that use of the seeds were not to produce shoot or pre-shoot like food or feed. The seeds had been analysed before entering in the market. Results were negative for *Salmonella* and *E. coli.*

[45] The Italian producer did not provide complete traceability data, because it stated that its product is not sold as food for direct consumption. Usually this producer sells seeds for vegetable garden and intensive farming of forages or, at most, for ornithological feed.

Date	Reference	Notifying Country	Reason for notification	Microbiological hazard	Product	Origin	Distribution	Reported cases[43] (a)
Aug 2009	2009.1082	Finland	food alert – foodborne outbreak suspected	*Salmonella* Bovismorbificans	alfalfa seeds[46]	Italy via Sweden	Finland, Sweden, Estonia	About 40 persons affected. Of these, about 20 cases ate ready to eat sprouts manufactured from the Finish company. 1out of 10 samples were positive for *Salmonella* Bovismorbificans. Two other *Salmonella* serotypes were also found in this seed sample: *S.* Umbilo and *S.* Szentes but these were not linked to any cases. The *Salmonella* Bovismorbificans strains from patients and from the seed sample were PFGE-genotyped and were identical confirming the epidemiological result. Eight Finnish persons who travelled to Estonia were affected. Four of them were tested and positive to the same type of *S.* Bovismorbificans as last summer in the outbreak in Finland. Also five Estonian persons who ate sprouts in Estonia had *S.* Bovismorbificans in November-December.
May 2010	2010.0679	Denmark	food information -official control on the market	*Salmonella* Newport	beetroot sprouts	Netherlands	Denmark	NR
Jun 2011	2011.659	Germany	food news – official control on the market	suspicion STEC	sprouts mixture	Germany	Germany	2 female workers of the producer fall ill with diarrhoea and one of them was tested positive for EHEC
Jun 2011	2011.0752	Germany	food alert – food poisoning	suspicion STEC	organic sprouts mixture	Germany	Germany	At least 114 cases

[46] The Italian producer stated that they produce and market the seeds exclusively for agriculture; the labels on the products stated "Do not use for food, feed or oil purpose". Before trading, the product has been analyzed for pesticides residues, *Salmonella* and *E. coli* with negative results.

Date	Reference	Notifying Country	Reason for notification	Microbiological hazard	Product	Origin	Distribution	Reported cases[43] (a)
Jun 2011	2011.0758	Germany	food alert – official control on the market	VTEC (positive/25g)	beetroot sprouts	Netherlands but seeds used for sprouting were bought from Italy and France	Germany, Italy, France, UK, Ireland	NR
Jun 2011	2011.0842	France	food alert - foodborne outbreak suspected[47]	suspicion VTEC O104:H4	fenugreek seeds	Egypt packaged in United Kingdom, via Germany and Netherlands	Several MSs and third countries.	(EFSA Technical Report http://www.efsa.europa.eu/en/supporting/doc/176e.pdf) On 24 June, France reported12 a cluster of patients with bloody diarrhoea, after having participated in an event in the Commune of Bègles near Bordeaux on the 8th of June. As of 28th June, eight cases of bloody diarrhoea and a further eight cases with HUS have been identified. Eleven of these patients, seven women and four men, between 31 and 64 years of age, had attended the same event in Bègles. Infection with *E. coli* O104:H4 has been confirmed for four patients with HUS (ECDC, 2011). Six of the cases reported having eaten sprouts at the event on the 8th of June.
Oct 2011	2011.1288	France	food alert – official control on the market	*Salmonella* spp.	seeds for sprouts and shoots	Germany via Switzerland	Germany, France, UK and Austria	NR

(a) NR: not reported

[47] Commission implementing decision of 6 July 2011 on emergency measures applicable to fenugreek seeds and certain seeds and beans imported from Egypt (2011/402/EU) http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:179:0010:0012:EN:PDF

C. DATA REPORTED IN THE ZOONOSES DATABASE ON OCCURRENCE OF ZOONOTIC AGENTS IN SPROUTED SEEDS (2004-2010)

Table 9: Reported occurrence of zoonotic agents in sprouted seeds in the EU in accordance with Directive 2033/99/EC[48], 2004-2010[49]

Year	Zoonotic agent	Country	Sampling frame [a]	Sample unit	Number of units tested	Number of positive units	Prevalence (%)	Identified *Salmonella* serovars [a, b]
2005	VTEC	Latvia	at retail	batch	29	0	0	NA
2005	VTEC	Slovenia	NR	single	45	0	0	NA
2006	VTEC	Slovenia	NR	single	30	0	0	NA
2005	*L. monocytogenes*	Ireland	at retail	single	16	0	0	NA
2005	*L. monocytogenes*	Latvia	at retail	batch	29	2	6.9	NA
2005	*L. monocytogenes*	Slovenia	NR	single	45	2	4.4	NA
2006	*L. monocytogenes*	Slovenia	NR	single	30	0	0	NA
2007	*L. monocytogenes*	Czech Republic	at processing plant	batch	18	0	0	NA
2007	*L. monocytogenes*	Ireland	at retail	single	4	0	0	NA
2007	*L. monocytogenes*	Netherlands	at retail	single	1722	4	0.2	NA
2008	*L. monocytogenes*	Portugal	at retail	batch	25	0	0	NA
2009	*L. monocytogenes*	Czech Republic	at processing plant	batch	1	0	0	NA
2009	*L. monocytogenes*	Czech Republic	at retail	batch	3	0	0	NA
2009	*L. monocytogenes*	Hungary	NR	single	88	1	1.1	NA
2010	*L. monocytogenes*	Hungary	at processing plant	single	3	0	0	NA
2010	*L. monocytogenes*	Hungary	at retail	single	89	0	0	NA
2010	*L. monocytogenes*	Slovakia	at processing plant	batch	2	0	0	NA
2005	*Salmonella* spp.	Ireland	NR	single	22	1	4.5	*S.* Fresno *S.* Fanti
2005	*Salmonella* spp.	Slovenia	at retail	single	45	0	0	NA
2005	*Salmonella* spp.	Germany	NR	NR	56	0	0	NA
2006	*Salmonella* spp.	Czech Republic	NR	batch	2	0	0	NA
2006	*Salmonella* spp.	Hungary	NR	NR	114	0	0	NA
2006	*Salmonella* spp.	Italy	NR	single	2	0	0	NA
2006	*Salmonella* spp.	Netherlands	NR	NR	52	0	0	NA
2006	*Salmonella* spp.	Estonia	at retail	single	3	0	0	NA
2006	*Salmonella* spp.	Ireland	NR	NR	3	0	0	NA
2006	*Salmonella* spp.	Slovenia	NR	single	30	0	0	NA
2006	*Salmonella* spp.	Poland	NR	batch	66	0	0	NA

[48] OJ L 325, 12.12.2003, p. 31–40
[49] Preliminary data for 2010

Year	Zoonotic agent	Country	Sampling frame [a]	Sample unit	Number of units tested	Number of positive units	Prevalence (%)	Identified *Salmonella* serovars [a, b]
2006	*Salmonella* spp.	Austria	NR	single	20	12	0.6	*S.* Mbandaka *S.* Bere
2006	*Salmonella* spp.	Italy	NR	NR	2	0	0	NA
2006	*Salmonella* spp.	Estonia	at processing plant	single	2	0	0	NA
2007	*Salmonella* spp.	Czech Republic	NR	batch	17	0	0	NA
2007	*Salmonella* spp.	Hungary	NR	NR	101	0	0	NA
2007	*Salmonella* spp.	Poland	NR	NR	84	0	0	NA
2007	*Salmonella* spp.	Estonia	at retail	single	2	0	0	NA
2007	*Salmonella* spp.	Germany	NR	NR	135	3	2.2	NR
2007	*Salmonella* spp.	Netherlands	NR	NR	581	9	1.5	*S.* Senftenberg
2007	*Salmonella* spp.	Romania	NR	NR	4	0	0	NA
2007	*Salmonella* spp.	Ireland	NR	single	4	0	0	NA
2007	*Salmonella* spp.	Poland	NR	NR	65	0	0	NA
2007	*Salmonella* spp.	Portugal	NR	single	26	0	0	NA
2008	*Salmonella* spp.	Latvia	NR	single	7	0	0	NA
2008	*Salmonella* spp.	Estonia	at retail	single	7	0	0	NA
2008	*Salmonella* spp.	Hungary	NR	single	44	0	0	NA
2008	*Salmonella* spp.	Portugal	NR	batch	25	0	0	NA
2008	*Salmonella* spp.	Romania	at retail	batch	5	0	0	NA
2008	*Salmonella* spp.	Germany	NR	single	229	12	5.2	NR
2008	*Salmonella* spp.	Romania	at processing plant	batch	3	0	0	NA
2009	*Salmonella* spp.	Hungary	NR	single	56	0	0	NA
2009	*Salmonella* spp.	Netherlands	at retail	NR	174	1	0.6	*S.* Weltvreden
2009	*Salmonella* spp.	Romania	at processing plant	batch	1	0	0	NA
2009	*Salmonella* spp.	Romania	at retail	batch	3	0	0	NA
2009	*Salmonella* spp.	Estonia	NR	single	1	0	0	NA
2009	*Salmonella* spp.	Germany	NR	single	150	0	0	NA
2010	*Salmonella* spp.	Germany	NR	single	65	1	1.5	NR
2010	*Salmonella* spp.	Hungary	NR	NR	65	0	0	NA
2010	*Salmonella* spp.	Slovenia	NR	NR	9	0	0	NA
2010	*Salmonella* spp.	Estonia	at retail	single	7	0	0	NA
2010	*Salmonella* spp.	Romania	at retail	batch	2	0	0	NA
2010	*Salmonella* spp.	Hungary	NR	single	3	0	0	NA

[a] NR: not reported
[b] NA: Not applicable

D. FOOD CONSUMPTION DATA ON SPROUTED SEEDS

Table 10: Reported food descriptors considered for the extraction of data on consumption of sprouted seeds from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011g).

Country	Dietary survey	EFSA food descriptor	Original national food descriptor [a]	Number of eating occasions
United Kingdom	NDNS	Mung bean (*Phaseolus aureus*)	beansprouts-cooked	151
Germany	National_Nutrition_Survey_II	Soya beans (*Glycine max*)	sprout, soya, fresh, flesh, fried	132
Ireland	NSIFCS	Legume vegetables	beansprouts, mung, raw	101
United Kingdom	NDNS	Bamboo shoots (*Bambusa vulgaris*)	bamboo shoots canned drained	101
France	INCA2	Soya beans (*Glycine max*)	soya beans, sprouted, raw	89
United Kingdom	NDNS	Mung bean (*Phaseolus aureus*)	beansprouts mung raw	76
Belgium	Diet_National_2004	Soya beans (*Glycine max*)	sprout, soya	62
Ireland	NSIFCS	Bamboo shoots (*Bambusa vulgaris*)	bamboo shoots, canned, drained	56
Finland	FINDIET_2007	Bamboo shoots (*Bambusa vulgaris*)	bamboo shoots	46
United Kingdom	NDNS	Mung bean (*Phaseolus aureus*)	beansprouts fried	45
Netherlands	VCP_kids	Mung bean (*Phaseolus aureus*)	mung bean sprouts boiled w/o salt	41
Netherlands	DNFCS_2003	Soya beans (*Glycine max*)	sprouts, bean - fresh - flesh - stir fried/sauted	35
France	INCA2	Soya beans (*Glycine max*)	soya beans, sprouted	28
Sweden	Riksmaten_1997_98	Bamboo shoots (*Bambusa vulgaris*)	bamboo shoots canned drained	21
Sweden	Riksmaten_1997_98	Mung bean (*Phaseolus aureus*)	mung bean sprouts	21
Spain	NUT_INK05	Legumes, nuts and oilseeds	sprout, soya canned, cooking method not applicable	14
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, canned, not applicable, flesh, fried	14
Sweden	NFA	Alfalfa spouts, fresh (*Medicago sativa*)	alfalfa sprouts	13
Finland	DIPP	Alfalfa spouts, fresh (*Medicago sativa*)	beansprouts and alfalfa sprouts, average	12
Italy	INRAN_SCAI_2005_06	Soya beans (*Glycine max*)	soy, soybeans, mature seeds, sprouted, raw	10
Sweden	NFA	Bamboo shoots (*Bambusa vulgaris*)	bamboo shoots canned drained	9
United Kingdom	NDNS	Mung bean (*Phaseolus aureus*)	beansprouts fried in pufa	9
Netherlands	DNFCS_2003	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo - canned - canned in water/brine - flesh - stir fried/sauted	8
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, fresh, flesh, cooked (n.s.)	8
United Kingdom	NDNS	Broccoli (*Brassica oleracea* var. *italica*)	broccoli-sprouting raw	8
Finland	FINDIET_2007	Mung bean (*Phaseolus aureus*)	beansprouts and alfalfa sprouts average	7
Finland	DIPP	Bamboo shoots (*Bambusa vulgaris*)	bamboo shoots, canned	7
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, canned, canned in water / brine, flesh, boiled	6
Germany	National_Nutrition_Survey_II	Stem vegetables (Fresh)	sprout, mungbean, fresh, flesh, raw	5
Germany	National_Nutrition_Survey_II	Alfalfa spouts, fresh (*Medicago sativa*)	sprout, lucerne / alfalfa, fresh, flesh, raw	4
Germany	National_Nutrition_Survey_II	Soya beans (*Glycine max*)	sprout, soya, undefined, flesh, boiled	4

Country	Dietary survey	EFSA food descriptor	Original national food descriptor [a]	Number of eating occasions
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, fresh, flesh, fried	4
Germany	National_Nutrition_Survey_II	Soya beans (*Glycine max*)	sprout, soya, fresh, flesh, raw	4
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, fresh, flesh, cooked without fat (n.s.)	4
Sweden	Riksmaten_1997_98	Mung bean (*Phaseolus aureus*)	mung bean sprouts canned drained	3
Sweden	NFA	Mung bean (*Phaseolus aureus*)	mung bean sprouts	3
Germany	National_Nutrition_Survey_II	Soya beans (*Glycine max*)	sprout, soya, canned, canned in water / brine, flesh, boiled	3
Belgium	Diet_National_2004	Soya beans (*Glycine max*)	soya, sprout	3
Finland	DIPP	Alfalfa spouts, fresh (*Medicago sativa*)	sprout, alfalfa	2
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, fresh, flesh, boiled	2
Finland	FINDIET_2007	Alfalfa spouts, fresh (*Medicago sativa*)	sprout alfalfa	2
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, canned, undefined, flesh, fried	2
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, canned, undefined, flesh, boiled	2
Germany	DONALD_2008	Mung bean (*Phaseolus aureus*)	mungbean sprouts, raw, edible part	2
Germany	DONALD_2007	Mung bean (*Phaseolus aureus*)	mungbean sprouts, tinned, drained	2
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, canned, canned in water / brine, flesh, reheated	2
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, canned, canned in water / brine, flesh, fried	2
Sweden	NFA	Mung bean (*Phaseolus aureus*)	mung bean sprouts canned drained	2
Belgium	Diet_National_2004	Vegetables and vegetable products (including fungi)	sprout n.s.	2
Sweden	Riksmaten_1997_98	Alfalfa spouts, fresh (*Medicago sativa*)	alfalfa sprouts	2
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, undefined, flesh, fried	2
Sweden	Riksmaten_1997_98	Soya beans (*Glycine max*)	soya bean sprouts	2
Germany	National_Nutrition_Survey_II	Stem vegetables (Fresh)	sprout n.s., fresh, flesh, raw	2
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, canned, canned in own juice, flesh, boiled	2
Netherlands	DNFCS_2003	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo - canned - not applicable - flesh - stir fried/sauted	2
Netherlands	DNFCS_2003	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo - canned - canned in vinegar or pickled - flesh	2
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, canned, canned in own juice, flesh, cooked (n.s.)	2
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo	2
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, undefined, undefined, boiled	2
Netherlands	DNFCS_2003	Soya beans (*Glycine max*)	sprouts, bean - fresh - flesh - fried	2
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, undefined, flesh, raw	2
Ireland	NSIFCS	Broccoli (*Brassica oleracea* var. *italica*)	broccoli, purple sprouting, boiled in unsalted water	2
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, undefined, flesh, cooking method not applicable	2
Ireland	NSIFCS	Legume vegetables	beansprouts, mung, boiled in salted water	2
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, undefined, flesh, cooked without fat (n.s.)	2
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, undefined, flesh, boiled	2
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, fresh, flesh, stewed	2

Country	Dietary survey	EFSA food descriptor	Original national food descriptor [a]	Number of eating occasions
Germany	National_Nutrition_Survey_II	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo, fresh, flesh, raw	2
United Kingdom	NDNS	Mung bean (*Phaseolus aureus*)	beansprouts canned	2
Belgium	Diet_National_2004	Bamboo shoots (*Bambusa vulgaris*)	sprout, bamboo	2
Belgium	Regional_Flanders	Bamboo shoots (*Bambusa vulgaris*)	bamboo shoot/sprout	2
Belgium	Diet_National_2004	Vegetables and vegetable products (including fungi)	stalk and sprout n.s.	1
Germany	National_Nutrition_Survey_II	Soya beans (*Glycine max*)	sprout, soya, fresh, flesh, cooked with fat (n.s.)	1
Spain	enKid	Legumes, nuts and oilseeds	bean sprouts	1
Germany	DONALD_2008	Bamboo shoots (*Bambusa vulgaris*)	bamboo shoots, tinned, drained	1
Germany	National_Nutrition_Survey_II	Soya beans (*Glycine max*)	sprout, soya, canned, undefined, flesh, boiled	1
Germany	DONALD_2006	Bamboo shoots (*Bambusa vulgaris*)	bamboo shoots, tinned, drained	1
Germany	DONALD_2006	Mung bean (*Phaseolus aureus*)	mungbean sprouts, tinned, drained	1
Germany	National_Nutrition_Survey_II	Vegetable sauce	sprout, bamboo, fresh, sauce, boiled	1
Germany	National_Nutrition_Survey_II	Soya beans (*Glycine max*)	sprout, soya, canned, canned in water / brine, flesh, reheated	1
Germany	National_Nutrition_Survey_II	Stem vegetables (Fresh)	sprout n.s., marinaded, flesh, cooking method not applicable	1
Germany	National_Nutrition_Survey_II	Stem vegetables (Fresh)	sprout n.s., fresh, flesh, stewed	1
Netherlands	VCP_kids	Bamboo shoots (*Bambusa vulgaris*)	bamboo shoots canned	1
Germany	National_Nutrition_Survey_II	Soya beans (*Glycine max*)	sprout, soya, canned, canned in water / brine, flesh, cooking method not applicable	1
Germany	National_Nutrition_Survey_II	Stem vegetables (Fresh)	sprout n.s., fresh, flesh, cooking method not applicable	1
Netherlands	DNFCS_2003	Alfalfa spouts, fresh (*Medicago sativa*)	sprout, alfalfa - fresh - flesh - raw	1
Germany	National_Nutrition_Survey_II	Stem vegetables (Fresh)	sprout n.s., fresh, flesh, cooked (n.s.)	1
Germany	National_Nutrition_Survey_II	Stem vegetables (Fresh)	sprout n.s., canned, not applicable, flesh, cooking method not applicable	1
Netherlands	DNFCS_2003	Soya beans (*Glycine max*)	sprouts, bean - fried	1
Netherlands	DNFCS_2003	Soya beans (*Glycine max*)	sprouts, bean - fresh - stir fried/sauted	1
Netherlands	DNFCS_2003	Soya beans (*Glycine max*)	sprouts, bean - fresh - minced - fried	1
Netherlands	DNFCS_2003	Soya beans (*Glycine max*)	sprouts, bean - fresh - flesh - reheated	1
Belgium	Diet_National_2004	Alfalfa spouts, fresh (*Medicago sativa*)	sprout, alfalfa	1
Germany	National_Nutrition_Survey_II	Soya beans (*Glycine max*)	sprout, soya, canned, canned in water / brine, flesh, cooked without fat (n.s.)	1
Germany	National_Nutrition_Survey_II	Soya beans (*Glycine max*)	sprout, soya, undefined, flesh, stir fried / sautéed	1
Netherlands	DNFCS_2003	Soya beans (*Glycine max*)	sprouts, bean - fresh - flesh - blanched	1
Germany	National_Nutrition_Survey_II	Soya beans (*Glycine max*)	sprout, soya, undefined, flesh, raw	1
Germany	National_Nutrition_Survey_II	Soya beans (*Glycine max*)	sprout, soya, undefined, flesh, cooked (n.s.)	1
Belgium	Diet_National_2004	Soya beans (*Glycine max*)	Soya sprout	1
Finland	STRIP	Legumes, nuts and oilseeds	bean sprout	1
Finland	DIPP	Lentils, green (*Lens culinaris* syn. *L. esculenta*)	sprout, lentils	1

[a] n.s. = not specified

Table 11: Average and 95th percentile consumption of sprouted seeds (in grams/day and grams/day per kg body weight) in the EU for total population and consumers according to age class, country, dietary survey and gender (EFSA, 2011g).

					Total population						Consumers only			
					Average consumption		P95 consumption				Average consumption		P95 consumption	
Age class	Country	Survey	Gender	Number of subjects	g/day	g/day per kg bw	g/day	g/day per kg bw	Number of consumers	Percentage of consumers	g/day	g/day per kg bw	g/day	g/day per kg bw
Adolescents	Belgium	Diet_National_2004	Male	281	0.3	0.0	0.0	0.0	6	2.1	12.3	0.2	32.8	0.5
Adolescents	Belgium	Diet_National_2004	Female	303	0.9	0.0	0.0	0.0	9	3.0	30.7	0.5	172.8	2.5
Adults	Belgium	Diet_National_2004	Male	654	0.4	0.0	0.0	0.0	15	2.3	16.5	0.2	105.0	1.5
Adults	Belgium	Diet_National_2004	Female	650	0.6	0.0	0.0	0.0	14	2.2	26.9	0.4	78.2	1.3
Elderly	Belgium	Diet_National_2004	Male	253	1.3	0.0	0.0	0.0	4	1.6	83.8	1.1	193.0	2.4
Elderly	Belgium	Diet_National_2004	Female	265	0.7	0.0	0.0	0.0	6	2.3	31.3	0.5	58.6	1.0
Very elderly	Belgium	Diet_National_2004	Male	377	0.0	0.0	0.0	0.0	1	0.3	6.5	0.1	6.5	0.1
Very elderly	Belgium	Diet_National_2004	Female	335	0.4	0.0	0.0	0.0	5	1.5	23.9	0.4	102.5	1.7
Toddlers	Belgium	Regional_Flanders	Male	18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Toddlers	Belgium	Regional_Flanders	Female	18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Belgium	Regional_Flanders	Male	320	0.0	0.0	0.0	0.0	1	0.3	10.0	0.7	10.0	0.7
Other children	Belgium	Regional_Flanders	Female	305	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Infants	Bulgaria	NUTRICHILD	Male	432	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Infants	Bulgaria	NUTRICHILD	Female	428	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Toddlers	Bulgaria	NUTRICHILD	Male	218	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Toddlers	Bulgaria	NUTRICHILD	Female	210	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Bulgaria	NUTRICHILD	Male	223	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Bulgaria	NUTRICHILD	Female	210	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adolescents	Cyprus	Childhealth	Male	115	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adolescents	Cyprus	Childhealth	Female	188	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Czech Republic	SISP04	Male	190	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Czech Republic	SISP04	Female	199	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adolescents	Czech Republic	SISP04	Male	156	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adolescents	Czech Republic	SISP04	Female	142	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adults	Czech Republic	SISP04	Male	793	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Age class	Country	Survey	Gender	Number of subjects	Total population				Number of consumers	Percentage of consumers	Consumers only			
					Average consumption		P95 consumption				Average consumption		P95 consumption	
					g/day	g/day per kg bw	g/day	g/day per kg bw			g/day	g/day per kg bw	g/day	g/day per kg bw
Adults	Czech Republic	SISP04	Female	873	0.0	0.0	0.0	0.0						
Adolescents	Germany	National_Nutrition_Survey_II	Male	500	0.2	0.0	0.0	0.0	7	1.4	15.7	0.3	21.8	0.4
Adolescents	Germany	National_Nutrition_Survey_II	Female	511	0.2	0.0	0.0	0.0	10	2.0	8.4	0.2	22.5	0.4
Adults	Germany	National_Nutrition_Survey_II	Male	4592	0.3	0.0	0.0	0.0	76	1.7	20.0	0.2	52.3	0.7
Adults	Germany	National_Nutrition_Survey_II	Female	5827	0.2	0.0	0.0	0.0	68	1.2	18.6	0.3	59.4	0.9
Elderly	Germany	National_Nutrition_Survey_II	Male	951	0.2	0.0	0.0	0.0	10	1.1	16.2	0.2	40.6	0.5
Elderly	Germany	National_Nutrition_Survey_II	Female	1055	0.4	0.0	0.0	0.0	12	1.1	38.6	0.5	270.0	3.3
Very elderly	Germany	National_Nutrition_Survey_II	Male	214	0.1	0.0	0.0	0.0	1	0.5	16.3	0.2	16.3	0.2
Very elderly	Germany	National_Nutrition_Survey_II	Female	276	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Toddlers	Germany	DONALD_2006	Male	50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Toddlers	Germany	DONALD_2006	Female	42	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Germany	DONALD_2006	Male	102	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Germany	DONALD_2006	Female	109	0.2	0.0	0.0	0.0	1	0.9	16.7	0.9	16.7	0.9
Toddlers	Germany	DONALD_2007	Male	50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Toddlers	Germany	DONALD_2007	Female	35	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Germany	DONALD_2007	Male	116	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Germany	DONALD_2007	Female	110	0.1	0.0	0.0	0.0	2	1.8	7.2	0.2	12.0	0.4
Toddlers	Germany	DONALD_2008	Male	48	0.0	0.0	0.0	0.0	1	2.1	1.7	0.1	1.7	0.1
Toddlers	Germany	DONALD_2008	Female	36	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Germany	DONALD_2008	Male	115	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Germany	DONALD_2008	Female	108	0.1	0.0	0.0	0.0	1	0.9	6.7	0.3	6.7	0.3
Other children	Denmark	Danish_Dietary_Survey	Male	256	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Denmark	Danish_Dietary_Survey	Female	234	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adolescents	Denmark	Danish_Dietary_Survey	Male	231	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adolescents	Denmark	Danish_Dietary_Survey	Female	248	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adults	Denmark	Danish_Dietary_Survey	Male	1302	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adults	Denmark	Danish_Dietary_Survey	Female	1520	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Elderly	Denmark	Danish_Dietary_Survey	Male	154	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Elderly	Denmark	Danish_Dietary_Survey	Female	155	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Age class	Country	Survey	Gender	Number of subjects	Total population				Number of consumers	Percentage of consumers	Consumers only			
					Average consumption		P95 consumption				Average consumption		P95 consumption	
					g/day	g/day per kg bw	g/day	g/day per kg bw			g/day	g/day per kg bw	g/day	g/day per kg bw
Very elderly	Denmark	Danish_Dietary_Survey	Male	11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Very elderly	Denmark	Danish_Dietary_Survey	Female	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adults	Spain	AESAN	Male	192	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adults	Spain	AESAN	Female	218	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adolescents	Spain	AESAN_FIAB	Male	47	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adolescents	Spain	AESAN_FIAB	Female	39	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adults	Spain	AESAN_FIAB	Male	473	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adults	Spain	AESAN_FIAB	Female	508	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Toddlers	Spain	enKid	Male	7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Toddlers	Spain	enKid	Female	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Spain	enKid	Male	77	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Spain	enKid	Female	79	0.1	0.0	0.0	0.0	1	1.3	5.0	0.1	5.0	0.1
Adolescents	Spain	enKid	Male	98	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adolescents	Spain	enKid	Female	111	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Spain	NUT_INK05	Male	205	0.2	0.0	0.0	0.0	2	1.0	18.4	0.6	28.2	0.9
Other children	Spain	NUT_INK05	Female	194	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adolescents	Spain	NUT_INK05	Male	329	0.2	0.0	0.0	0.0	4	1.2	14.0	0.2	18.4	0.3
Adolescents	Spain	NUT_INK05	Female	322	0.2	0.0	0.0	0.0	5	1.6	14.6	0.2	45.0	0.8
Adults	Finland	FINDIET_2007	Male	729	0.2	0.0	0.0	0.0	14	1.9	9.2	0.1	37.5	0.5
Adults	Finland	FINDIET_2007	Female	846	0.2	0.0	0.0	0.0	29	3.4	7.1	0.1	20.0	0.3
Elderly	Finland	FINDIET_2007	Male	229	0.0	0.0	0.0	0.0	2	0.9	3.1	0.0	5.0	0.1
Elderly	Finland	FINDIET_2007	Female	234	0.0	0.0	0.0	0.0	1	0.4	1.5	0.0	1.5	0.0
Toddlers	Finland	DIPP	Male	257	0.0	0.0	0.0	0.0	1	0.4	0.3	0.0	0.3	0.0
Toddlers	Finland	DIPP	Female	240	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Finland	DIPP	Male	495	0.0	0.0	0.0	0.0	4	0.8	3.3	0.2	8.8	0.7
Other children	Finland	DIPP	Female	438	0.1	0.0	0.0	0.0	11	2.5	3.4	0.2	17.3	0.7
Other children	Finland	STRIP	Male	134	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Finland	STRIP	Female	116	0.1	0.0	0.0	0.0	1	0.9	12.5	0.4	12.5	0.4
Other children	France	INCA2	Male	239	0.1	0.0	0.0	0.0	3	1.3	4.8	0.2	7.1	0.3

					Total population						Consumers only			
					Average consumption		P95 consumption				Average consumption		P95 consumption	
Age class	Country	Survey	Gender	Number of subjects	g/day	g/day per kg bw	g/day	g/day per kg bw	Number of consumers	Percentage of consumers	g/day	g/day per kg bw	g/day	g/day per kg bw
Other children	France	INCA2	Female	243	0.1	0.0	0.0	0.0	3	1.2	8.6	0.5	14.3	0.9
Adolescents	France	INCA2	Male	449	0.1	0.0	0.0	0.0	7	1.6	9.3	0.2	18.5	0.3
Adolescents	France	INCA2	Female	524	0.1	0.0	0.0	0.0	11	2.1	5.5	0.1	16.7	0.5
Adults	France	INCA2	Male	936	0.2	0.0	0.0	0.0	21	2.2	9.6	0.1	25.0	0.3
Adults	France	INCA2	Female	1340	0.3	0.0	0.0	0.0	44	3.3	8.9	0.1	25.7	0.4
Elderly	France	INCA2	Male	111	0.5	0.0	0.0	0.0	3	2.7	18.8	0.2	21.4	0.2
Elderly	France	INCA2	Female	153	0.1	0.0	0.0	0.0	2	1.3	5.4	0.1	7.1	0.1
Very elderly	France	INCA2	Male	40	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Very elderly	France	INCA2	Female	44	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adults	United Kingdom	NDNS	Male	766	1.3	0.0	10.2	0.1	97	12.7	10.6	0.1	30.0	0.4
Adults	United Kingdom	NDNS	Female	958	1.2	0.0	7.6	0.1	134	14.0	8.3	0.1	25.6	0.4
Other children	Greece	Regional_Crete	Male	438	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Greece	Regional_Crete	Female	401	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adults	Hungary	National_Repr_Surv	Male	437	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adults	Hungary	National_Repr_Surv	Female	637	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Elderly	Hungary	National_Repr_Surv	Male	80	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Elderly	Hungary	National_Repr_Surv	Female	126	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Very elderly	Hungary	National_Repr_Surv	Male	28	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Very elderly	Hungary	National_Repr_Surv	Female	52	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adults	Ireland	NSIFCS	Male	475	0.5	0.0	3.6	0.0	59	12.4	3.8	0.0	14.2	0.2
Adults	Ireland	NSIFCS	Female	483	0.6	0.0	3.6	0.1	67	13.9	4.6	0.1	16.5	0.2
Infants	Italy	INRAN_SCAI_2005_06	Male	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Infants	Italy	INRAN_SCAI_2005_06	Female	7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Toddlers	Italy	INRAN_SCAI_2005_06	Male	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Toddlers	Italy	INRAN_SCAI_2005_06	Female	16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Italy	INRAN_SCAI_2005_06	Male	94	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Italy	INRAN_SCAI_2005_06	Female	99	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adolescents	Italy	INRAN_SCAI_2005_06	Male	108	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adolescents	Italy	INRAN_SCAI_2005_06	Female	139	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

					Total population						Consumers only			
					Average consumption		P95 consumption				Average consumption		P95 consumption	
Age class	Country	Survey	Gender	Number of subjects	g/day	g/day per kg bw	g/day	g/day per kg bw	Number of consumers	Percentage of consumers	g/day	g/day per kg bw	g/day	g/day per kg bw
Adults	Italy	INRAN_SCAI_2005_06	Male	1068	0.0	0.0	0.0	0.0	4	0.4	7.8	0.1	8.9	0.1
Adults	Italy	INRAN_SCAI_2005_06	Female	1245	0.0	0.0	0.0	0.0	5	0.4	8.7	0.1	16.7	0.2
Elderly	Italy	INRAN_SCAI_2005_06	Male	133	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Elderly	Italy	INRAN_SCAI_2005_06	Female	157	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Very elderly	Italy	INRAN_SCAI_2005_06	Male	69	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Very elderly	Italy	INRAN_SCAI_2005_06	Female	159	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Latvia	EFSA_TEST	Male	95	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other children	Latvia	EFSA_TEST	Female	94	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adolescents	Latvia	EFSA_TEST	Male	237	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adolescents	Latvia	EFSA_TEST	Female	233	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adults	Latvia	EFSA_TEST	Male	651	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adults	Latvia	EFSA_TEST	Female	655	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adults	Netherlands	DNFCS_2003	Male	352	0.9	0.0	5.6	0.1	23	6.5	13.1	0.2	30.1	0.4
Adults	Netherlands	DNFCS_2003	Female	398	0.5	0.0	3.6	0.0	21	5.3	8.5	0.1	22.7	0.3
Toddlers	Netherlands	VCP_kids	Male	165	0.2	0.0	0.0	0.0	5	3.0	6.4	0.5	15.2	1.3
Toddlers	Netherlands	VCP_kids	Female	157	0.1	0.0	0.0	0.0	5	3.2	1.8	0.1	3.7	0.3
Other children	Netherlands	VCP_kids	Male	489	0.1	0.0	0.0	0.0	17	3.5	3.1	0.2	9.3	0.5
Other children	Netherlands	VCP_kids	Female	468	0.1	0.0	0.0	0.0	13	2.8	3.2	0.2	19.9	1.2
Adults	Sweden	Riksmaten_1997_98	Male	585	0.1	0.0	0.0	0.0	9	1.5	6.5	0.1	11.6	0.1
Adults	Sweden	Riksmaten_1997_98	Female	625	0.2	0.0	0.0	0.0	18	2.9	6.7	0.1	25.7	0.5
Other children	Sweden	NFA	Male	744	0.1	0.0	0.0	0.0	4	0.5	9.8	0.4	34.3	1.3
Other children	Sweden	NFA	Female	729	0.0	0.0	0.0	0.0	8	1.1	3.3	0.1	7.5	0.3
Adolescents	Sweden	NFA	Male	518	0.1	0.0	0.0	0.0	4	0.8	13.5	0.3	32.0	0.7
Adolescents	Sweden	NFA	Female	500	0.1	0.0	0.0	0.0	4	0.8	8.9	0.2	12.5	0.3

E. REVIEW LEGISLATION

Hygiene package

Regulation (EC) No 852/2004[50] on the hygiene of foodstuffs provides general rules for food business operators for the production and processing of all food throughout the food chain. General implementation of procedures is based on the HACCP principle, together with application of good hygiene practice. Provisions for primary production cover e.g. the transport, storage and handling of primary products at the place of production and transport to an establishment. According to Regulation guides to good practice should be developed to encourage the use of appropriate hygiene practices at farm level. Guides may include e.g. the use of water, organic waste, the proper disposal of waste, protective measures to prevent introduction of contagious diseases transmissible to humans through food, procedures, practices and methods to ensure that food is produced, handled, stored and transported under appropriate hygienic conditions etc.

Use of water

The provision for food business operators to possibly use clean water is referred to in several parts of the hygiene Regulations (e.g. Annex I, point 5 (c) for primary production, and Annex II, chapter VII of Regulation 852/2004[50] on the hygiene of foods). For producing or harvesting plant products or producing primary products of animal origin the water used has to be potable or clean whenever necessary. Clean water shall not contain micro-organisms, harmful substances or toxic marine plankton in quantities capable of directly or indirectly affecting the health quality of food.

The Directive 98/83/EC[51] on the quality of water intended for human consumption establishes chemical and microbiological parameters for monitoring. In the scope of this directive water intended for human consumption may be defined as all water used in any food-production undertaking for the manufacture, processing, preservation or marketing of products or substances intended for human consumption unless the competent national authorities are satisfied that the quality of the water cannot affect the wholesomeness of the foodstuff in its finished form.

General food law

Article 14 on food safety requirements of Regulation (EC) No 178/2002[52] of the European Parliament and of the Council laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, states that unsafe food shall not be placed on the market. Unsafe food is considered to be food that is injurious to health or unfit for human consumption. According to the article food that complies with specific Union provisions, like microbiological criteria set in Regulation 2073/2005[53], is deemed to be safe.

However, as prescribed in Article 14 (8) of the Regulation, the competent authorities of Member States may take appropriate measures to impose restrictions where there are reasons to suspect, on a case-by-case basis, that despite conformity with the Union legislation, the food in question is unsafe.

Moreover, in case of emergency, or if official control analysis reveals that a foodstuff is likely to constitute a serious risk to human health, the procedure set out in Article 54 of Regulation 178/2002[52] could also apply and national measures could be adopted on an interim basis.

[50] OJ L 139, 30.4.2004, p.1-54
[51] OJ L 330, 5.12.1998, p.32-54
[52] OJ L 31, 1.2.2002, p.1-24
[53] OJ L 338, 22.12.2005, p.1-26

Animal by-products

Regulations (EC) No 1774/2002[54] laying down health rules concerning animal by-products not intended for human consumption and (EC) No 181/2006[55] on organic fertilisers and soil improvers other than manure lay down health rules on the classification of manure (excrements and urine from farmed animals) and the possibilities to apply it to land, as well as on the production, placing on the market and use of organic fertilisers which have been produced from animal by-products.

Furthermore, Regulation (EC) No 1774/2002[54] lays down rules for the transformation of animal byproducts into biogas and for their composting.

Waste

Article 13 of Directive 2008/98/EC[56] on waste states on protection of human health and the environment that Member States shall take the necessary measures to ensure that waste management is carried out without endangering human health, without harming the environment and, in particular:

(a) without risk to water, air, soil, plants or animals;

(b) without causing a nuisance through noise or odours; and

(c) without adversely affecting the countryside or places of special interest.

Organic production

The Regulation (EC) No 834/2007[57] and Regulation (EC) No 967/2008[58] on organic production and labelling of organic products provides the basis for the sustainable development of organic production while ensuring the effective functioning of the internal market, guaranteeing fair competition, ensuring consumer confidence and protecting consumer interests.

[54] OJ L 273, 10.10.2002, p.1-95
[55] OJ L 29, 2.2.2006, p. 31–34
[56] OJ L 312, 22.11.2008, p. 3–30
[57] OJ L 189, 20.7.2007, p. 1–23
[58] OJ L 264, 3.10.2008, p. 1–2

GLOSSARY

A seed is defined from the botanical point of view as a unit of sexual reproduction developed from the fertilised ovule (Hickey and King, 2000). In the scope of this scientific Opinion the following definitions were considered:

- Cotyledon: first leaf or one of the first leaves developed in the embryo within the seed. The cotyledon stage refers to the developmental stage where the cotyledons are completely unfolded and the first true leaf is not expanded.

- Cress: sprouted seeds obtained from the germination and development of true seeds in soil or in hydroponic substrate, to produce a green shoot with very young leaves and/or cotyledons. Cress is sold as the entire plants in its substrate or soil. In the present Opinion "cress" refers to this particular mode of production and not to some botanical species.

- Crop seed: seeds used to grow the plants that produce seeds for sprouting and consumption.

- Seed: seeds that are going to be germinated.

- Seed distributor: any person responsible for the distribution of seeds (handling, storage and transportation) to various markets and sprout producers. Seed distributors may deal with single or multiple seed producers and processors and they can be producers and processors themselves.

- (Seed) Lot: a definite quantity of some commodity (seed) manufactured or produced under conditions, which are presumed uniform (CAC, 2004).

- Consignment: a consignment is a quantity (of some commodity) delivered at one time (CAC, 2004).

- Inspection lot: the material for which the sampling plan must decide whether to accept or reject.

- Seed producer: any person responsible for the management of activities associated with the primary production of seeds, including post harvest practices.

- Seed processor: any person responsible for the seed processing activities including receiving the harvested seeds from seed producers, storing, cleaning, segregation, grading, and bagging of seeds. Seed conditioning and seed scarification are also a part of the business operation of seed processors and occur to maximise the rates of seed germination.

- Shoots: sprouted seeds obtained from the germination and the development seeds to produce a green shoot with very young leaves and/or cotyledons. The shoots and the leaves are harvested at the end of the production process and the final product does not include the seed teguments and the roots.

- Sprouted Seed: any seed that has been sprouted (germinated) for human consumption as ready to eat or ready to use produce. This includes seeds grown in water as well as soil.

- Sprouts: sprouted seeds obtained from the germination of true seeds and their development in water. Sprouts are collected before the development of leaves. The final product still contains the seed.

- Sprout lot: a quantity of sprouts produced and handled under uniform conditions with as little variation as possible and harvested on the same day (e.g., sprouts produced from a single seed lot, germinated, grown and harvested at the same time using the same treatment and growing methods and type of equipment).

- Spent irrigation water: water that has been in contact with sprouts during the sprouting process.