FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Supporting Document 1

Proposal P 1004 – Primary Production & Processing Standard for Seed Sprouts

TECHNICAL PAPER

A through-chain analysis of food safety hazards and control measures in the production and supply of seed sprouts for human consumption

January 2010

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1. Introduction

1.1 Purpose

The purpose of this paper is to provide a through-chain analysis of food safety hazards and measures that can be used to control food safety hazards in the production and processing of seed sprouts for human consumption. It is prepared to provide technical and scientific information to support risk management decision making aimed at maximising seed sprout safety.

1.2 Structure of this paper

This analysis firstly describes seed sprouts as a food commodity and the sprout production process. It then describes possible food safety hazards that may contaminate seeds and sprouts during their production, processing, storage and transportation. It also describes the associated food safety control measures and practices that, if implemented effectively, would lead to prevention and/or minimisation of the identified hazards.

1.3 Methods applied in the preparation of this paper

This analysis employs the principle of Hazard Analysis Critical Control Point (HACCP) to identify:

- potential food safety hazards;
- where these hazards may occur during growth, harvest, processing, and transportation of seeds and seed sprouts; and
- control measures and practices that can be used to prevent the introduction of the identified hazards and/or to minimise the proliferation of such hazards during seed sprout production, storage and transportation.

A range of domestic and international references have been used to inform the preparation of this through-chain analysis including particularly:

- the FSANZ First Assessment Report for Proposal P1004;
- the NSW Food Authority 'Plant Products Food Safety Scheme' (2004) and 'Plant Products Food Safety Manual' (2007)
- the Guidelines prepared by the Australian New Zealand Sprouters Association (2008);
- the Codex Alimentarius 'Code of Hygienic Practice for Fresh Fruits and Vegetables' (2003);
- the US Food and Drug Administration's Guidance for industry: reducing microbial food safety hazards for sprouted seeds and guidance for industry: sampling and microbial testing of spent irrigation water during sprout production (1999);
- the Canadian Food Inspection Agency 'Code of Practice for Hygienic Production of Sprouted Seeds' (2001);
- the Food Safety Authority of Ireland 'Code of Practice for Food Safety in the Fresh Produce Supply Chain in Ireland' (2001); and
- the Ontario Ministry of Agriculture and Food 'Sprouted Seeds Food Safety Risk Assessment' (2002).

1.4 Seed sprouts

Seed sprouts are germinated seeds[1] that are commonly consumed raw. A wide range of seeds can be used to produce sprouts. These include alfalfa, broccoli, cress, lentils, mungbeans, onion, peas, radish, snow peas, soybeans, sunflower and others. In Australia, bean sprouts, alfalfa sprouts (germinated lucerne seeds) and snow pea sprouts are the main type of seed sprouts produced and consumed.

Microgreens, a recently new term, refer to tiny young plants that are intermediates between seed sprouts and baby plants (see Attachment 1 for a comparison between seed sprouts and microgreens). Unlike most of the seed sprouts, only leaves and stems of microgreens are harvested for human consumption, not the root portion. For clarification purpose, microgreens are excluded from this standard development proposal.

2. Seed sprout production in Australia

The seed sprout production chain starts from seed production in the field where the seed crop is grown. The next stage is seed processing where seeds are received, cleaned, graded and stored. The final stage is sprout production, which occurs at a food production establishment where seeds are germinated and sprouts are harvested.

Figure 1 is a simplified process flow of seed sprout production and supply. The steps involved can be grouped into 3 phases according to operation boundaries of the business set-up:

- Phase 1 is seed production where fields are prepared, seed crops are planted and grown, and seeds are harvested and then transported to a seed processing site.

- Phase 2 is seed processing where seeds are received from seed producers, seeds are stored, cleaned, graded, bagged, and processed seeds are stored and transported to sprout production sites.

- Phase 3 is sprout production where seeds sourced from seed processors/suppliers are received and stored, seeds are germinated, and seed sprouts produced as human food are harvested, packed, stored and transported to food retail establishments.

2.1 On-farm seed production

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. Crops involved may have annual or perennial production cycles and may not exclusively be grown for seed production. For example, beans, such as mung beans and soybeans, are annual crops and the seeds are harvested once per annum in autumn. Lucerne (alfalfa) on the other hand, is a perennial crop and is subject to grazing or repeated harvests for hay, with lucerne seeds harvested once per year.

As alfalfa sprouts and mung bean sprouts are two of the main seed sprouts produced in Australia, this section outlines the production systems used for lucerne seed (perennial crop) and mung bean (annual crop) production.

[1] This Proposal is concerned with seeds from legumes, pulses, brassicas, bulb and root vegetables, and oilseeds such as sunflower seeds which, when sprouted, are used and consumed as salad vegetables. Sprouted forms of cereal grains (wheat, barley, oats etc.) which are used in the brewing industry or in juice manufacture (e.g. wheat grass) are excluded from the scope of this Proposal.

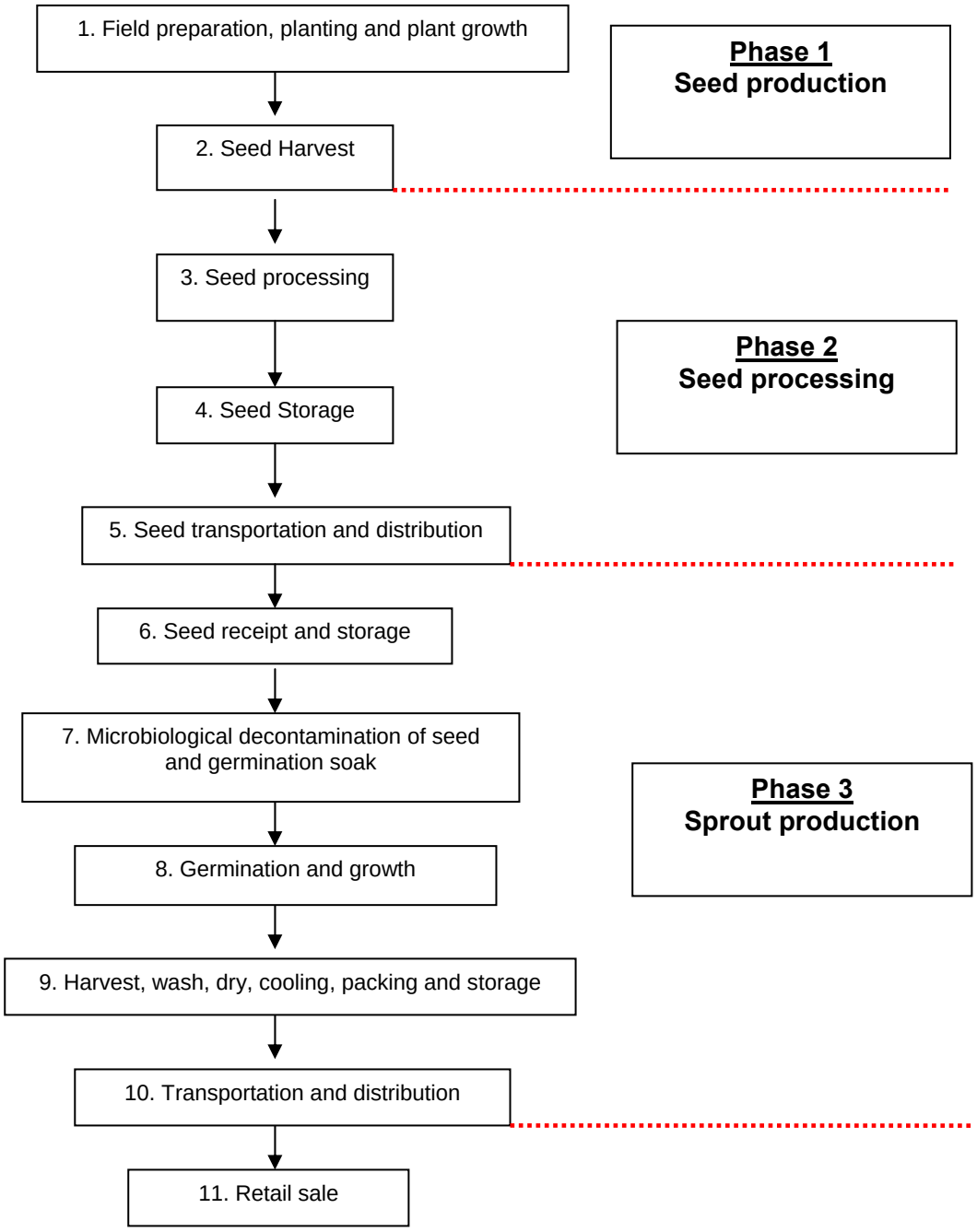

Figure 1: Seed sprout production and supply chain

2.1.1 Lucerne production in Australia[2]

Lucerne is a perennial plant which has a life-span of 6-15 years depending on variety and crop management. It is grown predominantly as a fodder crop, either grazed directly, or made into silage or hay first. Lucerne pastures are drought-resistant and produce green feed in all seasons.

[2] Information on production practices supplied by Lucerne Australia

More than 80% of lucerne seed production in Australia takes place in South Australia with the remaining occurring in NSW and Victoria (Hassall & Associates Pty Ltd, 2001; De Barro, 2005). Lucerne seed production within South Australia is centred in the region of Keith, Bordertown and Jamestown. In NSW, lucerne production takes place from Howlong through to Corowa.

The volume of lucerne seed produced in Australia varies from 4,000 to 7,000 tonnes per year. While most of this seed is exported as pasture seed, a proportion of lucerne seed is used to produce alfalfa sprouts for human consumption. Industry data indicates that an estimated 300 tonnes of lucerne seeds have been used for sprout production in Australia in 2006 and approximately 600 tonnes of exported lucerne seeds (~10% of all exported seeds) are used for sprout production in the international market. With an estimated market price of $AUD5.0 to $AUD5.50 per kilogram of lucerne seeds, the value of lucerne seeds used for sprout production in Australia is approximately $AUD1.5 million.

2.1.1.1 Paddock management

In Australia, lucerne seed is only harvested once a year during the months of February to April. Lucerne growers use a number of strategies to manage the production of lucerne hay and lucerne seeds. This involves grazing and hay cutting. To maximise agricultural output, it is a common practice to allow animals (sheep and cattle) to graze on lucerne fields on a rotational basis. Paddocks may be grazed in the final quarter of the year, which removes most of the vegetation from the paddock and encourages flowering of the plant. Grazing animals are excluded from the lucerne field at least 60 days prior to the harvest.

An alternative approach to paddock management is where animals may be excluded from paddocks in the months July to August, and the crop is cut for hay between October to early December. Using this approach means the amount of time that paddocks are kept free of grazing animals prior to seed harvesting could be as much as 240 days, depending on crop management strategy.

Lucernes are cultivated either by dryland agriculture or irrigation agriculture. Dryland lucerne production is uniquely dependent on natural rainfall. For irrigated lucerne production, crops may be flood irrigated between one to six times per season, or three to ten times for spray irrigation, depending on local conditions.

2.1.1.2 Seed Harvest

The lucerne plant flowers in January and February, at which time pollination occurs and the seed is set. Once set, the lucerne seeds develop within an enclosed seed pod, with each pod containing up to 12 or more seeds.

Harvesting of lucerne seeds can be undertaken by two methods:

- cutting and windrow curing followed by threshing with a combine harvester; or
- chemical desiccation followed by direct harvesting of the standing crop.

Windrowing involves cutting the crop just above the crown of the plant and laying the foliage in rows (windrows) on the ground. The plant material is allowed to dry for a number of days, until the moisture content of the foliage falls to approximately 12-18%.

Once the plant material is suitably dry, it is picked up using a harvester which works close to the ground. Inevitably, the harvester will also pick up extraneous material from the ground including soil and other potential contaminants. The plant material is then threshed inside the harvester to separate the seed from the other material (e.g. stalks).

For direct harvesting, crops are sprayed with a chemical desiccant/defoliant, allowed to dry, then collected using a header and harvested.

Harvested seed is generally collected in mobile field bins, which are either stored on-site or transported to a seed processor for cleaning and packing.

2.1.2 Mungbean production

Mungbeans are a specialised food crop. They are produced for sale as whole beans, sprouted or processed into flour. They are predominantly produced in Australia for the export market.

The bulk of the mungbean production in Australia is in central and southern Queensland and northern NSW. Production volumes vary from 30,000-50,000 tonnes annually, depending on seasonal factors, the varieties cultivated and farm management practices. The crop produced is graded as sprouting grade seeds (premium grade), cooking grade beans, processing grade beans and manufacturing grade beans.

In 2006-07 it was estimated that the Australian mungbean industry produced 38 ,974 tonnes of beans, of which 1,325 tonnes were used for sprouts. The majority of Australian mungbean production is processing grade seed.

2.1.2.1 Crop management

Mungbeans are a warm-season annual pulse grown mostly in rotation with other crops such as cereals. Plants have a short growth period (75-90 days) which means that they can easily be included in crop rotations. Sowing times vary depending on the location and variety grown.

Mungbean crops are managed with the aim of producing premium grade seed. Factors that need to be considered to maximise the yield include:

- choice of paddock (e.g. no soil variation, adequate soil moisture profile)
- seed variety
- time of planting
- planting rates
- pests and diseases
- seasonal variability

Mungbeans may be grown under dry land or irrigated crop production systems.

2.1.2.2 Seed Harvest

Mungbeans have an indeterminate flowering habit. This means that they do not have a defined flowering period and consequently, can have flowers, green pods and black pods present on the plant at the same time. Harvest occurs when more than 90% of pods are mature and dry.

To minimise damage to seeds, they are harvested at seed moisture contents of 14-16%. A desiccant is often used before harvest to kill any green leaves and the few remaining green pods.

The mungbean is an annual, semi-erect to erect or sometimes twining deep-rooted plant. Ten to twenty-five flowers are borne in ancillary clusters. The seed pods are curved and pointed and each pod contains 8-20 seeds. Mung bean seeds are mechanically harvested by combine harvesters.

Following harvest, beans are trucked to a grading shed where they are cleaned, graded and bagged as soon as possible (seed processing).

2.2. Seed processing

Seed processing includes receiving seeds from seed producers, establishing identity of seed lot, seed cleaning and segregation, storage and transportation of cleaned seeds.

Seed harvested from the field may contain extraneous material such as soil, weed seeds and other debris. This is removed during seed cleaning process, whereby the seed received from the field is passed through a series of sieves (4-5 screens of different pore sizes) and then further cleaned via use of a gravity table, where seeds are separated by their weight. Processed seed is packed into 40 kg bags, or larger containers for the bulk seed market, and stored on-site prior to shipping. Seed purchased by sprouters is generally required in 25 kg bags.

2.2.1 Alfalfa

The accepted market quality of sowing lucerne seed is[3]:

- minimum rate of germination: 85%
- minimum normal seedling: 60%.

For sprouting purposes, sprout producers specify that seed should have a 4-day minimum germination rate of approximately 90% and a maximum hard/abnormal/dead seed count of approximately 10%. Germination rates of harvested lucerne seeds are variable, and the suitability of seeds for sprouting cannot be assured until a seed germination test is performed.

Where there is a high hard-seed count, the germination rate can be increased by scarifying the seeds during processing or by leaving seed in storage. Scarification is a process whereby the seed coat is broken or scratched. This makes it permeable to water and gases and thus aids germination. During seed processing this can be achieved by a mechanical process using spinning abrasive discs against which seed is dropped then collected.

Seeds used for production of alfalfa sprouts are largely grown and processed in Australia.

[3] De Barro, J. (2006) Presentation from Lucerne Australia, Presented at the workshop of “Food Safety and Sprouts”, held at the Tiffins on the Park, Adelaide, 20 July 2006.

2.2.2 Mungbeans

The accepted market quality of sprouting mungbean seed as defined by the Australian Mungbean Association – Standards for Export Mungbeans[4] as:

- size range (2 mm): >98% (75% must be in 0.8 mm range)
- purity: >99% (other seeds: 0.3% and maximum soil content: 0.1%)
- minimum rate of germination[5]: >90%
- over soaks[6]: 10%
- moisture: 12%
- Charcoal Rot: absent
- Salmonella: not detected[7] (equivalent to not detected in 25 grams)
- E. coli: not detected[8] (equivalent to <10 E. coli / gram)
- sprout test: suitable[9]

Seeds used for production of mung bean sprouts are largely grown and processed in Australia.

2.3 Sprout production

Seed sprouts can generally be subdivided into three groups:

- green sprouts (e.g. alfalfa sprouts, onion sprouts, radish sprouts)
- bean sprouts/bean shoots (produced primarily from mung beans)
- shoots (e.g. snow pea shoots).

The scope of this proposal is concerned with seed sprouts produced from seeds of brassicas, bulb, grass, herbs and spices, nuts, legumes and pulses, oilseeds, root vegetables and others, which are consumed as salad vegetables, garnish, stir fry vegetables or vegetables in soup. Sprouted forms of cereal grains which are used in the brewing industry or in juice manufacture or microgreens are excluded from the scope of this proposal.

2.3.1 Australian sprout producers

The Australian sprout production sector consists of approximately 40 known sprout producers operating in five states throughout Australia. Around two thirds of these sprout producers have been in operation for 16 years or longer with many being family owned and operated. While the majority of sprout businesses produce a range of products, a number of operations are single product producers, producing just mung bean sprouts or snow pea sprouts.

The sprouting industry has an estimated annual turnover of approximately $AUD30 million, employing approximately 300 people and producing more than twelve product lines.

[4] www.mungbean.org.au/pdf/AMAExportStandards2009.pdf
[5] Rate of germination excludes hard seeds for varieties of Berken, Delta Emerald and Satin.
[6] Over soaks refer to the percentage of mung beans which imbibe after submerging in water at 32ºC for 1 hour.
[7] *Salmonella* test is sometimes conducted using 48 hours + irrigation waters collected from germination tests on sample seeds where *Salmonella* is not detected in 25 grams of irrigation water.
[8] *E. coli* test is sometimes conducted using 48 hours + irrigation waters collected from germination tests on sample seeds where *E. coli* is < 10 per ml of irrigation water.
[9] Suitable is defined in accordance with the Australian Mungbean Association's approved procedures.

Mungbean sprouts are the predominant product (by volume) produced followed by snow pea sprouts and then alfalfa sprouts.

2.3.2 Sprout growing

Methods used for the germination and growth of seed sprouts, including water cycles used, vary depending on the type of sprout being produced and the size/sophistication of the sprouting operation. The basic process involves applying water to seeds and placing them in a warm humid environment for a period of 1-14 days (NACMCF, 1999a)(NACMCF, 1999). Sprouting may take place in temperature-controlled environments, which maintain air temperatures at 20-30°C, or at ambient temperatures. Once the sprout has reached the required size it is harvested, chilled and packaged ready for storage and distribution. The typical production steps undertaken are outlined in Figure 2 and discussed further below.

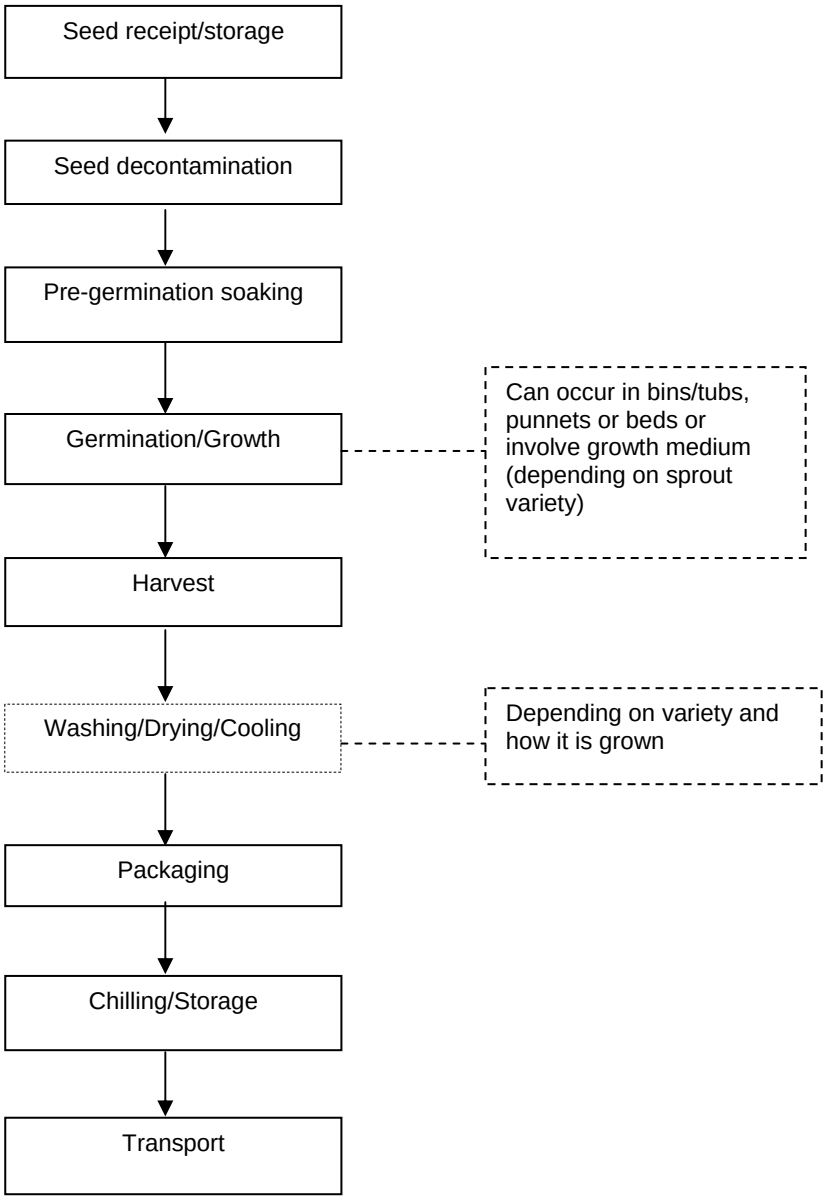

Figure 2: Typical production steps during production of seed sprouts

2.3.3 Seed decontamination

Prior to sprouting, seed should be washed to remove any dirt or debris. It is then recommended that seeds are treated with an antimicrobial agent, such as chlorine, prior to the sprouting process. Recommended seed decontamination regimes vary, however a treatment of 20,000 ppm calcium hypochlorite has been commonly suggested for alfalfa seed.

Following seed disinfection the seed is again rinsed to remove the antimicrobial agent used.

2.3.4 Pre-germination soak

Soaking is undertaken to improve germination. Seed is commonly soaked in potable water for 3 to 10 hours (depending on seed variety) at ambient temperature. Sufficient quantities of water need to be used during the soaking process as the seed swells and may double its volume during this time.

2.3.5 Germination/Growth

The sprout growing process varies depending on the variety of seed being germinated and the sophistication of the sprouting operation. Examples of typical protocols for alfalfa sprouts, bean sprouts and snow pea shoots are outlined below.

2.3.5.1 Alfalfa sprouts

Alfalfa sprouts (including mixes) may be grown in large rotating drums/tumblers (for larger operations) or in trays or punnets over a 3-6-day period. The sprouts are continually watered during this time and runoff water (spent irrigation water) removed. For businesses that have temperature controlled rooms, the temperature for growth is kept at 18-21°C and the irrigation water at 20-22 °C.

2.3.5.2 Bean sprouts and shoots

Bean shoots are generally grown in bins, buckets or on beds over a 5 to 6 day period to allow for shoot development. In large scale operations, bean sprouts are grown in temperature controlled rooms at 20-28 °C. Water is generally applied every 1-2 hours.

Shorter ‘crunchy’ style bean sprouts are grown under similar conditions to bean shoots but for only a 24-48 hour period.

2.3.5.3 Snow pea shoots

Snow pea shoots are generally grown on trays, using soil/compost mix or other supporting medium. They are usually grown in green houses or exposed to light for a period of time, once the seed has germinated, to encourage green growth. Snow pea shoots are grown over an 8-12 day period, depending on the shoot and leaf development required.

2.3.6 Harvest, packaging and storage

Seed sprouts are generally harvested by hand once they have reached the desired size (some mechanical harvesting of bean shoots may occur in large scale operations). For green sprouts and bean sprouts/shoots, the whole product is collected. For snow pea shoots the product is cut away from the seed and root development. Some products may be grown in punnets and not require harvesting per se.

Green and bean sprouts are generally washed before packaging, often using cooled water to start chilling the product before storage. Water is drained away (spinning or shaking may be used to help dry off product) before the product is hand packaged into plastic punnets/tubs or bags.

Packaged product is placed into cool rooms (<5°C) and stored and transported at refrigeration temperatures.

3. Hazards and control measures associated with seed sprouts

Biological, chemical or physical hazards impacting seed sprout safety at the time of consumption can be introduced during seed sprout production and supply chain. While any type of seed sprout has the potentially to become contaminated[10], foodborne illness data from the last 30 years indicate that only alfalfa and mungbean sprouts, and to a less degree cress, clover and radish sprouts, have been implicated in causing human illness (Attachment 3). The following analysis is based on the available information of production and processing of alfalfa and mungbean sprouts. It is assumed that hazards and control measures are similar for other types of sprouts.

Microbiological pathogens are the principal hazards affecting seed sprout safety. Significant pathogens associated with foodborne illness outbreaks resulting from consumption of contaminated seed sprouts are pathogenic *Salmonella* species and Shiga-toxin producing *Escherichia coli* (STEC). To a lesser extent, *Bacillus cereus* and *Yersinia enterocolitica* have been involved in previous foodborne outbreaks resulting from consumption of contaminated seed sprouts. Potential microbial hazards evidenced by positive detection in seed sprouts include *Clostridium*, *Cryptosporidium*, *Giardia*, *Listeria monocytogenes* and *Shigella*.

Chemical hazards potentially affecting seed sprout safety may originate from environmental contaminants, agrichemicals, chemical processing aids or others. Physical hazards potentially affecting the safety of seed sprouts include metal, stone, glass and plastics. There have been no reported incidences of chemical or physical hazards associated with consumption of seed sprouts. However, in order to inform the risk management decisions, an analysis of where potential chemical and physical hazards may be introduced in seed sprout production chain and what control measures are available to minimise the contamination of sprouts by such hazards has been provided (Attachment 2).

3.1 Hazards and control measures associated with seed production

Seeds used for sprout production have been implicated as a main source of contamination in epidemiological investigations following previous outbreaks (Harris *et al*., 2003). Trace back investigations following the 2005/06 outbreaks occurred in Western Australia and Victoria found seeds produced in South Australia were contaminated by *Salmonella* Oranienburg that shared the same pattern of molecular trace as the outbreak strain (Attachment 2). Pathogenic microorganisms such as *Salmonella* species, STEC, and to a less extent *Cryptosporidium*, *Giardia* and *Yersinia* are found in animal faces. These microbial pathogens may contaminate the seeds if untreated animal manure is used for seed crop production or animal grazing is mixed with seed crop production in the same field. Lucerne crop is traditionally grown for animal grazing in Australia. Production of lucerne seeds can be achieved by not allowing animal access to the field for the 14 weeks leading to maturity of the lucerne crop. While such practice maximise agriculture production, it increases the

10 Harris, L.J., J.N. Farber, L.R. Beuchat, N.E. Parish, T.V. Suslow, E.H. Garrett & F.F. Busia (2003) Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh cut produce, Chapter III in: Analysis and evaluation of preventive control measures for the control and reduction/ elimination of microbial hazards on fresh and fresh-cut produce. A report of the Institute of Food Technologists for the Food and Drug Administration of the United States Department of Health and Human Services, Comprehensive Reviews in Food Science and Food Safety Vol 2 http://www3.interscience.wiley.com/cgi-bin/fulltext/119191777/PDFSTART

chances of pathogen contamination on lucerne seed.

Contaminated lucerne seed presents a potential food safety problem when it is used for producing alfalfa sprouts. On the other hand, such seeds are perfect for sowing to produce new lucerne crop. Control measures identified to prevent or minimise seed contamination by microbial pathogens (Attachment 2) are suitable for seeds produced for sprouting only, and should not be regarded as equally applicable for seeds produced for other purposes.

Other sources that may introduce pathogens to seeds during seed production include contaminated soil, contaminated water, and contaminated agricultural machineries. Seed growers should identify the sources of water used on the farm, and assess its microbial and chemical quality and the suitability for its intended use, and identify corrective actions to prevent or minimise contamination from livestock, sewage treatment, and human habitation. Where necessary, growers should have the water tested for microbial and chemical contamination. The frequency of testing will depend on the water source and the risks of environmental contamination. If the water source is found to be contaminated, corrective actions should be taken to ensure that the water is suitable for its intended use.

Potential food safety hazards, sources where these hazards may be harboured, practical control measures and food safety practices that can prevent or minimise the opportunity of such hazards contaminating seeds during seed production are summarised in Table 1 (Attachment 2).

3.2 Hazards and control measures associated with seed processing

During processing, seeds can become contaminated by microbial pathogens through the mixing of different harvest lots, contaminated equipment, activities of rodents, birds, other animals or pests, or via infected workers. Seed scarification, a seed treatment by either mechanical or chemical means to improve germination rate, if carried out, creates cuts on seed surface that present additional space and environment to harbour microbial pathogens (Attachment 2). During transportation and distribution, seeds may become contaminated by biological or chemical hazards because of rodent activities or dirty vehicles (Attachment 2).

Control measures and food safety practices identified by relevant food safety agencies in the US, Canada and Ireland, by alfalfa agronomists at the University of California, by Codex, and by Australian Freshcare Code of Practice to prevent and minimise the chance of seed contamination by such hazards are summarised in Table 1 (Attachment 2).

3.3 Hazards and control measures associated with sprout production

Sources of potential food safety hazards that may contaminate sprouts during sprout production, packing, handling, storage and transportation can be the seeds used, equipment, water and medium supporting sprout growth. Chemicals used or present in a sprout production environment and metal, glass, wood or plastics objects present in or used for sprout production and handling may present food safety hazards.

Sprout production requires a warm environment with high moisture to stimulate seed germination and plant growth. Such conditions create a perfect environment for rapid growth of microorganisms. Often, the microbial growth is associated with the growth of seed sprouts as the nutrients are plentiful. This applies to pathogenic microorganisms if present. If even a few cells of microbial pathogens survive the microbiological decontamination treatment or originate from contaminated equipment or contaminated water or growth media, they can grow to high numbers during seed germination and sprout growth phase because of the favourable growth environment and conditions where water, nutrients and temperature are desirable for microbial activity.

Control measures and food safety management practices to inactivate microbial pathogens from seeds and the environment and to monitor the likelihood of microbial contamination as recommended by a wide range of government agencies, industry guidelines are summarised in Table 1 (Attachment 2). Among them, the most highly recommended preventative measure is seed decontamination to eliminate microbial pathogens. Literature indicates that current seed treatments, i.e. microbiological decontamination of seeds, cannot guarantee total elimination of microbial pathogens (Codex, 2003). The next highly recommended food safety control in this part of the seed sprout supply chain is to have in place an appropriate monitoring procedure to ensure the integrity of the sprout production system and to prevent microbial pathogen contaminated sprouts being sold to consumers.

3.4 Hazards and control measures associated with seed sprout retailing

Hazards that can affect seed sprout safety at retail are very much the same as those of other ready-to-eat foods, provided that seed sprouts delivered to the retail site are not contaminated. Environmental pathogens such as *Listeria monocytogenes* and highly transmittable pathogens such as norovirus are likely to be introduced to seed sprouts at retail according to a Canadian risk assessment. The likelihood of chemical and physical contamination is relatively rare at retail sale of seed sprouts despite the possibility that such hazards could be introduced through contact with dirty surfaces.

Effective cleaning and appropriate sanitisation program for the retail display environment, pest control and hygienic food handling are among those control measures and food safety practices identified in Table 1 (Attachment 2).

4. Summary

Principal hazards affecting seed sprout safety are microbiological pathogens which can be introduced at various points in the production and supply chain of seed sprouts for human consumption. While seed is considered the likely source of contamination, recommended control measures so far are placed on microbiological decontamination of seeds and to a lesser extent monitoring of spent irrigation water for the presence of microbial pathogens at sprout production premises.

Effective implementation of preventative measures during the phase of seed production and seed processing will reduce the chance of contaminated seeds reaching sprout producers, however if these measures are not in place then microbiological decontamination of seeds is required.

Attachment 1: Seed sprouts and microgreens

Seed sprouts are germinated seeds of brassicas, bulb, grass, herbs and spices, nuts, legumes and pulses, oilseeds, root vegetables and others, and are consumed as salad vegetables, garnish, stir fry vegetables or vegetables in a soup. Seed sprouts are often consumed raw or lightly cooked.

A wide range of seeds can be used to produce seed sprout (Table 1). Common varieties of seed sprouts sold in Australia are listed under the second column in Table 1. Among them, mung bean sprouts or shoots, alfalfa sprouts (germinated lucerne seeds) and snow pea sprouts or shoots[11] are the main varieties of seed sprouts produced and sold in Australia.

Table 1: Seeds used for production of seed sprouts

Seeds have and can be used for sprout production[12]	**Common variety of seed sprouts available on Australian market**
adzuki bean, alfalfa, almond, amaranth, annatto seed, anise seed, arugula; barley, basil, broccoli, brown rice, buckwheat; cabbage, canola seed, caragana, cauliflower, celery, chia seed, chickpea, chive, cilantro (coriander, dhania), clover, corn, cowpea, cress; dill; fennel, fenugreek, flax seed; garden cress, garlic; hemp seed; kale, kamut, kat; lentil, lemon grass, lettuce, lima bean, lupin; milk thistle, millet (pearl millet), mizuna, mung bean, mustard; navy bean; oats, onion; pea (black-eyed, green, pigeon, snow), peanut, pinto bean, psyllium, pumpkin; quinoa; radish, rye; sesame, soybean, spelt, sunflower; tatsoi, triticale; watercress, wheat, wheat berry; and yam	Alfalfa; broccoli chickpea, chive, corn, cowpea garlic lentil mung bean, mustard onion pea (snow, blue) radish soybean, sunflower wheat

As this standard development proposal deals largely with food safety problems associated with consumption seed sprouts as vegetables and garnish, sprouted cereal grains used for brewing (e.g. malts of barley, oats, sorghum, wheat etc.) or for juice making (e.g. wheat grass) are excluded from the scope of this Proposal.

[11] Snow pea sprouts or snow pea shoots have long been considered seed sprouts in Australia despite the fact that the tissue above the root system is normally cut for human consumption at the harvest time.

[12] Seed varieties in this list have been sourced from various Internet sites with Wikipedia as the main source of information. The list is not intended to be exhaustive because of the constant change in consumer preference, manufacturers' innovation and food preparation.

Microgreens, young plants used generally as a garnish or as an ingredient in salads. Microgreens have been described as the smallest form of salad greens, leafy vegetables or herbs. While microgreens and seed sprouts are similar to young plants, they do differ in a number of aspects. The comparison described in the following table separates microgreens from seed sprouts.

Table 2: Some of the differences between seed sprouts and microgreens

	Seed sprouts	**Microgreens**
Growth medium	In most circumstances, seed sprouts do not require a medium to support its root system but water only.	In most circumstances, microgreens require a medium (such as soil, perlite) to support its root system.
Growth environment	Seed sprouts grown under little light and in a highly moist environment	Microgreens grown under light and in a less moist environment
Growth time	5 – 6 days for most seed sprouts	8 – 21 days for most microgreens
Nutrients for growth	In most circumstances, seed sprouts require no added nutrients for growth	In most circumstances, microgreens require added nutrients for growth
Cotyledon leaves	In most circumstances, cotyledon leaves of seed sprouts at the time of harvest are underdeveloped	At the time of harvest, cotyledon leaves of microgreens are fully developed
Plant development stage	An early stage of plant development between seed and first true leave	A development state that is younger than a baby plant but older than a seed sprout
Harvest	In most circumstances, seed sprouts are harvested before first true leaf is emerged	Microgreens are harvested after at least the first set of true leaves is emerged
Consumption	In most circumstances, the whole plant is consumed including the root system.	Only the leaves and stems are consumed, but not the roots

For clarification purpose, seed sprouts [13] under this standard development proposal, are germinated seeds of brassicas, bulb, grass, herbs and spices, nuts, legumes and pulses, oilseeds, root vegetables and others, which are consumed as salad vegetables, garnish, stir fry vegetables or vegetables in soup. Microgreens, sprouted cereal grains used for brewing or for juice making are excluded from the scope of seed sprouts under this standard development proposal.

[13] Seed sprouts are referred as sprouted seeds in North America.

Attachment 2: Potential food safety hazards and control measures that prevent or minimise contamination of seeds or seed sprouts from identified hazards

Production stage	Hazards	Source of hazards	Control Measures	Food Safety Management Practice, Knowledge and Skill Requirements
SEED PRODUCTION				
1. Field preparation, planting and plant growth	Major[14]: Pathogenic *Salmonella* spp, Shiga toxin-producing *E. coli* Minor[15]: *Cryptosporidium*, *Giardia* and *Yersinia*	Animal faces and manure present in field where the crop is grown[16]	Prevent grazing animals and wild animals from entering the seed growth field; manure, biosolids and other natural fertilizers should only be used upon treatments which achieve a high level of pathogen reduction[18]; crops be protected from contamination by human, animal, domestic, industrial and agricultural wastes[19 and 20]	Seeds for sprout production should be grown under good agricultural practices in order to minimise the likelihood that they will contain pathogenic bacteria Maintain good records of inputs and activities applied to the field;
	Major: Pathogenic *Salmonella*, Shiga toxin-producing *E. coli* Minor: *Bacillus cereus*, *Clostridium*, *Listeria monocytogenes*	Contaminated soil or machinery used for crop growth[17]	Ensure soil and environment are suitable for growing seeds that are intended for sprout production for human consumption[21] ; and ensure equipment and machinery are maintained and used to minimise and/or avoid the contamination of seeds[22]	Establish good agricultural practices in seed production and supply to meet sprout producer's safety specification on seeds
	Major: Pathogenic *Salmonella*, Shiga toxin-producing *E. coli* Minor: *Cryptosporidium*, *Giardia* and *Yersinia*	Contaminated water for crop irrigation	Ensure water quality is suitable for its intended purpose	Knowing the history of the land and possible impact of adjacent land;
	Possible chemical hazards: Environment contamination or applied agricultural chemicals may pose a risk to seed sprout safety	Environmental contamination and agricultural chemicals used for crop production	Ensure that environment and agricultural chemicals including pesticides used for seed production are intended for sprout production for human consumption. Ensure that regulatory limits for environmental contaminants and agricultural chemicals in food are met. Limits for environmental contaminants are set as low as reasonably achievable[23]. Agricultural chemical limits reflect legitimate use in accordance with registered use patterns of those chemicals (registered through the Australian Pesticides and Veterinary Medicines Authority (APVMA)).	Pesticides and agricultural chemicals are applied in accordance with product instruction, by qualified personnel, and with the knowledge that the seeds produced will be used as food following a sprouting step

[14] Seeds have been implicated as a source of contamination in epidemiological investigations following the outbreaks (Harris *et al*., 2003).
[15] Microbial pathogens, such as *Clostridium*, *Listeria monocytogenes*, and parasites such as *Cryptosporidium* and *Giardia* have been detected on seed sprouts (Harris *et al*., 2003).
[16] Suslow, T.V., Cliver, D. & Meyer, D. (1998) Progress in defining microbial risk reduction [practices for animal manure and manure-based composts, Perishable Handling Quarterly Issue No 95 p15-17
[17] Ontario Ministry of Agriculture and Food (2002) Sprouted Seeds – 5.0 Sprouted Seeds Food Safety Risk Assessment, http://www.omafra.gov.on.ca/english/food/inspection/fruitveg/risk_assessment_pdf/sproutedseeds/50ra.pdf accessed 6 February 2009
[18] Code of hygienic practice for fresh fruits and vegetables (CAC/RCP 53-2003) (Codex, 2003)
[19] Australian Mungbean Association (2007) Code of Hygienic Practice for Mungbeans
[20] Freshcare (2009) Freshcare Code of Practice on food safety and quality, The national on-farm assurance program for Australian Growers, 3rd edition
[21] Soils should be evaluated through laboratory testing for microbiological and chemical hazards. If the evaluation concludes that such hazards were at levels that may compromise the safety of the seeds, control measures should be implemented to reduce hazards to acceptable levels. If this cannot be achieved by the available control measures, growers should not use these soils for primary production.
[22] Equipment and tools should function according to the use for which they are designed for without damaging the seed. Such equipment and machinery should be maintained in good working order.
[23] (limits already included in Standard 1.4.1

Production stage	Hazards	Source of hazards	Control Measures	Food Safety Management Practice, Knowledge and Skill Requirements
SEED PRODUCTION				
2. Seed harvest (include cutting, swathing, combining and transport)	Major[24]: Pathogenic *Salmonella* spp, Shiga toxin-producing *E. coli* Minor: *Bacillus cereus*, *Clostridium*, *Listeria monocytogenes, Cryptosporidium*, G*iardia* and *Yersinia* Possible chemical hazards: Desiccants applied may pose a risk to seed sprout safety.	Contaminated soil being picked up during harvest[25] Contaminated machinery and equipment used at harvest Desiccants used to dry crop	Ensure soil and environment are suitable for growing seeds that are intended for sprout production for human consumption Harvesting equipment should be adjusted to minimise soil intake and seed damage and should be cleaned from any debris or earth Bins and equipment used for harvest are cleaned (sanitised when necessary) and kept in dry condition; and bins filled with seeds are covered to prevent potential microbial contamination Maintain sanitation in drying yards, and exposure of seeds to mist, high humidity or fog be avoided Seed produced for the production of sprouts for human consumption should be segregated from product to be seeded or planted for animal feed and clearly labelled Desiccants used to dry the crop prior to seed harvest needs to be appropriate for seeds destined for sprouting purpose. Products used as desiccants for crops must be approved and registered with the APVMA and used in accordance with those approved registered use patterns.	Seeds for sprout production should be grown/harvested under good agricultural practices in order to minimise the likelihood that they will contain pathogenic bacteria Good agricultural practice to minimise soil pick up during seed harvest; to minimise seed damage during harvest[26]; and to minimise microbial contamination and growth during thrashing, drying and transportation of seed Knowledge of registered use pattern for agricultural chemicals

[24] Microbial pathogens can be picked up from soil during seed harvest. When this occurs, not only the seed surface but also the crevices and cracks of damaged seeds may get contaminated. Dirty harvest equipment and bins will contaminate the seeds. Wet harvest equipment and bins will encourage microbial growth. Uncovered bins filled with seeds can get contaminated by airborne microbial pathogens, bird droppings. Harvest has the potential to spread localised contamination throughout the seed lot (Ontario Ministry of Agriculture, 2002, Mueller, UC Davis).
[25] Mueller, S. Alfalfa seed production in the western united states. http://alfalfaseed.ucdavis.edu/pages/Western_alf_seed_production.htm accessed 28 July 2009
[26] Charkowski et al., 2003; Wade et al., 2003 ; Fett, 2006b

Production stage	Hazards	Source of hazards	Control Measures	Food Safety Management Practice, Knowledge and Skill Requirements
SEED PROCESSING				
3. Seed processing (include seed conditioning, cleaning, segregation, grading and scarification)	Major[27]: Pathogenic *Salmonella* spp, Shiga toxin-producing *E. coli* Minor: *Bacillus cereus*, *Clostridium*, *Listeria monocytogenes, Cryptosporidium*, G*iardia* and *Yersinia*	Cross contamination of different harvest lots and spread of localised contamination to other lots	Minimisation of mixing different harvest lots, and seed produced for the production of sprouts for human consumption should be segregated from product to be seeded or planted for anuimal feed and clearly labelled, diseased and damaged seed should not be supplied for sprout production	Seeds that may be used for sprouting should be processed, stored and transported in a manner that minimises the likelihood that the seeds will be contaminated with pathogens
		Equipment used in seed processing	Equipment used for seed processing be kept clean	Maintain good records of stock and blending of seed lots
		Rodent and pest activities	Containers, silos and premises used for seed processing, storage and handling are controlled to prevent potential contamination from activities of rodents and pests	Consider to supply only seeds that have not been scarified for sprouting purpose
		Infected workers	Workers with infectious diseases be kept away from working on seed handling and processing	Prevent potential biological and chemical contamination on seeds destined for sprouting purpose by following appropriate good manufacturing guidelines developed for seed processing and handling
		Seed scarification process	If seeds are scarified, equipment must be maintained clean to prevent microbial contamination	Workers are trained in knowledge and skills on hygienic food handling including those that will prevent biological and chemical contamination on seeds during seed processing
	Potential chemical hazards: Chemicals used during seed handling and processing may contaminate seeds	Chemicals used at seed processing establishment	Ensure any chemicals that may contact seed are approved for use for seeds destined for sprouting industry. A range of processing aids is permitted for use in foods generally (Standard 1.3.3 of the Food Standards Code). A number of processing aids are specifically listed as approved for use as washing agents in Standard 1.3.3 of the Code	Knowledge of permitted processing aids and any maximum permitted levels associated with their use

[27] Seeds at this stage can become contaminated by microbial pathogens through mixing of different harvest lots, contaminated equipment, activities of rodents, birds, other animals or pests, or via infected workers; and seed scarification, if carried out, creates cuts on seed surface that present additional space and environment to harbour microbial pathogens.

Production stage	Hazards	Source of hazards	Control Measures	Food Safety Management Practice, Knowledge and Skill Requirements
SEED PROCESSING				
4. Seed storage (include silo storage, bagging and storage of bagged seeds)	Major: Pathogenic *Salmonella* spp Minor: Other pathogenic microorganisms carried by rodents, pests or infected workers may contaminate seed. Potential chemical hazards: Seeds could be contaminated during seed storage by chemical hazards.	Animals (faeces), rodents (faeces and urine), birds (droppings) and insects, and possibly infected workers Agricultural and industrial wastes and chemicals	Facilities and equipment used for seed storage and handling be maintained in clean, dry condition and inaccessible to rodents, birds, vermin and pest[28] Bags used for seed storage should prevent external moisture to from entering the internal space of the bag, i.e. solid wall bags are desirable, and do not use contaminated or recycled bags Agricultural and industry waste and chemicals are isolated from seed storage space to prevent potential chemical contamination	Seeds for sprout production should be handled and stored under good agricultural practices in order to minimise the likelihood that they will contain pathogenic bacteria Frequent inspection of storage facility using an appropriate device such as a black light stain detector to detect rodent urine stain should be practiced Prevent potential chemical contamination on seeds destined for sprouting purpose by following appropriate guidelines of chemical use in seed storage and handling Maintain good records of inventory and stock turnover Workers involved in handling seed storage be trained on hygienic food handling knowledge and skill; and workers with infectious diseases be kept away from working on handling seed storage

[28] Canadian Food Inspection Agency (2001) Code of Practice for the hygienic production of sprouted seeds. http://www.inspection.gc.ca/english/fssa/frefra/safsal/sprointe.shtml accessed 04 June 2006

Production stage	Hazards	Source of hazards	Control Measures	Food Safety Management Practice, Knowledge and Skill Requirements
SEED PROCESSING				
5. Transportation and distribution of seeds (including inspection, establishing identity of seed lot, loading and transportation of seeds, unloading and storage)	Major: Pathogenic *Salmonella* Minor: Other pathogenic microorganisms carried by rodents, pests or infected workers may contaminate seeds.	Rodents, pests, birds, water and possibly cross contamination due to mixing of seed lots may occur during seed transportation and distribution because of rodent activities or dirty vehicles used for seed transportation and distribution.	Containers, vehicles and storage facilities be cleaned and sanitised before use At all times seeds, equipment, storage bins and shipping bags be protected from activities of rodents and birds Not mix seed lots to upgrade the seed or increase the size of the lot Seed supplier should inspect all bags upon delivery for evidence of contamination[29], and bags with evidence of contamination should not be used. Seed distributors should analyse each lot for the presence of microbial pathogens of concerns[30] using internationally accepted analytical methods. Lots of seeds for which positive results are obtained shall not be used for sprout production. Other lots being produced under similar condition which present a similar hazard shall not be used for sprouting. These lots should be held and detained until they are disposed of properly.	Seeds for sprout production should be transported under good agricultural practices in order to minimise the likelihood that they will contain pathogenic bacteria Seeds for sprouting purpose should be transported and distributed under good hygienic practices to ensure they are safe and suitable for human consumption upon delivery Maintain good record of the use of transport vehicle; and producers of seed for sprout production must ensure that trace-back records and recall procedures are in place to effectively respond to health risk situations Provide a certificate of analysis of key microbial pathogens or indicator organisms for the lot of seeds delivered that meets the specification of the sprout producer
	Potential chemical hazards: Seed could be contaminated by chemicals during seed transportation and distribution.	Potential cross contamination from chemical sources during transportation and distribution where mixed loads or unclean vehicles or equipment are used	Vehicle transporting seeds for sprouting purpose should not transport goods at the same time that may contaminate the seeds	Workers involved in seed transportation and distribution be trained on hygienic food handling knowledge and skill and workers with infectious diseases be kept away from working on seed transportation and distribution

[29] Visually examine bags and pallets for signs of possible contamination including insects, water stains, and presence of rodent or bird droppings, or foreign material
[30] Both Mueller and Canadian Code of Practices (2001) recommended testing for *Salmonella*, Shiga toxin-producing *E. coli* and *Listeria monocytogenes* for each seed lot

Production stage	Hazards	Source of hazards	Control Measures	Food Safety Management Practice, Knowledge and Skill Requirements
SPROUT PRODUCTION				
6. Seed receipt and storage	Major[31]: Pathogenic *Salmonella*, Shiga-toxin-producing *E. coli* Minor: Pathogenic microorganisms carried by rodents, pests or infected workers may contaminate seeds. Potential chemical hazards: Seeds may get contaminated by chemicals during seed receipt and storage.	Seeds being contaminated by soil, water, manure, animal faces, rodents, birds during its production, processing and/or transportation and distribution Activities of rodents, pests, and possibly from workers with infectious diseases during seed receipt and storage Chemicals used or stored in the sprout production environment	Sprout producers should obtain from seed producers or distributors, where appropriate, certificates of analysis for microbial pathogens of concerns; sprout producers should examine physical damage and signs of contamination of seeds received; if seed is found to be damaged, contaminated or potentially contaminated, it should not be used for the production of sprouts for human consumption; each seed lot is analysed for the presence of microbial pathogens of concern, these should not be used until results of analysis are available[32 and 33] Seed should be handled and stored in a manner that will prevent damage and contamination Maintain storage facility in a clean and dry condition and inaccessible to pests and other sources of contamination. Sprout producers may obtain assurance from seed producers or distributors that chemical residues of each incoming lot are within the limits established by the relevant authorities and chemicals are stored away from seed storage area. A range of processing aids is permitted for use in foods generally (Standard 1.3.3 of the Food Standards Code). A number of processing aids are specifically listed as approved for use as washing agents in Standard 1.3.3 of the Code	Implementation of HACCP based food safety program and the program be audited at least annually by an accredited third party auditor to ensure compliance with all relevant government regulations and industry guidelines and applicable customer requirements[34] Regular inspection of storage facility using an appropriate device such as a black light stain detector to detect rodent urine stain should be conducted Maintain good records of inventory (reflect product information such as seed supplier, lot number, country of origin) and stock turnover to facilitate recall procedures Workers involved in handling seed receival and storage be trained in hygienic food handling knowledge, skill and practice; and workers with infectious diseases be kept away from working on receiving and handling seed storage Prevent potential chemical contamination on seeds by following appropriate good manufacturing practices (GMP) guidelines Knowledge of permitted processing aids and any maximum permitted levels associated with their use

[31] Contamination from microbial pathogens during seed production, processing, storage, transportation and distribution may have not been picked up by control measures implemented by seed producers, seed processors, or seed suppliers/distributors.
[32] NSW Food Authority (2007) Plant products safety manual. http://www.foodauthority.nsw.gov.au/_Documents/industry_pdf/Plant+Products+Manual.pdf accessed 26 June 2009
[33] Woolworth (2007) WQA Product Category Requirement - Produce, http://www.woolworths.com.au/resources/16.%20wqa%20category%20produce%20june%202007.pdf Accessed 08 September 2009
[34] ANZSA Guidelines, 2008; NSWFA Manual, 2007; FDA, 1999; Department of Health (SA) and Primary Industries and Resources SA (May 2006) Guidelines for the safe production of sprouts in South Australia.

Production stage	Hazards	Source of hazards	Control Measures	Food Safety Management Practice, Knowledge and Skill Requirements
SPROUT PRODUCTION				
7. Microbiological decontamination of seeds and pre-germination soak	Major: Pathogenic *Salmonella* spp, Shiga-toxin-producing *E. coli*	Seeds being contaminated by soil, water, manure, animal faces, rodents, birds during its production, processing and/or transportation and distribution	Seeds be treated prior to the sprouting process to reduce and/or eliminate microbiological pathogens[35]	An effective cleaning and sanitisation program for the sprouting facility be implemented Seed disinfection is conducted effectively and consistently on every batch of seeds by appropriately trained workers using appropriate equipment
	Minor: *Cryptosporidium*, G*iardia* and other water-borne microbial pathogens or parasites	Water used for sprout production	Water used for sprout production be maintained pathogen free	
	Minor: Pathogenic microorganisms carried by rodents, pests or infected workers may contaminate seeds.	Rodent and insect activities, or workers with infectious diseases, or cross contamination	Sprouting facility be constructed and maintained in such a way that it will not allow access of rodents, insects or pests or animals; and storage, seed rinsing and microbiological decontamination, germination and packaging areas be physically separated from each other to prevent cross contamination	Implement HACCP based food safety program incorporating good manufacturing practices, good hygiene practices in seed handling, microbiological decontamination of seeds and pre-germination soak to prevent potential biological, chemical and physical hazards from contaminating seeds
	Potential chemical hazards: Chemicals used at sprout production facility may contaminate seed sprouts.	Chemicals or waste at sprout production facility	Only registered chemicals are used for their intended purpose; chemicals are stored away from microbiological decontamination area and pre-germination soak area.	
	Potential physical hazards: Seeds may get contaminated by physical hazards during seed disinfection and germination soak.	Metal, glass, wood, plastics etc	Take appropriate procedures to prevent physical contamination to occur	Workers involved in sprout production be appropriately trained in hygienic food handling knowledge, skills and practice; and workers with infectious diseases be kept away from working on seed disinfection and pre-germination soak

[35] Due to the difficulty of obtaining seeds which can be guaranteed as pathogen free, it is necessary to treat the seeds prior to sprouting to reduce and/or eliminate microbial pathogens such as *Salmonella* and Shiga toxin-producing *E. coli* and others. The process involves an initial rinse of seeds before microbiological decontamination treatment to remove dirt and increase the efficiency of the decontamination treatment; and appropriate rinse after seed decontamination treatment to reduce disinfectant to an appropriate level. Microbiological decontamination of seeds may follow a risk based category approach where small seeds like alfalfa seeds are treated with a high level of active disinfectant, and large seeds like mung bean seeds be treated with a low level of active disinfectant, see NSWFA Manual (2007) and ANZSA Guidelines (2008) for further information.

Production stage	Hazards	Source of hazards	Control Measures	Food Safety Management Practice, Knowledge and Skill Requirements
SPROUT PRODUCTION				
8. Germination and growth	Pathogenic *Salmonella* and Shiga toxin-producing *E. coli*, *Bacillus cereus*, *Listeria monocytogenes*, *Yersinia*, and protozoa like *Cryptosporidium* and G*iardia*[36]	Seeds, equipment, water, medium supporting sprout growth, such as unsterilised soil, or other inputs, such as nutrients supporting sprout germination or growth	Sprouting equipment and water be maintained pathogen free Sprouting facility be constructed and maintained in such a way that it will not allow access of rodents, insects or pests or animals Solid media to support sprout growth be appropriately treated to eliminate microorganisms that are pathogenic to human Sprout producer should have in place a sampling/testing plan to regularly monitor for microbial pathogens at one or more stages after the start of germination. The analysis can be performed on spent irrigation water or on finished sprouts[37]	Implement GMP, good hygien practices (GHP) and a food safety program to prevent microbial pathogens contaminating workplace, seeds and seed sprouts Disrupt the chance of formation of large microbial populations by implementing a frequent rinse program in sprout growth Implement an appropriate microbial testing program for equipment, water, spent water, sprouts and others to monitor possible contamination from microbial pathogens
	<u>Potential chemical hazards</u>: Chemicals used at sprout production facility may contaminate seed sprouts.	Chemicals or waste at sprout production facility	Only registered chemicals are used for their intended purpose; chemicals are stored away from seed germination and sprout growth area	Develop an appropriate corrective procedure in case of positive and doubtable testing outcome to contain the contamination and to prevent contaminated sprouts from reaching consumers
	<u>Potential physical hazards</u>: Seeds may get contaminated by physical hazards during seed germination and growth.	Metal, glass, wood, plastics etc	Take appropriate procedures to prevent physical contamination to occur	Maintain food operation records Workers involved in sprout production be appropriately trained in hygienic food handling knowledge, skills and practice; and workers with infectious diseases be kept away from working on seed germination and growth

36 Current seed treatments, i.e. microbiological decontamination of seeds cannot guarantee total elimination of microbial pathogens. If even a few cells of microbial pathogens survive the microbiological decontamination treatment or originate from contaminated equipment or contaminated water or growth media, they can grow to high numbers during seed germination and sprout growth phase because of the favourable growth environment and conditions where water, nutrients and temperature are desirable for microbial activity (Codex, 2003).

37 NSWFA Manual, 2004; ANZSA Guidelines, 2008; Codex, 2003; FDA, 1999; Canadian Code of Practice, 2001; Woolworth WQA Product Category Requirement, 2007; SA Guidelines, 2006

Production stage	Hazards	Source of hazards	Control Measures	Food Safety Management Practice, Knowledge and Skill Requirements
SPROUT PRODUCTION				
9. Harvest, washing/drying/ cooling/packing/ storage	Pathogenic *Salmonella* and Shiga toxin-producing *E. coli*, *Bacillus cereus*, *Listeria monocytogenes*, *Yersinia*, and protozoa like *Cryptosporidium* and G*iardia*[38]	Water, packing material, workers with infectious disease, cross contamination from a previously contaminated batch	Implementation of an effective cleaning and sanitisation program for the equipment and facilities used and an effective pest and rodent control program to prevent microbial contamination A final water rinse, and where appropriate rinse with cold water, should be implemented to reduce sprout temperature and slow down microbial growth Where appropriate, sprouts should be kept under cold temperature (e.g. 5^{0}C) to minimise microbial growth Sprout producers should monitor the temperature of storage areas regularly and effectively Sprout producers should have in place a sampling/testing plan to regularly monitor for microbial pathogens at one or more stages after the start of germination. The analysis can be performed on spent irrigation water or on finished sprouts[39]	Implement good manufacturing practice, good hygiene practice and a food safety program for sprout harvest, wash, dry, cooling, packing and storage to prevent potential biological, chemical and physical hazards (such as glass, metal, wood, plastic, and others) from contaminating sprouts Minimise microbial growth by ensuring sprouts after harvest are cooled down rapidly and maintained at refrigerated temperature Maintain good operational records to enable rapid and accurate responses to traceability investigations
	<u>Potential chemical hazards</u>: Chemicals used at sprout production facility may contaminate seed sprouts.	Chemicals or waste at sprout production facility	Only registered chemicals are used for their intended purpose; chemicals are stored away from sprout harvest, washing, drying, cooling, packing and storage area	Workers involved in sprout production be appropriately trained in hygienic food handling knowledge, skills and practice; and workers with infectious diseases be kept away from working on seed germination and growth
	<u>Potential physical hazards</u>: Seeds may get contaminated by physical hazards during sprout harvest, wash, dry, cooling, packing, or storage.	Metal, glass, wood, plastics etc	Take appropriate procedures to prevent physical contamination from occurring	

[38] Current seed treatments, i.e. microbiological decontamination of seeds cannot guarantee total elimination of microbial pathogens. If even a few microbial pathogens survive the microbiological decontamination treatment or from contaminate equipment or contaminated water or growth media, they can grow to high numbers during seed germination and sprout growth phase because of the favourable growth environment and conditions where water, nutrients and temperature are desirable for microbial activity (Codex, 2003).

[39] (NSWFA Manual, 2007; ANZSA Guidelines, 2008; Codex, 2003; FDA, 1999; Canadian Code of Practice, 2001; Woolworth WQA Product Category Requirement, 2007; Food Standards Code 1.6.1

Production stage	Hazards	Source of hazards	Control Measures	Food Safety Management Practice, Knowledge and Skill Requirements
SPROUT PRODUCTION				
10. Transportation and distribution	Pathogenic *Salmonella* and Shiga toxin-producing *E. coli*, *Bacillus cereus*, *Listeria monocytogenes*, *Shigella* and others	Inadequate temperature control during transportation and distribution	Regular and effective monitoring of temperature of transport vehicles should be carried out and sprout producers must ensure sprouts are distributed at 5^0C or below	Implement good food transportation and distribution system and practices including good cold chain management practices to prevent sprouts from contamination of pathogenic microorganisms, chemical hazards and physical hazards, and to ensure sprouts transported and distributed for human consumption remain safe and suitable for human consumption upon delivery Maintain good records of transportation vehicles, delivery and distribution Provide the purchaser appropriate compliance analysis that shows that key microbial pathogens and/or indicator organisms comply with regulatory or contractual microbiological specification[40] Workers involved in sprout transportation and distribution are trained to be aware of the likelihood of biological, chemical and physical hazards contaminating sprouts and ways to prevent the occurrence of such contamination during sprout transport and distribution
		Cross contamination due to other material being co-transported	Vehicle transporting sprouting should not transport goods at the same time that may contaminate the seeds	
		Rodent and pest activities	Implementation of an effective cleaning and sanitisation program for the equipment and facilities used and an effective pest and rodent control program to prevent microbial contamination	
		Workers who carry an infectious disease	Workers with infectious diseases be kept away from working on sprout transport and distribution	
	<u>Potential chemical hazards</u>: Chemicals used for cleaning, pest control, disinfection or temperature control may contaminate sprouts during transport and distribution.	Chemicals used for cleaning, pest control, disinfection or temperature control	Only registered chemicals are used for their intended purpose	
	<u>Potential physical hazards</u>: Sprouts may get contaminated by physical hazards during sprout transportation and distribution.	Metal, glass, wood, plastics etc	Take appropriate procedures to prevent physical contamination to occur	

[40] Woolworth WQA Product Category Requirement, 2007; FreshSpec - Produce specification for sprouts, 2007 (The Australian Chamber of Fruit and Vegetable Industries Ltd (2007) FreshSpec – Produce Specifications – Sprout Specifications, http://www.freshmarkets.com.au/FreshSpec/SproutsSpecifications.html, accessed 7 July 2009)

Production stage	Hazards	Source of hazards	Control Measures	Food Safety Management Practice, Knowledge and Skill Requirements
Post-production				
11. Retail handling (storage, handling, display and sale at retail outlets)	Pathogenic microorganisms like *Listeria monocytogenes*, or norovirus Possible chemical or physical hazards	Workers who handle the product with an infectious disease Presence of insects, pests, rodents Contact with dirty surfaces at retail outlets	Workers who have an infectious disease that may be transmitted via food are kept away from handling sprouts Implementation of an effective cleaning and sanitisation program for the equipments and facilities used and an effective pest and rodent control program to prevent microbial contamination Workers involved in sprout production are appropriately trained in hygienic food handling knowledge, skills and practice Only registered chemicals are used for their intended purpose during fresh produce retail sale and handling. Surfaces intended for contact with sprouts be kept clean.	Implement good retail food safety practices to prevent contamination or cross contamination of sprouts from biological, chemical and physical hazards

Attachment 3: Microbiological Hazards Associated with Seed Sprouts

1. ***Outbreaks of food-borne illness associated with seed sprouts***

Between 1988 and 2008 there have been over 40 reported outbreaks worldwide attributed to consumption of contaminated seed sprouts (Appendix 1). The most commonly reported aetiological agents in these outbreaks have been various serovars of *Salmonella* spp. and enterohaemorrhagic *E. coli* (EHEC). *Bacillus cereus* and *Yersinia enterocolitica* have also been responsible for outbreaks of food-borne illness associated with seed sprouts (Cover and Aber, 1989; Portnoy *et al*., 1976). Alfalfa and mung bean sprouts have been the most commonly reported seed sprouts implicated in outbreaks of food-borne illness.

The majority of sprout-associated outbreaks have been reported in the United States, however, outbreaks have also occurred in Canada, Sweden, Finland, Denmark, United Kingdom, Japan and Australia. The largest reported outbreak occurred in Japan in 1996, with over 10,000 notified cases and was attributed to consumption of radish sprouts contaminated with *E. coli* O157:H7 (Michino *et al*., 1999; Watanabe *et al*., 1999).

An outbreak due to *S.* Oranienburg occurred in Western Australia during November 2005-January 2006 that was epidemiologically linked to consumption of alfalfa sprouts. This was later confirmed microbiologically, with *S.* Oranienburg being isolated from the implicated alfalfa sprouts. A total of 125 cases of salmonellosis were reported, resulting in 11 hospitalisations.

In May 2006, another outbreak of *S. Oranienburg* was reported in Victoria, with a total of 15 cases attributed to consumption of alfalfa sprouts. In the outbreak, *S.* Oranienburg was isolated from the implicated alfalfa sprouts as well as from seed obtained from the sprouting facility. Molecular typing of the *S. Oranienburg* isolates from both the Victorian and Western Australian outbreaks showed indistinguishable patterns by pulsed field gel electrophoresis (Pers Comm. Martyn Kirk, OzFoodNet 23 April 2009). Trace back of seeds associated with these outbreaks found that the seed originated from the same Australian state but from different seed suppliers.

A number of contributing factors have been identified in reported sprout-associated outbreaks. In the US, a multi-state outbreak of *S. Mbandaka* associated with alfalfa sprouts occurred in Oregon, Washington, Idaho and California in 1999 with 89 confirmed cases (Gill *et al*., 2003). The cases of salmonellosis were linked to two geographically separate sprout growers. A single lot of contaminated seed was identified which was used by five sprout growers during the outbreak period. Onsite investigations of the sprouting facilities associated with cases of salmonellosis identified that no form of seed sanitising was being employed prior to sprouting. No cases of illness were linked to the other three sprout growers that used the same lot of seed, all of whom employed seed sanitisation (20,000 ppm $Ca(OCl)_2$ or 500 ppm NaOCl).

However, there have been reports of sprout-associated outbreaks where seed sanitising has been undertaken (Brooks *et al*., 2001; Gill *et al*., 2003; Proctor *et al*., 2001). A multi-state outbreak of *E. coli* O157:NM associated with alfalfa sprouts occurred in Minnesota and Colorado in 2003 (Ferguson *et al*., 2005). Trace-back investigations identified a common seed distributor who supplied seed (originally sourced from Australia) from the same lot to both implicated sprout growers. During the on-site inspection of the Minnesota sprouting facility, a number of issues were identified that may have contributed to the outbreak including: use of lower hypochlorite concentration for seed disinfection than that

recommended by FDA (15,000 ppm rather than the recommended 20,000 ppm for 15 min), inadequate agitation of disinfection solution, and weekly testing of spent irrigation water rather than by production lots as recommended. No deficiencies were identified at the sprouting facility implicated in the Colorado outbreak. *E. coli* O157 was not detected from samples taken at the sprouting facility, although a sample of the seed implicated in the outbreak was not available for testing.

Another multi-state alfalfa sprout-associated outbreak occurred in the US in 1999, in which there were 157 reported cases of *S. Munchen* (Proctor *et al*., 2001). Outbreaks were epidemiologically, and microbiologically, linked to sprouting facilities that sanitised seed in 20,000 ppm calcium hypochlorite ($Ca(OCl)_2$) for 15 min prior to sprouting. These outbreaks illustrate that employing seed sanitising in isolation may not reliably prevent cases of food-borne illness from occurring.

2. Reported prevalence and levels of pathogens/microorganisms in seed sprouts

Microbiological surveys of seed sprouts, both domestically and internationally, have identified the presence of a variety of food-borne pathogens including *Salmonella* spp., EHEC, *B. cereus*, *Cryptosporidium* spp. and *Giardia* spp (Beuchat, 1996; Kim *et al*., 2004; Prokopowich and Blank, 1991; Robertson *et al*., 2002; Samadpour *et al*., 2006).

In Australia, the Health Department of Western Australia undertook a microbiological survey of seed sprouts between January to March 2000 (Department of Health WA, 2002). In total, 261 sprouts (pre-packed and bulk) were sampled and included alfalfa, mung bean, bean sprouts, sunflower sprouts, snow pea shoots, onion sprouts and "other sprouts". Samples were assessed as being acceptable/unacceptable based on whether they exceeded set criteria for total plate count (TPC), coliforms, *L. monocytogenes*, *Salmonella* spp., *E. coli*, *B. cereus* and coagulase-positive staphylococci. Of the pathogens tested, *L. monocytogenes* was detected in eight samples (at levels <5 cfu/g), and *Salmonella* spp. was detected in one sample (Table 1). *E. coli* was detected at >100 cfu/g in seven samples, however, none of these isolates were found to be toxigenic. Coagulase-positive staphylococci was detected at levels >100 cfu/g in two samples of sprouts.

Table 1: Summary of results from WA survey of sprouts at retail (Department of Health WA, 2002)

Sprout type	n	*E. coli* ≥100 cfu/g (%)	Coagulase +ve Staph. ≥100 cfu/g (%)	*B. cereus* ≥100 cfu/g (%)	*Salmonella* (%)	*L. monocytogenes* (%)
Alfalfa	110	3 (2.7)	-	-	-	6 (5.5)
Bean sprout	42	-	-	1 (2.4)	-	-
Mung bean	20	-	-	-	-	1 (5.0)
Onion sprout	7	-	-	1 (14.3)	-	1 (14.3)
Snow pea	57	2 (3.5)	1 (1.8)	2 (3.5)	-	-
Sunflower	9	-	-	-	-	-
Other sprouts	13	2 (12.5)	1 (6.3)	3 (6.3)	1 (6.3)	-
Total	261	7 (2.7)	2 (0.8)	7 (0.4)	1 (0.4)	8 (3.1)

A similar survey was conducted in the ACT during April to June 2001, where 62 samples of various seed sprouts were analysed for *E. coli*, coagulase-positive staphylococci, *B. cereus*, *Salmonella* spp. and *L. monocytogenes* (Millard and Rockliff, 2001). Again, samples were classified as satisfactory, marginal, unsatisfactory or potentially hazardous based on the level of organisms detected. *Salmonella* spp. and *L. monocytogenes* was not detected in any of the samples. *E. coli* was detected in 11 samples (11.1%), with only one of these samples (mung bean sprouts) having over 100 cfu/g. Testing for pathogenic strains of *E. coli* was not undertaken. Coagulase-positive staphylococci was detect in one sample of snow pea shoots

at levels deemed 'potentially hazardous', with >10^4 cfu/g, however, on further testing of a sample from the same manufacturer and retail outlet (although different lots), levels were considered satisfactory i.e. <100 cfu/g. For *B. cereus*, two samples were positive with ranges of 50-150 cfu/g reported.

Results from microbiological surveys of sprouts undertaken by the NSW Food Authority prior to, and following, the implementation of the NSW Plant Products Food Safety Scheme also demonstrate that sprouts are occasionally contaminated with pathogenic micro-organisms (NSWFA, 2008). In a limited sample of 30 seed sprout products in 2005, no *L. monocytogenes* or *Salmonella* spp. were detected, while *E. coli* was detected at levels <100 cfu/g in two samples. In 2006 (n=36), five samples of seed sprouts tested positive for *E. coli*, with two of these samples containing >100 cfu/g sample. One sample of broccoli sprouts tested positive for verotoxin-producing *E. coli* (VTEC) and was therefore rated as potentially hazardous. The survey was expanded in 2008 to a total of 122 samples of seed sprouts and included testing for *B. cereus*. Overall, no *L. monocytogenes*, *Salmonella* spp. or *E. coli* was detected. *B. cereus* was detected at levels of 100 – 1000 cfu/g in four samples and at 5500 cfu/g in one sample.

The National Enteric Pathogen Surveillance Scheme (NEPSS) collects data on isolates of *Salmonella* spp. and other pathogens submitted by primary diagnostic laboratories throughout Australia. Between 2000 and 2005 there were 13 *Salmonella* spp. isolates from seed sprouts submitted to laboratories (Table 2). While this data does not provide information on the prevalence of *Salmonella*-contaminated seed sprouts in Australia (only reports positive samples) it does give information on the range of *Salmonella* serovars associated with these products.

Table 2: *Salmonella* isolates from seed sprouts, NEPSS data 2000 – 2005

Year	Sample	Number of isolates	Serovar
2000	Sprouts	1	*S.* Zanzibar
2001	Lucerne seeds	1	*S.* Saintpaul
2002	-	-	
2003	Sprouts	8	*S.* Agona; *S.* Chester; *S.* Choleraesuis bv Kunzendorf Australia; *S.* Havana (2); *S.* Oranienburg; *S.* Orion; *S.* subsp I ser 16:1,v:-
	Mustard seed	1	*S.* subsp IIIa ser 38:z53:-
2004	Sprouts	1	*S.* Oranienburg
2005	Bean sprouts	1	*S.* Infantis

As summarised by Harris *et al*. (2003), numerous international surveys have also detected pathogens in seed sprouts. It is difficult, however, to directly compare results between surveys due to differences in the number and type of samples analysed, the stage of production where samples were taken, and the methodologies used to isolate and/or enumerate the organisms.

In a microbiological survey of seed sprouts in Norway, Robertson *et al*. (2002) detected *Cryptosporidium* and *Giardia* from 9% and 2% samples respectively of mung bean sprouts (n=149), with reported level levels of 2-6 oocysts/100 g sprouts. *Cryptosporidium* and *Giardia* are highly infectious, with ingestion of one oocyst being considered sufficient to cause illness in humans (US FDA, 2008). Protozoan parasites were also detected in six out of eight 100 g samples of unsprouted mung bean seeds – all of the positive samples contained *Cryptosporidium* (range 1-5 oocysts/100 g), with three samples also being positive for *Giardia* (1 cyst/100 g). *Cryptosporidium* was also detected in one sample of radish sprouts (n=6). No *Cryptosporidium* or *Giardia* was isolated from alfalfa sprouts, however the sample size was very low (n=6).

3. Potential pathogen contamination during seed production

While there is little specific data in the scientific literature on how seeds used for sprouting become contaminated with microbiological pathogens during on-farm production, and the relative contribution of potential sources of contamination, epidemiological investigations suggest contaminated seed is the likely source of most, if not all, sprout-associated outbreaks.

Grazing animals such as cattle and sheep are known reservoirs of *Salmonella* spp. and EHEC and infected animals may shed large numbers of these organisms in their faeces. A number of studies have shown that these pathogens can persist in animal faeces for significant periods of time. For example in Ireland, Bolton *et al*. (1999) found that when bovine faeces inoculated with *E. coli* O157:H7 (initial concentration of approximately 10^8 CFU/g) was applied to grassland, numbers of organisms reduced by 4-5 $\log_{10}$ within 50 days, however *E. coli* O157:H7 could still be recovered from surrounding soil for up to 99 days (the duration of the study period). In a similar study undertaken in New Zealand, Sinton *et al*. (2007) observed that the time required for a 1-$\log_{10}$ (90%) decrease in numbers of *Salmonella* spp in bovine faeces during summer was 58 days. Survival of pathogens in the environment is extremely complex and is affected by many factors such as temperature, intensity of sunlight (UV), and moisture, hence inactivation of pathogens in animal faeces would vary significantly between geographic regions depending on environmental conditions.

Attachment of pathogens to seeds

Few studies have investigated the mechanisms by which seeds become associated with human pathogens such as *Salmonella* spp. and EHEC. Theoretically, seeds can become contaminated at any stage of production – from while they are being formed through to immediately prior to sprouting.

Pathogens may be able to enter seeds by a variety of routes such as the vascular system, pollen germ tube and the dorsal suture of the silique (seed pod) or hilum of the mature seed, (Delaquis *et al*., 1999; Harman, 1983; Mundt and Hinkle, 1976; Thayer *et al*., 2003). Cracks or openings in the seed coat increases the opportunity for bacterial attachment, and may enhance the potential for penetration into the seed (Charkowski *et al*., 2001; Fett, 2006b; Wade *et al*., 2003). The prevalence of seeds with cracks or other imperfections is highly variable, with rates of 3-85% being reported in alfalfa seeds (Wade *et al*., 2003). Cooley *et al*. (2003) also demonstrated that pathogens can become associated with seed via direct contact with contaminated material such as chaff.

Cooley *et al*. (2003) found that following inoculation of the roots and shoots of thale cress (*Arabidopsis thaliana*) with *S.* Newport and *E. coli* O157:H7, pathogens were able to be recovered from the flowers and seeds of the mature plants. Both pathogens were found to persist longer on plants grown gnotobiotically (sterile agar) compared to those grown in sterile and non-sterile soil respectively. This suggests the role of competing microflora in reducing the colonisation and persistence of the pathogens tested. Pathogens were detected deep within the primary root system, but not in the vasculature (bacteria were not detected systemically throughout the plant). The authors concluded that movement of pathogens to flowers and seed was therefore most likely via the plant exterior i.e. epiphytically. Results from other studies, however, have shown that *Salmonella* spp. and *E. coli* have the ability to enter plant tissue and move through the vascular system (Dong *et al*., 2003; Itoh *et al*., 1998).

Prevalence and levels of pathogens in seed

Very little data is available on the prevalence and levels of *Salmonella* and EHEC in seed destined for sprouting. This is, in some part, due to limitations in methodologies to isolate bacterial pathogens from contaminated seeds, where contamination is usually sporadic, at low levels and non-uniformly distributed within a sample (Inami and Moler, 1999; Stewart *et al*., 2001b; Wu *et al*., 2001). Reported levels of pathogens from naturally contaminated seeds range from 0.7 MPN/kg to 100 MPN/kg (Fu *et al*., 2008; NACMCF, 1999b). The level of contamination in two lots of alfalfa seed associated with an outbreak of *S. Munchen* in the US in 1999 was reported to be 16.2 ± 1.9 MPN/kg (n=5) and 13.2 ± 3.5 MPN/kg (n=3) (Fu *et al*., 2008).

Seed cleaning/processing

There are many opportunities for cross contamination to occur during seed processing. This may be via contact with contaminated material such as chaff (stalks etc) or with equipment that has residual contamination from previous contaminated lots (NACMCF, 1999a). Rodents, birds and other pests can harbour *Salmonella* and EHEC, and may therefore be a source of contamination if allowed access to the seed.

Seeds are sometimes scarified during processing to assist germination. Scarification involves intentionally damaging the seed coat. It has been suggested that this may provide additional sites for pathogens to attach to, and enter the seed, and potentially be protected from exposure to sanitising agents (Fett, 2006b). Results from studies investigating the efficacy of chemical treatments on reducing levels of pathogens from scarified and non-scarified seeds have been inconclusive. Holliday *et al*. (2001) found that there was a reduction in the efficacy of sanitising using scarified alfalfa seeds from one supplier compared to control (non-scarified) seeds, although this effect was not observed using seeds from a second supplier.

Survival of *Salmonella* and EHEC on contaminated seeds

Studies have demonstrated that once seeds are contaminated, *Salmonella* and EHEC can survive for long periods of time under normal seed storage conditions (Jaquette *et al*., 1996; Taormina and Beuchat, 1999). In a study by Jaquette *et al*. (1996) using artificially contaminated alfalfa seeds, populations of *S. Stanle*y were found to decrease by approximately 0.7-$\log_{10}$ during storage at 8°C for 9 weeks. Storage of seed at 8°C for one week and then 21°C for 8 weeks resulted in reduction in of *S. Stanley* from initial levels of 339 cfu/g to 8 cfu/g (1.6- $\log_{10}$ reduction). Taormina *et al*. (1999) investigated the survival of *E. coli* O157:H7 on artificially contaminated alfalfa seeds (initial concentration of approximately 10^3 cfu/g), whereby the pathogen could be recovered by enrichment from 25 g samples of seed after storage for 38 weeks at either 25 or 37°C. When seed was stored at 5°C, populations of *E. coli* O157:H7 remained relatively stable during the study period of 54 weeks.

Seed sanitising

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. Consensus amongst the scientific literature is that sanitising reduces, but does not necessarily eliminate pathogens from contamination seed. Statistical analyses

of published seed sanitisation studies to reduce levels of *Salmonella* and EHEC have revealed a high degree of variability in the results (Montville and Schaffner, 2004).

A summary of published studies into the efficacy of physical and chemical treatments for reducing levels of microbial pathogens from seeds is provided in Appendix 2. There are very few disinfection treatments that consistently achieve a substantial (i.e. > 5-$\log_{10}$) reduction in pathogen numbers. Some disinfection treatments can have a negative effect on seed germination, which needs to be considered when determining the most appropriate method of treatment. In a review of published studies on reductions of *Salmonella* and *E. coli* O157:H7 levels in seeds treated with 20,000 ppm calcium hypochlorite ($Ca(OCl)_2$), Montville and Schaffner (2004) found that the most likely level of inactivation (mode) was 2.5 $\log_{10}$, with a range of 1.0 – 6.5 $\log_{10}$. Chemical sanitisers, such as chlorine, have reduced efficacy against naturally resistant pathogens such as oocysts of *Cryptosporidium* and *Giardia*, and bacterial spores (Venczel *et al*., 1997).

The variability in reported efficacy of seed sanitisation may be due to a range of factors such as differences in: the initial pathogen load on contaminated seeds (either naturally or artificially contaminated), physiological status of the test microorganism (fresh laboratory cultures versus environmentally stressed micro-organisms), type and condition of the seed, treatment time and concentration of active compound, use of buffers, agitation of seeds during treatment and methods used to detect pathogens (e.g. direct plating on selective media versus enrichment in broth). While the same disinfection treatment may have been used in published studies, protocols for the application of the treatment and conditions for the growing of the sprouts often vary, which may affect the results observed.

As previously discussed, pathogens may be protected from chemical sanitising agents due to their location in cracks or other openings in the seed coat, or incorporation into biofilms (Fett, 2006b). Results from a study by Chrkowski *et al*. (2001) found that the efficacy of alfalfa seed sanitisation with $Ca(OCl_2)$ varied significantly between different seed lots. When separated based on seed characteristics, it was observed that wrinkled alfalfa seeds had higher levels of total aerobic bacteria and were more difficult to sanitise compared with smooth seeds. When seeds were artificially inoculated with *S.* Newport and then treated with $Ca(OCl)_2$, no *Salmonella* was recovered from smooth seeds however > 10^3 CFU/seed was recovered from wrinkled seeds. However, when sanitised seeds were sprouted, *Salmonella* was recovered from batches grown from both smooth and wrinkled seed. This highlights the limitations of sanitising treatments in eliminating pathogens such as *Salmonella* from contaminated seed.

4. Potential pathogen contamination during sprout production

Germination/growth of sprouts

As previously discussed, seed has been identified as the likely source of contamination in many reported outbreaks of food-borne illness associated with seed sprouts. Other possible sources of contamination during the sprouting process include water, pests, growing medium (e.g. soil).

Regardless of sprouting method, studies have demonstrated the growth of *Salmonella* and *E. coli* O157:H7 during the germinating process, with increases of 2–5 $\log_{10}$ within 48 hours being reported in the literature (Charkowski *et al*., 2002; Fu *et al*., 2008; Gandhi *et al*., 2001; Howard and Hutcheson, 2003; Liao, 2008; Liu and Schaffner, 2007; Montville and Schaffner,

2005; Palmai and Buchanan, 2002; Pao *et al*., 2005; Stewart *et al*., 2001a; Stewart *et al*., 2001b).

Methodologies used to investigate the growth of pathogens during the sprouting process vary widely, which may affect observed rates of growth. For example, studies have used naturally or artificially contaminated seed. For seeds that have been artificially contaminated prior to sprouting, there are differences in the methods used to inoculate the seed e.g. growth phase of the bacterial culture used, length of time and conditions in which inoculated seeds are dried before sprouting, and differences in the inoculum size. If the initial inoculum level in seeds is high (>10^4 cfu/g), a reduced potential for growth may be observed as levels may quickly reach the maximum population density (Montville and Schaffner, 2005). The probability of naturally contaminated seeds having levels of contamination as high as this is also extremely low. Stewart *et al*. (2001a) found that the maximum level of *E. coli* O157:H7 reached in alfalfa sprouts grown from seeds with low (1.9 $\log_{10}$ cfu/g) or high (3.9 $\log_{10}$ cfu/g) inoculums was 5–6 $\log_{10}$ cfu/g.

Methods used to sprout seeds in the laboratory often differ from that used commercially. For example in the laboratory small volumes of seeds may be sprouted in glass jars or “mini-drums”, with different methods and/or frequencies of irrigation utilised. Fu *et al*. (2008) demonstrated that irrigation frequency significantly affected the level of growth of *Salmonella* in alfalfa sprouts grown using a small-scale rotating drum. Decreasing the irrigation frequency from 20 minutes every 2 h to 20 min every 4 h resulted in a 2-$\log_{10}$ increase in levels of *Salmonella*. Increasing the temperature during sprouting from 20°C-30°C also resulted in a 2-$\log_{10}$ increase in *Salmonella* levels.

Pao *et al*. (2005) undertook a study to investigate the growth of *B. cereus* during the production of different sprout types. Results for sprouts grown in glass jars using naturally contaminated seed showed that levels of *B. cereus* increased by > 5 $\log_{10}$ for radish and broccoli sprouts, however no significant growth was observed for alfalfa, lentil or mung bean sprouts. When seeds were sprouted using a “home-sprouting” drum with automatic watering, levels of *B. cereus* increased by 3 $\log_{10}$ in radish, broccoli and mung bean sprouts, with no growth observed in alfalfa or lentil sprouts.

A number of studies have shown that *Salmonella* spp. and *E. coli* O157:H7 can become internalised in the tissue of seed sprouts during germination (Itoh *et al*., 1998; Warriner *et al*., 2003). In a study by Itoh *et al*. (1998) *E. coli* O157:H7 was found to be attached to the inner tissue and stomata of cotyledons as well as the outer surface of radish sprouts. *E. coli* O157:H7 was isolated from sprouts after being surface-sterilised with mercuric chloride ($HgCl_2$), further suggesting internalisation. Warriner *et al*. (2003) also demonstrated the ability of *E. coli* and *Salmonella* spp. to become internalised in mung bean sprouts during germination. Treating the sprouts with 20,000 ppm sodium hypochlorite removed the majority of bacteria from the surface of hypocotyls, although viable organisms were recovered from the sprout tissue.

Microorganisms can also become incorporated into biofilms on the sprout surface (Fett, 2000; Fett and Cooke, 2003b; Warriner *et al*., 2003). Biofilms are a complex structure of microorganisms adhered to a surface (usually inert) and encapsulated in self-produced extracellular material such as exopolysaccharides, lipids and proteins. This structure provides protection to microorganisms from antimicrobial agents such as chemical sanitisers (Costerton *et al*., 1995).

When viewing alfalfa, broccoli, clover and sunflower sprouts by scanning electron microscopy, biofilms have been found to be most abundant on the cotyledon surface compared with hypocotyls and roots (Fett, 2000).

Several studies have shown that levels of bacterial pathogens in spent irrigation water during the germinating process is strongly correlated to levels found in the contaminated seed sprouts (Costerton *et al*., 1995; Howard and Hutcheson, 2003; Johnston *et al*., 2005; Liu and Schaffner, 2007; Stewart *et al*., 2001b). In an analysis of published data, Montville and Schaffner (2005) found that the average concentration of pathogens was slightly higher in sprouts than in spent irrigation water (mean 0.5 $\log_{10}$, range -0.75-2.25 $\log_{10}$). Liu and Schaffner (2007) demonstrated that *S.* Stanley could be detected in irrigation water within 12 hours of germination of alfalfa sprouts.

Various treatments have been studied to reduce the levels of pathogens either during the sprouting process or post harvest. These treatments include the use of chemical agents, competitive exclusion (e.g. plant-associated pseudomonads), bacteriophages and irradiation (Fett, 2002c; Fett, 2006a; Kocharunchitt *et al*., 2009; Rajkowski and Thayer, 2000). To date, however, most of these treatments have not been shown to result in consistent levels of pathogen reduction. This may be due to a number of factors such as protection of microbial pathogens in biofilms, or internalisation into the sprout tissue. One method that has been found to reduce levels of pathogens in sprouts is treatment by irradiation, with a minimum gamma radiation dose of 0.5 kGy shown to be effective in eliminating *Salmonella* from naturally contaminated alfalfa sprouts (Rajkowski and Thayer, 2000).

Sprout harvesting

Contamination of seed sprouts can also occur during harvesting. Possible sources of contamination include equipment, rinse waters and workers themselves. Gandhi *et al*. (2001) found that the transfer of *Salmonella* to non-contaminated sprouts via hands directly after harvesting contaminated sprouts (7.9 $\log_{10}$ cfu/g) was approximately 5 $\log_{10}$ cfu/g. While the level of *Salmonella* in the contaminated sprouts used in this study is considered higher than that observed under commercial conditions, it demonstrates the potential for pathogens to be transferred between batches of seed sprouts during harvesting.

Depending on the type of seed sprout being produced, they are often rinsed prior to packaging. While studies have demonstrated that there is limited/no reduction in pathogens from rinsing sprouts in water containing sanitisers, there is an opportunity for significant cross contamination if wash/rinse water becomes contaminated.

5. Retail/consumer

Once packaged, sprouts are generally stored under temperature control (<4°C) to limit the growth of microorganisms. Gandhi *et al*. (2001) found that levels of *S.* Stanley in contaminated alfalfa sprouts reduced slightly (0.09 $\log_{10}$) when stored at 4°C. In the survey of seed sprouts at retail undertaken by WA Department of Health in 2001, 78% (203/261) of samples were stored under refrigeration. Of the refrigerated samples, 75% (154 samples) were recorded to have temperatures above 10°C.

Most seed sprouts are consumed raw and therefore will not receive any form of heat treatment prior to consumption (which would inactivate pathogens if present).

APPENDIX 1

Reported sprout-associated outbreaks (adapted from Taormina *et al*., 1999)

Year	Pathogen	No. of culture-confirmed cases	Location	Type of sprout	Likely source of contamination	Reference
1988	*S.* Saintpaul	143	United Kingdom	Mung bean	Seed	(O'Mahony *et al*., 1990)
1988	*S.* Saintpaul, *S.* Havana, *S.* Muenchen	148	Sweden	Mung bean		
1988	*S.* Virchow	7	United Kingdom	Mung bean		(O'Mahony *et al*., 1990)
1989	*S.* Gold-Coast	31	United Kingdom	Cress	Seed and/or sprouter	(Joce *et al*., 1990)
1990	*S.* Anatum	15	US (Washington)	Alfalfa		(CDC, 1990)
1992	*S. enterica* 4,5,12:*b*:-	272	Finland	Mung	ND	(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 6 US States	Alfalfa	Seed	(Mahon *et al*., 1997)
1995-1996	*S.* Newport	>133	7 US States Canada Denmark	Alfalfa	Seed	(van Beneden *et al*., 1999)
1996	*S.* Stanley	30	US (Virginia)	Alfalfa	Seed	(Barret and Chaos, 1996; CDC, 1996)
1996	*E. coli* O157:H7	>10,000	Japan	Radish	Seed	(Watanabe *et al*., 1999)
1996	*S.* Montevideo and *S.* Maleagridis	~500	California, Nevada (USA)	Alfalfa	Seed and/or sprouter	(Mohle-Boetani *et al*., 2001; NACMCF, 1999a; Taormina *et al*., 1999)
1997	*S.* Anatum and *S.*Infantis	109	Kansas, Missouri (USA)	Alfalfa	Seed	(Taormina *et al*., 1999)
1997	*E. coli* O157:H7	79	4 US States	Alfalfa	Seed	(Breuer *et al*., 2001)
1997	*S.* Meleagridis	78	Canada	Alfalfa	Seed	(Sewell and Farber, 2001)
1997-1998	*S.* Senftenberg	60	US (California, Nevada)	Alfalfa	Seed and/or sprouting drum	(Mohle-Boetani *et al*., 2001)
1998	*S.* Havana/*S.* Cubana	40	US (California)	Alfalfa	Seed	(CDC, 1998; NACMCF, 1999a)
1998	*E. coli* O157:NM	8	California, Nevada	Alfalfa, Clover	Seed and/or sprouter	(CDC 1998; Mohle-Boetani *et al*., 2001)
1999	*S.* Mbandaka	83	8 US states	Alfalfa	Seed	(CDC 1998; NACMCF, 1999a)
1999	*S.* Muenchen	157	10 US states	Alfalfa	Seed	(Proctor *et al*., 2001)
1999	*S. paratyphi* var Java	51	Canada	Alfalfa	Seed	(Stratton *et al*., 2001)
1999	*S.* Saintpaul	36	US (California)	Clover	ND	(CDC 1998)
1999	*Salmonella* spp.	34	US (Michigan)	Alfalfa	ND	(CDC 1998)
1999	*S.* Typhimurium	120	Colorado (USA)	Alfalfa	Seed	(Winthrop *et al*., 2003)

Year	Pathogen	No. of culture-confirmed cases	Location	Type of sprout	Likely source of contamination	Reference
2000	*S.* Enteritidis phage type 4b	27	The Netherlands	Mung beans	Seed	(van Duynhoven *et al*., 2002)
2000	*S.* Enteritidis	75	US	Mung beans	ND	(CDC, 2000)
2000	*S.* Enteritidis	8	Canada	Alfalfa	ND	(Harris *et al*., 2003)
2001	*S.* Enteritidis PT 913	84	Canada	Mung bean	Seed	(Honish and Nguyen, 2001)
2001	*S.* Kottbus	31	California	Alfalfa	Seed	(Mohle-Boetani *et al*., 2002)
2001	*S.* Enteritidis PT1	26	US (Hawaii)	Mung bean	Seed and/or sprouter	(CDC, 2002)
2002	*S.* Abony	13	Finland	Mung bean	ND	(Ministry of Agriculture and Forestry, 2003)
2003	*S.* Saintpaul	16	US	Alfalfa	ND	(CDC, 2003)
2003	*E. coli* O157:H7	7	US	Alfalfa	ND	(CDC 2003)
2003	*E. coli* O157:NM (H-)	13	US	Alfalfa	ND	(CDC 2003)
2003	*S.* Chester	26	US	Alfalfa	ND	(CDC 2003)
2004	*E. coli* O157:NM	2	US	Alfalfa	ND	(CDC 2003)
2004	*S.* Bovismorbificans	35	US	Alfalfa	ND	(CDC, 2004)
2005-2006	*S.* Oranienburg	126	Australia (WA)	Alfalfa	Seed	(OzFoodNet, 2006)
2006	*S.* Oranienburg	15	Australia (Vic)	Alfalfa	Seed	(OzFoodNet, 2007)
2006	*S.* Braenderup	4	US	Mung bean	ND	(CDC, 2006)
2007	*S.* Weltevreden	45	Norway Denmark Sweden	Alfalfa	Seed	(Emberland *et al*., 2007)

APPENDIX 2

Physical and chemical treatments for the inactivation of pathogens on inoculated sprouting seeds (Fett, 2006b)

Treatment	Conditions	Time	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*	4.0	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)
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*	2.0	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*	2.0	No effect	(Himathongkham *et al*., 2001)
Ammonia, gas	300 mg/L	22 h	Mung bean	*Salmonella*	5.0	No effect	(Himathongkham *et al*., 2001)
Ammonia, gas	300 mg/L	22 h	Alfalfa	*E. coli* O157:H7	3.0	No effect	(Himathongkham *et al*., 2001)
Ammonia, gas	300 mg/L	22 h	Mung bean	*E. coli* O157:H7	6.0	No effect	(Himathongkham *et al*., 2001)
$Ca(OH)_2$ (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)61,62
$Ca(OCl)_2$ (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*	2.0	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.*	5.0	No effect	(Fett, 2002b)
$Ca(OCl)_2$	16,000 ppm	10 min	Mung bean	*E. coliO157:H7*	3.9	No effect	(Fett, 2002b)
Chlorine dioxide, acidified	500 ppm	10 min	Alfalfa	*E. coliO157:H7*	>2.4, survivors present	Significant reduction	(Taormina and Beuchat, 1999)
Citrex™	20,000 ppm	10 min	Alfalfa	*Salmonella*	3.6	No effect	(Fett and Cooke, 2003a)
Citrex™	20,000 ppm	10 min	Alfalfa	*E. coli* O157:H7	3.4	No effect	(Fett and Cooke, 2003a)
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*	3.0	Slight reduction	(Weissinger *et al*., 2000)
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)

Treatment	Conditions	Time	Seed Type	Bacterium	Logarithmic Reduction - (CFU/G)	Seed Germination	Reference
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*	3.0	No effect	(Lang *et al*., 2000)
Radiation, gamma	Various		Alfalfa	*Salmonella*	D-value of 0.97 kGy	Dosage dependent	(Thayer *et al*., 2003)
Radiation, gamma	Various		Alfalfa	*E. coli* O157:H7	D-value of 0.60 kGy	Dosage dependent	(Thayer *et al*., 2003)
Radiation, gamma	Various		Broccoli	*Salmonella*	D- value of 1.10 kGy	Dosage dependent	(Rajkowski *et al*., 2003)
Radiation, gamma	Various		Broccoli	*E. coli* O157:H7	D- value 0f 1.11 kGy	Dosage dependent	(Rajkowski *et al*., 2003)
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	5.0	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)
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)
Supercritical CO_2	4000 psi, 50 C	60 min	Alfalfa	*E. coli,* generic	1.0	No effect	(Mazzoni *et al*., 2001)
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)
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)

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