INTRODUCTION:
Fresh produce is an important addition to a healthy diet, however, some produce, including sprouts, the germinating form of seeds and beans, have been implicated in foodborne illness. In July 1999, the U.S Food and Drug Administration (FDA) issued an advisory to the public, “Consumers Advised of Risks Associated with Raw Sprouts” (Ref. 4) based on the continued reports of illness from consuming raw sprouts. In October 1999, FDA released two guidance documents to provide recommendations to seed suppliers and sprout producers to reduce microbial food safety hazards common to the production of sprouts. “Guidance for Industry: Reducing Microbial Food Safety Hazards for Sprouted Seeds” (Ref. 5) identified the most important steps that should be implemented to reduce the risk of raw sprouts as a vehicle for foodborne illness. The second document, “Guidance for Industry: Sampling and Microbial Testing of Spent Irrigation Water During Sprout Production” (Ref. 6) provides sampling instructions and validated methods for testing sprout irrigation water to determine whether the pathogens Salmonella and E. coli O157:H7 are present.
Sprouts may include alfalfa, clover, sunflower, broccoli, mustard, radish, garlic, dill and pumpkin as well as mung, kidney, pinto, navy, soy beans and wheat berries (wheat grass). Raw and lightly cooked sprouts, especially alfalfa, clover and mung bean sprouts, have been associated with foodborne illness in a number of outbreaks.
Microorganisms already on the seeds or introduced during the sprouting process grow quickly during the ideal conditions of germination and sprouting. The water from frequent irrigation, pH of the water and plant tissue, days to complete sprouting and the nutrients available from the seeds and sprouts are very favorable to bacterial growth. If pathogens are present, they can increase exponentially by 2-6 logs in the first several days of sprouting. There is no step in the production of raw sprouts such as cooking or pasteurization to reduce or eliminate pathogens before consumption. In addition, many sprout producers are unaware that raw sprouts have been the vehicle for foodborne illness.
Sprouts are produced by placing the seed in a warm, humid environment for approximately 3-7 days for germination and growth, depending on the type of seed chosen. Some types of seeds take up to 10 days to sprout and some types of seeds and beans are sprouted for a shorter time (24 hours) to produce a slightly sprouted product.
Contamination of seeds appears to be sporadic and usually at low levels. Seeds are easier to sanitize or disinfect than sprouts because contamination levels are lower, there is less debris present and seeds are generally more resistant to treatments than delicate sprouts. In addition, the roots may take up bacteria into the sprout tissue which makes the pathogens inaccessible to any sanitizer.
Based on levels of pathogenic bacteria found on seeds associated with foodborne outbreaks, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) recommends treatments that achieve a 5 log reduction in pathogen levels for food safety (Ref. 3). Methods to achieve this level of assurance are not as well accepted as they are with other kill steps such as cooking meats or pasteurizing juices. Therefore, the process used should be validated by laboratory testing to keep contaminated sprouts from being sold.

PATHOGENS OF CONCERN (HAZARDS):
Foodborne outbreaks associated with sprouts have identified Escherichia coli O157:H7, various Salmonella serotypes and Bacillus cereus as the common causative agents. Sprouts contaminated with foodborne pathogens such as Salmonella or E. coli O157:H7 show no changes in appearance, smell or taste. Since 1996, raw sprouts have been increasingly implicated in foodborne outbreaks. Between January 1996 and December 2003, there were 25 reported outbreaks in the United States associated with sprouts from commercial growers, 19 of which were due to various Salmonella serotypes and 6 due to E. coli O157:H7. Alfalfa, clover sprouts and mung bean have been implicated most often; however, since all kinds of sprouts are produced under similar conditions, all raw (uncooked) sprouts may pose a risk. In all of the reported outbreaks, the likely source of the pathogen was contaminated seed. However, in one large 1996 outbreak, poor sanitation and unhygienic practices at the sprouting facility may also have contributed to the contamination of sprouts.
The sprouting process is very challenging to control from a food safety perspective due to a number of reasons. First, pathogens can survive for extended periods of time on seeds under dry storage conditions. Salmonella can survive for weeks, months or longer. Other natural microorganisms have survived for up to three years during dry storage.
Pathogens such as *E. coli* O157:H7 can be taken up by the roots of the sprouts and then be found in the tissue of the sprout (radish). *E. coli* O157:H7 can multiply $10^3 - 10^5$ (from 1,000 to 100,000 times) during germination and sprouting. Extensive formation of biofilms has been identified on sprouts. Biofilms protect entrapped foodborne pathogens from the antimicrobial activity of sanitizers and disinfectants.

**CURRENT RETAIL SPROUTING INDUSTRY BEST PRACTICES:**

Often seeds are not identified as seed for sprouting during harvest but are considered an agricultural commodity that will probably be used to plant and grow additional fields of that crop. Consequently, the seeds may be mishandled or stored under conditions unsuitable for a food item. Sprouts are considered a ready-to-eat food with little additional processing. **There is no single treatment so far that has been shown to completely eliminate pathogens on seeds or sprouts that cause foodborne illness without affecting germination or yield.** Therefore, every precautionary measure should be taken to prevent high levels of bacteria from growing on the seeds or sprouts. Using the following retail sprout industry "Best Practices" will help ensure a safe and healthy product.

<table>
<thead>
<tr>
<th>PROCESS STEP</th>
<th>SOURCE OF CONTAMINATION</th>
<th>CONTROL MEASURES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receiving (Seeds or Sprouts)</td>
<td>Bacterial contamination</td>
<td>Approved source (purchase specifications - grown for human food, grown under Good Agricultural Practices (GAPs) including manure management, labeled with lot number for traceback to source Stored and handled under sanitary conditions during distribution Inspection for torn bags or containers, rodent evidence (feces, urine - fluoresces in UV light) Product condition (not wet or moldy)</td>
</tr>
<tr>
<td>Seed Storage at Retail</td>
<td>Cross-contamination</td>
<td>Stored in clean, sanitized bins/containers Seeds protected after opening Have SSOPs in place (cleaning &amp; sanitizing, maintenance, pest control, etc.)</td>
</tr>
<tr>
<td>Seed Treatment (Soaking &amp; Rinsing)</td>
<td>Unsafe water Physical contamination Bacterial contamination</td>
<td>Use a public water supply or test private well water on a regular basis Screen for stones and other debris Protect all seeds from contamination especially if scarification is done to change germination <strong>Disinfection treatment</strong></td>
</tr>
<tr>
<td>Germination (Sprouting)</td>
<td>Dirty equipment Unsafe water Unsafe soil (if used)</td>
<td>Hot &amp; cold water available Use potable irrigation water for sprouting seeds</td>
</tr>
</tbody>
</table>
| **Post-Germination** (Harvesting/Packaging or Repackaging) | **Storage & Display** | **DISINFECTION TREATMENT:**
Seeds for sprouting should receive a treatment (such as 20,000 ppm calcium hypochlorite) that has been approved for reduction of pathogens in seeds or sprouts. Some treatments can be applied at the sprouting facility or applied earlier in the seed production process. However, at least one approved antimicrobial treatment should be applied immediately before sprouting. Sprouters should carefully follow all label directions when mixing and using antimicrobial chemicals.

**VARIANCE APPLICATION AND HACCP PLAN:**
Without a kill step to destroy pathogens that may be present on sprouts, other controls must be in place to assure that sprouts are safe to consume. When sprouting is done in a retail food store or food service establishment and sprouts are offered for sale or service directly to the consumer, the FDA Food Code requires the food establishment to obtain a variance from the regulatory authority, based on an approved HACCP Plan (See 2005 Food Code Section 3-502.11 (H) Variance Requirements).
As specified under Section 8-201.14 of the 2005 Food Code (Ref. 7), information to be included in the HACCP plan includes:

- A list of the types of seeds being sprouted in the food establishment
- A flow diagram or process description for each seed type that identifies the control measures, critical control points (CCPs) and critical limits, the methods and frequencies these CCPs are monitored, how and when management verifies that this has been done, what corrective action will be done in the case of a problem and what records will be kept for documentation that the process has been done correctly
- Training on safe sprouting practices for employees
- Control measures showing which approved treatment method or combination of methods were used to achieve a reduction in pathogens and showing how the irrigation water from each batch of germinating sprouts is tested. There is zero tolerance for *Salmonella* and *E. coli* O157:H7 in raw sprouts.

**REDUCED MICROBIAL RISK THROUGH INTERVENTIONS:**
Few alternative interventions have been found which will be effective against all pathogens associated with sprout outbreaks, be effective for all types of seeds and which will not affect germination rates, sprouting time, length of sprouts (yield) or quality of sprouts. Often a treatment that is effective for one type of seed may not be effective for

<table>
<thead>
<tr>
<th>for sprouts)</th>
<th>Clean &amp; sanitize all surfaces that irrigation water and sprouts contact Wash hands before and after handling sprouts No broken or cracked utensils or equipment Building enclosed <strong>Testing irrigation water for Salmonella and E. coli O157:H7</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Airborne contamination</td>
<td>Bacterial growth Ill employees with infections</td>
</tr>
<tr>
<td>Irrigation water and sprouts contact</td>
<td>Wash hands before and after handling sprouts</td>
</tr>
<tr>
<td>No broken or cracked utensils or equipment</td>
<td>Building enclosed <strong>Testing irrigation water for Salmonella and E. coli O157:H7</strong></td>
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</tr>
<tr>
<td><strong>Post-Germination</strong> (Harvesting/Packaging or Repackaging)</td>
<td>Unsafe water Ill employees with infections Inadequate label information Unsafe packaging materials</td>
</tr>
<tr>
<td><strong>Storage &amp; Display</strong></td>
<td>Bacterial Growth Cross-contamination</td>
</tr>
<tr>
<td>Store/display at 41°/5°C or less</td>
<td>Protect sprouts from contamination</td>
</tr>
</tbody>
</table>
other seeds. Some seeds are rough, wrinkled or have larger rough areas where the seed attaches to the pod during development (the hilum). Bacteria can easily attach to these rough areas and disinfection is more difficult. In some cases, the treatment reduces the level of pathogens leaving some injured bacteria that may show up in microbiological testing. Using an enrichment culture technique allows the bacteria to heal themselves and start to grow again showing that the disinfectant was not completely effective against that pathogen.

Seed disinfection is usually chosen over a treatment for sprouts because of the fragile nature of the sprout. In addition, contamination on the seeds or in the irrigation water can be taken up into the interior of the sprout tissue during the sprouting process where chemical disinfection treatment is ineffective. Disinfection appears to be more effective to remove any pathogens from the seed before sprouting begins but care must be taken so the sprouts are not recontaminated later by water, equipment or employees. It is also important to follow the specific instructions for any sanitizing/disinfection compounds and to apply the chosen treatment method to each batch of seeds.

Inconsistent application has been identified as a factor contributing to several outbreaks. Experimental treatments and technologies under development may provide alternate microbial intervention processes for sprouts in the future. Possibilities include: ethanol, hydrogen peroxide, ozone or ozonated water, calcium hydroxide, chlorous acid, dry heat, hot water, irradiation, UV light, ultrasound, pulsed light, high hydrostatic pressure, pulsed electrical or magnetic fields, gaseous antimicrobial compounds, vacuum infiltration of sodium or calcium hypochlorite, and non-pathogenic competitive exclusion.

Approval for irradiation of seeds is found in 21 Code of Federal Regulations (CFR) 179.26 (b)(10). Ozone in the gaseous or aqeous phase was declared Generally Recognized as Safe (GRAS) in 2001. Chemical compounds that are used in disinfectant treatments for seeds must be approved by the U.S. Environmental Protection Agency (EPA) for that purpose and used according to the instructions for use on the label. Only calcium hypochlorite has been approved for seed disinfection to date.

VERIFICATION (TESTING):

Because no single treatment has been found to completely eliminate pathogens, FDA recommends microbial testing of spent irrigation water. Verification testing can be done on-site in the retail establishment if adequate equipment and qualified personnel are available or private laboratories can be contracted to perform the necessary tests. A seed disinfection treatment done in conjunction with microbial testing reduces the likelihood that contaminated product will be sold. Testing should be done for both the pathogens of concern, *E. coli* O157:H7 and *Salmonella*. There are reliable rapid tests kits that can be used but an enrichment step is required. Enrichment helps identify pathogens that may have been injured by the treatment method but not destroyed. It allows the bacteria to recover under conditions favorable to their growth. This method requires the use of a basic microbiology laboratory (media preparation area, flasks, pH meter, balance, autoclave and incubator). Testing sprout irrigation water is a reliable indicator of pathogen growth on the sprouts.

Samples should be taken by individuals trained to use aseptic (sterile) techniques and delivered to the testing laboratory that same day. Private laboratory personnel can either collect samples themselves or train employees in the retail food establishment in sampling technique. Analytical methods should be AOAC-approved screening tests or formal confirmatory tests which have been validated. Additional information about sampling and testing is found in FDA's "Guidance for Industry: Sampling and Microbial Testing of Spent Irrigation Water During Sprout Production".

REFERENCES:

8. Annex 1 - SURVEILLANCE SAMPLING OF SPROUTS:

Microbiological surveys where no known illnesses were involved have identified a number of pathogens in sprouts including *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus* and *Aeromonas hydrophilia*. Risks from viruses and protozoa which do not multiply during sprouting, might be reduced because of the extensive washing the
sprouts receive. Pathogens that are not competitive enough to reach high levels such as *Staphylococcus aureus* are not considered to be a problem compared to other fresh product.

**Annex 2 - NACMCF RECOMMENDATION FOR 5 LOG REDUCTION OF PATHOGENS:**

Limited quantitative data is available on the level of foodborne pathogens present in seeds used for sprouting. Quantitative analyses performed on seeds associated with illness attributed to sprout consumption found pathogen levels ranging from <1 to 6 CFU/100 g of seed. (Ref. 2) Therefore the worst case scenario for seed contamination was assumed to be 1 pathogen/10 g of seed. It was also assumed that fifty kg of seeds is the amount of starting materials for each batch of sprouts. This yields an initial level of 5,000 pathogens per batch of sprouts. Thus, a 1-log treatment reduction will yield 500 pathogens/batch; a 2-log treatment 50 pathogens/batch; a 3-log treatment 5 pathogens/batch; a 4-log treatment 0.5 pathogens/batch (one batch out of every two will contain a pathogen); and a 5-log treatment 0.05 pathogens/batch (one batch out of every twenty will contain a pathogen). It is realized that the actual extent of risk reduction achieved will likely be greater than this because the extent of initial contamination and the amount of seed used per batch of sprouts will typically be less than the values assumed in the current worst case calculation. (Ref. 3)