FRESH PRODUCE SAFETY CENTRE

AUSTRALIA & NEW ZEALAND

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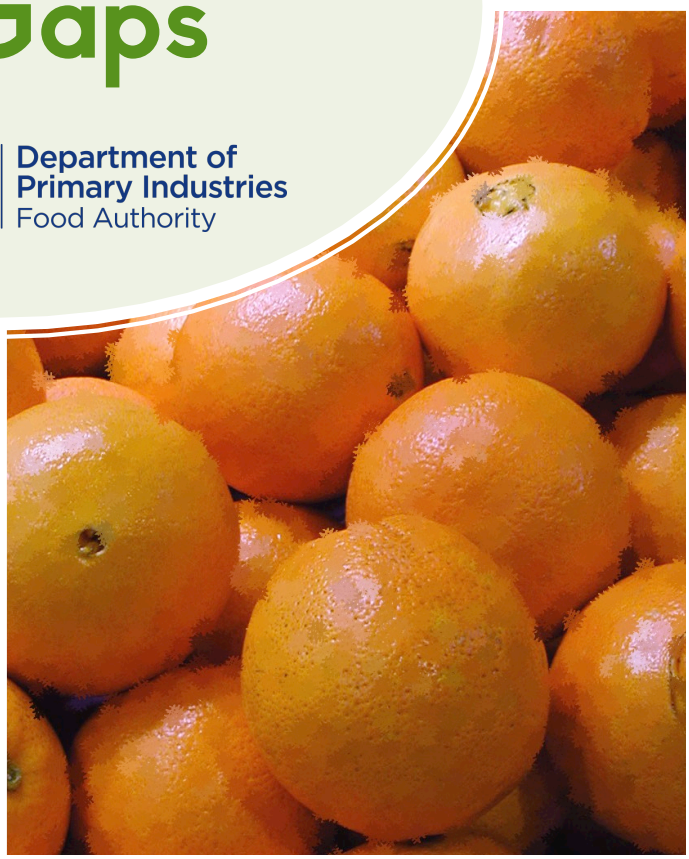
Understanding the Gaps

PIPFRUIT NEW ZEALAND

NSW GOVERNMENT Department of Primary Industries Food Authority



gsf golden state foods

Snap Fresh Foods

FRESH SELECT "If it's fresh, it must be Select"

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Disclaimer

Contents

1 Recommendations

This project was commissioned by the Fresh Produce Safety Centre to understand the gaps in current food safety knowledge.

Five priority areas were identified through industry consultation. The objective of this project was to review the literature, summarise what was known in the five areas, and identify priority areas that needed more research and/or development.

The following are the key recommendations from this review.

1.1 Fresh produce microbial contamination levels

Data is required providing a clear view on the level and type of fresh produce microbial contamination in Australia and New Zealand. Trace back of contamination detections will enable root cause identification and help focus industry efforts on managing key risk areas.

1.2 Agricultural water

Improved information on water quality risk assessment, testing and water source management is required to help growers identify water use risks.

The potential for pathogen transfer from agricultural water to produce surfaces needs to be better understood. The dynamics of transfer will determine the residual pathogen populations on the produce.

Pathogen survival and growth on produce types in production environments needs further investigation. Identifying the pathogen and commodity characteristics that increase or decrease pathogen survival and growth would help growers to assess risks from water use.

The ability of pathogens to remain viable and grow on different produce types through distribution systems and storage environments requires further investigation.

Industry guidelines and communication strategies need to be developed on effective water sanitation methods. This is required to improve industry understanding of how to remediate or treat water. User-friendly information on sanitation strategies, products and use methods for water sanitation management is required to enable growers and packers to correctly manage water treatment.

1.3 Organic inputs and composting

Data on the types of manures used in Australian and New Zealand composts is required. This will enable assessment of the pathogen types and effectiveness of composting treatments in reducing risks to an acceptable level.

Research on the effect of different animal feedstock on pathogen types, populations and survival in the manure are required to complement risk assessments and effective composting treatments.

Research is required to evaluate how long pathogens persist in Australian and New Zealand agricultural soils and production systems. Production environment variables may be different from studies in the published literature.

Research is required to determine if other food safety pathogens such as viruses, protozoans and nematodes are present on Australian and New Zealand fresh produce.

Industry guidelines and communication strategies are required to better communicate and inform Australian and New Zealand growers on correct composting practices.

1.4 The storage environment and transport

Research is needed to determine the prevalence and persistence of *L. monocytogenes* in incidental condensate within fresh produce storage environments.

Extension materials are needed to provide guidance on good practice in managing storage and transport facilities.

1.5 Interaction of sanitisers and fungicides

Research is needed to understand sanitiser and fungicide use patterns in Australian and New Zealand fresh produce industries. This information may be used to prioritise which produce types or sanitiser and fungicide use patterns need to be evaluated for compatibility

Industry guidelines and communication strategies are required to growers and packers with information on regulatory requirements and management practices for postharvest sanitizer and fungicide compatibility.

2 Fresh produce microbial contamination levels

What do we know about the level of microbial contamination on Australian and New Zealand fruit and vegetables? This literature review will consolidate existing knowledge and information on the microbial risks for fresh produce grown using different soil types and soil preparation, planting material, organic inputs, irrigation methods, harvesting methods, seasonal and regional impacts and other general production variables.

Key points

What the literature tells us

- Microbial testing of fresh produce is generally disaggregated and conducted on a commercial-in-confidence basis.
- Industry test data suggests there may be a low level of microbial pathogen presence but does not:
  - indicate if the detections were at microbial population levels likely to cause foodborne illness
  - identify the specific cause that led to the contamination
  - identify areas of increased risk for industry
- On-farm water management during production and poor worker hygiene are suspected to be key sources of fresh produce contamination.
- There is little microbial test data available to verify whether fresh produce in Australia and New Zealand is, or is not, a significant cause of foodborne illness.
- Foodborne illness outbreaks have been associated with the consumption of fresh produce grown in Australia and New Zealand.

Research Gaps

- Data is required providing a clear view on the level and type of fresh produce microbial contamination in Australia and New Zealand. Trace back of contamination detections will enable root cause identification and help focus industry efforts on managing key risk areas.

2.1 Background

A range of microflora inhabit the surface of raw fruits and vegetables. The composition of this population is highly variable and unpredictable, since it is influenced by the growing environment, the type and age of the plant and its fruit, the location of the growing area and interactions with humans (eg pruning, spraying, irrigating, picking).

While most of these microbes are likely to be innocuous, some may be pathogenic to the plant and a few may be pathogenic to humans. The resident non-human pathogenic microflora can act as competitors for human pathogens, are alternatively increase survival due to alterations in pH and the

presence of exudates in rots[1, 2]. For example, a study of 500 samples of healthy or rotted fruit and vegetables found that *Salmonella* was twice as common on produce affected by bacterial soft rot (eg *Erwinia carotovora*, *Pseudomonas* spp.) compared to healthy samples[3].

A review by Beuchat[4] provides an overview of foodborne pathogens in different vegetable and fruit products. The frequency of *Salmonella,* enterovirulent *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter*, *Cyclospora*, and others varied widely between studies. Often no pathogens were detected. However, other surveys have found high percentages of samples contaminated with pathogens. It is concluded that the presence of pathogenic microorganisms on raw fruits and vegetables varies considerably depending on a wide range of factors.

More recent studies confirm the variability in types and numbers of human pathogens isolated. Contaminated samples range from zero to relatively high frequencies (> 50%), depending upon the target pathogen, type of produce and circumstances; isolation rates are not consistent.

Harris et al.[5] conducted a comprehensive review on this topic, including extensive information on the detection, survival and growth of different pathogenic bacteria on various fruit and vegetables. The review highlights that prevalence studies can provide a snapshot assessment of contamination at a particular location on a particular produce item at a particular time of year, but rarely provide information on the source of contamination. The conditions that led to contamination often cannot be described because produce for testing was sourced at the packhouse or retail.

There are fewer surveys on the presence of parasites or viruses because of the lack of detection methods. Not all of the pathogenic microbes that might be expected on fresh produce have been found. Moreover, some pathogens that are present have never been linked to actual illness.

Many factors will influence microbial test results even for identical produce / pathogen / condition combinations. These include:

- Procedure for sampling
- Location of source (field, packing shed, processing plant, retail, food service, home)
- Number of pieces, weight and size of samples
- Distribution of samples in test lot
- Protection of samples for transport to laboratory
- Handling samples between collection and analysis
- Protection against cross-contamination
- Temperature and time between selection and analysis of sample
- Processing of samples

[1] Conway, W. S., Leverentz, B., Saftner, R. A., Janisiewicz, W. J., Sams, C. E., Leblanc, E., 2000. Survival and growth of Listeria monocytogenes on fresh-cut apple slices and its interaction with Glomerella cingulata and Penicillium expansum. *Plant Dis*. **84**,177-181.

[2] Riordan, D. C. R., Sapers, G. M., Annous, B. A., 2000. The survival of Escherichia coli O157:H7 in the presence of Penicillium expansum and Glomerella cingulata in wounds on apple surfaces. *J. Food Prot*. **63**,1637-1642.

[3] Weissinger, W. R., Beuchat, L. R., 2000. Comparison of aqueous chemical treatments to eliminate Salmonella on alfalfa seeds. *J. Food Prot*. **63**,1475-1482.

[4] Beuchat, L. R. 1998. Surface decontamination of fruits and vegetables eaten raw: A review. Food Safety Unit, World Health Organization. WHO/FSF/FOS/98.2.

[5] Harris L.J., Farber, J.N., Beuchat, L.R., Parish, M.E., Suslow, T.V., Garrett, E.H. and Busta, F.F., 2003. Chapter III Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Comprehensive reviews in food science and food safety* **2** (Supplement), 78-141.

- Area or portion to be tested (whole piece, skin only, diced, cut)
- Ratio of produce to wash fluid or diluent, selection and temperature of wash fluid or diluent
- Soaked or not soaked before processing
- Type of processing (washing, rubbing, stomaching, homogenising, macerating, blending)
- Time of processing

The method used for testing has the greatest influence, eg testing by plate count, immunological methods, microscopy and molecular methods. All of these approaches involve further decision-making, eg what culturing media and conditions to use, what genes to target, what stains to use in microscopy. These detection methods may only result in a presumptive finding, or identification to a genus or species level, but further confirmation tests may be required to, for example, confirm species, confirm virulence, identify a serotype or confirm the isolate is viable.

2.2 Occurrence of human pathogens on Australian and New Zealand produce

Many businesses in the fresh produce supply chain conduct testing with commercial laboratories in order to comply with quality assurance system certification requirements. The results are commercial-in-confidence and there is no mechanism for aggregation and analysis of the results. However, data on microbe levels on fresh produce is available from FSANZ and FreshTest.

2.2.1 Survey by FSANZ

FSANZ funded a national coordinated survey of the prevalence of microbiological contamination in fresh horticultural produce for the period 2005 – 2007[6]. A total of 369 samples were analysed. These included lettuce, seed sprouts, strawberries, parsley and basil. The survey collected samples from three points in the fresh produce supply chain: the field, the farm gate and retail. The exception was seed sprouts, which were collected prior to germination, at the end of the production and at retail. Samples were analysed for the presence of *E. coli* (including verocytotoxin producing *E. coli* (VTEC) or *E. coli* O157:H7), *Listeria* spp. and /or *L. monocytogenes*, *Salmonella* spp.

VTEC were detected in two samples (seed sprout and parsley), *L. monocytogenes* was detected in four strawberry samples and *Salmonella* spp. was detected in one strawberry sample. For strawberries, pathogens were detected at all stages along the produce supply chain. For seed sprouts, pathogens were detected after sprouting and at retail. No pathogens were detected on lettuce samples (Table 1).

[6] Food Standards Australia New Zealand, 2010. Microbiological survey of fresh horticultural produce in Australia 2005-2007, accessed 31 August 2015, http://www.foodstandards.gov.au/science/surveillance/documents/Report%20on%20the%20Microbiological%20survey%20of%20fresh%20horticultural%20produce%20in%20Australia.pdf

Table 1 - Results from survey of human pathogenic microbes on Australian fresh produce by FSANZ

Produce	Location	Pathogen											
		E. coli (MPN>3/g)		*E. coli* O157:H7		VTEC		*Salmonella*		*Listeria*		*L. monocytogenes*	
		samples	detections	samples	detections	samples	detections	samples	detections	samples	detections	samples	detections
Lettuce	Field	37	0	28	0	9	0	37	0	31	0	37	0
	Packed	19	0	19	0			19	0	10	0	19	0
	Retail	78	4	60	0	9	0	78	0	72	0	78	0
	Total	***134***	***4***	***107***	***0***	***18***	***0***	***134***	***0***	***113***	***0***	***134***	***0***
Seed sprouts	Pre-germ	13	0	4	0	9	0	13	0	13	0	13	0
	Harvest	34	4	24	0	10	1	34	0	24	0	34	0
	Retail	54	3	37	0	8	0	54	0	48	2	54	0
	Water	12	2	1	0			3	0	3	0	3	0
	Total	***113***	***9***	***66***	***0***	***27***	***1***	***104***	***0***	***88***	***2***	***104***	***0***
Strawberries	Field	31	5	28	0	3	0	31	1	27	0	31	0
	Packed	22	2	19	0	3	0	22	0	19	2	22	2
	Retail	52	2	49	0	3	0	52	0	48	2	52	2
	Total	***105***	***9***	***96***	***0***	***9***	***0***	***105***	***1***	***94***	***4***	***105***	***4***
Parsley	Field	3	0			3	0	3	0	3	0	3	0
	Packed	3	0			3	1	3	0	3	0	3	0
	Retail	9	1	3	0	3	0	9	0	6	0	9	0
	Total	***15***	***1***	***3***	***0***	***9***	***0***	***15***	***0***	***12***	***0***	***15***	***0***
Basil	Retail	2	1	1	0			2	0	2	0	2	0
	Total	***2***	***1***	***1***	***0***			***2***	***0***	***2***	***0***	***2***	***0***
All		**369**	**24**	**273**	**0**	**63**	**2**	**360**	**1**	**309**	**6**	**360**	**4**

FSANZ concluded that the frequency of pathogenic bacteria on the fresh produce sampled was very low. The presence of VTEC and Salmonella spp. could result from contamination in the field (from manure, contaminated water, livestock, wild animals and birds) or harvesting (from cross contamination of equipment or farm workers).

2.2.2 FreshTest data

FreshTest Australia[1] is an Australian Chamber of Fruit and Vegetable Industries (ACFVI) initiative to provide Australian wholesalers and growers with sampling and testing services for chemical and microbial residues. The program was established in Australia's central market system in 2001 to provide ACFVI members and their grower suppliers with access to a cost effective system that meets their quality assurance system requirements for chemical and microbial testing.

FreshTest has grown to become the largest and most comprehensive testing program for fresh produce in Australia, testing over 55,000 samples across 240 products since starting. FreshTest data is commercial-in-confidence information but the program can collate their information to identify trends or issues at a commodity or industry level. Adverse detections require the business submitting the sample to complete a follow up investigation report.

A range of sample analyses may be tested for:

- Total plate count
- Generic *E. coli*
- Fecal coliforms
- *Listeria* spp. (positive results typed for confirmation as *L. monocytogenes*)
- *Salmonella* spp.,
- Enterobacteriacea
- Coagulase + staphylococci
- *Bacillus cereus*.

FreshTest reports results that exceed critical limits for water (Table 2) or on fresh produce (Table 3).

Table 2 – Maximum Microbial Indicator Limits Prescribed for Water Quality in Agriculture/Food Processing (FreshTest, 2015)

Test Organism	Potable Water (CFU/100mL)	Non-potable Water (CFU/100mL)
E. coli	<1	<126
Coliforms	<1	<100
Thermotolerant coliforms	<1	<100

Water Testing: (FreshTest use membrane filtration method – microbial limits as follows)

[1] FreshTest Australia, viewed 16 September 2015, https://www.freshtest.com.au/

Table 3 – Maximum Microbial Limits Prescribed for Fresh Produce (FreshTest, 2015)

Organism	Criterion (CFU/g)
E. coli	<10
Salmonella	not detected per 25 g
Listeria	not detected per 25 g
Listeria monocytogenes	not detected per 25 g
Faecal coliforms	<100
Coagulase +ve staphylococci	<100

An FSANZ Proposal in 2011[2] included a summary of FreshTest data for 3507 fresh produce samples. The study was undertaken to determine whether fresh produce regarded as 'high-risk' from experiences in the USA was also high-risk in Australia. Samples included lettuce and salad leaves (n=277), strawberries (n=313), mushrooms (n=154), tomatoes (n=227) and cucumber (n=187). Unfortunately, this report does not distinguish between imported and domestically grown produce. Due to its limited scope, the study cannot be assumed to be representative of all horticultural products. However, despite these limitations, the results appear to confirm that contamination of fresh produce with pathogens is very low (Table 4).

Table 4 - Summary results from 3507 samples of fresh produce (FreshTest, 2011)

Tested pathogens	No. samples tested	Positive samples	
		Number	Percentage
Listeria monocytogenes	2480	3	0.1
Salmonella spp.	2003	4	0.2
Coagulase positive staphylococci	1396	7	0.5
E. coli	2288	133	3.9
Fecal coliforms	1404	173	12.3

Table 5 shows the percentage of samples of fresh produce that exceeded the FreshTest microbial limits for the period of 2005-2015. Results below 5% have been excluded from this table.

The results do not identify the level of microbial populations on samples for adverse detections, only that the population in the sample exceeded the indicator threshold. The results do indicate that:

- A small but significant percentage of fresh produce carries residual human pathogens
- Curry leaves (81.8%) and lime leaves (79.0%) have high rates of detection but only a small sample size. These are hand harvested suggesting the source is workers handling product
- Water has a high adverse detection rate - Corrective Action responses reported back to FreshTest following an adverse detection notification suggest that the reasons for this include
    - poor sampling technique

[2] Food Standards Australia New Zealand (FSANZ) (2011) Supporting Document 2, Review of foodborne illness associated with selected ready-to eat fresh produce, Proposal P1015 Primary Production & Processing Requirements for Horticulture, http://www.foodstandards.gov.au/code/proposals/documents/P1015%20Horticulture%20PPPS%201CFS%20SD2%20Illness%20review.pdf accessed 15 June 2015

- sample not chilled or provided to FreshTest in a timely manner
- non-sterile container being used
- water taken from a rain water tank (FreshTest pers. comm)

- More than 30% of some fresh herbs, sprouts and watercress had microbial contamination above the standard limits. This is of concern, as all of these products may be eaten uncooked. Possible sources include cross contamination from the production environment and/or poor personal hygiene by workers.
- Ginger adverse detection rate is over 28%. Although it could have been assumed in the past that ginger was always cooked, this is no longer the case. It is possible that current methods used to grow, harvest and clean ginger are not being fully effective at avoiding contact with pathogens or removing them after harvest.
- Compost adverse detection rate is 13.7%. This suggests poor composting techniques are being used. As well as inadequate compost management (turning, temperature monitoring), it is possible that recontamination from other sources could be occurring after composting is completed.

Table 5 - Percentage of samples tested over the period 2005-2015 that exceeded the FreshTest maximum microbial limits (adverse detections)

Product		Adverse detections (%)	*E. coli*	*Staphylococcus* spp.	Faecal coliforms	Std Plate Count	Coliforms	*Listeria* spp.	*L. monocytogenes*	*Salmonella* spp.	Enterobacteriaceae	*Bacillus cereus*	Total Plate Count
Curry Leaves	*	81.8	X	X	X								
Lime Leaves	*	79.0	X	X	X								
Water - pre-harvest	*	70.0	X			X	X						
Water - ice	*	62.5	X			X	X						
Water - irrigation		43.9	X		X	X	X						
Sweet Corn	*	39.5	X	X	X								
Water - potable		39.1	X			X	X						
Lemon Thyme	*	36.4	X		X								
Watercress	*	33.3	X	X	X				X	X			
Water - Bore water	*	33.3				X	X						
Sprouts		32.3	X	X	X				X	X			
Water		32.0	X		X	X	X						
Ginger	*	28.6	X		X								
Herbs	*	22.2	X				X						
Mint		21.9	X		X								
Oregano	*	20.8	X		X								
Thyme	*	20.7	X		X								
Marjoram	*	20.0	X		X								
Dragon Fruit	*	18.8						X					
Chives		18.3	X	X	X				X	X			

Product		Adverse detections (%)	*E. coli*	*Staphylococcus* spp.	Faecal coliforms	Std Plate Count	Coliforms	*Listeria* spp.	*L. monocytogenes*	*Salmonella* spp.	Enterobacteriaceae	*Bacillus cereus*	Total Plate Count
Parsley		14.8	X	X	X		X	X	X	X	X		
Tarragon	*	14.3	X		X								
Coriander		13.9	X	X	X					X			
Compost		13.7				X	X						
Sage	*	13.3	X		X								
Rhubarb	*	10.7			X			X	X				
Spinach		10.7	X		X		X						
Sweet Potatoes		10.3	X	X	X								
Lettuce Salad Mix		10.0	X		X							X	X
Silverbeet		9.9	X		X						X		
Rocket	*	9.9	X				X						
Endive	*	9.5	X										
Radish	*	8.9	X					X				X	
Rosemary		8.0	X		X								
Dill	*	6.5	X		X								
Lemon Grass	*	6.4	X		X								
Beans		6.2	X		X			X					
Choy		5.8	X		X								
Beetroot		5.6	X		X						X		
Lettuce		5.4	X	X	X	X		X					X

(note * indicates less than 50 samples were tested)

2.3 Foodborne illness associated with fresh produce

It is rare that a food is specifically linked to human illness using epidemiological or laboratory evidence. In most reported outbreaks, a food or several foods are suspected based on investigating common foods that affected people had consumed in a certain timeframe prior to the outbreak. The spatial distribution of cases of foodborne illness also assists in identifying the probable causal food and reasons for its contamination. Often the suspected foods are not available for testing once an outbreak is recognised.

2.3.1 Australia

The Government funded organisation OzFoodNet (www.ozfoodnet.gov.au) records foodborne illness outbreaks in Australia. Information on outbreaks resulting from contaminated food can be accessed through the OzFoodNet Outbreak Register. Currently it has data on the major causes of foodborne disease outbreaks for 2001 to 2008.

OzFoodNet and partner agencies follow up and investigate outbreaks (two or more related cases) of illnesses caused by the following food-borne pathogens:

- *Campylobacter*
- *Salmonella* spp.
- Typhoid (*Salmonella typhi*)
- *Shigella*
- Shiga Toxin-producing *Escherichia coli*
- Haemolytic Uraemic Syndrome (eg Enterohaemorrhagic *E. coli* infections)
- *Listeria monocytogenes*
- Hepatitis A
- Norovirus - most common cause of gastroenteritis – many ways of transmission including person to person, surfaces, food and water.

OzFoodnet keeps records only on major microbial issues. The total number of food complaints, food samples analysed and number of marginal and non-compliant results is not recorded or reported. Any trends in food safety from regulatory food sampling and complaint investigation are therefore unknown. Retailers also do not disclose statistics on rejections based on food safety concerns.

OzFoodnet recorded a total of 22 foodborne illness outbreaks associated with fresh produce between July 2006 to October 2010 (Table 6).

Table 6 - Foodborne illnesses traced back to fresh produce. July 2006 to October 2010, OzFoodnet 2010[3]

Pathogen	Incidents	Hospitalised	Deaths	Comment
Norovirus	8	0	0	
Unknown	8	1	0	
Salmonella spp.	4	13	0	
Shigella spp.	1	3	0	Baby Corn
Listeria spp.	1	8	2	Melon, 2010
Total	**22**	**25**	**2**	

With the 22 total cases, the following crops were implicated:

- Eight x salads
- Eight x salads in sandwich or cold salad
- One x baby corn
- Two x melon
- One x orange mango drink
- One x cashew nut
- One x chillies.

Outbreaks of foodborne illnesses are also reported in the media. An analysis of media reports can be used to estimate the number of actual illnesses associated with fresh produce (Table 7). The number of media reports is somewhat lower than official records, indicating that not all outbreaks make it into the news.

[3] OzFoodNet Working Group, 2010; Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: annual report of the OzFoodNet network

Table 7 - Media reports of foodborne illnesses traced back to fresh produce. July 1998 to October 2014, Confidential source.

Pathogen	Incidents	Comment (bold font indicates more than one incident)
Salmonella spp.	13	**Almonds**, macadamia nuts, **pistachio** nuts, nuts general, lettuce, **cucumber**, hand cut fruit, sandwiches, alfalfa **sprouts**, rockmelons, **pawpaw**, herbs, snake beans
Listeria spp.	2	Melon, 2010 plus 1
E. coli	2	Salad, seed sprouts
Cyclospora spp.	1	Rockmelon
Norovirus	1	Mixed fruit and vegetable salad
Campylobacter spp.	1	Tomato / cucumber salad
Shigella spp.	1	Baby Corn
Total outbreaks	**21**	

The data in Table 6 and Table 7 suggest that there is a relatively low level of foodborne illness associated with fresh produce, whether officially or according to media reports. This was confirmed by an FSANZ study, which found only five foodborne illness outbreaks that could be traced back to fresh produce. Two of these involved imported product. Of the three other cases, two were linked to rockmelons and the final to papaya.

Table 8 - Foodborne illnesses traced back to fresh produce in Australia (FSANZ, 2011).

Food	Pathogen	Year	Origin	Cases	Evid	Cause	References
Semi-dried tomato	Hepatitis A	2009	Imported	>500	E, L	n.d.	OzFoodNet 2010, Donnan et al. 2011
Baby corn	*Shigella* spp.	2007	Imported	55	E	Implicated corn in Denmark and Australia imported from Thailand and traced to same packing shed. Poor hygienic practice during de-silking process implicated as source of contamination.	OzFoodNet 2008, Lewis et al. 2009
Rockmelon	*Listeria* spp.	2010	Domestic	9	E, L	n.d.	OzFoodNet 2010, Astridge 2011
	Salmonella spp.	2006	Domestic	115	E, L	Inconclusive; use of non-potable water in processing ready-to-eat melons likely failure point.	Munnoch et al. 2009
Papaya	*Salmonella* spp.	2006	Domestic	26	E, L	Inconclusive; use of non-potable river water to wash fruit likely failure point.	(Gibbs et al. 2009)

n.d. - not determined; **E** - epidemiological study; **L** - laboratory confirmed link between outbreak strain and food

2.3.2 New Zealand

In New Zealand, incidents and outbreaks of notifiable communicable diseases are collated in a secure database called EpiSurv on behalf of the Ministry of Health[4]. Notifiable diseases that are reported to EpiSurv include foodborne diseases, such as campylobacteriosis and salmonellosis[5]. Outbreaks of acute gastroenteritis, where two or more people become ill from a suspected common source, are also reported to EpiSurv (eg norovirus).

Annual summary reports are generated from the data collated in EpiSurv. This includes summaries of sporadic infection and outbreaks[6]. Annual reports are also published that specifically focus on outbreaks where "foodborne" is one of the reported modes of transmission (more than one mode of transmission can be reported for an outbreak)[7]. Several reports have included information on produce-related outbreaks.

A total of five outbreaks of foodborne illness have been linked to fresh produce over the last 17 years. These are the only outbreaks that have been confirmed with robust epidemiological and/or microbiological data.

Table 9 - Foodborne illnesses traced back to fresh produce in New Zealand

Food	Pathogen	Year	Evidence	Cause	References
Blueberries	Hepatitis A	2002	Case control study, 90% similar HAV strains from patients faeces and blueberries	Blueberry plants adjacent to toilets, inadequate hand washing facilities, bare hands picking, infected person	Calder et al. 2003
Carrots	*Salmonella* Saintpaul	2005	Case control study	Washing in stream water	Neuwelt et al. 2006
Watermelon	*Salmonella typhimurium* PT1	2009	Case control study	No cooling, wild animal faeces in packhouse, growing area close to septic tank and effluent disposal trenches	McCallum et al. 2010
Fresh fruit salad	Norovirus	2011	Case control study	Infected food handler	Lim et al. 2012*
Lettuces or carrots	*Yersinia pseudotuber-culosis*	2014	Case control study	Not identified	Health Intelligence Team 2014, MPI 2014

* Additional details retrieved from EpiSurv report

[4] https://surv.esr.cri.nz/episurv/iindex.php, accessed 25 May 2015
[5] http://www.health.govt.nz/our-work/diseases-and-conditions/notifiable-diseases, accessed 25 May 2015
[6] https://surv.esr.cri.nz/surveillance/surveillance.php accessed 25 May 2015
[7] http://www.foodsafety.govt.nz/science-risk/human-health-surveillance/foodborne-disease-annual-reports.htm accessed 25 May 2015

McIntyre et al[8] identified the causes of the blueberry and carrot outbreaks as poor GAP / GHP and contaminated pre harvest water sources respectively:

- Blueberries 2002 - outbreak of Hepatitis A infection, involving 43 cases, was associated with consumption of fruit originating from a single orchard. Research into this outbreak identified a number of likely causes including inadequate hand washing facilities (no running water, soap or hand towels), bare hands picking (ie no gloves) and the presence of a child on the farm who later developed symptoms consistent with a Hepatitis A infection[9].

- Carrots - The 2005 *Salmonella* Saintpaul outbreak in Auckland and Waikato was linked to the consumption of uncooked carrots grown in Ohakune, which had been washed in river water by the producer. The river water tested high for coliform counts, although *Salmonella* Saintpaul was not isolated during investigative water testing[10].

Further information on the incidence, economic cost and burden of disease from foodborne illness in New Zealand is also available[11]. These analyses are not specific to fresh produce.

[8] McIntyre L., Cressey, P., and Lake, R. (2008) Discussion document on pathogens in fruits and vegetables in New Zealand. http://www.foodsafety.govt.nz/elibrary/industry/discussion-document-pathogens-research-projects/index.htm

[9] Calder L, Simmons G, Thornley C, Taylor P, Pritchard K, Greening G, Bishop J (2003) An outbreak of hepatitis A associated with consumption of raw blueberries. Epidemiology and Infection 131(1):745–751

[10] Neuwelt P, Simmons G, Thornley C and Mohiuddin J (2006) Salmonella outbreak in the Auckland and Waikato regions. New Zealand Public Health Surveillance Report; 4: 6

[11] http://www.foodsafety.govt.nz/science-risk/risk-assessment/risk-ranking.htm accessed 25 May 2015

3 Agricultural water

What do we know about the critical limits for food safety pathogens in water applied to crops during production? How have these limits been determined, how reliable are they and could they be challenged by further research? Are there any specific products, seasonal or regional influences that may impact these critical limits? How do they impact and what measures, if any, can be implemented to mitigate any additional risk imposed?

Key points

What the literature tells us

- There is a large body of research examining the occurrence and survival of human pathogens in agricultural water.
- Reported occurrence and survival rates vary widely. Persistence is strongly influenced by variables such as temperature, available moisture (wetness and Relative Humidity), crop type and presence/absence of other microbes.
- Although *E. coli* is often used as an indicator of contamination, other pathogens (eg viruses) may survive longer and are less well researched.
- The method used to apply irrigation water (eg drip vs. overhead) has a major impact on the potential for produce contamination.
- It is not clear what reservoirs of pathogens exist, particularly the prevalence and importance of biofilms.
- In most cases, pathogens on the surface of fresh produce die off rapidly once the crop dries, especially if conditions are sunny, hot and dry.
- Although a number of disease outbreaks have been linked to agricultural water, there are few, if any, proven causative connections between food borne illness and contamination by irrigation.

Research Gaps

- Improved information on water quality risk assessment, testing and water source management is required to help growers identify water use risks.
- The potential for pathogen transfer from agricultural water to produce surfaces needs to be better understood. The dynamics of transfer will determine the residual pathogen populations on the produce.
- Pathogen survival and growth on produce types in production environments needs further investigation. Identifying the pathogen and commodity characteristics that increase or decrease pathogen survival and growth would help growers to assess risks from water use.
- The ability of pathogens to remain viable and grow on different produce types through distribution systems and storage environments requires further investigation.

- Industry guidelines and communication strategies need to be developed on effective water sanitation methods. This is required to improve industry understanding of how to remediate or treat water. User-friendly information on sanitation strategies, products and use methods for water sanitation management is required to enable growers and packers to correctly manage water treatment.

3.1 Background

Water used for irrigation and as a carrier for plant protection products, fertilisers and for frost protection is a potential source of contamination of fresh produce. Water may be directly contaminated by faeces from wild or domestic animals (eg ducks in the dam) or indirectly by leaching and runoff from composting sites, septic tanks, sewage systems or pasture used by livestock.

Water can provide a means for pathogens to adhere to plant surfaces, or become internalised through above ground structures or roots. There is a very large body of literature on these topics, and this is included in the appendices of this report. Despite this, even where contaminated water has been suspected of causing a food safety outbreak, the resulting trace back investigation has failed to irrefutably demonstrate the mechanism by which this occurred.

There are also many ways of reducing risk, including using drip rather than overhead irrigation and preventing build-up of biofilms that can harbour pathogens inside irrigation equipment.

3.2 Influence of water source on pathogen load

Pathogen load and contamination risk vary with the water source used. Indicator pathogens may be tested to verify that water is suitable for irrigation[1].

Reticulated water is the best quality, based on strict drinking water standards for levels of microbial contaminants. It is seldom used for irrigation because most farms are not located near a supply and it is costly. In Australia, water from reticulated supplies is available in some vegetable production regions. However, the cost of this water as a sole source for irrigation is prohibitive in most circumstances, given that horticultural crops generally require between 3 and 6 ML per hectare of water to reach optimum yield. Growers more often use reticulated water for spray mixes and hand washing.

Groundwater pumped **from deep bores** is considered relatively safe, as direct faecal contamination is unlikely, unless the bore (extraction point) is very close to a major faecal contamination source and the geology of the area allows easy drainage into groundwater (eg karsts, fissures)[2] .

Surface water (dams, lakes, rivers and streams) and **shallow groundwater bores** carry risks that increase with the proximity to high-density domestic or wild animal populations, animal product processing facilities and human dwellings and their wastes. Grazing activities can increase bacterial counts in runoff

[1] Tyrrel, S. F., Knox, J. W., Burton, C. H. and Weatherhead, E. K., 2004. *Assuring the microbiological quality of water used to irrigate salad crops: an assessment of the options available.* Horticultural Development Board Report FV248. Accessed 1 October 2105 at http://horticulture.ahdb.org.uk

[2] Groves, S. J., Davies, N., Aitken, M. N., 2002. A review of the use of water in UK agriculture and the potential risks to food safety. UK Food Standards Agency Project B17001. Accessed on 1 October 2015 at tna.europarchive.org

water particularly following heavy rainfall and if stocking rates are high, the ground is sloping and vegetation cover is poor. The highest risks occur when animals are allowed in the water. Contamination from animals can persist for one year after livestock have been removed from the location, although the level of contamination declines with time[3]. Standing surface water such as in **on-farm dams** may also have high levels of pathogens due to either pollution of the original water source (eg a river) or from direct faecal inputs from wildlife or domestic animals (eg flocks of birds).

Similarly **spring water** can be susceptible to faecal contamination due to animal excreta in runoff from land after heavy rainfall. *E. coli* counts have been observed to rise from near zero to $6x10^3$ colony forming units (cfu/100 ml) after such events[2]. Inadequate installation or improper maintenance of water extraction points can increase the risk.

Birds or other wild animals may contaminate **stormwater (rainwater)** collected **in tanks or dams** by soiling the surfaces the water is collected from. Stormwater would not be a major source for irrigation but is used for hydroponic production, especially where large water volumes can be collected from structure roofs and water use is lower than for field production.

Recycled water, if used raw or inadequately treated (so not in compliance with recycled water regulations), can easily contaminate fresh produce. Use of recycled water requires wastewater management plans to be prepared for the Environment Protection Authority (EPA). Recycled water is generally strictly monitored, so that this type of water may be safer than some other sources.

In summary, the greatest risks to water sources are

- Faecal contamination from farm animals and wild animals, especially if they have access to a water source.
- Human sewage.
- Contaminated soil, manures or sludges that enter water via erosion and percolation.

3.3 Risks associated with irrigation method

The risk of microbial contamination of the edible portion of a crop can be directly linked to the method of irrigation. Methods include surface furrow/flood, above surface or subsurface drip or overhead irrigation (centre pivots, irrigation guns). These can be ranked according to the likelihood of water contaminating the edible part of the crop.

[3] Hooda, P. S., Edwards, A. C., Anderson, H. A., Miller, A., 2000. A review of water quality concerns in livestock farming areas. *The Science of the Total Environment* **250**, 143-167

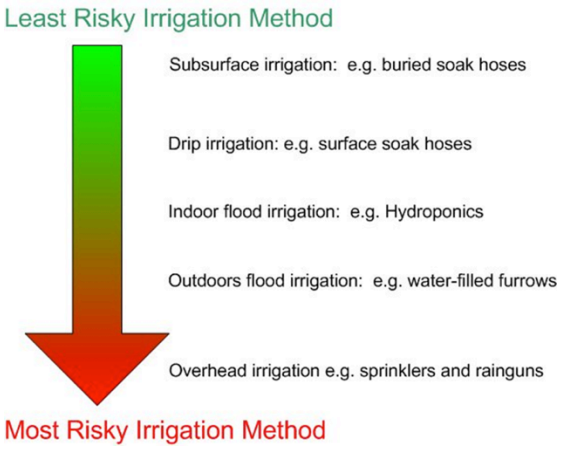

Figure 1 - Safest to most risky irrigation methods in terms of potential to contaminate a product grown close to or in contact with the soil (Reproduced from Food Standards Authority, 2015)

The effect of irrigation method on potential contamination of crops has been widely researched:

- Song et al.[4] compared irrigation using flooded furrows with subsurface drip in terms of their microbial safety. Melons, lettuce and capsicums were irrigated using water contaminated with *E. coli, Clostridium* and a bacteriophage (bacterial virus). No pathogens were found on capsicums irrigated by either method. However, flood irrigation resulted in significant contamination on melons and lettuce.
- Overhead irrigation increased the risk of *E. coli* contamination of lettuce compared to furrow and subsurface drip[5]. Product samples were positive for *E. coli* up to 7 days when using overhead irrigation, whereas only one product sample was found positive for *E. coli* when using furrow or subsurface drip irrigation methods.
- Bacteria survived longer in soil irrigated by furrow than soil irrigated by other methods, possibly due to soil remaining wet for a longer period after an irrigation event. Survival ranged from an estimated 17 days in winter months to 5 days during the warmer summer periods.
- Hutchison et al.[6] determined the implications of overhead irrigation using contaminated water on baby spinach and lettuce grown in two different soil types in the United Kingdom. Pathogens of interest were *E. coli, Campylobacter, L. monocytogenes* and *Salmonella enteriditis*. Although the irrigation water contaminated the above ground parts of produce, the number of bacteria became too low to count after 2 weeks of dry, sunny weather for both crop types.
- Armon et al.[7] estimated that if drip irrigation was used to apply contaminated irrigation water, the microbial risks of a food disease outbreak were 100-1000 times lower than if overhead sprinklers applied the same water.

[4] Song , I., Stine, S.W., Choi, C.Y. and Gerba,C.P., 2006. Comparison of crop contamination by microorganisms during subsurface drip and furrow irrigation. *Journal of Environmental Engineering-Asce* **132**,1243-1248.

[5] Fonseca, J.M., Fallon, S.D., Sanchez, C.A. and Nolte, K.D. 2011. *Escherichia coli* survival in lettuce fields following its introduction through different irrigation systems, *Journal of Applied Microbiology*, **110**, 893-902.

[6] Hutchison, M.L., Avery, S.M., Monaghan, J.M., 2008. The air-borne distribution of zoonotic agents from livestock waste spreading and microbiological risk to fresh produce from contaminated irrigation sources. *Journal of Applied Microbiology* **105**(3), 848–857.

[7] Armon,R., Dosoretz C.G., Brodsky M and Oron G. 2002. Surface and subsurface irrigation with effluents with different qualities and presence of *Cryptosporidium oocysts* in soil and crops. Wat.Sci.Techn. 46(3) 115-122

- Park et al[8] concluded in their review that overhead irrigation using contaminated water was one of the highest risks for causing contamination on leaves.

- UC Davis (Post Harvest Technology) supports these findings, noting that overhead irrigation can potentially apply contaminated water directly to the edible portions of fruits and vegetables.

3.4 Pathogen occurrence and survival in agricultural water

One of the factors limiting contamination by irrigation water is the absolute amount of water that adheres to the plant surface. So, for example, 10.8mL of recycled water can be retained per 100g of lettuce following overhead irrigation, but only 0.36mL per 100g of cucumber[9]. An Australian study also found water retention within this range, with three types of cabbage holding an average of 3.3–8.9mL per 100g and broccoli 1.9mL per 100g (A Hamilton, Research Fellow, Deakin University, pers. comm. 2005).

Another factor limiting contamination is the rapid die-off of most organisms once the plant dries. For example, *Listeria* spp. applied to spinach fields died off rapidly in the 24-48 hours following irrigation[10].

However, *Listeria* spp. can form biofilms, which attach to solid surfaces, such as the inside of irrigation equipment. This means that while the pathogen may die off relatively quickly under field conditions, it may survive in irrigation infrastructure, re-contaminating the crop every time it is used[11].

Similarly, *E. coli* is believed to possibly enter a 'dormant' state under adverse environmental conditions[12]. Rainfall events or other disturbance can re-suspend dormant bacteria held in sediments etc., potentially resulting in rapid elevation of *E. coli* levels in irrigation water[13].

Some organisms can survive quite extended periods in groundwater. For example, *Bacillus megaterium* and *Staphylococcus aureus* survived 10 to 100 days in groundwater[14], while other researchers have reported survival times of >400 days, with low temperatures prolonging survival[15]

Survival in surface water may be shorter than in groundwater, especially if water is warmer. The number of viable *E. coli, Yersinia enterocolitica,* and *Campylobacter jejuni* in irrigation water all declined over a four day period[16], especially under warm conditions.

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[8] Park, S., Szonyi, B., Gautam, R., Nightingale, K., Anisco, J. and Ivanek, R., 2012. Risk factors for microbial contamination in fruits and vegetables at the pre harvest level: a systematic review. *Journal of Food Protection* **75**, 2055-2081

[9] Shuval, H., Lampert, Y. and Fattal, B. 1997. Development of a risk assessment approach for evaluating wastewater reuse standards for agriculture. *Wat. Sci. Technol.*, **35**, 15-20.

[10] Weller D., Wiedmann M. and Strawn L. 2015. Spatial and temporal factors associated with an increased prevalence of *Listeria monocytogenes* in New York spinach fields. *Appl. Environ. Microbiol*. doi:10.1128/AEM.01286-15.

[11] Chae, M.S. and Schraft, H. 2000. Comparative evaluation of adhesion and biofilm formation different *Listeria monocytogenes* strains. I*nternational Journal of Food Microbiology,* **62**, 103-111.

[12] van Elsas, J. D. Semenov, A. V. Costa, R. and Trevors, J. T. 2011. Survival of *Escherichia coli* in the environment: Fundamental and public health aspects. *ISME J.*, **5**, 173-183.

[13] Davies, C. M., Long, J. A., Donald, M., and Ashbolt, N. J. 1995. Survival of faecal microorganisms in marine and freshwater sediments. *Appl. Environ. Microbiol.,* **61,** 888-1896

[14] Filip Z. and Demnerova K. 2009. Survival in groundwater and FT-IR characterization of some pathogenic and indicator bacteria. In: Jones JAA, Vardanian TG, Hakopian C (ed) *Threats to Global Water Security. Springe*r, Dordrecht, The Netherlands, pp 117–122

[15] Nevecherya I.K., Shestakov V.M., Mazaev V.T., Shlepnina TG 2005. Survival rate of pathogenic bacteria and viruses in groundwater. *Water Resources* ,**32**, 209–214

The Center for Produce Safety in the USA have summarised research on agricultural water[17]. The review suggests a number of factors increase the risk of contaminants occurring and surviving in agricultural water:

- Weather conditions causing runoff/erosion carrying faeces, especially if combined with favourable temperatures.
- Presence of sediments suspended in water or streambeds
- Biofilms in irrigation infrastructure
- Use of overhead irrigation
- Liquid spray applications prepared using contaminated water – although the role of surfactants, spreaders etc. is not clear.
- Low temperatures (sometimes high temperatures, depending on species and strain)
- Available nutrients in water
- Protection from UV light
- Absence of microbes which compete with or predate upon human pathogens

Again, sampling methods, samples size and analytical methods were mentioned as having an impact on the detection levels of enteric pathogens in water and on crops. Consistent with other reports, it was mentioned that *E. coli* may not be the most reliable indicator of the actual level of contamination because other pathogens eg *Salmonella* or viruses, may survive longer. Conversely, detection of high levels of *E. coli* does not necessarily mean that produce is unsafe, as some non-pathogenic strains may survive longer than those that cause illness.

Human pathogens have been reported in water sources used for agriculture Australia and New Zealand. For example, *C. jejuni*, enterohaemorrhagic *E. coli* and *Salmonella* spp. were detected in ponds and creeks taken in the Brisbane area[18]. The New Zealand study identified *Campylobacter* in rivers, shallow ground water, drinking water and stormwater sources[19].

Salmonella contamination of tomatoes (in 2002 and 2005) and peppers (in 2008) affected hundreds of people in the USA across multiple states. The outbreaks were linked to contaminated ponds containing irrigation water[20]. The tomato outbreaks of 2002 and 2005 were both traced to the same water source but occurred over a three-year period[21]. Although the water did not contact the fruit through irrigation, it may have been used to apply pesticides.

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[16] Terzieva S.I. and McFeters G.A.1991. Survival and injury of *E. coli, Campylobacter jejuni,* and *Yersinia enterocolitica* in stream water. *Can J Microbiol*.., **37**:785–790 .

[17] www.pma.com/~/media/pma-files/food-safety/cps/cps-research-reportag-water-200813version-11final.pdf

[18] Ahmed, W., S. Sawant, F. Huygens, A. Goonetilleke, and T. Gardner. 2009. Prevalence and occurrence of zoonotic bacterial pathogens in surface waters determined by quantitative PCR. *Wat. Res*. **43**, 4918-4928.

[19] Savill, M.G., J.A. Hudson, A. Ball, J.D. Kiena, P. Scholes, R.J. Whyte, R.E. McCormick and D. Jankovic. 2001. Enumeration of Campylobacter in New Zealand recreational and drinking waters. *J. Appl. Microbiol.,* **91**, 38-46.

[20] Hanning, IB., Nutt, J.D., Ricke S.C., 2009. Salmonellosis Outbreaks in the United States Due to Fresh Produce: Sources and Potential Intervention Measures, *Foodborne Pathogens and Disease* **6**, 635-648.

[21] Green, S.K., Daly, E.R., Talbot, E.A., 2008. Recurrent multistate outbreak of *Salmonella* Newport associated with tomatoes from contaminated fields, 2005. *Emerging Infectious Diseases*, **136**, 157-165.

Erickson[22] compiled international research on the fate of enteric pathogens and indicators in agricultural water sources. The table summarising this information is included in the Appendices (Section 7.1) of this document.

3.5 Reducing risk from irrigation water

3.5.1 Managing how water is applied

Contaminated irrigation water poses the greatest foodborne illness risk when it comes in contact with the edible part(s) of a plant that is eaten raw (peeled or unpeeled) close to harvest. The persistence of pathogenic microorganisms on and in fruits and vegetables in the field is variable and is subject to factors such as sunlight, rainfall, the characteristics of the fruit or vegetable and its native microbial population, the microbial ecology of the soil and the initial concentration and characteristics of the pathogen itself.

The WHO (2003, 2006) summarised measures that can be expected to reduce contamination of fresh produce by human pathogens carried in irrigation water. Although intended for smallholder farmers, the information gives an approximate indication of the effectiveness of different irrigation / water management strategies (Table 10).

[22] Erickson M. 2013. A Systems Approach for Produce Safety: A Research Project Addressing Leafy Greens. Center for Food Safety, University of Georgia. Accessed 1 October 2015, at www.ugacfs.org/producesafety

Table 10 - Pathogen reductions expected for various treatment processes of potentially contaminated irrigation water

Control measure	Pathogen reduction (log units)	Notes
Wastewater treatment	6 – 7	Pathogen reduction depends on type and degree of treatment
On farm options		
Crop restriction	6 – 7	No crops grown which may be eaten uncooked. May not be a commercial option
Three tank system	1 – 2	One pond is being filled, one is settling, settled water from third used for irrigation
Simple sedimentation	0.5 – 1	Sedimentation for approx 18 hours
Simple filtration	1 – 3	Value depends on filtration system used
Method of water application		
Furrow irrigation	1 – 2	Crop density and yield may be affected
Drip irrigation	2 – 4	2-log reduction for low growing crops, 4 log reduction for taller crops
Reduction of splashing	1 – 2	Adjustment of irrigation equipment for small droplet size, mulching
Pathogen die off	0.5 – 2 per day	Withholding period between last irrigation and harvest (depends on crop type, climate etc)
Postharvest		
Washing in clean water	1 – 2	Washing salad crops, vegetables and fruit in clean water
Washing with sanitiser	2 – 3	Washing in high microbial quality water with sanitiser
Cooking	5 – 6	Up to consumer preference

Pathogen die off after irrigation is usually very rapid, although rates will depend on humidity, crop type etc. The WHO (1989) estimated die off rates of various human pathogens on crops:

Enteroviruses	<60 but usually <15 days
Faecal coliforms	<30 but usually <15 days
Salmonella spp.	<30 but usually <15 days
Vibrio cholerae	<5 but usually <2 days
Protozoa	<10 but usually <2 days

3.5.2 Microbial water quality standards

There are a range of standards in place in Australia and New Zealand regarding the microbial quality of drinking water and water used for recreation such as swimming. Recreational water quality may be analogous to some of the standards used for irrigation water, particularly close to harvest, if water contacts the edible part and the product is eaten uncooked.

The median bacterial content in samples of fresh or marine waters taken over the bathing season should not exceed:

- 150 faecal coliform organisms/100 mL (minimum of five samples taken at regular intervals not exceeding one month, with four of five samples containing less than 600 organisms/100 mL)
- 35 enterococci organisms/100 mL (max number in any one sample: 60–100 organisms/100mL).

Pathogenic free-living protozoans should be absent. It is not necessary to analyse water for these pathogens unless the temperature is greater than 24°C.

ANZECC 2000 guidelines for irrigation water provide the following trigger values for thermotolerant coliforms in irrigation water:

- Raw human food crops in direct contact with irrigation water (eg via sprays, irrigation of salad vegetables): <10 cfu / 100 mL
- Raw human food crops not in direct contact with irrigation water (edible product separated from contact with water, eg by peeling, use of drip irrigation); or crops sold to consumers cooked or processed: <1000 cfu / 100 mL

The irrigation water guidelines do not include preventative or corrective actions for 'pathogen contaminated' irrigation water, or distinguish between crop risks. Despite this, regional councils in New Zealand have adopted the ANZECC 2000 guidelines. They use it to support resource consent applications (people wanting to take water for irrigation must apply for consent to do so).

International standards for irrigation water vary considerably, including both the indicator organism tested and the numbers present in water (Table 11).

Table 11 - Selected international standards and guidelines for indicator organisms in irrigation water for crops likely to be eaten uncooked.

Issuing body	Indicator bacteria	Performance criteria
World Health Organisation, Treated wastewater	Faecal coliforms	≤ 1000 CFU/100 ml (calculated as a geometric mean)
State of California USA, Recycled irrigation water	Total coliforms	≤ 2.2 MPN CFU/100 ml in previous 7 days of test results. Not more than one sample to exceed 23 MPN CFU/100 ml in previous month
Canadian Agricultural Ministry, Irrigation water	Faecal coliforms or *E. coli* and also Total coliforms	≤ 100 CFU of faecal coliforms or *E. coli* per 100 ml ≤1000 CFU of total coliforms per 100 ml
Tesco Stores Nurture Scheme, Irrigation water	*E. coli* and also Total coliforms	≤ 1000 CFU/100 ml for both indicators (calculated as geometric mean if multiple samples are taken)
Marks & Spencer Field to Fork, Irrigation water	*E. coli*	≤ 1000 CFU/100 ml
Australian Government Raw human food crop in direct contact with irrigation water eg via sprays, irrigation of salad	Thermotolerant (faecal) coliforms	<10 CFU/100 ml based on median value of thermotolerant coliforms from a number of readings generated over time from a regular monitoring program. Cause should be investigated when 20% of

Issuing body	Indicator bacteria	Performance criteria
crops		results exceed the median guideline value.
Australian Government, irrigation Raw human food crops not in direct contact with irrigation water (edible product separated from contact with water, eg by drip irrigation); or crops sold consumers cooked or processed	Thermotolerant (faecal) coliforms	<1000 CFU/100 ml based on median value of thermotolerant coliforms from a number of readings generated over time from a regular monitoring program. Cause should be investigated when 20% of results exceed the median guideline value.
Texas*** Recycled irrigation water (not permitted on foods that may be consumed raw, only irrigation types that avoid reclaimed water contact with edible portions of food crops are acceptable)	Faecal coliforms	20/100ml MPN/100 ml (30 day geometric mean)
Israel, Standards for reuse in irrigation	*E. coli*	<10 CFU/100ml.
CFU: Colony Forming Units. Test results are usually reported in CFU. ** MPN: Most Probable Number. This is a statistical estimate of the bacterial numbers within a sample rather than an absolute count (which is made by the filter method). This involves testing a number of small volumes of a sample and statistically estimating the probable number of bacteria contained within it.		

3.5.3 Recycled water

In many countries, guidelines for the use of recycled water require zero faecal coliform bacteria/100 ml for water used to irrigate crops that are eaten raw, achieved through secondary treatment, filtration and disinfection of recycled water. The United States Environmental Protection Agency (USEPA) and the US Agency for International Development (USAID) have taken this approach, and consequently have recommended strict guidelines for wastewater use USEPA, 1992). For unrestricted irrigation (including high-risk crops likely to be eaten raw), no detectable faecal coliform bacteria are allowed in 100 ml (compared with the 1989 WHO guidelines of <1000 faecal coliform bacteria/100 ml), and for irrigation of commercially processed crops the guideline limit is <200 faecal coliform bacteria/100 ml (a guideline limit on the presence of nematode eggs is set by the WHO only).

In the USA, the setting of Standards is the responsibility of individual states, and different states take different approaches (some specify treatment processes, others specify water quality standards). A range of standards are in use[23]. For unrestricted irrigation of food crops these range from 10–1000 faecal coliform bacteria/100 ml for surface irrigation and 2.2–200 faecal coliform bacteria/100 ml for overhead irrigation. California has some of the strictest standards, requiring <2.2 total coliform bacteria/ 100 ml for irrigation of food crops.

[23] Cooper, R.C. and Olivieri, A.W., 1998. Infectious disease concerns in wastewater reuse. In: Asano T, ed. Wastewater reclamation and reuse. Lancaster, PA, Technomic Publishing, 1998: 489–520.

Many countries, including in the European Union, have used advice from the 1989 WHO guideline rather than adopting the stricter USA approach[24]. France published guidelines in 1991 that are similar to those of WHO in defining analogous water categories (called A, B and C in the WHO guidelines) and microbial limits but complemented them with strict rules for application[25]. For example, for category A in the French guidelines, the quality requirement must be complemented by the use of irrigation techniques that avoid wetting fruit and vegetables.

The Australian National Guidelines for Water Recycling set out recommendations based on determination of acceptable or tolerable risk. The guidelines use disability adjusted life years (DALYs) to convert the likelihood of infection or illness into burdens of disease, and set a tolerable risk as 10^{-6} DALYs per person per year. The tolerable risk is then used to set health-based targets that, if met, will ensure that the risk remains below 10^{-6} DALYs per person per year.

In identifying hazards, it is impractical to set human health-based targets for all microorganisms that might be present in a source of recycled water; therefore, the guidelines specify the use of reference/ indicator pathogens instead — *Campylobacter* for bacteria, rotavirus and adenovirus for viruses, and *Cryptosporidium parvum* for protozoa and helminths. Dose–response information from investigations of outbreaks (data more readily available than human feeding studies) could be used to determine how exposure to a particular dose of a hazard relates to incidence or likelihood of illness.

The Australian critical limits for recycled water are as follows:

1. *E. coli* limits for commercial food crops consumed raw or unprocessed (the same as for drinking water in New Zealand): <1 per 100 mL. At this level use is unrestricted.

2. Water with secondary treatment, > 25 days lagoon retention and disinfection and E. coli <100 cfu/100 mL can be used for commercial food crops under the following conditions:

    - Crops with limited or no ground contact and eaten raw (eg tomatoes, capsicums) — drip irrigation and no harvest of wet or dropped produce
    - Crops with ground contact with skins removed before consumption (eg watermelons) — if spray irrigation, minimum 2 days between final irrigation and harvest
    - Pathogen reduction between harvesting and sale 0.5 log CFU/day

3. Water with secondary treatment, no lagoon retention and disinfection and E. coli <100 cfu/100 mL can be used for commercial food crops under the following conditions:

    - All above-ground crops with subsurface irrigation
    - Crops with no ground contact and skins removed before consumption (eg, citrus, nuts)
    - No harvest of wet or dropped produce
    - If overhead irrigation, minimum 2 days between final irrigation and harvest

[24] Bontoux L. 1998. The regulatory status of wastewater reuse in the European Union. In: Asano T, (Ed.), *Wastewater reclamation and reuse.* Technomic Publishing, Lancaster, USA, pp 1463–1476.

[25] Bontoux, L and Courtois, G., 1998. The French wastewater reuse experience. In: Asano T, (Ed.), Wastewater reclamation and reuse. Lancaster, PA, Technomic Publishing, pp 489–520.

4. Water with secondary treatment or primary treatment with lagoon detention and *E. coli* <1000 cfu/100 mL can be used for commercial food crops under the following conditions:
    - Crops with no ground contact and heavily processed (eg grapes for wine production, cereals)
    - Crops cooked/processed before consumption (eg potatoes, beetroot)
    - No harvest of wet or dropped produce consumption (eg citrus, nuts) – no spray irrigation
    - Crops with no ground contact and skin removed before consumption
    - Raised crops (eg apples, apricots, grapes) – drip irrigation and no harvest of wet, or dropped produce
    - Pathogen reduction between harvesting and sale 0.5 log units/day

New Zealand does not have general recycled water guidelines but guidelines exist for grey water (household wastewater excluding sewage). The New Zealand Environment Research Foundation has published New Zealand Municipal Wastewater Monitoring Guidelines (NZWEDRF, 2002). This document provides guidance to developing **risk based monitoring programmes for municipal wastewater discharges**. Although discharge to food gathering areas is considered (eg shellfish beds), horticultural production or irrigation are not addressed.

Apart from recycled water guidelines presented above, the proposed / reviewed US Agricultural Water Standards may provide guidance for Australia and New Zealand.

4 Organic inputs and composting

Is the current 90-day pre harvest restriction for applying untreated organic inputs justified for Australia and New Zealand? What are the breakdown rates of microbial contaminants on fresh produce under a variety of input, soil and climatic conditions? Under what conditions could the 90-day restriction be reduced or extended?

Key points

What the literature tells us

- Pathogen survival times in soil amended with organic materials vary widely.
- A number of environmental variables impact significantly on pathogen survival.
- Occurrence of human pathogens in organic inputs varies greatly, with the number present at the start significantly affecting the time until they are no longer detectable.
- Outbreak data suggest that *E. coli* and *Salmonella* spp. are the two bacterial groups found in organic materials that pose the greatest risk.
- The survival of other foodborne pathogens on fresh produce, including viruses, protozoa and nematodes, is not well understood.
- Composting critical limits that destroy *E. coli* and *Salmonella* spp. may not control other food safety pathogens.
- Inputs to composting and the processes used may affect the safety of the end product.
- Pathogen survival in laboratory studies cannot necessarily be extrapolated to field situations.

Research Gaps

- Data on the types of manures used in Australian and New Zealand composts is required. This will enable assessment of the pathogen types and effectiveness of composting treatments in reducing risks to an acceptable level.
- Research on the effect of different animal feedstock on pathogen types, populations and survival in the manure are required to complement risk assessments and effective composting treatments.
- Research is required to evaluate how long pathogens persist in Australian and New Zealand agricultural soils and production systems. Production environment variables may be different from studies in the published literature.

- Research is required to determine if other food safety pathogens such as viruses, protozoans and nematodes are present on Australian and New Zealand fresh produce.
- Industry guidelines and communication strategies are required to better communicate and inform Australian and New Zealand growers on correct composting practices.

4.1 Background

Composted organic materials from a range of sources are used as organic amendments (fertilisers and soil conditioners) during fruit and vegetable production. Sources include farm waste, manures, household wastes, municipal green wastes and other biodegradable wastes (eg textiles, paper, wood), as well as sludges (eg from food processing or as biosolids). Some growers may also use uncomposted organic materials.

The microbial content in the different forms of organic solid and liquid / sludge type soil amendments and other organic inputs used as fertilisers, for pest and disease control, soil improvement or as biostimulants will vary depending on its origin, composition and treatment.

The increased use of crop production inputs that are derived from biodegradable materials in agricultural production, especially horticulture, has two important drivers:

- Increased interest by growers in integrated or organic production of fruits and vegetables with reduced or no inputs of synthetic fertilisers and pesticides.
- A significant focus by government policies and programs on recycling organic products.

Characterising the makeup and quality of different organic products is challenging. However, understanding the content of organic products applied to soil is essential for ensuring reliable performance as well as understanding risks.

This section deals with hazards, risks, and control measures for organic inputs and composting. The focus is on avoiding contamination of fresh produce with human pathogens that may be carried through to harvested produce.

4.1.1 Definitions

“Soil amendments” are any chemical, biological, or physical material intentionally added to the soil to improve its chemical, physical or biological condition or increase water holding capacity. In this document, we use the term soil amendments to refer to soil amendments that consist, in whole or in part, of materials of animal origin, including manure, animal by-products and table waste. More detailed definitions of terminology are included in the appendices of this report.

4.2 Soil borne pathogens

The microbes responsible for causing "human diseases resulting from any pathogen or parasite, transmission from the soil, even in the absence of other infectious individuals" [43] can be divided into two groups: Edaphic pathogenic organisms (EPOs) and soil transmitted pathogens (STPs).

EPOs are potential pathogens that are true soil organisms. That is, their usual habitat is the soil. This list includes most bacterial pathogens and all the fungal pathogens. However, most of these bacteria still need a definitive host or group of hosts, as they cannot multiply or survive indefinitely in the soil.

STPs are organisms that, while they may be able to survive in soil for extended periods of time, are not true soil organisms. Rather, these are obligate pathogens that must infect a host in order to complete their life cycles.

It should be noted that, as with many classifications and groupings, strict categorisation has its limitations. A continuum is rather likely to exist with some overlap of organisms between the two groupings (eg strongyloidiasis and shigellosis). Jeffery and van der Putten[43] have grouped human pathogens in soils (Table 12).

Table 12 - Soil borne infectious diseases and some of their causal organisms, split into EPOs and STPs depending on their relationship with the soil.

Edaphic pathogenic organisms (EPOs)		Soil Transmitted Pathogens (STPs)	
Disease	**Causal organism**	**Disease**	**Causal organism**
Actinomycetoma	*Actinomyces israelii*	Poliovirus	
Anthrax	*Bacillus anthracis*	Hantavirus	
Aspergillosis	*Aspergillus* sp.	Amoebiasis	*Entamoeba histolytica*
Blastomycosis	*Blastomyces dermatitidis*	Ascariasis	*Ascaris lumbricoides*
Botulism	*Clostridium botulinium*	Balantidiasis	*Balantidium coli*
Campylobacteriosis	*Campylobacter jejuni*	Cyclosporiasis	*Cyclospora cayetanensis*
Coccidioidomycosis	*Coccidiodes immitis*	Cryptosporidiasis	*Cryptosporidium parvum*
Gas Gangrene	*Clostridium perfringens*	Echinococcosis	*Echinococcus multicularis*
Histoplasmosis	*Histoplasma capsulatum*	Gastroenteritis	*Escherichia coli*
Leptospirosis	*Leptospira interrogans*	Giardiasis	*Giardia lamblia*
Listeriosis	*Listeria monocytogenes*	Hookworm	*Ancylostoma duodenale*
Mucormycosis	*Rhizopus* sp.	Isosporiasis	*Isospora belli*
Mycetoma	*Nocardia* sp.	Lyme disease	*Borrelia* sp.
Sporotrichosis:	*Sporothrix schenckii*	Pinworm	Genus Enteriobius
Strongyloidiasis	*Strongyloides stercoralis*	Q Fever	*Coxiella burnetii*
Tetanus	*Clostridium tetani*	Salmonellosis	*Salmonella enterica enterica*
Tularemia	*Francisella tularensis*	Shigellosis	*Shigella dyseneriae*

[43] Jeffery, S. and van der Putten, W. 2011. Soil-borne human diseases. European Commission, EUR 24893 EN – 2011.

Edaphic pathogenic organisms (EPOs)		Soil Transmitted Pathogens (STPs)	
Yersiniosis	*Yersinia enterocolitica*	Strongyloidiasis	*Strongyloides stercoralis*
		Toxoplasmosis:	*Toxoplasma gondii*
		Trichinellosis	*Trichinella spiralis*
		Whipworm	*Trichuris trichiura*
			Pseudomonas aeruginosa

Most human pathogens in agricultural soils originate from faecal contamination. Faecal contamination in soil amendments may be introduced from farm manures, human biosolids or faeces in municipal green waste. Wildlife, rodents or pets can also introduce faeces to growing sites. Pathogens found in soil due to such natural contamination with organic materials include pathogenic *E. coli*, *Salmonella* spp., *Campylobacter* spp., *Clostridium perfringens*, *Clostridium difficile*, *Cryptosporidium* spp., *Giardia* spp., *Toxoplasma gondii*, *L. monocytogenes*, *Y. enterocolitica*, *Y. pseudotuberculosis*. Many of these pathogens can also be detected in livestock.

4.3 Pathogen survival in soils

The prevalence, persistence and infectivity of a range of pathogens after disposal on agricultural land has been extensively studied and reviewed. Enteric pathogens can move both horizontally and vertically on and in soil. They can spread onto produce, surface waters and ground waters adjacent to production areas[44]. A systematic review of risk factors for contamination of fruits and vegetables with soilborne *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7 confirmed that soil conditions influence pathogen survival and, therefore, microbial contamination at harvest[45].

Some studies have found that certain pathogens survived in the soil for as much as one year or longer. However, most faecal pathogens from human and animal wastes usually die more quickly[46]. Pathogen die-off in soils is affected by many factors, including time, temperature, pH, moisture, relative humidity, tillage, sunlight, predators and microbial competition in the soil.

Factors that may increase pathogen survival include:

- Crop factors. These include structural features that aid attachment of pathogens[47], internalisation of pathogens[48] and root exudates that provide nutrients to soil borne pathogens.

[44] Cooley, M., Carychao, D., Crawford-Miksza, L., Jay, M.T., Myers, C., Rose, C., Keys, C., Farrar, J., Mandrell, R.E., 2007. Incidence and tracking of *Escherichia coli* O157:H7 in a major produce production region in California. PLoS ONE 11:E1159.

[45] Park, S., Szonyi, B., Gautam, R., Nightingale, K., Anisco, J. Ivanek, R., 2012. Risk factors for microbial contamination in fruits and vegetables at the pre harvest level: a systematic review. *Journal of Food Protection* **75**, 2055-2081.

[46] Mubiru, D. N. et al. 2000. Mortality of Escherichia coli O157:H7 in Two Soils with Different Physical and Chemical Properties". Plant and Soil Sciences Faculty Publications. Paper 7.

[47] Saldaña, Z., E. Sánchez, J. Xicohtencatl-Cortes, J.L. Puente, and J.A. Girón. 2011. Surface structures involved in plant stomata and leaf colonization by Shiga-toxigenic Escherichia coli O157:H7. Frontiers Microbiol. 2. Article 119.

- The nature of the organic amendment. Greater percolation into soil occurs from a liquid slurry source, while a solid source may result in greater spread over the soil surface[49].
- Moist, compared to dry, environments.
- Lack of microbial competition.
    - Reducing the number of organisms that can survive in low organic matter soils may reduce competitive pressure on enteric pathogens, increasing survival[50].
    - When microbial competition was high, *E. coli* O157:H7 died off within 140-150 days, while low microbial competition allowed survival for more than 240 days (Figure 2).
    - If organic materials are applied after soil fumigation, lack of microbial competition may extend survival of added pathogens[51]. The use of composts and chicken manure products after fumigation to 'reinvigorate' the soil is relatively common in vegetable production
    - While soil microbial competition usually reduces human pathogen loads[52], one study reported enhanced survival of enteric pathogens in the presence of specific soil organisms[53].
    - Soil macrofauna such as nematodes can feed on enteric pathogens[54, 55, 56].

[48] Erickson, M.C., C.C. Webb, J.C. Diaz-Perez, S.C. Phatak, J.J. Silvoy, L. Davey, A.S. Payton, J. Liao, L. Ma, and M.P. Doyle. 2010. Infrequent internalization of Escherichia coli O157:H7 into field-grown leafy greens. J. Food Prot. 73:500-506.

[49] Forslund, A., Markussen, B., Toenner-Klank, L., Bech, T.B., Jacobsen, O.S., Dalsgaard, A., 2011. Leaching of *Cryptosporidium parvum* oocysts, *Escherichia coli*, and a *Salmonella enterica* serovar Typhimurium bacteriophage through intact soil cores following surface application and injection of slurry. *Applied and Environmental Microbiology* 77, 8129-8138.

[50] Semenov, A.V., E. Franz, L. van Overbeek, A.J. Termorshulzen, and A.H.C. van Bruggen. 2008. Estimating the stability of Escherichia coli O157:H7 survival in manure-amended soils with different management histories. Environ. Microbiol. 10:1450-1459.

[51] Ibekwe, A.M., S.K. Papiernik, C.M. Grieve, and C.-H. Yang. 2010. Influence of fumigants on soil microbial diversity and survival of E. coli O157:H7. J. Environ. Sci. Hlth. Part B 45:416-426.

[52] van Elsas, J.D., P. Hill, A. Chroňáková, M. Grekova, Y. Topalova, D. Elhottová, and V. Krištůfek. 2007. Survival of genetically marked Escherichia coli O157:H7 in soil as affected by soil microbial community. The ISME J. 1:204-214.

[53] Cooley, M.B., W.G. Miller, and R.E. Mandrell. 2003. Colonization of Arabidopsis thaliana with Salmonella enterica and enterohemorrhagic Escherichia coli O157:H7 and competition by Enterobacter asburiae. Appl. Environ. Microbiol. 69:4915-4926.

[54] Kenney, S.J., G.O. Anderson, P.L. Williams, P.D. Millner, and L.R. Beuchat. 2005. Persistence of Escherichia coli O157:H7, Salmonella Newport, and Salmonella Poona in the gut of a free-living nematode, Caenorhabditis elegans, and transmission to progeny and uninfected nematodes. Int. J. Food Microbiol. 101:227-236.

[55] Erickson, M.C., M. Islam, C. Sheppard, J. Liao, and M.P. Doyle. 2004. Reduction of Escherichia coli O157:H7 and Salmonella enterica serovar Enteritidis in chicken manure by larvae of the black soldier fly. J. Food Prot. 67:685-690.

[56] Huamanchay, O. L. Genzlinger, M. Iglesias, and Y.R. Ortega. 2004. Ingestion of Cryptosporidium oocysts by Caenorhabditis elegans. J. Parasitol. 90:1176-1178.

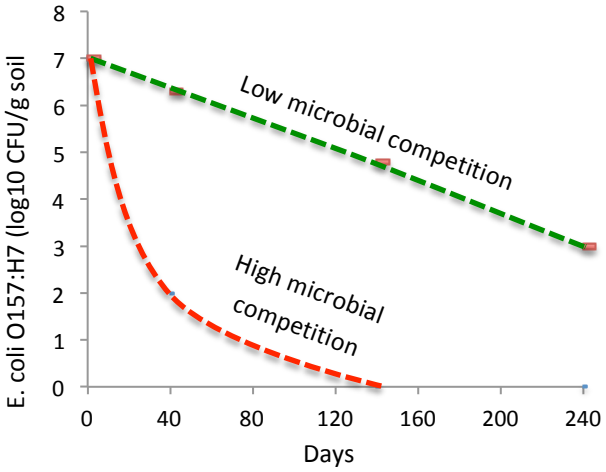

Figure 2 - Influence of microbial competition on survival of E. coli O157:H7 in manure applied to soil[57]

- Application of herbicides. These do not harm human pathogens but may kill other soil borne organisms, potentially increasing survival compared to non-herbicide treated plots[58].
- Cool, stable soil temperatures[59]. Fluctuations of soil temperature under tropical conditions cause metabolic stress, decreasing survival of pathogens[60, 61].
- Use of non-pH stabilised manure. Lower pathogen survival was observed for produce grown in soil mixed with alkaline-pH-stabilised manure due to release of ammonia under the increased pH conditions[62].
- Sandy, rather than clay, soil structure[63].
- Sunlight. The effect of sunlight on killing pathogens depends on the season and pathogen location in the soil profile[64].

[57] University of Maryland, 2010. Improving the Safety and Quality of Fresh Fruits and Vegetables: A Training Manual for Trainers

[58] Fischer-Arndt, M., D. Neuhoff, L. Tamm, and U. Kopke. 2010. Effects of weed management practices on enteric pathogen transfer into lettuce (Lactuca sativa var. capitata). Food Control 21:1004-1010.

[59] Literature on the effect of soil temperature in pathogen survival reviewed for this section can be found under a separate heading in the References section of this report

[60] Ongeng, D., C. Muyanja, J. Ryckeboer, D. Springael, and A.H. Geeraerd. 2011. Kinetic model-based prediction of the persistence of Salmonella enterica serovar Typhimurium under tropical agricultural field conditions. J. Appl. Microbiol. 110:995-1006.

[61] Ongeng, D., C. Muyanja, J. Ryckeboer, A.H. Geeraerd, and D. Springael. 2011. Rhizosphere effect on survival of Escherichia coli O157LH7 and Salmonella enterica serovar Typhimurium in manure-amended soil during cabbage (*Brassica oleracea*) cultivation under tropical field conditions in sub-Saharan Africa. Int. J. Food Microbiol. 149:133-142

[62] Wong, J. W., Selvam, A., 2009. Reduction of indicator and pathogenic microorganisms in pig manure through fly ash and lime addition during alkaline stabilization. *Journal of Hazard Mater* **169**, 882–889.

[63] Ibekwe, A. M., Papiernik, S. K., Grieve, C. M., Yang, C. H., 2011. Quantification of persistence of *Escherichia coli* O157:H7 in contrasting soils. *Int. J. Microbiol.* 2011:421379.

[64] Palacios, M.P., P. Lupiola, M.T. Tejedor, E. Del-Nero, A. Pardo, and L. Pita. 2001. Climatic effects on Salmonella survival in plant and soil irrigated with artificially inoculated wastewater: preliminary results. Wat. Sci. Technol. 43(12):103-108.

Many of these studies were conducted in Europe or northern areas of the USA. While these conditions may be comparable to those found in New Zealand, the persistence of enteric pathogens in tropical and subtropical agricultural soils such as in Australia is less well researched. It is not clear whether survival rates in these soils (and crops) are in a similar range to those reported for temperate climates.

Naganandhini et al. conducted a study that used conditions possibly more comparable to Australia[65]. They compared survival patterns of STEC strain (O157-TNAU) with non-pathogenic (MTCC433) and genetically modified (DH5α) strains on different tropical agricultural soils and on a vegetable growing medium (coco peat) under controlled conditions in India. Survival rates in soils in this study were 40-60 days.

4.4 Occurrence of pathogens in soil amendments

CSIRO[66] and the Recycled Organics Unit[67] have reviewed the use of organic soil amendments in Australian agriculture. Both reviews highlight the wide range of products and uses as well as a lack of consistency in the production and composition of many organic products. They note a general lack of understanding of the potential benefits and risks associated with organic soil amendments. Similar studies are not available for New Zealand, but the situation is likely to be similar.

The Australian Standard AS-4454 (2012) 'Composts, soil conditioners and mulches' states requirements for pasteurisation and defines biological stability and compost maturity. However, not all products sold commercially necessarily meet this standard. Raw or semi-processed manures or incorrectly composted organic mixes containing manures and/or biosolids as well as source-separated urban organic waste materials may be sold to fresh produce growers. Some of these may have a high-risk profile because their pathogen status is not verified through testing. Growers may not be aware of the food safety risks associated with the amendments they use.

In Canada and the US, some investigations following foodborne illness outbreaks linked to the consumption of raw fruits and vegetables have identified manures as the source of contamination. Examples include:

- *L. monocytogenes* on cabbage in Canada[68].
- *Salmonella* and *E. coli* O157:H7 on apples used to make apple juice in the US[68].

[65] Naganandhini S. John Kennedy Z., Uyttendaele M., and Balachandar D. 2015. Persistence of Pathogenic and Non-Pathogenic Escherichia coli Strains in Various Tropical Agricultural Soils of India. PLoS One. 2015; 10(6): e0130038. Published online 2015 Jun 23. doi: 10.1371/journal.pone.0130038

[66] Quilty J.R. and Cattle S. R. 2011. Use and understanding of organic amendments in Australian agriculture: a review. Soil Research, 2011, 49, 1–26.

[67] Recycled Organics Unit 2012. Organics Recycling in Australia. Industry Statistics 2012.

[68] Tauxe, R., 2001. Oral information at the Food Chain 2001 Conference 14.-16.3.2001, Uppsala, Sweden
Nguyen-the, C., Carlin, F., 1994. The microbiology of minimally processed fresh fruits and vegetables. *Crit. Rev. Food Sci. Nutr*. **34**, 371-401.

Field contamination may also be due to water runoff from nearby pastures or exposure to droppings from wild animals / birds[69] .

To understand the potential risk of different manure sources, Table 13 provides an overview of human pathogens identified in intestinal tracts of animals or in animal faeces. Some pathogens may not be listed because they have not been frequently reported in the literature. The majority of studies on faecal contamination of soils, soil amendments, water and plants focus on *E. coli* first, and *Salmonella* spp. second, as indicator organisms and pathogens.

Information on the survival of viruses in soils is relatively scarce in food safety related literature.[70]

Some bacteria, like *C. perfringens,* can survive in soils for months and outlast normal cooking procedures. Juneja et al.[71] note that it is a frequent cause of food poisoning, although fortunately with mild symptoms in healthy people. The incidence is not high in Australia or New Zealand, and is primarily related to consumption of meat, not fresh produce.

[69] Hilborn, E.D., Mermin, J.H., Mshar, P.A., Hadler, J.L., Voetsch, A., Wojtkunski, C., Swartz, M., Mshar, R., Lambert-Fair, J.A., Farrar, M., Glynn, M.K., Slutsker, L., 1999. A multistate outbreak of *Escherichia coli* O157:H7 infection associated with consumption of mesclun lettuce. *Arch. Intern. Med*. **159**, 1758-1764.

Rice, D.H., Hancook, D.D., Besser, T.E., 1995. Verotoxigenic *E.coli* O157 colonisation of wild deer and range cattle. *Vet. Rec*. **137**, 524

[70] Noble, R., Jones, P.W., Coventry, E., Roberts, S,R., Martin, M., Alabouvette, C., 2004. Investigation of the Effect of the Composting Process on Particular Plant, Animal and Human Pathogens known to be of Concern for High Quality End-Uses. Published by: The Waste & Resources Action Programme, The Old Academy, 21 Horse Fair, Banbury, Oxon OX16 0AH

[71] Juneja, V. K., Novak, J. S., Huang, L., & Eblen, B. S. 2003. Increased thermotolerance of Clostridium perfringens spores following sublethal heat shock. Food Control, 14(3), 163-168. doi:DOI: 10.1016/S0956-7135(02)00060-9 Public

Table 13 – Overview of human pathogens identified in the intestinal tracts of animals or in animal faeces (surveys conducted in many countries)

Cattle	Sheep	Pigs	Poultry
E. coli O157:H7	*E. coli* O157:H7	*E. coli* O157	*E. coli* O157
E. coli, non-O157 STEC (*E. coli* O26 and O111)		*E. coli*, non-O157 STEC	
E. coli EHEC, STEC, EPEC			
E. coli, CTX-M positive			
Salmonella spp.	*Salmonella* spp.	*Salmonella* spp.	*Salmonella* spp.
Campylobacter spp.	*Campylobacter spp.*	*Campylobacter spp.*	*Campylobacter* spp.
Cryptosporidium spp.	*Cryptosporidium parvum*	*Cryptosporidium* spp.	*Cryptosporidium* spp.
Giardia spp.	*Giardia intestinalis*	*Giardia* spp.	*Giardia* spp.
Yersinia spp.		*Yersinia enterocolitica*	
Listeria spp.	*Listeria* spp.	*Listeria* spp.	*Listeria* spp.
Leptospira spp.			
adenovirus		adenovirus	
enterovirus		enterovirus	
reovirus		reovirus	
norovirus		norovirus	
Mycotuberculosis avium subsp. *paratuberculosis*		hepatitis E virus	
Coxiella burnetii		*Arcobacter*	
	Clostridium perfringens	*Clostridium perfringens*	*Clostridium perfringens*
		Shigella spp.	

L. monocytogenes has been found in samples from organic soil amendments, and in high levels if amendments contained biosolids[72, 73,74,75,76,77]. Premier[78] reports that chicken manure may be a carrier of the pathogen, but appears not to be a principal means of *L. monocytogenes* contamination of leafy vegetables in Australia. This may be due to low

[72] Husu, J.R., 1990. Epidemiological studies on the occurence of *Listeria monocytogenes* in the faeces of dairy cattle. *J. Vet. Med*. B **37**, 276-282.
[73] Van Renterghem, B., F. Huysman, R. Rygole, and W. Verstraete. 1991. Detection and prevalence of Listeria monocytogenes in the agricultural ecosystem. J. Appl. Bacteriol. 71:211-217.
[74] Strauch, D., 1991. Survival of microorganisms and parasites in excreta, manure and sewage sludge. *Rev. Sci. Tech. Off. Int. Epiz*. **10**, 816-846.
[75] De Luca, G., Zanetti, F., Fateh-Moghadm, P., Strampi, S., 1998. Occurrence of *Listeria monocytogenes* in Sewage Sludge. *Zent. Bl. Hyg. Umweltmed* **201**, 269-177.
[76] Bernagozzi, M., Bianucci, F., Sachetti, R., Bisbini, P., 1994. Study of the prevalence of Listeria spp. in surface water. *Zbl. Hyg*. **196**, 237-244.
[77] Dijkstra, R., 1989. Ecology of Listeria. *Microbiol. Alim. Nutr*. **7**, 353-359
[78] Premier, R., 2010. Reducing *Listeria monocytogenes* contamination from salad vegetable farms. Horticulture Innovation Australia Limited, Project No: VG07079.

populations of the pathogen in chicken manures, the infrequent use of chicken manure in high-risk crops or adequate composting and/or withholding periods.

Premier reports that cow and sheep manure can be heavily contaminated with *L. monocytogenes* especially if the animals have been fed on improperly fermented silage or hay or there are infected animals in the herd. The use of fresh or incorrectly composted cow and sheep manure may therefore pose a high-risk for the food safety of fresh produce.

Food borne illnesses caused by *Listeria* have not been directly linked to fresh produce in Australia or New Zealand. *Listeria monocytogenes* has been found on fresh cut melons but was believed to be a result of contamination from handlers and improper handling[79].

The use of incorrectly composted manures on farms has not been directly linked to foodborne illness outbreaks in either Australia or New Zealand. This lack of trace-back does not necessary mean that illnesses have not occurred or that risks do not exist. Identifying the food causing an outbreak and the initial event that contaminated that food is very difficult. If a food is confirmed as the vehicle of infection in an outbreak, trace-back is also made difficult by the fact that contamination may occur within the supply chain. Other reasons contamination has not been linked include:

- Separation of intensive animal holdings and production sites of high-risk fresh produce
- Fast die-off of pathogens under production conditions
- Intervals commonly used between application of contaminated amendments and harvest
- Many growers have an HACCP based food safety system in place with control steps

4.5 Survival of pathogens in soil amendments

A range of additional factors can influence pathogen survival after amendment to soils

- Volume, timing and method of application, (eg surface application or incorporation),
- Soil type
- Time of year (soil moisture and temperature variations, exposure to sunlight)
- Tillage practices
- Oxygen levels
- pH
- Salinity
- Ammonium and nutrient content
- Carbon / nitrogen ratio
- Microbial competition.

In general, the higher the temperature and the lower the moisture content and the longer the storage or treatment time of manures or compost, the less likely it is that pathogens will survive for extended times.

[79] FSANZ 2011. Review of foodborne illness associated with selected ready-to-eat fresh produce. Proposal P1015, Supporting Document 2. Primary Production & Processing Requirements for Horticulture (December 2011)

Investigations have shown that populations of foodborne pathogenic bacteria and enteroviruses[80] decrease within days after their introduction into the soil. A residual population may survive for weeks or months.

Spore-forming organisms are more resistant to die-off, including during composting. Studies on this issue have been reviewed by the EU Scientific Committee on Food[81] and are used as a basis for EU policies on food safety. The US FDA will complete its review process on the topic in late 2015 to revise food safety legislation.

4.5.1 Survival of bacteria

Most human pathogens rely on available moisture for survival, so have only short persistence times of one or more days under dry conditions. Survival times at low temperatures are usually longer than at higher temperatures. Four weeks at 30°C is sufficient to kill many pathogens (Table 14) in soils and organic amendments, while cooler temperatures can support survival of some pathogens for several weeks or months in soils or faeces / manures.

In irrigated temperate horticultural crops (lettuce, berries), soil temperatures will seldom be above 25°C. Higher temperatures may occur in the top 5-10 cm if air temperatures are above 35°C for extended periods. The best field soil temperature range for many tropical crops, including melons, is between 21°C and 35°C. This means that pathogen die-off times may differ in temperate and tropical climates.

Survival in water is usually longer than in soils. Table 14 shows pathogen survival times in a range of environments. Pathogens survive much longer in liquid slurry than solid compost. Variations in survival times can be attributed to the specific circumstances under which trials were undertaken and interaction between factors such as:

- Soil and crop type as well as management practices
- Physical, biological and chemical properties of soils and amendments (including moisture)
- Soil and air temperatures, including variability in temperature
- Type and age of faeces
- Surface application or incorporation
- Variation in pathogen strains and age
- Whether the study was conducted in the field or a laboratory and the study methodology eg initial load of inoculum used

[80] Goyal, S.M., and C.P. Gerba. 1979. Comparative adsorption of human enteroviruses, simian rotavirus and selected bacteriophages to soils. Appl. Environ. Microbiol. 38:241-247.

[81] European Comission Scientific Committee on Food, 2002. Risk Profile on the Microbiological Contamination of Fruits and Vegetables Eaten Raw. Health & consumer protection directorate-general, SCF/CS/FMH/SURF/Final

There is a lack of data on pathogen survival times in soils under typical commercial production conditions for high-risk crops and production systems (eg where manures and composts are used) in Australia and New Zealand.

Table 14 – Survival of pathogens from animal faeces in the environment[82] [83]

Material	Temp	Duration of survival					
		Giardia	*Crypto-sporidium*	*Salmonella*	*Campylo-bacter*	*Yersinia entercolitica*	*E. coli O157:H7*
Reference		21–23 d	24–25 d	26–28 d	29–31 d	30–37 d	32–36 d
Water	frozen	<1 d	>1 yr	>6 mos	14-45 d	>1 yr	>300 d
	cold (5C)	77 d	>1 yr	>6 mos	12 d	>1 yr	>300 d
	warm (30C)	14 d	70 d	>6 mos	4 d	10 d	84 d
Soil	frozen	<1 d	>1 yr	>84 d	14–56 d	>1 yr	>300 d
	cold (5C)	50 d	56 d	84–196 d	14 d	>1 yr	100 d
	warm (30C)	14 d	28 d	28 d	7 d	10 d	2 d
Cattle faeces	frozen	<1 d	>1 yr	>6 mos	14–56 d	>1 yr	>100 d
	cold (5C)	7 d	56 d	84–196 d	7–20 d	30–100 d	>100 d
	warm (30C)	7 d	28 d	28 d	7 d	10–30 d	10 d
Slurry		1 yr	>1 yr	13–75 d	>112 d	12–28 d	10–100 d
Compost		14 d	28 d	7–14 d	7 d	7 d	7 d
Dry surface		1 d	1 d	1–7 d	1 d	1 d	1 d

Ongeng[84] reviewed literature on the fate of *E. coli* and *S. enterica* in manure amended soils. They discuss methodological issues involved in undertaking survival studies, based on a comparative analysis of experimental results obtained from research conducted under controlled environmental conditions and results obtained from field experiments. Their review highlights the variability of *E. coli* and *Salmonella* survival depending on conditions. They report that in many trials with vegetables, mainly lettuce and cabbage, pathogens successfully colonised or even invaded the plants.

The review does not report on pathogen survival times in and on vegetables over a timeframe that would be equivalent to the interval between harvest and consumption. There are many studies that report survival times on produce, but variability is inherent in these data.

Pathogen survival in composted products is shorter. A study investigating survival during composting, reported that strains of pathogenic *E. coli*, *Salmonella typhimurium* and *S.*

[82] Olson, M. E. 2001. Human and animal pathogens in manure. In Livestock Options for the Future National Conference, Winnipeg, Manitoba, Canada, June 25-27, 2001. Agriculture and Agri-Food Canada.
[83] University of Maryland, 2010. Improving the Safety and Quality of Fresh Fruits and Vegetables: A Training Manual for Trainers
[84] Ongeng D., Geeraerd A.H., Springael D., Ryckeboer J., Muyanja C., and Mauriello G. 2013. Fate of Escherichia coli O157:H7 and Salmonella enterica in the manure-amended soil-plant ecosystem of fresh vegetable crops: A review

enteriditis were not detectable after 1 hour at 55°C in green waste compost, despite being introduced at much higher concentrations than would be expected naturally[85].

Franz[86] studied the dynamics of *E. coli* O157:H7 and *S. typhimurium* survival in 36 different soil types and assessed contamination of iceberg lettuce. He noted that most published data included only a limited number of soil types. Franz found pathogen survival ranging from 54 to 105 days in the soils. Survival increased with a field history of low-quality manure use (mineral fertiliser and raw slurry) and in ''*conventional systems compared to high-quality manure application and 'organic' systems*". He identified inadequate manure storage time (<30 days) as a significant factor for contamination risk.

Franz reports that the best predictor of *E. coli* O157:H7 survival in mineral soils is the level of dissolved organic carbon per unit of biomass carbon (positive correlation). The parameters of soil type or soil management were not significant determinants of pathogen survival in this study. Increasing manure storage time to a minimum of 30 days together with an application-to-planting interval of at least 60 days was most successful in reducing the number of contaminated lettuce heads.

Heading lettuce varieties are ready for harvest 50 to 80 days after sowing depending on variety and temperature. Loose-leaf lettuce comes to harvest maturity in about 30 to 50 days. Crisphead / iceberg lettuce harvest usually occurs 70 to 90 days after seeding[87]. Franz conservatively recommends an interval of 60 days between uncomposted manure application and planting.

Kudva et al.[88] studied the growth and survival of *E. coli* O157:H7 in sheep and cattle faeces under various experimental and environmental conditions. A manure pile collected from experimentally inoculated sheep was left outside under fluctuating environmental conditions. *E. coli* O157:H7 survived in the manure pile for 21 months, and the concentrations of bacteria recovered ranged widely from $<10^2$ to 10^6 CFU/g at different times. A second *E. coli* O157:H7-positive sheep manure pile, which was periodically aerated by mixing, remained culture positive for four months (120 – 123 days). An *E. coli* O157:H7-positive cattle manure pile was culture positive for only 47 days. This study emphasises the difference between source animals and manure treatment. Also, as the manure was not spread into a field, the pathogens were not subject to the environmental variables that enhance die-off.

In the same study, *E. coli* O157:H7 was inoculated into faeces, untreated slurry, or treated slurry in the laboratory and incubated at -20, 4, 23, 37, 45, and 70°C. *E. coli* O157:H7 survived best in manure incubated without aeration at temperatures below 23°C. Under

[85] Noble, R., Jones, P.W., Coventry, E., Roberts, S,R., Martin, M., Alabouvette, C., 2004. Investigation of the Effect of the Composting Process on Particular Plant, Animal and Human Pathogens known to be of Concern for High Quality End-Uses. Published by: The Waste & Resources Action Programme, The Old Academy, 21 Horse Fair, Banbury, Oxon OX16 0AH

[86] Franz, E. 2007. Ecology and Risk Assessment of E. coli O157:H7 and Salmonella Typhimurium in the Primary Production Chain of Lettuce. PHD Doctoral Thesis, Wageningen University, the Netherlands.

[87] Blaesing D. 2015. General production knowledge acquired from growers and agronomsists.

[88] Kudva I.T., Blanch K., Hovde C.J.. 1998. Analysis of Escherichia coli O157:H7 survival in ovine or bovine manure and manure slurry. Appl Environ Microbiol. 1998 Sep;64(9):3166-74.

warm conditions survival times ranged from 24 h to 40 days. In addition, Kudva et al. found that the Shiga toxin type 1 and 2 genes in *E. coli* O157:H7 had little or no influence on bacterial survival in manure or manure slurry. Kudva et al. did not report on the feeding regime or animal health of the livestock from which the manure was obtained.

The potential for long-term survival of *E. coli* O157:H7 in manure, especially from sheep, emphasises the need for correct composting and/or site/crop-specific withholding periods for fresh manures. While the above study showed that *E. coli* might survive longer in sheep than in cattle manure, it did not include information on viruses, which may have different survival times. *Cryptosporidium*, which can be found in faeces from both animals, and *Yersinia,* that can be found in cattle manure, may survive longer than *E. coli* (Table 14).

Avery et al[89] investigated generic *E. coli* die-off in cattle, sheep and pig faeces deposited directly onto pasture. *E. coli* levels in freshly deposited faeces from all three species were similar (around 7 log CFU/g). Random 5 cm deep soil cores were taken. *E. coli* originating from cattle, sheep, and pigs reduced significantly over 38, 36, and 26 days respectively. In this study, the manure was on the top of the soil and exposed to sunlight and drying.

Researchers in Guelph, Canada used sentinel vials filled with a mixture of liquid dairy or liquid swine manure and soil to study pathogen survival[90]. Each vial was inoculated with a known population of: *Salmonella* or Shiga toxin-producing *E. coli*. Pathogens in vials placed on the surface had a faster die-off rate than those in the paired treatment that were buried. Pathogens in vials on the sandy loam soil surface had the fastest die-off, ranging from less than four days to 10 days. When buried in this soil, the die-off ranged from 4 to 28 days. Pathogens in vials on the loam soil surface had die-off rates ranging from <4 days to 26 days compared with the buried vials, where die-off rates ranged from 4 to 64 days.

4.5.2 Survival of viruses

The survival of enteric viruses in the environment and adherence to plants has not been studied intensively. One reason may be that viruses (and protozoa) are most often associated with contaminated water or food handlers. They are also difficult to study because they need living cells to replicate. Limited research has been conducted on the ability of viral pathogens to internalize into plant tissue via root uptake. In a review paper, Hirneisen et al.[91] could report on only seven virus studies conducted between 1982 and 2010. They summarise that, similar to studies with human pathogenic bacteria, growth substrate played a large role in the ability of viruses to internalize through root uptake. Studies using hydroponic solution as the growth substrate showed greater viral uptake than with plants grown in soil.

[89] Avery, S.M., Moore A., and Hutchison M.L.. 2004. Fate of Escherichia coli originating from livestock faeces deposited directly onto pasture. Lett. Appl. Microbiol. 38:355-359.
[90] Warriner K. 2014. Die off rates of human pathogens in manure amended soils under natural climatic conditions. Final Report. Center for Produce Safety, California.
[91] Hirneisen, K.A. Sharma M.and Kniel K.E. 2010. Human Enteric Pathogen Internalization by Root Uptake into Food Crops. Foodborne Pathogens and Disease Volume 9, Number 5, 2012

Adsorption rates of virus to soil with water and transport through soils have been studied more intensively. This is due to concerns about drinking water, rather than transmission on fresh produce. If a suitable host is not present, the adsorption rate of individual viruses to soil is correlated with cation exchange capacity, specific surface areas, organic content and pH of the soil [92]. Soil that did not adsorb viruses in the cited study had the coarsest texture and highest pH. The abovementioned study was conducted with non-enteric viruses. Some evidence exists that enteric viruses also attach to soil particles but have shorter survival times in groundwater than non-enteric pathogenic viruses[93].

Duboise et al.(1979)[94] conducted an extensive literature review (301 references) of the behaviour of viruses in soils. While this review was published over 30 years ago, the emphasis of their summary discussion was on the need for site-specific data to understand and predict viral behaviour in individual soils (or situations). This statement remains relevant today.

4.6 Organic biostimulants, pesticides and biopesticides

The food safety risks associated with organic biostimulants, biopesticides and liquid compost products have not been extensively investigated. Although commercial products do not usually contain substances of faecal origin, compost teas and extracts may be contaminated. Compost teas that contain carbohydrate sources, such as molasses, provide an environment where pathogens can grow.

4.7 Composts and composting

The potential risks of organic inputs containing or originating from animal waste have been described in previous sections. Municipal wastes that contain organic materials from public greens and parks may contain excrement from dogs, cats and even humans. Soil amendments made from wastes that do not contain manures or biosolids can still carry human pathogens. Hazards in municipal composts may include but not be limited to *Salmonella* spp., pathogenic *E. coli*, *C. jejuni* and *C. coli*, *L. monocytogenes*, *Y. enterocolitica*, *Shigella dysenteriae*, *Bacillus cereus*, *C. perfringens*, *Staphylococcus aureus*, *C. botulinum*, hepatitis viruses, norovirus, Arboviruses and Caliciviruses.[95] .

Correct composting procedures can inactivate bacterial pathogens effectively. However, the survival of human pathogenic viruses, protozoa and pathogenic nematodes in compost has not been clearly determined[96].

[92] Burge, W. D. and Enkiri. N. K. 1978. Virus adsorption by five soils. J. Environ. Qual. 7:73-76
[93] Powelson, D.K., and C.P. Gerba. 1994. Virus removal from sewage effluents during saturated and unsaturated flow through soil columns. Water Res., 28:2175-2181.
[94] Duboise, S. M., Moore B. E., Sorber C. A., and Sagik B. P. 1979. Viruses in Soil Systems. CRC Critical Reviews in Microbiology 9:245-285.
[95] Cook, E.M., 1991. Epidemiology of foodborne illness: UK. In: Foodborne Illness, a Lancet Review, 16-23, Ed. Waites, W.M. and Arbuthnott, J.P. Edward Arnold, London.
[96] University of Maryland, 2010. Improving the Safety and Quality of Fresh Fruits and Vegetables: A Training Manual for Trainers

Moisture content, temperature and treatment time are important factors determining whether composting processes will kill human pathogens. Minimum compost temperatures of 55-65°C for periods of 3 to 14 days, depending on the composting process (turned windrow, in-vessel, static aerated piles), will kill foodborne pathogens that do not form spores if the composting process is managed and monitored carefully[74].

It is generally recommended that windrows maintain a core temperature of 55°C for 15 days with at least 5 turnings. Due to the need for proper mixing and consistent high temperatures, pathogen reduction in windrow composting has sometimes been found to be less consistent than when using well-managed, aerated static pile or in-vessel systems.

Some composting operators may perform microbial analyses of the composts to determine if the procedure was effective in eliminating pathogens. *E. coli* and *Salmonella* spp. are generally used as indicators. If these bacteria are present, high-risk exclusion periods should apply, as used for untreated manures.

Although human pathogens may be present in composts, ten years of research on *Salmonellae* and Enterobacteriaceae (*E. coli* and other coliform bacteria) in Austria have never found evidence of any compost-derived human disease problem[97].

Passive composting treatments require very little inputs. Organic waste is simply held under natural conditions. The piles are not turned and oxygen is depleted, resulting in anaerobic conditions that slow the composting process. Given enough time, environmental factors such as temperature, ultraviolet radiation and humidity will inhibit pathogen growth and eventually kill them. However, passive compost must be regarded as untreated manure.

4.8 Compost derived products

A considerable amount of work has been carried out to develop improved methods for preparation and use of compost extracts and teas for use as fertilisers and to assist crop protection. Commercial companies in the United States have done most of this work.

Compost teas should only be made with fully mature compost to reduce pathogens. Adding sugar or molasses materials during the steeping process has been reported to increase the incidence of pathogens in the compost tea. Compost tea must be aerated during the "steeping" process and used immediately after steeping to reduce the risk of pathogen contamination. Compost teas should not be applied to edible parts of a crop because the risk of pathogen contamination is not well enough understood to prescribe withholding periods.

With the increased interest in compost derived products there is a need for research on the risk of the colonisation of aerated and non-aerated compost teas and extracts made from

[97] Hogg, D., Barth, J., Favoino, E., Centemero, M., Caimi, V., Amlinger, F., Devliegher, W., Brinton, W., Antler, S., 2002. Comparison of compost standards within the EU, North America and Australasia. The Waste and Resources Action Programme, Banbury, UK.

mature compost to understand the risk of contaminating produce with human pathogens and to develop withholding periods.

4.9 Biosolids

In Australia, biosolids are regulated under a specific statutory framework in each State. Generally the key piece of legislation is the State's head environment protection Act. Each State in Australia also has a biosolids guideline. This is a specific guideline that sets out the best practice requirements for biosolids use.

A particular feature of biosolids guidelines is that they deal exclusively with application of biosolids to land, either directly or indirectly, rather than the use of biosolids in compost mixtures. The contaminant and stabilisation grade of biosolids are used to determine allowable uses for land application or use in composting. The highest grade (AA) has unrestricted use. Biosolids products that are not contaminant or stabilisation graded are automatically classified 'Not suitable for use'.

In **New Zealand** microbial standards for grade 'A' biosolid are:

- *E. coli* < 100 MPN/g
- *Campylobacter* < 1/25 g
- *Salmonella* < 1/25 g
- Enteric viruses < 1 PFU/4 g
- Helminth ova < 1/4 g.

Grade 'B' biosolids do not need to comply with these microbiological standards because they cannot be used directly for edible crops.

The guidelines propose that grade B biosolids can be applied to land that will be used for horticultural production and for crops that are eaten raw and unpeeled if a withholding period of 1 year is observed, specifically: salad crops, fruit, other crops for human consumption that may be eaten unpeeled or uncooked; or orchards where dropped fruit is not harvested, crops that will be peeled or cooked before eating. Where grade B biosolids are applied to orchards where dropped fruit is not harvested, the fruit should not be harvested for at least six months after application. Similarly, crops that will be peeled or cooked should not be harvested for at least six months after application of grade 'B' biosolids. There are no recommendations regarding the sowing or planting of such crops.

5 The storage environment and transport

What does the current research literature tell us about the potential for on-farm cool-rooms, refrigerated transport and other storage to be a source of microbial contamination or enable cross contamination of fresh produce? What are the key food safety messages to managers of coolroom, transport and other refrigerated and storage facilities?

Key points

What the literature tells us

- There are few recorded examples of fresh produce being contaminated after packing and palletisation.
- Microbial contamination is possible through rodent faeces or contaminated water in coolrooms. This has occurred when product has been packed in bins used for long-term storage.

Research Gaps

- Research is needed to determine the prevalence and persistence of *L. monocytogenes* in incidental condensate within fresh produce storage environments.
- Extension materials are needed to provide guidance on good practice in managing storage and transport facilities.

5.1 Background

Little is known about the likelihood of fresh produce becoming contaminated after packing and palletisation. The majority of contamination sources are associated with growing practices on the farm as well as harvest and packing processes. Once products have been packed and palletised, they are much more protected from external sources of contamination.

There is ample evidence that some human pathogens are able to grow and spread in the storage environment. For example, high humidity and short-term non-refrigerated storage permit growth of pathogenic *E. coli*, *L. monocytogenes*, and *Salmonella* on leafy greens and herbs. *L. monocytogenes* is unusual among food-borne pathogens in that it can grow at refrigeration temperatures, including fresh produce, as has been demonstrated for asparagus, cauliflower and broccoli stored at 4◦C [98].

[98] Berrang, M.E., Brackett, R.E., and Beuchat, L.R., 1989. Growth of Listeria monocytogenes to genes on fresh vegetables stored under controlled atmosphere. *Journal of Food Protection*. 53 (10): 702-705.

Low temperatures can extend survival times, while high humidity and condensation inside cool rooms provide moisture that can allow pathogens to grow. However, in most cases it appears that the initial microbial contamination occurred during production or packing of the product. The pathogen itself did not originate within the cool room, transport vehicle or other storage facility, even though an outbreak may be associated with that facility.

This review focuses on what is known about contamination risks potentially introduced during storage and transport, including receival and storage prior to retail sale.

5.2 Microbial contamination

Microbial contamination and cross contamination can potentially occur during cooling as well as in short-term storage and handling areas prior to transport to the first customer.

Insects and vermin, poor worker hygiene, improper handling, poor facility design and inadequate maintenance of storage areas can lead to contamination. Rats and mice inside storage facilities can chew through packaging materials or enter bins, contaminating the fresh produce with urine and faeces.

Despite this, the number of cases of where fresh produce has been contaminated with human pathogens during storage is extremely limited. For example:

- A large outbreak of *Y. pseudotuberculosis* in Finland in 2003 was associated with stored carrots[99]. The outbreak was epidemiologically traced to a carrot farm that had stored carrots in open bins in an unenclosed barn accessible to rodents and wildlife.

- A second outbreak of *Y. pseudotuberculosis* in 2004 was also traced back to farm storage. In this case what initially appeared to be a contamination during storage proved to be contamination during harvest: it was speculated that a small infected shrew picked up by harvesting equipment had contaminated the carrots, with growth of the pathogen simply facilitated by long-term storage over winter[100].

- A third outbreak in 2006 was microbiologically linked to a carrot distributor's storage facility. The outbreak was associated with poor quality carrots that had been stored on farm for six months and for a further four months at the distributor's facility. Again, contamination with *Y. pseudotuberculosis* occurred before packing.

[99] Food Standards Australia New Zealand, 2011. Supporting Document 2, Review of foodborne illness associated with selected ready-to-eat fresh produce, Proposal P1015, Primary Production and Processing Requirements for Horticulture, FSANZ, Canberra.

[100] Kangas S, Takkinen J, Hakkinen M, Nakari UM, Johansson T, Henttonen H, Virtaluoto L, Siitonen A, Ollgren J, Kuusi M (2008) Yersinia pseudotuberculosis O:1 traced to raw carrots, Finland. Emerging Infectious Diseases 14(12):1959–1961

- A multi-state outbreak of listeriosis was attributed to whole cantaloupes sourced from Jensen's Farms[101]. The pathogen had proliferated during cold storage, possibly due in part to harbourages in refrigeration units and other areas where water had pooled. As previously, initial contamination occurred before harvest, although pathogen numbers increased in the storage environment.

5.2.1 Water borne pathogens

Some products are cooled after packing and palletisation. Cooling methods for packed product include forced air systems, vacuum cooling, and even hydrocooling. Hydrocooling is used for products such as asparagus, which may be packed in plastic or wooden packaging to facilitate this process. Water can be both a source and a mode of transfer of pathogens, so is the basis of many production risk factors[102]. Water used for hydrocooling must be sanitised to ensure it is not a source of microbial contaminants of packed product.

The Australian Building Code Board (ABCB[103]) emphasises that increasing levels of insulation and air tightness change underlying building physics: less energy flow through the building fabric also means less moisture flow – so when materials get wet they stay wet longer. Indicators of dampness include condensation on surfaces or in structures, visible mould, mouldy odour and a history of water damage, leakage or penetration[104].

Condensation often occurs in refrigerated environments. If relative humidity is high then even small fluctuations in temperature can cause condensation, which increases the risk of microbial growth on wet surfaces. In general, microbes can develop on surfaces that are wet or have sustained high relative humidity (typically >85%), as well as which provide a sufficient nutrient supply and are at temperatures between 4 and 40°C[105].

James[106] suggests that poor sanitation in storage facilities can lead to the formation of biofilms. Biofilms are dense layers of bacteria that accumulate and proliferate on surfaces like stainless steel and plastic. They also attach to each other through polymeric materials, effectively slimes, produced by the microbes themselves. Biofilms can trap other bacteria, debris and nutrients. Poor sanitation/cleaning programs allow biofilms to build up and become established. Nonpathogenic and pathogenic bacteria can form biofilms. Organisms in the film tend to be resistant to cleaners and sanitizers.

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[101] FDA, Food and Drug Administration, 2011, Environmental Assessment: Factors Potentially Contributing to the Contamination of Fresh Whole Cantaloupe Implicated in a Multi-State Outbreak of Listeriosis, http://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm276247.htm, accessed 04/06/2015.

[102] Suslow, T.V., Oria, M.P., Beuchat, L.R., Garrett, E.H., Parish, M.E., Harris, L.J., Farber, J.N., Busta, F.F. 2003. Production practices as risk factors in microbial food safety of fresh and fresh-cut produce, *Comprehensive Reviews in Food Science and Food Safety* **2**, 38-77.

[103] Australian Building Code Board [The], 2014. Handbook: Condensation of Buildings. Located at http://www.www.abcb.gov.au/ accessed 04/06/2015.

[104] Sepännen, O., Kurnitski, J., 2009. Moisture control and ventilation, WHO Guidelines for Indoor Air Quality: Dampness and Mould, World Health Organisation, Geneva. Located at http://www.ncbi.nlm.nih.gov/books/NBK143947/ accessed 04/06/2015.

[105] ABCB (Australian Building Codes Board). (2014). Condensation in Buildings: Handbook (2nd Edition). Accessed 12 September 2015 at: http://www.abcb.gov.au/education-events-resources/publications/abcb-handbooks

[106] James, J., 2006. Overview of microbial hazards in fresh fruit and vegetable operations. Chapter 1. In: Microbial Hazards Identification in Fresh Fruit and Vegetables, pp 1-36. James, J., Ed., Wiley, New York.

Water from contaminated cool room walls, ceilings or refrigeration units may potentially drip onto stored produce. This is most likely to contaminate products in open bins or trays, rather than packed product inside sealed cartons. While there are no documented cases of this occurring, the proliferation of certain organisms inside storage facilities suggests that spread through water may have increased contamination in some cases.

5.2.2 Air borne pathogens

Microbes can be transported by ventilation systems throughout building facilities. James[107] notes that refrigeration units are thought to spread bacteria and mould throughout warehouses, hence routine servicing of air filters and refrigeration systems is required. Gil et al.[108] also states that as cold air systems blow particulates and mould spores into the air, there is a risk that pathogens may be spread along with the spores from one pallet or bin to another.

Despite air-borne microbes being identified as a risk, this review has not found any cases where ventilation systems were demonstrated to have spread human pathogens onto otherwise clean, palletised product.

5.2.3 Sanitation of storage environments

In response to the 2011 cantaloupe outbreak linked to Jensen's Farm, the US Food and Drug Administration (FDA) studied practices in cantaloupe packing and storage sheds[109]. A pathogenic listeria strain was found in only one of the seventeen facilities tested, although non-pathogenic strains were found at eight facilities.

Similarly, Taverner et al.[110] assessed the effectiveness of sanitation methods and general hygiene / cleaning of Australian citrus packing and storage facilities. Comparatively clean swabs were collected throughout the cool rooms tested, leading them to conclude that cleaning was adequate and contamination by this method unlikely.

Gil et al.[111] suggest that if contamination does occur in the storage area, the entire facility should be cleaned and disinfected to reduce any potential pathogen contamination[112].

[107] James, J., 2006. Overview of microbial hazards in fresh fruit and vegetable operations. Chapter 1. **In**: Microbial Hazards Identification in Fresh Fruit and Vegetables, pp 1-36. James, J., Ed., Wiley, New York.

[108] Gil, M.I., Selma, M.V., Suslow, T., Jacxsens, L., Uyttendaele, M., Allende, A., 2015. Pre- and Postharvest Preventive Measures and Intervention Strategies to Control Microbial Food Safety Hazards of Fresh Leafy Vegetables, Critical Reviews in Food Science and Nutrition, 55(4), 453-468.

[109] Food and Drug Administration, 2015. Summary Report: FY 2013 Inspection, Environmental Sampling and Sample Collection (Pre and Post-Process) at Cantaloupe Packinghouses Assignment (DFPG #13-19), http://www.fda.gov/Food/FoodborneIllnessContaminants/ucm455940.htm Accessed 25/08/2015.

[110] Taverner, P., Cunningham, N., and Steciuk, K., 2012. *Sanitation Survey of Citrus Packing sheds*, South Australian Research and Development Institute, http://mvcitrus.org.au/mvcb/wp-content/uploads/2012/09/Sanitation-Survey-on-Packing-Sheds-2012.pdf, accessed 25/08/2015

[111] Gil, M.I., Selma, M.V., Suslow, T., Jacxsens, L., Uyttendaele, M., Allende, A., 2015. Pre- and Postharvest Preventive Measures and Intervention Strategies to Control Microbial Food Safety Hazards of Fresh Leafy Vegetables, *Critical Reviews in Food Science and Nutrition*, **55**(4), 453-468.

Cleaning should include special attention to drains, cooling coils, drip pans, ice machines and other areas that are routinely cold and wet are regularly checked, sanitized and swabbed to prevent the survival of microbial foodborne hazards[113].

[112] IFPA, International Fresh-Cut Produce Association, 2001. Sanitary plant and equipment design. Wash water sanitation. **In**: Food Safety Guidelines for the Fresh-cut Produce Industry, 4th ed., pp 75-95 and pp 107-120, Gorny, J. R., Ed., Alexandria (VA).

[113] Korsten, L., and Zagory, D., 2006. Pathogen survival on fresh fruit in ocean cargo and warehouse storage. In: Microbial hazard identification in Fresh Fruits and Vegetables, pp 221-243, James, J. A., Ed., Wiley, New York.

6 Interactions between sanitisers and fungicides

What do we know of the interaction between fungicides and sanitisers and the potential impact on food safety? What are the current industry use patterns and strategies to maximise efficacy of both fungicides and sanitisers and are these different to the ideal strategies? What further R&D might be necessary to maximise both? What sanitisers are currently used and what is their registration / approval status?

Key points

What the literature tells us

- There is limited information available on interactions between fungicides and sanitisers, apart from research conducted on citrus.
- Some fungicides and sanitisers do interact, rapidly reducing the effectiveness of the sanitiser.
- Sanitisers must be registered if they claim to control plant pathogens. Sanitisers do not need to be registered if they are used only as processing aids to control human pathogens

Research Gaps

- Research is needed to understand sanitiser and fungicide use patterns in Australian and New Zealand fresh produce industries. This information may be used to prioritise which produce types or sanitiser and fungicide use patterns need to be evaluated for compatibility.
- Industry guidelines and communication strategies are required to growers and packers with information on regulatory requirements and management practices for postharvest sanitizer and fungicide compatibility.

6.1 Background

Sanitisers are added to water during postharvest operations to help prevent the multiplication and spread of human pathogens. Sanitisers may therefore be added to postharvest fungicide solutions to control bacteria, particularly human pathogenic bacteria that may be carried in the water.

The efficacy of both the sanitiser and the fungicide may be affected if they are chemically incompatible. If the efficacy of the sanitiser is reduced there is an increased risk of cross-contamination by human or plant pathogens. If the efficacy of the fungicide is reduced there is an increased risk of postharvest disease. This section of the review examines the impacts of these potential interactions.

- **Sanitisers** are chemicals that kill both plant pathogens/food spoilage organisms and human pathogens. Sanitisers can be used for sanitising equipment, or to treat water that is used to wash produce. Sanitisers "*reduce, but do not necessarily eliminate, microorganisms*"[114].

- **Fungicides** are compounds that target fungal (ie yeast or mould) plant pathogens/food spoilage pathogens and can be used pre or postharvest. They "*control, destroy, make harmless or regulate the effect of a fungus.*"[115].

6.1.1 Registration of sanitisers

Holmes and Harrup [116] describe Australian requirements for registration of sanitisers, summarised as follows:

- Not all sanitisers require registration.
- Products do not require registration if used as processing aids to control organisms that do not contribute to the deterioration of the harvested produce.
- A product must be registered if used for postharvest washing **and** considered an agricultural chemical. A product is considered an agricultural chemical if "*represented, imported, manufactured, supplied or used as a means of directly or indirectly: Destroying, stupefying, repelling, inhibiting the feeding of, or preventing infestation by or attacks of, any pest in relation to a plant, place or thing*". In this instance, the term 'pest' referred to spoilage causing organisms **but not** human pathogens.
- Registration is therefore required for sanitisers used to control plant pathogens and for products that claim control of both plant and human pathogens.

[114] Ryther R. 2014. Development of a Comprehensive Cleaning and Sanitising Program for Food Production Facilities, In: Food Safety Management: A Practical Guide for the Food Industry. Motarjemi, Y., Lelieveld, H. Eds. Elsevier Inc., USA.
[115] EPA New South Wales, 2013. http://www.epa.nsw.gov.au/pesticides/pestwhatrhow.htm (accessed 31st May 2015)
[116] Holmes, R., Harrup, P. 2003. Clean and Safe Handling Systems for Fresh Vegetables and Tomatoes. Horticulture Australia Limited, Project Number: VX99004.

This is similar to the registration requirements in New Zealand: "*Cleansers, disinfectants, sanitisers, and water conditioners are considered agricultural compounds if they are intended to be used to maintain hygienic conditions for animals or plants*" [117]

Some substances eg chlorine are permitted by FSANZ as processing aids (Table 15). However, if a product claims to control both plant and human pathogens it may require registration.

Information regarding registration requirements for sanitisers used for washing fresh produce is not readily available. This could be problematic for producers and suggests that industry requires clearer information on which products can be used, products that require registration and products that are exempt from registration.

Sanitisers registered in Australia:

A search of the Australian Pesticides and Veterinary Medicines Authority (APVMA) PubCRIS (Public Chemical Registration Information System (APVMA, 2015[118])) database search (31 May 2015) found that seven **'sanitiser'** products were registered for postharvest use in fruit and/or vegetables. These included the following active ingredients:

- Chlorine present as calcium hypochlorite
- Chlorine present as chlorine dioxide

Five **'microbiocide'** products were registered for postharvest use in fruit and/or vegetables. These included the following active ingredients:

- Hydrogen peroxide | Peracetic acid
- Hydrogen peroxide | Peroxyacetic acid
- Bromochlorodimethylhydantoin

The Appendices provide a list of registered products and the plant pathogens they are considered to control.

Sanitisers registered in New Zealand:

A search of the ACVM Register (8th June 2015) identified five products registered in New Zealand as biocides and/or viricides where the label indicated these could be used in postharvest dump tanks (ACVM, 2015[119]). The primary purpose nominated for these products was for the control of plant fungal pathogens. These products used the following active ingredients:

- 3-bromo-1-chloro-5,5-dimethylhydantoin (Product registration numbers P008011, P008400 and P008679)

[117] http://www.foodsafety.govt.nz/elibrary/industry/Scope_Agricultural-Chemicals_Medicines.htm
[118] APVMA (2015). Public Chemical Registration Information System Search. https://portal.apvma.gov.au/pubcris
[119] APVMA, Agricultural Compounds and Veterinary Medicines register, https://eatsafe.nzfsa.govt.nz/web/public/acvm-register (accessed 8 June 2015).

- Hydrogen peroxide (Product registration number P007819)
- Bitter orange oil (Product registration number P007997)

Processing aids permitted by FSANZ:

Table 15 lists processing aids permitted by FSANZ that may be used as bleaching, washing or peeling agents, providing that the final food contains no more than the corresponding maximum permitted level specified in the table[120]. In addition, Sodium chlorite is permitted as an "anti-microbial agent for meat, fish, fruit and vegetables" (clause 14).

Table 15 - Processing aids permitted by FSANZ (Standard 1.3.3)

Substance	Food	Maximum permitted level (mg/kg)
Benzoyl peroxide	All foods	40 (measured as benzoic acid)
Bromo-chloro-dimethylhydantoin	All foods	1.0 (available chlorine) 1.0 (inorganic bromide) 2.0 (dimethylhydantoin)
Calcium hypochlorite	All foods	1.0 (available chlorine)
Chlorine	All foods	1.0 (available chlorine)
Chlorine dioxide	All foods	1.0 (available chlorine)
Diammonium hydrogen orthophosphate	All foods	GMP
Dibromo-dimethylhydantoin	All foods	2.0 (inorganic bromide) 2.0 (dimethylhydantoin)
2-Ethylhexyl sodium sulphate	All foods	0.7
Hydrogen peroxide	All foods	5
Iodine	Fruits, vegetables and eggs	GMP
Oxides of nitrogen	All foods	GMP
Ozone	All foods	GMP
Peracetic acid	All foods	GMP
Sodium chlorite	All foods	1.0 (available chlorine)
Sodium dodecylbenzene sulphonate	All foods	0.7
Sodium hypochlorite	All foods	1.0 (available chlorine)
Sodium laurate	All foods	GMP
Sodium metabisulphite	Root and tuber vegetables	25
Sodium peroxide	All foods	5
Sodium persulphate	All foods	GMP
Triethanolamine	Dried vine fruit	GMP

[120] FSANZ, 2000. FSANZ Standard 1.3.3

6.1.2 What sanitisers are currently used and for what purpose?

Sanitisers can be used to kill or reduce human pathogens on produce, surfaces and equipment or water eg water used for cleaning and/or packing[121]. Sanitisers are also used to help destroy spoilage organisms eg fungal spores. Sanitisers can only reduce the concentration of microorganisms and cannot be relied on to kill all pathogens or spoilage organisms present.

Water is used during or after harvest for washing produce to remove dirt and surface contaminants. It can be a potential source of cross-contamination by plant and human pathogens, especially if the water is re-circulated. Re-circulated water that does not contain a sanitiser or is not frequently changed can transfer human pathogens and spoilage organisms to previously clean product.

Chlorine, the most commonly used sanitiser, is primarily used to limit cross-contamination during washing operations rather than decontaminate produce *per se*[122]. Other studies[123, 124] support this view, suggesting that despite common perceptions that sanitisers are used to reduce pathogen load on the produce, their main effect is in maintaining the microbial quality of processing water ie, that the use of sanitisers is focussed on avoidance of cross-contamination.

Although chlorine is the most commonly used sanitiser, there is some concern about potential toxicity of reaction products. Degradation of chlorine may lead to the accumulation of chlorinated trihalomethanes, haloacetic acids, and chloramines[125] that are implicated with human disease.

In New Zealand, hypochlorite is used for washing fresh produce, such as lettuce and carrots. Typical concentrations used are 50-200 ppm with produce contact for 1-2 minutes[126]. There is no central source of information to advise what sanitisers are approved for use during or after harvest. It is likely that chemical sanitisers, particularly inexpensive compounds with chlorine as an active ingredient, are still the most commonly used and applied in flumes or dump tanks. Sanitisers that come into contact with fresh produce are considered to be processing aids, and only processing aids permitted by Standard 1.3.3 of the Australia New

[121] Parish, M.E., Beuchat, L.R., Suslow, T.V., Harris, L.J., Garrett, E.H., Farber, J.N., Busta, F.F. 2003. Methods to reduce/eliminate pathogens from fresh and fresh-cut produce. *Comprehensive reviews in food science and food safety*, 161-173

[122] Doyle, M.P., Erickson, M.C. 2008. Problems with fresh produce. *Journal of Applied Microbiology* **105**, 317-330

[123] Gil, M.I., Selma, M.V., Lopez-Galvez, F., Allende, A., 2009. Fresh-cut product sanitation and wash water disinfection: Problems and solutions. *International Journal of Food Microbiology* **134**, 37-45.

[124] Lopez-Galvez, F., Gil, M.I., Truchado, P., Selma, V., Allende, A. 2010. Cross-contamination of fresh-cut lettuce after a short-term exposure during pre-washing cannot be controlled after subsequent. *Food Microbiology* **27**, 199-204.

[125] Francis , G. A. , Gallone , A., Nychas, G. J., Sofos, J. N., Colelli, G., Amodio, M. L., Spano, G., 2012. Factors Affecting Quality and Safety of Fresh-Cut Produce, *Critical Reviews in Food Science and Nutrition* **52**(7), 595-610.

[126] McIntyre, L., Cressey, P., Lake, R., 2008. Discussion document on pathogens in fruits and vegetables in New Zealand. http://www.foodsafety.govt.nz/elibrary/industry/discussion-document-pathogens-research-projects/index.htm

Zealand Food Safety Code can be used[127]. This Standard includes a list of 20 washing agents permitted for fresh fruits and vegetables, of which six contain a chlorine component.

In Australia, Holmes and Harrup[128] surveyed vegetable growers and found that:

- 73% of respondents wash or handle at least some of their produce in water.
- 27% add a sanitiser to their water (mainly chlorine-based agents).
- Premier[129] found that "*of 30 growers* [of leafy vegetables] *contacted, 27 were using chlorine, with more than half of these using calcium hypochlorite and the rest using sodium hypochlorite*."

Based on studies conducted in the Australian citrus industry, the following sanitisers are used or at least available:

- Calcium hypochlorite
- Chlorine dioxide
- Bromochloro-dimethylhydantoin
- Peroxyacetic acid

Tancred [130] lists the following sanitisers used (or at least available) in pome fruit and/or stone fruit:

- Chlorine dioxide (Vibrex / Vibrex Horticare)
- Bromochloro-dimethylhydantoin (Nylate)
- Peroxyacetic acid (Tsunami)
- Quaternary ammonium (Deccosan 315) [note that this is registered for use on surfaces only]

Anecdotal evidence suggests that swimming pool chlorine (eg calcium hypochlorite) is widely used as a water sanitiser in Australian produce packing sheds. The active ingredients and concentrations of registered 'pool sanitiser' and 'pool chlorine' products vary (based on APVMA PubCRIS search). However, not all swimming pool products require registration for general use[131]. We do not know the extent these products are being used in the fresh produce industry or at what concentrations.

[127] FSANZ, 2015. Australia New Zealand Food Standards Code - Standard 1.3.3 - Processing aids. Version in force 26 February 2015. Food Standards Australia New Zealand, Canberra. http://www.comlaw.gov.au/Series/F2008B00616 (accessed: 8 June 2015).

[128] Holmes, R., Harrup, P. 2003. Clean and Safe Handling Systems for Fresh Vegetables and Tomatoes. Horticulture Australia Limited, Project Number: VX99004.

[129] Premier, R. 2013. Evaluation of vegetable washing chemicals. Horticulture Australia Limited, Project Number: VG09086

[130] Tancred, S. 2013. Australian Fruit Grower, August 2013, pp 19.

[131] APVMA 2014. Agricultural and Veterinary Chemicals Code (Listed Chemical Product – Home Swimming Pool and Spa Products) Standard 2014 (http://www.comlaw.gov.au/Details/F2014L00838)

6.2 Interactions between sanitisers and fungicides

6.2.1 Postharvest fungicides used on fresh produce

With the exception of citrus, there are few studies on what postharvest fungicides are used commercially on specific produce lines or which sanitisers are usually combined with them.

However, the following postharvest fungicides are both registered and reported as being currently used for citrus:

- Imazalil
- Thiabendazole
- Guazatine
- Fludioxonil
- Pyrimethanil
- Iprodione

Postharvest fungicide groups registered and used on apples and pears include[132, 32]:

- Imazalil
- Iprodione
- Fludioxonil
- Thiabendazole

The APVMA database suggests that other fungicide products are available. However, at least some are restricted for use in individual states or for specific uses eg disinfection of hard surfaces. The labels for each fungicide generally do not provide information on sanitiser compatibility.

6.2.2 Effects of fungicides on sanitisers

There is limited information available on the interaction between postharvest sanitisers and fungicides apart from the research commissioned by the citrus industry in Australia and the USA. Publications related to this research include citrus industry newsletters and fact sheets.

Australian citrus packers often combine postharvest sanitisers with fungicides. Sanitisers are used to control sour rot (*Geotrichum candidum*) spores in water, reducing spread between harvested fruit. Taverner et al.[133] suggest that the combination of sanitisers and fungicides can be neutral, additive, synergistic or antagonistic to their effectiveness. The response varies with sanitiser active ingredients, fungicide active ingredient and fungicide formulation.

[132] Holmes, R. 2011. Through Chain Rot Management in Apples. Horticulture Australia Limited project number AP08043.

[133] Taverner, P., Cunningham, N.M., Leo, A.T. (no date) Current and Emerging Strategies for Sour Rot Management of Citrus in Australia.

Some growers combine two fungicides with different modes of action to improve control[134] [135]. In citrus at least, the effect of these combinations is unknown. Some citrus growers also add mineral salts (sodium carbonate or sodium bicarbonate) to improve sour rot control.

Peroxyacetic acid

According to Taverner et al., Peroxyacetic acid (PAA) is stable after one hour when combined with six different fungicides, but the PAA decreased when Generally Recognised as Safe (GRAS) compounds (Sodium bicarbonate or Potassium sorbate) were added (Table 16). These losses were relatively modest (eg 20ppm) or stable after topping up the solution concentrations.

The antimicrobial efficacy of organic acids such as PAA is strongly affected by the quantity of the acid that is present in the undissociated form. The undissociated form is strongly favoured at lower pH – each decrease in pH by one unit causes a 10-fold increase in the undissociated acid concentration, and therefore, the antimicrobial action.

The addition of sodium bicarbonate increases pH, as does potassium sorbate. Accordingly, it is likely that anything that increases the pH of the mixture will likely decrease the efficacy of organic acid based biocides.

[134] Tancred, S. 2013. The art of postharvest management Part 2. Australian Fruit Grower Magazine. August 2013, pp 18-21

[135] Taverner, P., Cunningham, N., Steciuk, K., Lucas, N. 2008. Delivering postharvest decay, food safety and market access solutions for export citrus. Horticulture Australia Limited, Project Number: CT03015.

Table 16 - PAA concentration and pH of Tsunami combined with various fungicides and salts (Taverner et al.[133])

Fungicide	Salt	pH	PAA concentration (ppm)	
			5 minutes	1 hour
No Fungicide	No Salt	4.0	80	80
	Sodium bicarbonate	7.7	80	60
	Potassium sorbate	6.5	80	60
Magnate 750WG	No salt	3.3	80	80
	Sodium bicarbonate	7.5	80	
	Potassium sorbate	6.2	80	60
Fungaflor 500EC	No salt	4.8	80	80
	Sodium bicarbonate	7.8	80	60
	Potassium sorbate	6.6	80	60
Scholar	No salt	4.0	80	80
	Sodium bicarbonate	7.9	80	80
	Potassium sorbate	6.4	80	70
Tecto SC	No salt	4.3	80	80
	Sodium bicarbonate	7.6	60z	80
	Potassium sorbate	6.4	80	60
Philabuster	No salt	4.4	60z	80
	Sodium bicarbonate	8.0	80	60
	K Sorbate	6.4	80	60
Penbotec	No salt	4.4	80	80
	Na Bicarbonate	7.7	80	60
	K Sorbate	6.5	80	80

z Tsunami concentration topped up to 80 ppm after 5 min measurement was taken

Chlorine

Chlorine-based sanitisers tend to be, in general, more reactive than PAA when combined with a range of postharvest fungicides[136, 137]. The effectiveness of sanitisers when mixed with fungicides varies with product combinations. For some, the sanitiser concentration was reduced to unacceptable levels within 30 seconds[138]. For full details see the Appendices to this section.

[136] Taverner P.D , Cunningham N.C. 2004. Compatibility of postharvest fungicides with Tsunami. Packer Newsletter 75: 4.

[137] Kanetis L., Förster H., and Adaskaveg J.E. 2008. Optimising efficacy of new postharvest fungicides and evaluation of sanitising agents for managing citrus green mould. Plant Disease 261-269.

[138] Taverner, P., Cunningham, N., Steciuk, K., Lucas, N. 2008. Delivering postharvest decay, food safety and market access solutions for export citrus. Horticulture Australia Limited, Project Number: CT03015.

In the USA, the interaction of sanitisers and postharvest fungicides has been assessed for managing citrus green mould[139]. Effectiveness of the sanitisers (sodium hypochlorite and hydrogen peroxide/peroxyacetic acid (HPPA)) for inhibiting germination of conidia depended on the pH of the solution and the exposure time. Earlier work in the US[140] found that "*peroxyacetic acid was compatible and efficacious with all fungicides tested. Sodium hypochlorite was incompatible with azoxystrobin, imazalil, or pyrimethanil, but not with fludioxonil or TBZ*."

McKay et al. [141] studied the interactions and efficacy of postharvest fungicide combinations with sanitisers at a range of temperatures. They concluded that fludioxonil and thiabendazole are stable in solutions of chlorine or SBC-chlorine, whereas imazalil and pyrimethanil are not. Other results were that "*SBC* [sodium bicarbonate] *applied at 3% by itself or in combination with chlorine at ambient temperature significantly reduced the incidence of sour rot of inoculated fruit. When combined with propiconazole, a slight additive effect of the SBC or SBC-chlorine and propiconazole treatments was observed*."

Chlorine sanitisers are not compatible with DPA (Diphenylamine) (Tancred, 2013)[142]. The guidelines for postharvest drenching of apples and pears (DPI, 2007)[143] states that chlorine in pre-treatment water can break down the DPA. Therefore fruit must be well drained before drenching with DPA solution.

A condensed summary of fungicide active constituent and sanitiser compatibility is shown in Table 17.

Table 17 - Fungicide active and sanitiser compatibility (Source: Cunningham[144])

Fungicide constituent	Compatibility after 4 hours			
	Calcium hypochlorite	Chlorine dioxide	Peroxyacetic acid	Chlorobromo-dimethyl-hydantoin
Imazalil	✗✗	✓	✓✓	✗✗
Guazatine	✗✗	✓	✓✓	✓
Thiabendazole	✓✓	✓✓	✓✓	✓✓
Key	✓✓ Loses no concentration after 4 hours ✓ Loses some concentration after 4 hours ✗✗ Loses concentration rapidly after 4 hours			

[139] Kanetis, L., Forster, H., Adaskeveg, J.E. 2008. Optimizing Efficacy of New Postharvest Fungicides and Evaluation of Sanitizing Agents for Managing Citrus Green Mold *Plant Disease*, February 2008, 261-269.
[140] Kanetis, L, Forster, H.. Adaskaveg, J.E. 2005. New fungicide-sanitizer mixtures and recycling in-line drenches for postharvest decay control of citrus fruit. *Phytopathology* **96**, S169. (Abstract only). http://apsjournals.apsnet.org/doi/pdf/10.1094/PHYTO.2006.96.6.S166 Accessed 26th May 2015.
[141] McKay, A. H., Förster, H., Adaskaveg, J. E. 2012. Efficacy and application strategies for propiconazole as a new postharvest fungicide for managing sour rot and green mold of citrus fruit. *Plant Dis*. **96**, 235-242.
[142] Tancred, S. 2013. The art of postharvest management Part 2. Australian Fruit Grower Magazine. August 2013, pp 18-21
[143] Department of Primary Industries Victoria 2007. Guidelines for postharvest drenching of apples and pears.
[144] Cunningham, N. 2012. Fungicides and sanitisers – new products, new compatibility issues. Packer Newsletter, Volume 105, October 2012.

The '*Guidelines for the Management of Microbial Food Safety in Fruit Packing Houses'*[145] includes guidelines for cleaning and sanitising programs. It mentions "*the possible interaction of sanitisers with other chemical control agents (eg fungicides)*". However no further guidance is provided in regard to which are compatible or not.

Ekman et al. (2006)[146] produced a fact sheet/guide on '*Minimising the risk of microbial contamination of fresh produce'*, which includes a compatibility table for water sanitation options for fresh produce preparation. This publication drew on the early citrus research in this area by Taverner and Cunningham. Compatibility is defined as "greater than 50% of the sanitiser's active constitute persisted when mixed in solution with the fungicide formulation for four hours". The effect on fungicide efficacy was not reported.

The lack of information on sanitiser/fungicide/salt combinations indicates a gap in knowledge and further research and/or extension on interactions between sanitiser and fungicides is needed. Initial steps would include a survey of which sanitisers and fungicides are being used (active constituent and formulation), in which produce, what methods are used (eg dips, sprays) and how they are combined with or may impact on each other. This would enable industry research and extension priorities to be identified.

Anecdotal evidence suggests that many growers are not aware of existing information on the potential compatibility issues for combining sanitisers and fungicides. This could be addressed through communication and extension action.

In conclusion, there is evidence of reduced efficacy of some sanitisers when used in combination with fungicides. This increases the risk of cross-contamination from wash water and has the potential to impact on food safety.

[145] Western Australian Department of Agriculture, 2002. Guidelines for the management of microbial food safety in fruit packing houses. Bulletin 4567

[146] Ekman, J. Ledger, S., Premier, R., Hamilton-Bate, C., McApline, G., Lovell, J., Bennett, R., 2006. Minimising the risk of microbial contamination of fresh produce. https://producesafetycentreanz.files.wordpress.com/2015/02/minimising-microbes-on-fresh-produce.pdf

7 Appendices

7.1 Fate of enteric pathogens and indicators in agricultural water

(As compiled by Erickson[22])

Reference	Highlights
Arana, I., A. Irizar, C. Seco, A. Muela, A. Fernandez-Astorga, and I. Barcina. 2003. Gfp-tagged cells as a useful tool to study the survival of Escherichia coli in the presence of the river microbial community. **Microbial Ecol. 45:29-38.**	Flagellates ingest both viable and dead E. coli cells.
Artz, R.R.E. and K. Killham. 2002. Survival of Escherichia coli O157:H7 in private drinking water wells: influences of protozoan grazing and elevated copper concentrations. **FEMS Microbiol. Lett. 216:117-122.**	Grazing and other biological factors were studied using filtered and autoclaved water. Survival of E. coli O157:H**7** was primarily decreased by elevated copper concentrations.
Atwill, E.R., K.W. Tate, M.G.C. Pereira, J. Bartolome, and G. Nader. 2006. Efficacy of natural grassland buffers for removal of Cryptosporidium parvum in rangeland runoff. **J. Food Prot. 69:177-184.**	Grassland buffers were found to be an effective method for reducing animal agricultural inputs of waterborne C. parvum into drinking and irrigation water supplies.
Baudart, J., K. Lemarchand, A. Brisabois, and P. Lebaron. 2000. Diversity of Salmonella strains isolated from the aquatic environment as determined by serotyping and amplification of the ribosomal DNA spacer regions. **Appl. Environ. Microbiol. 66:1544-1552. .**	Isolation patterns of serotypes in water follow patterns of infection among humans and local fauna, suggesting a local terrestrial origin
Beaudeau, P., N. Tousset, F. Bruchon, A. Lefevre, and H.D. Taylor. 2001. In situ measurement and statistical modeling of Escherichia coli decay in small rivers. **Wat. Res. 35:3168-3178.**	Results suggested that predation by benthic micro-grazers was the main cause of E. coli die-off in small streams in temperate countries.
Blaustein, R.A., Y. Pachepsky, R.L. Hill, D.R. Shelton, and G. Whelan. 2013. Escherichia coli survival in waters: Temperature dependence. **Water Res. 47:569-578.**	There were three major patterns of E. coli inactivation taken from the literature: about half had a section of fast log-linear inactivation followed by a section of slow log-linear inactivation; about a quarter had a lag period followed by log-linear inactivation; and the remaining quarter were approximately linear throughout. E. coli survival rates are dependent on temperature, a dependency that is routinely expressed using an analogue of the Q_{10} model. There was a significant difference in inactivation rate at the reference temperature between the different water sources. At specific sites, the Q_{10} equation was more accurate in rivers and coastal waters than in lakes making the value of the Q_{10} coefficient appear to be site-specific.
Bolton, N.F., N.J. Cromar, P. Hallsworth, and H.J. Fallowfield. 2010. A review of the factors affecting sunlight inactivation of microorganisms in waste stabilization ponds: preliminary results for enterococci. **Wat. Sci. Technol. 61:885-890.**	Both UVA (320 – 400 nm) and UVB (280 – 320 nm) were found to affect inactivation of enterococci in waste stabilization ponds. Higher dissolved oxygen and higher pH increased the rate of inactivation by UVA.
Cahn, M., T.V. Suslow, A.O. Sbodio, and S.L. Kamal. 2009. Using vegetation and polymers to control sediments, nutrients and bacteria in irrigation run-off from vegetable fields. **HortSci. 44:1068-1069.**	Neither vegetation nor polyacrylamide reduced the amount of run-off from the fields or reduced the concentration of coliform and E. coli bacteria in the run-off.
Chao, W., R. Ding, and R. Chen. 1987. Survival of pathogenic bacteria in environmental microcosms. **Chin. J. Microb. Immunol. 20:339-348.**	Salmonella has been demonstrated to remain viable for longer than many other enteric bacteria in freshwaters suggesting that the aquatic environment may represent a relatively stable environment for these bacteria.
Chauret, C., K. Nolan, P. Chen, S. Springthorpe, and S. Sattar.	Oocyst survival in the St. Lawrence River was better in

Reference	Highlights
1998. Aging of Cryptosporidium parvum oocysts in river water and their susceptibility to disinfection by chlorine and monochloramine. **Can. J. Microbiol. 44:1154-1160.**	membrane-filtered water than in unfiltered water, suggesting that biological antagonism may play a role in the environmental fate of the parasite.
Cizek, A.R., G.W. Characklis, L.-A. Krometis, J.A. Hayes, O.D. Simmons III, S. Di Lonardo, K.A. Alderisio, and M.D. Sobsey. 2008. Comparing the partitioning behavior of Giardia and Cryptosporidium with that of indicator organisms in stormwater runoff. **Wat. Res. 42:4421-4438.**	15-30% of bacterial indicators (fecal coliform, E. coli and Enterococci) associated with settleable particles compared to 50% for C. perfringens spores. The settling velocities of C. perfringens were similar to the protozoan parasites with roughly 30% of Giardia and Cryptosporidium partitioning during dry weather and higher levels observed during wet weather events (Giardia to 60% and Cryptosporidium to 40%).
Clarkson, L.S., M. Tobin-D'Angelo, C. Shuler, S. Hanna, J. Benson, and A.C. Voetsch. 2010. Sporadic Salmonella enterica serotype Javiana infections in Georgia and Tennessee: a hypothesis-generating study. **Epidemiol. Infect. 138:340-346.**	Consumption of well water and reptile or amphibian contact in Georgia and Tennessee were associated with Salmonella infection.
Cooley, M., D. Carychao, L. Crawford-Miksza, M.T. Jay, C. Myers, C. Rose, C. Keys, J. Farrar, and R.E. Mandrell. 2007. Incidence and tracking of Escherichia coli O157:H7 in a major produce production region in California. **PLoS One 11:e1159.**	O157 was isolated at least once from 15 of 22 watershed sites over a 19-month period. The incidence of O157 increased significantly when heavy rain caused an increased flow rate in the rivers. Recurrence of identical and closely related O157 strains from specific locations in the Salinas and San Juan valleys suggests that transport of the pathogen is usually restricted.
Crabill, C., R. Donald, J. Snelling, R. Foust, and G. Southam. 1999. The impact of sediment fecal coliform reservoirs on seasonal water quality in Oak Creek, Arizona. **Water Res. 33:2163-2171.**	Seasonal fecal coliform levels were associated predominantly with sediment agitation during summer storms and recreational activity.
Curriero, F.C., J.A. Patz, J.B. Rose, and S. Lele. 2001. The association between extreme precipitation and waterborne disease outbreaks in the United States, 1948-1994. **Am. J. Public Hlth. 91:1194-1199.**	51% of waterborne disease outbreaks between 1948 and 1994 were preceded by precipitation events above the 90th percentile and 68% above the 80th percentile. Outbreaks due to surface water contamination showed the strongest association with extreme precipitation during the month of the outbreak; a 2-month lag applied to groundwater contamination events.
Czajkowska, D., A. Witkowska-Gwiazdowska, I. Sikorska, H. Boszczyk-Maleszak, and M. Horoch. 2005. Survival of Escherichia coli serotype O157:H7 in water and in bottom-shore sediments. **Polish J. Environ. Stud. 14:423-430.**	E. coli O157:H7 has been shown to survive for extended periods in sediment becoming undetectable only after 60 days at 24°C.
Dorner, S.M., W.B. Anderson, T. Gaulin, H.L. Candon, R.M. Slawson, P. Payment, and P.M. Huck. 2007. Pathogen and indicator variability in a heavily impacted watershed. **J. Wat. Hlth. 5:241.**	Storm and snowmelt events were samples at two locations. Peaks in pathogen numbers frequently preceded the peaks in numbers of indicator organisms and turbidity. As pathogen peaks did not correspond to turbidity and indicator peaks, the correlations were weak. Weak correlations may be the results of differences in the sources of the pathogens, rather than differences in pathogen movement through the environment.
Droppo, I.G., S.N. Liss, D. Williams, T. Nelson, C. Jaskot, and B. Trapp. 2009. Dynamic existence of waterborne pathogens within river sediment compartments. Implications for water quality regulatory affairs. **Environ. Sci. Technol. 43:1737-1743.**	Bacteria counts were consistently higher within sediment compartments (suspended and bed) than for the water alone, with the bed sediment found to represent a possible reservoir of pathogens for subsequent remobilization and transport to potentially high-risk areas. Current standard sampling strategies, however, are based on an assumption that bacteria are entirely planktonic and do not account for the potentially significant concentration of bacteria from the sediment compartments.

Reference	Highlights
Duffy, L. and G.A. Dykes. 2006. Growth temperature of four Campylobacter jejuni strains influences their subsequent survival in food and water. **Lett. Appl. Microbiol. 43:596-601.**	Study indicates that C. jejuni from animal sources growth at 37C, the body temperature of humans and cattle, are able to survive longer in water at ~4°C than those grown at 42°C, the body temperature of poultry. Specifically, it suggest that C. jejuni from cattle origin may represent a greater risk to public health from water sources than those of poultry origin. Used 4 different strains. Inoculated at 6 log CFU/ml. Stored at 3.5°C.
Edge, T.A., A. El-Shaarawi, V. Gannon, C. Jokinen, R. Kent, I.U.H. Khan, W. Koning, D. Lapen, J. Miller, N. Neumann, R. Phillips, W. Robertson, H. Schreier, A. Scott, I. Shtepani, E. Topp, G. Wilkes, and E. van Bochove. 2012. Investigation of an Escherichia coli environmental benchmark for waterborne pathogens in agricultural watersheds in Canada. **J. Environ. Qual. 41:21-30.**	Collected 902 water samples from 27 sites in four intensive agricultural watersheds across Canada. Waterborne pathogens were detected at agricultural sites in 80% of water samples with low E. coli concentrations (<100 cfu/100 ml). An approach was developed based on using the natural background occurrence of pathogens at reference sites in agricultural watersheds to derive provisional environmental benchmarks for pathogens at agricultural sites. The environmental benchmarks that were derived were found to represent E. coli values lower than geometric mean values typically found in recreational water quality guidelines.
Ensink, J.H.J., T. Mahmood, W. van der Hoek, L. Raschid-Sally, and F.P. Amerasinghe. 2004. A nationwide assessment of wastewater use in Pakistan: an obscure activity or a vitally important one? **Wat. Policy 6:197-206.**	It was estimated that 26% of the total domestic vegetable production of Pakistan was cultivated with wastewater and was attributed to the absence of alternative water sources, the reliability of the wastewater supply, the nutrient value, and the proximity to urban markets.
Foppen, J.W.A. and J.F. Schijven. 2006. Evaluation of data from the literature on the transport and survival of Escherichia coli and thermotolerant coliforms in aquifers under saturated conditions. **Wat. Res. 40:401-426.**	Sticking efficiencies of E. coli determined from field experiments were lower than those determined under laboratory conditions and were attributed to preferential flow mechanisms, heterogeneity in E. coli population, and/or the presence of organic and inorganic compounds in wastewater possibly affecting bacterial attachment characteristics.
Gaertner, J.P., T. Garres, J.C. Becker, M.L. Jimenez, M.R.J. Forstner, and D. Hahn. 2009. Temporal analyses of Salmonellae in a headwater spring ecosystem reveals the effects of precipitation and runoff events. **J. Water Health 7:115-121.**	Salmonellae was detected in water and sediment samples of the San Marcos River in Texas after three precipitation events, but failed to detect them immediately prior to the rainfall events.
Gagliardi, J.V., P.D. Millner,, G. Lester, and D. Ingram. 2003. On-farm and postharvest processing sources of bacterial contamination to melon rinds. **J. Food Prot. 66:82-87.**	High levels of coliforms and enterococci were found in melon production soils (furrows that were flood irrigated), in standing water at one field, and in irrigation water at both sites.
Garzio-Hadzick, A., D.R. Shelton, R.L. Hill, Y.A. Pachepsky, A.K. Guber, and R. Rowland. 2010. Survival of manure-borne E. coli in streambed sediment: Effects of temperature and sediment properties. **Wat. Res. 44:2753-2762.**	(4, 14, and 24°C). E. coli survived in sediments much longer than in the overlying water and was inactivated at slower rates when organic carbon contents were higher.
Greene, S.K., E.R. Daly, E.A. Talbot, L.J. Demma, S. Holzbauer, N.J. Patel, T.A. Hill, M.O. Walderhaug, R.M. Hoekstra, M.F. Lynch, and J.A. Painter. 2008. Recurrent multistate outbreak of Salmonella Newport associated with tomatoes from contaminated fields, 2005. **Epidemiol. Infect. 136:157-165.**	The outbreak strain was isolated from pond water used to irrigate tomato fields. Identification of that strain in irrigation ponds 2 years apart suggest persistent contamination of tomato fields. In the 2006 inspection, large numbers of geese and turtles were observed in ponds. .
Haley, B.J., D.J. Cole, and E.K. Lipp. 2009. Distribution, diversity, and seasonality of waterborne Salmonellae in a rural watershed. **Appl. Environ. Microbiol. 75:1248-1255.**	Salmonella abundance and diversity in the environment appeared to vary temporally and was strongly influenced by seasonal precipitation and water temperature. Detection of Salmonella occurred in instances when E. coli levels were not high.
Hellein, K.N., C. Battle, E. Tauchman, D. Lund, O.A. Oyarzabal, and J.E. Lepo. 2011. Culture-based indicators of fecal	The presence of C. jejuni and C. coli were rare in waterways but were prevalent in sewage and feces. Campylobacter-

Reference	Highlights
contamination and molecular microbial indicators rarely correlate with Campylobacter spp. in recreational waters. **J. Water Health 9:695-707.**	specific qPCR screening of environmental waters did not correlate with the Enterococcus culture method (EPA method 1600), nor with culture-independent, molecular-based microbial source tracking indicators (human polyomavirus, human Bacteroidales, and Methanobrevibacter smithii).
Ijabadeniyi, O.A., L.K. Debusho, M. Vanderlinde, and E.M. Buys. 2011. Irrigation water as a potential preharvest source of bacterial contamination of vegetables**. J. Food Safety 31:452-461.**	S. aureus, intestinal enterococci, Salmonella, and L. monocytogenes were recovered in South Africa from the Olifant and Wilge rivers and an irrigation canal using these water sources. Logistic regression analysis of the sampled data showed that chemical oxygen demand was statistically reliable to predict L. monocytogenes, turbidity reliable to predict intestinal enterococci, and fecal coliform and coliform reliable to predict Salmonella in irrigation water.
Jenkins, M.B., D.M. Endale, D.S. Fisher, M.P. Adams, R. Lowrance, G.L. Newton, and G. Vellidis. 2012. Survival dynamics of fecal bacteria in ponds in agricultural watersheds of the Piedmont and Coastal Plain of Georgia. **Wat. Res. 46:176-186.**	Both Salmonella and E. coli O157:H7 were measured when concomitant concentrations of commensal E. coli in three ponds were below the criterion for surface water impairment and these false negatives would put public health at risk. Complete mixing did not occur within the three ponds but residence times were around 3 months providing sufficient time for solar UV-radiation and microbial predation to decrease concentrations of fecal bacterial. Fecal indicator bacteria did decrease in a pond that had continuous in- and outflow fluxes whereas in the other two ponds that did not have continuous in- and outflow fluxes, reduction of fecal bacteria was not observed.
Jones, K. 2001. Campylobacters in water, sewage and the environment. **J. Appl. Microbiol. 90:68S-79.**	Campylobacter organisms were present in rivers all year round, but with lower numbers in the summer corresponding to elevated UV levels and higher temperatures. The presence of this pathogen in environmental samples can be taken as a sign of recent fecal contamination; however, there is not always a good correlation between the densities of the Campylobacter population and of the indicators.
Juhna, T., D. Birzniece, S. Larsson, D. Zulenkovs, A. Sharipo, N.F. Azevedo, F. Ménard-Szczebara, S. Castagnet, C. Féliers, and C.W. Keevil. 2007. Detection of Escherichia coli in biofilms from pipe samples and coupons in drinking water distribution networks. **Appl. Environ. Microbiol. 73:7456-7464.**	E. coli was found in the biofilms of drinking water pipes in Europe. After resuscitation in low-nutrient medium supplemented with pipemidic acid, suggesting that the cells were present in an active but nonculturable state.
Karim, M.R., F.D. Manshadi, M.M. Karpiscak, and C.P. Gerba. 2004. The persistence and removal of enteric pathogens in constructed wetlands. **Wat. Res. 38:1831-1837.**	Giardia cyst and Cryptosporidium oocyst concentration survived better in sediment than in the water column whereas die-off rates of all the bacteria and coliphage were greater in the water column than the sediment.
Keller, R., J.A. Tetro, V.S. Springthorpe, and S.A. Sattar. 2010. The influence of temperature on norovirus inactivation by monochloramine in potable waters: Testing with murine norovirus as a surrogate for human norovirus. **Food Environ. Virol. 2:97-100.**	The titre of murine norovirus (surrogate for human norovirus) remained essentially unchanged for at least 24 h in raw river water at both 4 and 25°C. The virus became undetectable in <2 h in monochloramine-containing samples held at 25°C, but its titre remained virtually unaltered at 4°C under the same conditions.
King, B.J., A.R. Keegan, P.T. Monis, and C.P. Saint. 2005. Environmental temperature controls Cryptosporidium oocyst metabolic rate and associated retention of infectivity. **Appl. Environ. Microbiol. 71:3848-3857.**	Although water quality did not affect oocyst inactivation, biological antagonism appears to be a key factor affecting oocyst removal from environmental waters.
King, B.J., D. Hoefel, D.P. Daminato, S. Fanok, and P.T. Monis. 2008. Solar UV reduces Cryptosporidium parvum oocysts	Oocysts were inactivated by solar UV with UV-B contributing the most germicidal wavelengths; however,

Reference	Highlights
infectivity in environmental waters. **J. Appl. Microbiol. 104:1311-1323.**	dissolved organic carbon content in environmental waters decreased solar inactivation.
Krometis, L-A.H., G.W. Characklis, P.N. Brummey, and M.D. Sobsey. 2010. Comparison of the presence and partitioning behavior of indicator organisms and Salmonella spp. in an urban watershed. **J. Water Health 8:44-59.**	Sedimentation appeared to be an important removal mechanism ; however, large fluctuations in detention pond performance between storm events and occasional net microbial exports in effluents indicate that they could not be relied on to achieve water quality objectives.
Krometis, L-A.H.,P.N. Drummey, G.W. Characklis, and M.D. Sobsey. 2009. Impact of microbial partitioning on wet retention pond effectiveness. **J. Environ. Engr. 135:758-767.**	Grab sampling results suggested that the two web ponds examined were relatively ineffective at reducing bacterial or particle concentrations under both storm and background conditions. There was some evidence that resuspended storm drain sediment served as a fecal coliform reservoir.
Lamendella, R., J.W. Santo Domingo, C. Kelty, and D.B. Oerther. 2008. Bifidobacteria in feces and environmental waters. **Appl. Environ. Microbiol. 74:575-584.**	The use of bifidobacteria species as potential markers to monitor human fecal pollution in natural waters may be questionable based on results in this study.
Masters, N., A. Wiegand, W. Ahmed, and M. Katouli. 2011. Escherichia coli virulence genes profile of surface waters as an indicator of water quality**. Wat. Res. 45:6321-6333.**	More types of virulence genes (VGs) associated with Escherichia coli strains were observed in water samples collected from three estuarine, four brackish, and 13 freshwater sites during the wet season. Eight VGs were found exclusively in the wet season, of which four were found in all three water types indicating their association with storm-water run-off. The number of VGs associated with extra-intestinal were significantly higher in only brackish and estuarine waters during the wet season compared to the dry season. No correlation was found between the number of E. coli and the presence of VGs in any of the water types; however, similarities in VG profiles were found at sites with similar land uses.
Maule, A. 1999. Environmental aspects of E. coli O157**. Int. Food Hyg. 9:21-23.**	E. coli O157 inoculated into river water at 8 log CFU/ml survived for up to 27 days at 18°C.
McBride, G.B. 2011. Explaining differential sources of zoonotic pathogens in intensively-farmed catchments using kinematic waves. **Wat. Sci. Technol. 63:695-703.**	Based on whether indicator (E. coli) arrives before, at same time, or after the pathogen (Campylobacter) can serve to indicate the potential predominant source in floodwater (by sediment entrainment, vial local land runoff, or from upstream releases).
Méndez-Hermida, F., J.A. Castro-Hermida, E. Ares-Mazás, S.C. Kehoe, and K.G. McGuigan. 2005. Effect of batch-process solar disinfection on survival of Cryptosporidium parvum oocysts in drinking water. **Appl. Environ. Microbiol. 71:1653-1654.**	Exposing oocysts in water to simulated sunlight (830 W/m^2) at 40°C for 6 and 12 h reduced oocyst infectivity..
Nichols, R.A.B., C.A. Paton, and H.V. Smith. 2004. Survival of Cryptosporidium parvum oocysts after prolonged exposure to still natural mineral waters. **J. Food Prot. 67:517-523.**	Both oocyst types remained viable after 12 weeks at 4°C, whereas at 20°C, approximately 30% of oocysts remained viable after 12 weeks incubation.
Ongerth, J.E. and F.M.A. Saaed. 2013. Distribution of Cryptosporidium oocysts and Giardia cysts in water above and below the normal limit of detection. **Parasitol. Res. 112:467-471.**	After seeding 50 L water with Cryptosporidium oocysts and Giardia cysts and analyzing five 10-L samples, the data conformed to the Poisson distribution and supported the interpretation that the absence of finding any target organisms was the result of their presence below the limit of detection. Their interpretation strongly suggested that analyzing fewer larger volume samples would provide more useful information.
Pachepsky, Y., J. Morrow, A. Guber, D. Shelton, R. Rowland, and G. Davies. 2011. Effect of biofilm in irrigation pipes on microbial quality of irrigation water. **Lett. Appl. Microbiol. 54:217-224.**	High E. coli concentrations in water remaining in irrigation pipes between irrigation events were indicative of E. coli growth. The population of bacteria associated with the biofilm on pipe walls was estimated to be larger than that in water in pipes.

Reference	Highlights
Pandey, P.K., M.L. Soupir, and C.R. Rehmann. 2012. A model for predicting resuspension of Escherichia coli from streambed sediments. **Wat. Res. 46:115-126.**	In a model developed to predict resuspension of Escherichia coli from sediment beds in streams, the resuspension rate was expressed as the product of the concentration of E. coli attached to sediment particles and an erosion rate that had been adapted from previous work on sediment transport. Using this model, inferred rates matched the predicted rates. The model's sensitivity to the parameters depended on the contributions of particle packing and binding effects of clay to the critical shear stress.
Patchanee, P., B. Molla, N. White, D.E. Line, and W.A. Gebreyes. 2010. Tracking Salmonella contamination in various watersheds and phenotypic and genotypic diversity. Foodborne Path. Dis. 7L1113	Eighty-six water samples collected from four different watershed systems, including those impacted by swine production, residential/industrial, crop agriculture, and forestry were cultured for Salmonella and further characterized by serotyping, antimicrobial susceptibility testing, and pulsed-field gel electrophoresis genotyping. Swine-production-associated isolates were distinctly different from the others. Overall, the findings suggest that all the various watersheds, including natural forest, remain important contributors of Salmonella contamination.
Ravva, S.V., C.Z. Sarreal, and R.E. Mandrell. 2010. Identification of protozoa in dairy lagoon wastewater that consume Escherichia coli O157:H7 preferentially. **PLoS One 5:e15671.**	Whereas two protozoa, Platyophyra and Colpoda, acted as predators of E. coli O157:H7, the protozoa, Vorticella microstoma internalized O157 but expelled vacuoles filled with live O157 cells.
Reinoso, R., S. Blanco, L.A. Torres-Villamizar, and E. Bécares. 2011. Mechanisms for parasites removal in a waste stabilization pond. **Microb. Ecol. 61:684-692**	Sunlight and water physicochemical conditions were the main factors influencing C. parvum oocysts removal both in anaerobic and maturation ponds, whereas other factors like predation or natural mortality were more important in facultative ponds. Sedimentation was a negligible factor for cyst removal in the studied ponds.
Rollins, D.M. and R.R. Colwell. 1986. Viable but nonculturable stage of Campylobacter jejuni and its role in survival in the natural aquatic environment**. Appl. Environ. Microbiol. 52:531-538.**	Stream water held at low temperature (4°C) sustained significant numbers of campylobacters for > 4 months. Microcosms, aerated with shaking, exhibited logarithmic decline in recoverable C. jejuni, while stationary systems underwent a more moderate rate of decrease to the nonculturable state.
Santo Domingo, J.W., S. Harmon, and J. Bennett. 2000. Survival of Salmonella species in river water. **Curr. Microbiol. 40:409-417.**	Salmonella serovars DT104, O78, and ML14 survived for 45 days in autoclaved river water at approximately 5 log CFU/ml (from an initial population of approximately 8 log CFU/ml) whereas plate counts of untreated or filtered river water supported fewer Salmonella. Direct counts were higher. Moreover, addition of a cell-free supernatant from viable cultures during the resuscitation period led to higher recoveries suggesting the presence of a not immediately culturable status in Salmonella.
Scott, L., P. McGee, J.J. Sheridan, B. Earley, and N. Leonard. 2006. A comparison of the survival in feces and water of Escherichia coli O157:H7 grown under laboratory conditions or obtained from cattle feces. **J. Food Prot. 69:6-11.**	E. coli O157:H7 (initial counts of 2.5-3.2 log CFU/g) survived up to 109 days in water, and the bacteria collected from inoculated cattle were detected up to 10 weeks longer than the laboratory-prepared culture suggesting that pathogen survival in low-nutrient conditions may be enhanced by passage through the gastrointestinal tract.
Searcy, K.E., A.I. Packman, E.R. Atwill, and T. Harter. 2005. Association of Cryptosporidium parvum with suspended particles: Impact on oocysts sedimentation. **Appl. Environ. Microbiol. 71:1072-1078.**	Direct microscopic observations showed that oocysts attached to suspended sediments and the rate of oocyst sedimentation depended primarily on the type of sediment with which the oocysts were mixed.
Seitz, S.R., J.S. Leon, K.J. Schwab, G.M. Lyon, M. Dowd, M.	Norovirus virus spiked in groundwater remained infectious

Reference	Highlights
McDaniels, G. Abdulhafid, M.L. Fernandez, L.C. Lindesmith, R.S. Baric, and C.L. Moe. 2011. Norovirus infectivity in humans and persistence in water. **Appl. Environ. Microbiol. 77:6884-6888.**	after storage at room temperature in the dark for 61 days. Norwalk virus RNA within intact capsids was detected in groundwater for 1,266 days, with no significant log reduction throughout 427 days and a significant 1.1 log reduction by day 1,266.
Sha, Q., D.A. Vattem, M.R.J. Forstner, and D. Hahn. 2013. Quantifying Salmonella population dynamics in water and biofilms. **Microb. Ecol. 65:6-67.**	Results demonstrate that pathogenic salmonellae were isolated from heterogeneous aquatic biofilms and that they could persist and stay viable in such biofilms in high numbers for some time. It is still unclear whether the pathogen can actually grow or just persist. Future studies should investigate the impact of potential pulse releases of these pathogens from biofilms in irrigation systems.
Shelton, D.R., J.S. Karns, C. Coppock, J. Patel, M. Sharma, and Y.A. Pachepsky. 2011. Relationship between eae and stx virulence genes and Escherichia coli in an agricultural watershed: Implications for irrigation water standards and leafy green commodities. **J. Food Prot. 74:18-23.**	No correlation was observed between E. coli concentrations and virulence genes; lower E. coli concentrations were not necessarily associated with decreased prevalence of eae and stx genes. E. coli concentrations in the Little Cove Creek watershed varied both spatially and temporally and was attributed to a combination of lower fecal inputs during the fall and winter, and several heavy rainfall events that ''flushed'' sediment-borne E. coli downstream.
Staley, C., K.H. Reckhow, J. Lukasik, and V.J. Harwood. 2012. Assessment of sources of human pathogens and fecal contamination in a Florida freshwater lake. **Water Res. 46:5799-5812.**	Pathogens and human source markers were detected in 55% and 21% of samples, respectively; however, markers rarely coincided with pathogen detection. The model showed associations between elevated temperature and rainfall with fecal coliform and enterococci concentrations, but not E. coli.
Staley, Z.R., J.R. Rohr, and V.J. Harwood. 2011. Test of direct and indirect effects of agrochemicals on the survival of fecal indicator bacteria**. Appl. Environ. Microbiol. 77:8765-8774.**	The pesticides, atrazine, malathion, and chlorothalonil, and inorganic fertilizer, did not have an effect on Escherichia coli and enterococcal survival in dark water systems. In light-exposed microcosms, atrazine significantly lowered E. coli densities in the water column but increased densities in the sediment compared to controls and appeared to be mediated by the effects of atrazine on algae.
Staley, Z.R., J.K. Senkbeil, J.R. Rohr, and V.J. Harwood. 2012. Lack of direct effects of agrochemicals on zoonotic pathogens and fecal indicator bacteria. **Appl. Environ. Microbiol. 78:8146-8150.**	By using sterile water and disinfected sediment, it was confirmed that there was no significant effect of fertilizer, atrazine, malathion, and chlorothalonil, on the survival of E. coli, Enterococcus faecalis, Salmonella enteria, human polyomaviruses, and adenovirus were detected regardless of whether the response variables used were the concentrations or the decay rates.
Tyrrel, S.F., J.W. Knox, and E.K. Weatherhead. 2006. Microbial water quality requirements for salad irrigation in the United Kingdom. **J. Food Prot. 69:2029-2035.**	Based on a survey conducted in 2003, half the total irrigated area in the United Kingdom was on lettuce crops whereas another third of the total irrigated area was applied to spinach and salad onion crops. The majority of United Kingdom salad crops (>99%) are irrigated by mobile overhead methods with surface water (rivers and streams) serving as the source for 71% of the irrigated salad crops. A significant proportion (37%) of water used for irrigation is abstracted and stored in farm reservoirs with the remainder being applied directly. For most salad cropping, the minimum period between the last irrigation and harvest is between 1 and 5 days. However, half the survey respondents growing salad onions, celery, and baby leaf salads reported harvesting their crops within 24 h of the last irrigation.
Vital, M., D. Stucki, T. Egli, and F. Hammes. 2010. Evaluating the	Results suggested that the extent of pathogen growth was

Reference	Highlights
growth potential of pathogenic bacteria in water. **Appl. Environ. Microbiol. 76:6477-6484.**	affected not only by the concentration but also by the composition of assimilable organic carbon.
Vital, M., F. Hammes, and T. Egli. 2008. Escherichia coli O157 can grow in natural freshwater at low carbon concentrations. **Environ. Microbiol. 10:2387-2396.**	E. coli O157 was able to grow in sterile freshwater at low carbon concentrations.
Walker, F.R. and J.R. Stedinger. 1999. Fate and transport model of Cryptosporidium. **J. Environ. Engr. 125:325-333.**	Manure and Cryptosporidium oocysts were modeled as surface pollutants and assumed to move in response to runoff events.
Wanjugi, P. and V.J. Harwood. 2013. The influence of predation and competition on the survival of commensal and pathogenic fecal bacteria in aquatic habitats. **Environ. Microbiol. 15:517-526.**	River water and sediments were disinfected by filtration of water and baking of sediments to remove indigenous protozoa (predators) and bacteria (competitors). The disinfection treatment significantly increased survival of E. coli, E. coli O157:H7 and Enterococcus faecalis in the water column but whereas survival of fecal indicator bacteria also increased in the sediments, survival of E. coli O157:H7 did not.
Wilkes, G., T. Edge, V. Gannon, C. Jokinen, E. Lyautey, D. Medeiros, N. Neumann, N. Ruecker, E. Topp, and D.R. Lapen. 2009. Seasonal relationships among indicator bacteria, pathogenic bacteria, Cryptosporidium oocysts, Giardia cysts, and hydrological indices for surface waters within an agricultural landscape. **Wat. Res. 43:2209-2223.**	Weak positive relationships between indicator bacteria (E. coli, C. perfringens, enterococci, total and fecal coliforms), pathogens (E. coli O157:H7, Campylobacter spp., Salmonella spp., Listeria monocytogenes), and parasite oocysts/cysts were found. Campylobacter, Salmonella, Giardia cysts and Cryptosporidium oocysts were most frequently detected in the fall. Rainfall and discharge were primarily associated with indicator bacteria densities and pathogen detection.
Wilkes, G., T.A. Edge, V.P.J. Gannon, C. Jokinen, E. Lyautey, N.F. Neumann, N. Ruecker, A. Scott, M. Sunohara, E. Topp, and D.R. Lapen. 2011. Associations among pathogenic bacteria, parasites, and environmental and land use factors in multiple mixed-use watersheds. **Water Res. 45:5807-5825.**	E. coli O157:H7 detections were infrequent, but detections were related to upstream livestock pasture density; 20% of the detections were located where cattle have access to the watercourses. Cooler water temperatures, which can promote bacteria survival and represent times when land applications of manure typically occur (spring and fall), may have promoted increased frequency of Campylobacter spp. Hydrological events that promote off farm/off field/in stream transport must manifest themselves in order for detection of Salmonella spp. to occur in surface water in this region. Fifty seven percent of L. monocytogenes detections occurred in spring, relative to other seasons. Cryptosporidium and Giardia oocyst and cyst densities were, overall, positively associated with surface water discharge, and negatively associated with air/water temperature during spring-summer-fall. Yet, some of the highest Cryptosporidium oocyst densities were associated with low discharge conditions on smaller order streams, suggesting wildlife as a contributing fecal source. Less rainfall may have been necessary to mobilize pathogens from adjacent land, and/or in stream sediments, during cooler water conditions; as these are times when manures are applied to fields in the area, and soil water contents and water table depths are relatively higher. Season, stream order, turbidity, mean daily temperature, surface water discharge, cropland coverage, and nearest upstream distance to a barn and pasture were variables that were relatively strong and recurrent with regard to discriminating pathogen presence and absence, and parasite densities in surface water in the region.
Won, G., T.R. Kline, and J.T. LeJeune. 2013. Spatial-temporal variations of water quality in surface reservoirs and canals used	Two irrigation canals and four surface reservoirs located in Ohio were sampled 227 times to investigate fluctuations in

Reference	Highlights
for irrigation. **Agric. Wat. Manag. 116:73-78.**	fecal indictor concentrations over the 2010 irrigation season. E. coli counts in canals averaged 2.5 log MPN/100 ml whereas average counts in reservoirs were approximately one order of magnitude lower (1.5 log MPN/100 ml). The E. coli concentrations in water increased following heavy rainfall events and it was concluded that a single water sample imprecisely reflected the quality of water over the course of the irrigation period. Environmental factors affecting the fluctuation in water quality included the type of water source and recent heavy precipitation events. The expected interval between testing and the time of harvest should be considered in developing irrigation water testing frequency guidelines.

7.2 Selected references for pathogens in water

7.2.1 Attachment of enteric pathogens to above ground plant surfaces

Alum, A., C. Enriquez, and C.P. Gerba. 2011. Impact of drip irrigation method, soil, and virus type on tomato and cucumber contamination. Food Environ. Virol. 4:78-85.

Arthurson, V., A. Sessitsch, and L. Jäderlund. 2011. Persistence and spread of Salmonella enterica serovar Weltebreden in soil and on spinach plants. FEMS Microbiol. Lett. 314:67-74.

Bae, D., K.S. Seo, T. Zhang, and C. Wang. 2013. Characterization of a potential Listeria monocytogenes virulence factor associated with attachment to fresh produce. Appl. Environ. Microbiol. 79:6855-6861. G

Baldotto, L.E.B. and F.L. Olivares. 2008. Phylloepiphytic interaction between bacteria and different plant species in a tropical agricultural system. Can. J. Microbiol. 54:918-931.

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Barak, J.D., A. Liang, and K-E. Narm. 2008. Differential attachment to and subsequent contamination of agricultural crops by Salmonella enterica. Appl. Environ. Microbiol. 74:5568-5570.

Berger, C.N., R.K. Shaw, F. Ruiz-Perez, J.P. Nataro, I.R. Henderson, M.J. Pallen, and G. Frankel. 2009. Interaction of enteroaggregative Escherichia coli with salad leaves. Environ. Microbiol. Rept. 1:234-239.

Dinu, L.-D. and S. Bach. 2011. Induction of viable for nonculturable Escherichia coli O157:H7 in the phyllosphere of lettuce: a food safety risk factor. Appl. Environ. Microbiol. 77:8295-8302.

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Ells, T.C. and L.T. Hansen. 2006. Strain and growth temperature influence Listeria spp. attachment to intact and cut cabbage. Int. J. Food Microbiol. 111:34-42.

Esseili, M.A., Q. Wang, and L.J. Saif. 2012. Binding of human GII.4 norovirus virus-like particles to carbohydrates of Romaine lettuce leaf cell wall materials. Appl. Environ. Microbiol. 78:786-794.

Fink, R.C., E.P. Black, Z. Hou, M. Sugawara, M.J. Sadowsky, and F. Diez-Gonzalez. 2012. Transcriptional responses of Escherichia coli K-12 and O157:H7 associated with lettuce leaves. Appl. Environ. Microbiol. 78:1752-1764.

Gutiérrez-Rodriguez, A. Gundersen, A.O. Sbodio, and T.V. Suslow. 2011. Variable agronomic practices, cultivar, strain source and initial contamination dose differentially affect survival of Escherichia coli on spinach. J. Appl. Microbiol. 112:109-118.

Ibekwe, A.M., P.M. Watt, P.J. Shouse, and C.M. Grieve. 2004. Fate of Escherichia coli O157:H7 in irrigation water on soils and plants as validated by culture method and real-time PCR. Can. J. Microbiol. 50:1007-1014.

Johannessen, G.S., R.B. Froseth, L. Solemdal, J. Jarp, Y. Wasteson, and L.M. Rorvik. 2004. Influence of bovine manure as fertilizer on the bacteriological quality of organic Iceberg lettuce. J. Appl. Microbiol. 96:787-794.

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7.2.2 Internalisation of enteric pathogens via above ground plant surfaces

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7.2.3 Internalisation of enteric pathogens via plant roots

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7.3 Definitions of terms used in Section 3

Organic material	Description
Soil amendment	Organic (biological) inputs to soils used to supply nutrients (as fertiliser) and or improve the biological, chemical or physical condition of the soil (soil conditioner). The inputs can be in solid, sludge / slurry or liquid form.
Soil Conditioner	Composted or pasteurised organic material including vermicast, manure and mushroom substrate that is suitable for adding to soils. This term also includes "soil amendment", "soil additive", "soil improver" and similar terms, but excludes polymers that do not biodegrade such as plastics, rubbers and coatings. Soil conditioners may be either "composted soil conditioners" or "pasteurised soil conditioners". Soil conditioner has not more than 20% by mass of particles with a maximum size above 16 mm. and results in beneficial effects. This term also includes "soil amendment", "soil additive", "soil improver" and similar terms, but excludes polymers which do not biodegrade such as plastics, rubbers and coatings.
Fertiliser	Any material of natural or synthetic origin (other than liming materials) that is applied to soils or to plants (usually the canopy) to supply one or more plant nutrients essential to their growth.
Composts	All types of recycled, organic materials, which are completely decomposed (biodegraded, rotted, humified) so that they are amorphous ie without a cellular structure characteristic of plants or animals. During the correct composting process, organic materials are pasteurised, microbially transformed and stabilised under aerobic and thermophilic conditions for a period of not less than 6 weeks[147]. Composts are destined for use as soil amendment, either as a fertiliser because of their nutrient content, and or as soil conditioner because of their positive effect on soil structure, biology and chemistry (in addition to the nutrient value).
Compost maturity	The degree of decomposition, pasteurisation and stabilisation at which compost is not phytotoxic or exerts negligible phytotoxicity in any plant growing situation when used as directed.
Stable stabilised, stability (of) compost	The degree of decomposition at which the rate of biological activity under conditions favourable for aerobic biodegradation has slowed and microbial respiration will not resurge under altered conditions, such as manipulation of moisture and oxygen levels or temperature.
Pasteurised / sanitised product	An organic product that has undergone pasteurisation but is relatively immature and lacking in stability. (Pasteurisation is a process whereby organic materials are treated to significantly

[147] Recycled Organics Unit 2007; Information sheet No. 3-6 Biosolids guidelines: Raw materials and compost product quality

Organic material	Description
	reduce the numbers of plant and animal pathogens and plant propagules.)
Vermicompost	Material that is egested from earthworms as casts then further decomposed and matured in a vermicomposting system.
Mulch	any pasteurised or composted organic product (excluding polymers which do not biodegrade such as plastics, rubbers and coatings) that is suitable for placing on soil surfaces. Fine mulch has more than 20% but less than 70% by mass of its particles with a maximum size above 16mm. Mulch has at least 70% by mass of its particles with a maximum size of greater than 16mm.
Manures	Animal excrement (urine, dung) that may contain various amounts of bedding such as sawdust, tree bark or straw.
Manure slurry	Animal excrement and water with only small amounts of bedding.
Biosolids	Organic solids or sludges produced by municipal sewage treatment processes. Solids become biosolids when they come out of an anaerobic digester or other treatment process and can be beneficially used. Until such solids are suitable for beneficial use they are defined as wastewater solids. The solids content in biosolids should be equal to or greater than 0.5% weight by volume (w/v). The solids component of biosolids is rich in organic matter and essential plant nutrients such as nitrogen and phosphorus. Thus, biosolids can be used as input materials for compost production. The term biosolids does not include untreated wastewater sludges, industrial sludges or the product created via the high temperature incineration of sewage sludge. It should also be noted that many other solid waste materials are not classified as biosolids, eg animal manures; food processing or abattoir wastes; solid inorganic wastes; and untreated sewage or untreated wastes from septic systems/sullage wastes.
Recycled organic waste, biowaste	Or (biodegradable waste) is any waste that is capable of undergoing anaerobic or aerobic decomposition (= composting), such as food and organic agricultural waste, paper, cardboard and waste from forestry (sawdust, wood chips) or municipal parks (tree cuttings, branches, grass, leaves - with the exception of street sweepings), and other wood waste not treated with heavy metals, pesticides or organic compounds, textiles made from natural fibres.
Municipal Solid Waste	Solid waste from households that is not necessarily made up entirely of organic materials.
Compost extract	The filtered product of compost mixed with any solvent (usually water), but not fermented. This term has been used in the past to define water extracts prepared using a very wide range of different methods. In the past, the terms “compost extract”, “watery fermented compost extract”, “amended extract”, “compost steepage” and “compost slurry” have all been used to refer to non-aerated fermentations. "Compost extract", "watery fermented compost extract" and "steepages" are approximate synonyms defined as a 1:5 to 1:10 (v:v) ratio of

Organic material	Description
	compost to water that is fermented without stirring at room temperature for a defined length of time. "Amended extracts" are compost extracts that have been fermented with the addition of specific nutrients or microorganisms prior to application.
Compost tea	The product of showering recirculated water through a porous bag of compost suspended over an open tank with the intention of maintaining aerobic conditions. The product of this method has also been termed "aerated compost tea" and "organic tea". In the past, the term "compost tea" has not always been associated with an aerated fermentation process. It is important to distinguish between compost teas prepared using aerated and non-aerated processes, therefore the terms aerated compost tea (ACT) and non aerated compost tea (NCT) are used in this review to refer to the two dominant compost fermentation methods. ACT will refer to any method in which the water extract is actively aerated during the fermentation process. NCT will refer to methods where the water extract is not aerated or receives minimal aeration during fermentation apart from during the initial mixing.
Organic biostimulants	The definition is still evolving: any substance or microorganism, in the form in which it is supplied to plants, seeds or the root environment with the intention to stimulate natural processes of plants benefiting nutrient use efficiency and/or tolerance to abiotic stress, regardless of its nutrient content, or any combination of such substances and/or microorganisms intended for this use.
Organic pesticides	Organic pesticides are substances that can be used in organic production under the relevant standard or code. They tend to have natural substances like soaps, lime sulphur, copper or hydrogen peroxide as ingredients. Not all naturally occurring substances that can control pests and disease are allowed in organic agriculture.
Biocides	Types of pesticides derived from natural materials such as animals, plants, bacteria, and certain minerals. For example, canola oil and baking soda have pesticidal applications and are considered biopesticides. Main groups are: microbial pesticides, Plant-Incorporated-Protectants (PIPs) and biochemical pesticides.

7.4 Sanitisers registered for use on fresh produce

Sanitisers:

- A search for product type "sanitiser" resulted in: 37 registered products, which included products not registered for use on fruit or vegetables ie these included products for use on hard surfaces and for sanitation of animal housing.
- The list of 37 products was searched for "vegetables – postharvest" and "fruit – postharvest", reducing the number to 7 products.

Microbiocides:

- A search for product type "microbiocide" resulted in 11 registered products, which included products not registered for use on fruit and vegetables.
- The list was searched for ""vegetables – postharvest" and "fruit – postharvest", reducing the number to 5 products.

Seven sanitiser and five microbiocide products registered for use in fruit and vegetables postharvest are listed in Table 18.

Table 18 - Sanitisers registered for postharvest use on fruit and vegetables (APVMA PubCRIS 31-5-2015)

Product name	Actives	Registered State	Pest
Sanitisers:			
KLORMAN WATERTECH CHLORINE PELLETS	Chlorine present as calcium hypochlorite	All	Bacteria, fungal leaf spot, fungus diseases, leaf spot diseases
FREXUS DURATION DRY CHLORINE	Chlorine present as calcium hypochlorite	All	Bacteria, fungal leaf spot, fungus diseases, leaf spot diseases
FREXUS DISINFECTION DRY CHLORINE	Chlorine present as calcium hypochlorite	All	Bacteria, fungal leaf spot, fungus diseases, leaf spot diseases
FREXUS BRIQUETTES DRY CHLORINE	Chlorine present as calcium hypochlorite	NSW	Bacteria, fungal leaf spot, fungus diseases, leaf spot diseases
VIBREX HORTICARE SANITISER	Chlorine present as chlorine dioxide	All	Bacteria
YM-FAB ACTIV 8 CALCIUM HYPOCHLORITE TABLETS	Chlorine present as calcium hypochlorite	All	Bacteria, fungal leaf spot, fungus diseases, leaf spot diseases
HYPOCHLOR CHLORINE CARTRIDGE	Chlorine present as calcium hypochlorite	All	Bacterial prevention, cleansing or disinfection, bacterial spot/blotch on mushroom, dip hygiene, postharvest dipping: fruit, soft rot, water treatment/sanitiser, disinfecting, ethylene degreening
Microbiocides:			
PINNACLE- PERACETIC ACID BIOCIDE	Hydrogen peroxide / peracetic acid	All	Bacterial growth
TIDAL SURGE, PERACETIC ACID MICRO BIOCIDE	Hydrogen peroxide / peroxyacetic acid	All	Bacterial growth
ADOXYSAN PERACETIC ACID BIOCIDE	Hydrogen peroxide / peroxyacetic acid	All	Bacterial growth
TSUNAMI ON FARM PERACETIC ACID BIOCIDE	Hydrogen peroxide / peroxyacetic acid	All	Bacterial growth
YM-FAB NYLATE HALOGEN BASED BROAD SPECTRUM BIOCIDE[a]	Bromochloro-dimethylhydantoin	All	Algae, bacteria, *Escherichia coli*, general disinfection, *Listeria* spp, rot diseases, *Salmonella* spp., slime, *Staphylococcus* spp., antiviral agent, detergent, floor, germicidal detergent, B-lactamase producing mould, protozoans.

[a] Host information includes “fruit and vegetable surface sterilisation”.

Table 19 - Compatibility guide (Taverner et al., 2008)

Fungicide (Formulation)	Sanitiser	pH (▲)†	Concentration of active ingredient (ppm)					
			Initial	30 sec	5 min	1 hr	4 hr	24hr
No Fungicide	Cal hypo	10.1 (+)	100 ■■■	100 ■■■	100 ■■■	100 ■■■	100 ■■■	100 ■■■
	Nylate®	5.7 (=)	30 ■■■	30 ■■■	30 ■■■	30 ■■■	30 ■■■	30 ■■■
	Tsunami®	3.8 (-)	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■
	Vibrex®	3.4 (-)	5 ■■■	5 ■■■	5 ■■■	5 ■■■	5 ■■■	5 ■■■
Tecto® (500 SC)	Cal hypo	10 (+)	100 ■■■	100 ■■■	100 ■■■	100 ■■■	100 ■■■	10 ■
	Nylate®	5.8 (=)	30 ■■■	30 ■■■	30 ■■■	30 ■■■	30 ■■■	9 ■
	Tsunami®	4.4 (-)	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■
	Vibrex®	3.8 (-)	5 ■■■	5 ■■■	5 ■■■	5 ■■■	5 ■■■	2 ■■
Bavistan®FL (500 SC)	Cal hypo	9.5 (+)	100 ■■■	50 ■■	0	0	0	0
	Nylate®	5.3 (-)	30 ■■■	30 ■■■	30 ■■■	30 ■■■	18 ■■	9 ■
	Tsunami®	4 (-)	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■
	Vibrex®	3.5 (-)	5 ■■■	5 ■■■	5 ■■■	5 ■■■	5 ■■■	2 ■■
Goldazim® (500 SC)	Cal hypo	9.8 (+)	100 ■■■	50 ■■	0	0	0	0
	Nylate®	4.9 (-)	30 ■■■	30 ■■■	30 ■■■	30 ■■■	30 ■■■	18 ■■
	Tsunami®	3.9 (-)	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■
	Vibrex®	3.7 (-)	5 ■■■	4 ■■■	4 ■■■	4 ■■■	4 ■■■	1 ■
Fungaflor® (500 EC)	Cal hypo	9.7 (+)	100 ■■■	10 ■	10 ■	0	0	0
	Nylate®	6.9 (=)	30 ■■■	0	0	0	0	0
	Tsunami®	4.8 (-)	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■
	Vibrex®	5.5 (-)	5 ■■■	5 ■■■	5 ■■■	4 ■■■	3 ■■	0.5 ■
Imazagard® (800 EC)	Cal hypo	10.2 (+)	100 ■■■	10 ■	10 ■	10 ■	1 ■	0
	Nylate®	6.9 (+)	30 ■■■	0	0	0	0	0
	Tsunami®	5.2 (-)	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■
	Vibrex®	5.8 (-)	5 ■■■	5 ■■■	5 ■■■	4 ■■■	3 ■■	0.5 ■
Magnate® (500 EC)	Cal hypo	10.3 (+)	100 ■■■	50 ■■	50 ■■	50 ■■	50 ■■	0
	Nylate®	6.5 (=)	30 ■■■	12 ■■	0	0	0	0
	Tsunami®	4.4 (-)	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■
	Vibrex®	3.9 (-)	5 ■■■	4 ■■■	4 ■■■	4 ■■■	2 ■■	0.5 ■
Magnate® (800 EC)	Cal hypo	10.2 (+)	100 ■■■	25 ■■	25 ■■	10 ■	0	0
	Nylate®	6.9 (=)	30 ■■■	0	0	0	0	0
	Tsunami®	5.8 (-)	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■	60 ■■
	Vibrex®	7 (+)	5 ■■■	2 ■■	2 ■■	1 ■	0.5 ■	0
Magnate® (750 WG)	Cal hypo	4.8 (-)	100 ■■■	10 ■	0	0	0 0	0
	Nylate®	3.5 (-)	30 ■■■	30 ■■■	30 ■■■	15 ■■	3 ■	0
	Tsunami®	3.2 (-)	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■	60 ■■
	Vibrex®	3.4 (-)	5 ■■■	2 ■■	2 ■■	1 ■	1 ■	0

*The list of fungicides/sanitisers used in this chart was derived after extensive consultation with the citrus postharvest steering committee, part of the 'Delivering postharvest decay, food safety and market solutions for export citrus' project, funded by the citrus growers of Australia. This chart aims to compliment the findings presented on the previous compatibility chart.

Fungicide (Formulation)	Sanitiser	pH (▲)†	Concentration of active ingredient (ppm)					
			Initial	30 sec	5 min	1 hr	4 hr	24hr
Panoctine® (liquid)	Cal hypo	9.3 (+)	100 ■■■	50 ■■	50 ■■	25 ■	25 ■	25 ■
	Nylate®	5.6 (=)	30 ■■■	30 ■■■	30 ■■■	18 ■■	12 ■	6 ■
	Tsunami®	4.9 (-)	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■
	Vibrex®	5 (-)	5 ■■■	5 ■■■	5 ■■■	5 ■■■	4 ■■	0.5 ■
Zanoctine® (liquid)	Cal hypo	9.2 (+)	100 ■■■	50 ■■	50 ■■	25 ■	10 ■	10 ■
	Nylate®	5.6 (-)	30 ■■■	30 ■■■	30 ■■■	18 ■■	12 ■	6 ■
	Tsunami®	4.8 (-)	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■
	Vibrex®	4.9 (-)	5 ■■■	5 ■■■	5 ■■■	5 ■■■	4 ■■	0.5 ■
Guazacure® (liquid)	Cal hypo	8.9 (+)	100 ■■■	20 ■	20 ■	20 ■	20 ■	20 ■
	Nylate®	6.6 (+)	30 ■■■	30 ■■■	18 ■■	18 ■■	12 ■	10 ■
	Tsunami®	4.4 (-)	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■	80 ■■■
	Vibrex®	3.6 (-)	5 ■■■	4 ■■■	4 ■■■	3 ■■	1.2 ■	0.5 ■

LEGEND

Mixture stable (no loss of active ingredient) ■■■
Increased active ingredient lost ■■
Rapid loss of active ingredient ■

† Decontaminated water (RO) is at pH (6.5±0.3). The symbol ▲ represents change in pH of solution once sanitisers and fungicides are added.

Active ingredients (a.i.) used in citrus postharvest fungicides

Despite the amount of products available to citrus packers there are a limited number of active ingredients (a.i.) used in citrus fungicides. The main a.i. used in Australia are:

Group A: Benzimidazole
Fungicide with actives containing Thiabendazole and Carbendazim belong to this group.

Group C: DMI – Imazidazole
Imazalil based fungicides.

Group X: unspecified – Guanidine
Fungicides containing Guazatine.

Ungrouped chemicals
Sodium ortho-phenylphenate (SOPP).

Fungicides registered for citrus postharvest will have only one of these four a.i. Many commercially available products differ in the type of inert ingredient used to formulate the product (see right). This chart therefore contains fungicides which have the the same a.i. but may differ in formulation. Packers should not make the assumption that if two products have the same a.i. they will have the same compatibility/incompatibility with any of the sanitisers listed on this chart.

Fungicide Formulations

Many fungicides are 'formulated' with more inert ingredients to facilitate application and effectiveness. Formulations include:

Emulsifiable concentrate (EC):
Contains the a.i. dissolved in a strong solvent (eg aromatic oil) and an emulsifier to allow mixing in water.

Suspension concentrate (SC):
Contains finely ground a.i. plus surfactants and stabilisers. Flowable form expressed as FL

Wettable powder/granules (WP/WG):
Contains finely ground a.i. powder or granules plus wetting and dispersal agents to enable suspension when mixed with water.

Liquid (L or LS):
Fungicide a.i. is soluble in water.

Formulations of Sanitisers used in this chart

Calcium hypochlorite (a.i.) available in granular or tablet form.
Tsunami® (a.i. peroxyacetic acid plus hydrogen peroxide) available as liquid concentrate.
Nylate® (a.i. bromo-chloro-dimethyl hydantoin) available in tablet form used with automatic dosing machine.
Vibrex® (a.i. chlorine dioxide – sodium chlorite plus acid activator) premix solution with water before adding to tank

Citrus packers should adhere to the instructions on the label of the fungicide/sanitiser before using any product in their line. This ensures correct dosage and application method. Some products may also have incompatibility warnings. The Australian Pesticides and Veterinary Medicines Authority (APVMA) www.apvma.gov.au has a current and complete list of postharvest fungicides and sanitisers registered for use on citrus in Australian packingsheds.