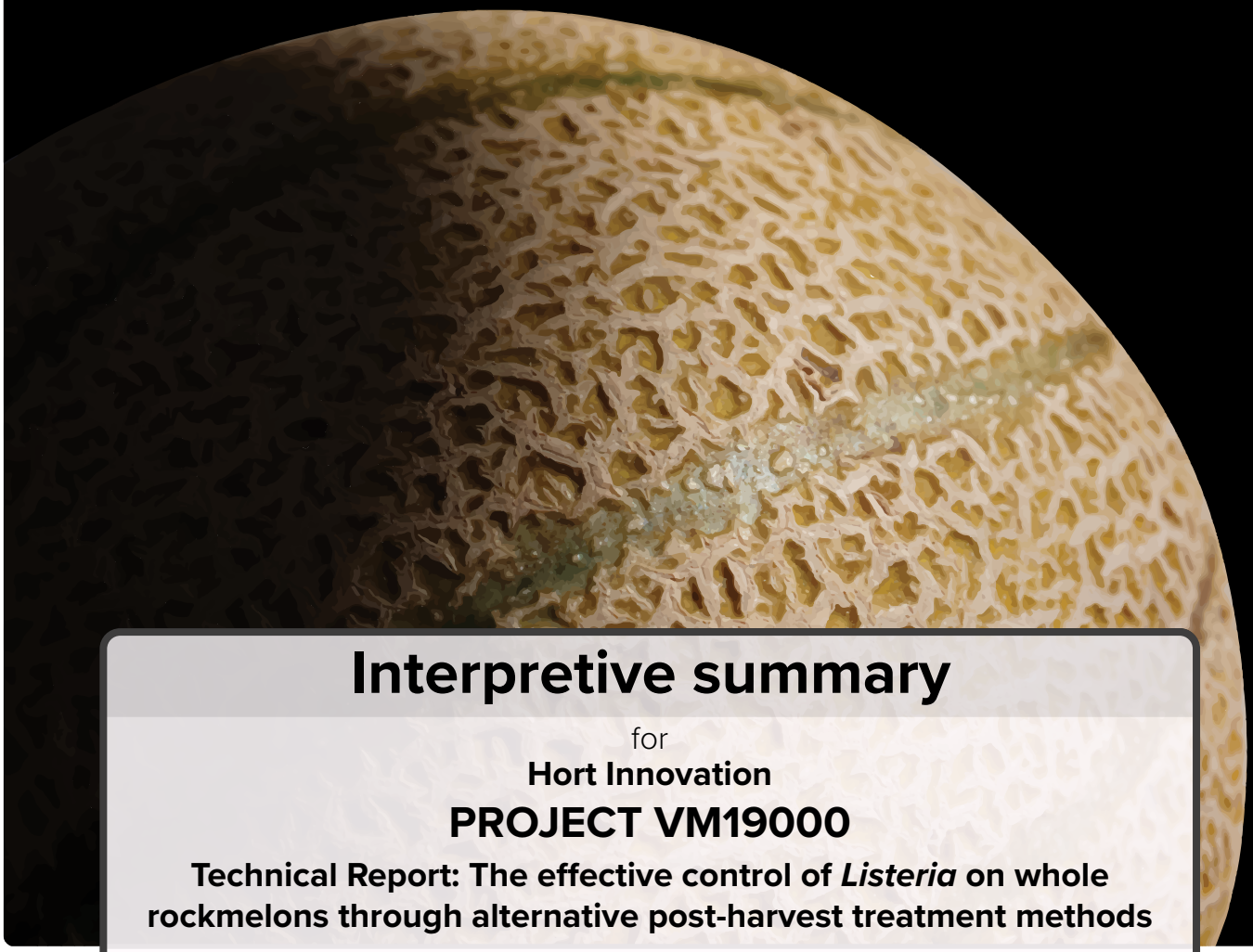
The effective control of *Listeria* on whole rockmelons through alternative post-harvest treatment methods



Interpretive summary

for

Hort Innovation

PROJECT VM19000

Technical Report: The effective control of *Listeria* on whole rockmelons through alternative post-harvest treatment methods

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The interpretive summary was developed by the science communication team including Michael Helman, Zoe Bartlett, and Tom Ross in consultation with Hort Innovation and industry stakeholders.

Hort Innovation
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MELON FUND
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The illlustrations and page layout for the interpretive summary were designed and produced by Communicatrium.

The *L. mono* cell imagery represented on page 9 was adapted from an electron micrograph from:

Fu Y, Deering AJ, Bhunia AK and Yao Y (2017). Pathogen biofilm formation on cantaloupe surface and its impact on the antibacterial effect of lauroyl arginate ethyl. *Food Microbiology*, 64: 139-144.

Hort Innovation Strategic levy investment | MELON FUND

This project has been funded by Hort Innovation using the melon research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au

CONTENTS

This booklet is a companion to the Hort Innovation technical report:

Project VM19000 Technical Report: The effective control of Listeria on whole rockmelons through alternative post-harvest treatment methods.

It highlights key information and directs readers to find more detail in the technical report.

This symbol provides page numbers from the technical report so you can easily find more detailed information that is important to you.

Study background

The Australian Melon Industry is one of the larger Australian fruit industries, with a well-established production base across most Australian states and territories that ensures a year-round supply.

An outbreak of listeriosis linked to the bacterium *Listeria monocytogenes* (*L. mono*) on rockmelons from a single Australian farm in 2018 had devastating outcomes, resulting in seven consumer deaths and a miscarriage, and large financial losses for producers.

To minimise the risk of listeriosis, food safety research and development to generate new knowledge and improve practice is a top priority for the industry.

The report

A scoping study was developed in consultation with farmers, packers, and other stakeholders and carried out by a team of experts from Hort Innovation, Tasmanian Institute of Agriculture, New South Wales Department of Primary Industries, University of Florida and private consultants.

The objective of the study was to bring together the relevant international scientific literature regarding how whole melons are treated after they are harvested to minimise the risk of listeriosis.

The study focussed on four areas:

1. **Outbreaks**

   How did melons become contaminated with *L. mono* during outbreaks; what key factors led to the contamination, and; after analysis of the outbreaks, what were the key recommendations to prevent further outbreaks?

2. **Best practice recommendations to industry**

   What are the key similarities and differences in recommendations from authoratative organisations for sanitisation of whole melons?

3. **Pre- and post-harvest interventions**

   What research exists describing the effectiveness of different interventions to reduce *L. mono* on whole melons from primary production to when melons leave the farm gate? What data gaps exist, what emerging technologies may be applicable to address those sanitisation needs, and; where is further research required?

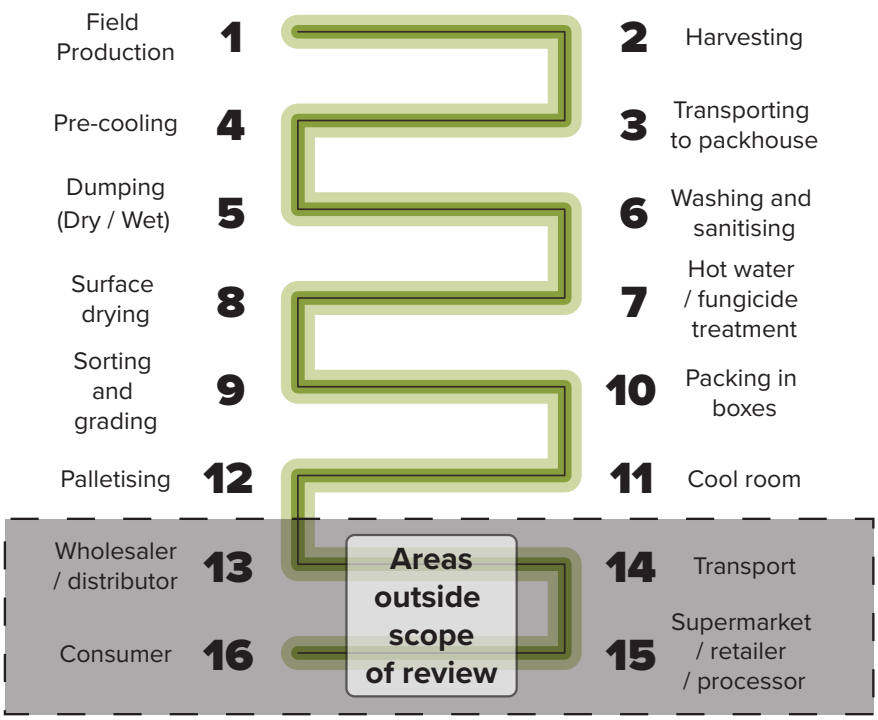
4. **Improving risk assessment**

   What research exists regarding growth rates, prevalence, or concentration of *L. mono* on and in rockmelons from primary production to consumption? Where along the supply chain does risk increase? What data gaps exist and where is further research required?

Project scope

The project only considered processes for whole melons before the melons leave the farm gate.

Processes for fresh cut melons and interventions after the melons leave the farm gate were beyond the scope of this study.



A generic rockmelon supply chain showing the steps included and excluded within the project scope

> 3 log reduction

There is a desire in the industry to identify interventions able to reliably achieve > 3 log reductions of *L. mono* on the surface of whole rockmelons.

However, the choice of any intervention needs to be assessed with consideration of the level of food safety risk reduction both to consumers and the industry against economic, legal, and fruit quality reasons that restrict the sanitiser concentration and contact times applied by the industry.

Currently in Australia, sanitisers are generally applied at manufacturer recommended concentrations for less than 2 min.

What is a log reduction?

A log reduction is a mathematical term used in this guide to describe the relative number of microbes a sanitiser can kill for a given time and concentration.

A '1 log reduction' is the same as saying a 10-fold, or 90% reduction, in cell numbers. Every additional log reduction reduces the cell numbers by 10-fold again.

For example, if a rockmelon had 100,000 cells on the surface (or '5 log') and the sanitiser produces a 3 log reduction (10% survival rate x 10% survival rate x 10% survival rate = 1 in a thousand) that means it would reduce the contamination to 100 cells on the surface.

But if there were only 10,000 ('4 log') cells on the surface then a sanitiser able to achieve a 3 log reduction could reduce the number to 10 cells on the surface.

>

Where the '>' symbol appears in front of a log_{10}CFU reduction value, this indicates the pathogen count was reduced to below the limit of detection, i.e., that it is possible that the actual log reduction was larger than the numerical value that we have reported.

Outbreaks are rare but have serious consequences.

Although *L. mono* is common in natural environments and can colonise food processing plants, listeriosis outbreaks from whole rockmelons are rare.

There are only three listeriosis outbreaks associated with whole melons reported in the international literature in over 40 years, but all resulted in fatalities.

Two of those outbreaks occurred in Australia, and one in North America.

A range of factors contribute to outbreaks, but contamination often occurs in the packhouse.

Investigations into the two largest outbreaks suggested that high frequency contamination of rockmelons with *L. mono* contributed to those outbreaks.

It was suggested that the contamination of the melons probably occurred in the packhouse after colonisation of the packhouse (and that remained undetected for many weeks) caused by:

- the introduction of contaminated 're-purposed' food processing equipment from another produce processing business (USA 2011 outbreak); or
- contamination from trucks that were transporting unsellable melons as feed to a cattle farm (USA 2011 outbreak); or
- failure to use sanitiser spray on melons (USA 2011 outbreak); or
- contamination in the field after adverse weather events (Australia 2018 outbreak).

It was also suggested that high prevalence but low level contamination occurred in the field after adverse weather events and was not eliminated during processing in the packhouse (Australia 2018 outbreak).

There is limited evidence to determine the efficacy of sanitisers under conditions currently used in Australia to kill or remove *L. mono* from the surface of whole rockmelons

While it is clear that sanitisers make an important contribution to product safety the review did not identify evidence that > 3 $\log_{10}$CFU reductions of *L. mono* on the surface of whole rockmelons could be achieved by sanitisers at currently used contact times and concentrations used by the Australian rockmelon industry.

There is also insufficient research to recommend optimal contact times specifically to kill *L. mono* on the surface of rockmelons for currently used sanitisers.

In the absence of more evidence, the review findings support the recommendations of NSW DPI (2019) for the use of chlorine (100ppm), peroxyacetic acid (80ppm), or chlorine dioxide (aqueous) (5ppm) with a contact time of 2 minutes.

Research for alternative sanitisation methods report increased reductions of *L. mono* on the surface of whole rockmelons.

There is evidence that technologies including, but not limited to, X-rays, octenidine dihydrochloride, hot water, superheated steam, and dry steam can produce > 3 $\log_{10}$CFU reductions in *L. mono* on the surface of whole rockmelons. Due to limited research, cost, practicality, and other considerations, not all of these are relevant for the Australian industry.

Melon safety guidelines should be reviewed by all stakeholders.

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Guidelines from the NSW Department of Primary Industries (listed below) in 2019 represent the most relevant and recent comprehensive advice provided to the Australian melon industry and should be reviewed by all stakeholders.

- Melon food safety toolbox: Practical resources for implementing best practice
- Melon food safety: A best practice guide for rockmelons and specialty melons

Further research is needed to minimise the risk of *L. mono* associated with Australian rockmelons.

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To reduce the likelihood of Australian rockmelons becoming contaminated with *L. mono*, future research should:

- further develop and communicate a holistic risk management strategy that includes growers assessing and responding to adverse weather events, or other unusual circumstances, and more effective and reliable hygienic handling of fruit from the field and during processing and transport
- determine the prevalence of *Listeria* spp. or *L. mono* on whole rockmelons and in environmental samples, relevant to risk, at different points in Australian rockmelon supply chains and from different geographic regions. While this is being undertaken in some parts of the industry, it would be beneficial for a database to be established where results can be collated by state, and nationally, to be able to demonstrate with confidence to risk assessors and consumers the currently apparent low prevalence of *L. mono* on rockmelons and in rockmelon growing sites in Australia
- investigate the potential for internalisation of *L. mono* into whole rockmelons at different points in the rockmelon supply chain (e.g. field, packhouse, consumer handling)
- assess the potential influence of weather events on the prevalence of *Listeria* spp. on/in fruit in the field and the growing environment and the potential persistence of *Listeria* spp. both in the soil and on whole melons in the field under different weather conditions. This assessment should include collaboration with farmers/producers regarding current practices to help frame science-based risk management decisions regarding harvest after ‘adverse’ weather events
- further investigate the ability of *L. mono* to colonise rockmelon packhouses from environmental sources or contaminated fruit
- improve quantitative knowledge of factors, such as temperature, surface moisture, relative humidity, extent of netting, or others, that influence the potential for growth of *L. mono* on rockmelons and how those factors vary throughout the supply chain
- investigate whether regular ‘in-house’ environmental monitoring (both factory and growing environment) is feasible and will reduce listeriosis risk from rockmelons, and if so, develop specific guidance on environmental testing programs including methods, sites, and frequencies.

The application of sanitisers on whole rockmelons should be optimised as part of a whole-of-supply chain approach.

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Due to the limited efficacy of currently used sanitisers to reliably eliminate potential contaminants on melons, the prevention of outbreaks from *L. mono* and other pathogens requires consistent implementation of a whole-of-supply chain approach. This means that events or changes that may lead to hazardous levels of contamination of rockmelons are recognised and processes and operations adjusted to accomodate the consequences of those events at every stage of the supply chain.

As part of this approach, the application of sanitisers should be optimised to minimise the risk of listeriosis from Australian rockmelons. This can be achieved through:

- research to determine minimum contact times at relevant concentrations for currently used sanitisers specifically to inactivate *L. mono* on the surface of whole rockmelons, with consideration of the level of risk reduction both to consumers and the industry against feasibility and economic, legal, and melon quality considerations
- validation of commercial sanitisation processes using industry-relevant conditions of sanitiser concentrations, contact times and other variables (such as organic load) on inoculated whole rockmelons
- research into hurdle applications (using combinations of methods), but not pursuing research into low penetration surface treatments such as UV and other light treatments alone
- further investigation into the efficacy of methods that have demonstrated relatively high effectiveness against *L. mono* such as ozone, X-ray, octenidine dihydrochloride, hot water, superheated steam, and dry steam including determination of their costs versus benefits
- investigation of the efficacy of high penetration technologies, such as X-rays, to eliminate potential internal contamination of rockmelons by *L. mono*

Future intervention studies into *L. mono* and rockmelons should consider different factors that may affect efficacy.

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It is recommended that any future intervention studies should assess and include detail about the:

- use of industry-relevant concentrations and contact times
- effect of increasing levels of organic matter and apply inoculations and treatments to whole melons (not portions)
- potential for, and consequences of, recontamination from the environment, including the packhouse itself, after the treatment
- effect on multiple pathogens and strains of pathogens with differing levels of resistance
- difference in the efficacy of the intervention at the rind and the stem scar
- potential impact of biofilm formation on the effectiveness of the intervention
- consequences for rockmelon quality and shelf life
- risk of re-contamination of fruit from environmental sources of *L. mono*

Characteristics

What is *L. mono*?

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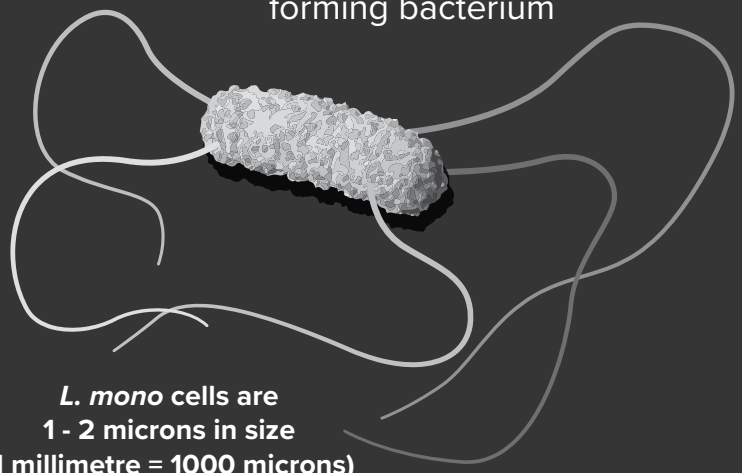
***L. mono* is a bacterium that can cause infection, illness and death in humans.**

Route of infection	Impact on health
Humans nearly always become infected from eating contaminated food	*L. mono* can cause listeriosis, a disease that can be mild (non-invasive) or severe (invasive)

Scientific name *Listeria monocytogenes*

Description Gram positive, non-spore forming bacterium



***L. mono* cells are 1 - 2 microns in size (1 millimetre = 1000 microns)**

L. mono grows under a range of conditions

L. mono has a specific set of characteristics that dictate whether it can survive and grow (reproduce) in specific environments, including on and in foods.

Unlike most other foodborne pathogenic bacteria, *L. mono* can not only survive, but can grow, on or in food that is refrigerated.

Grows at temperatures -1 to 45°C

- Fastest growth between 30–37°C
- Temperatures above 50°C are lethal. The higher the temperature the faster the lethality

Can grow equally well with or without oxygen

Grows across a pH range 4.3 – 9.6

Can grow at salt levels up to 11 – 12%

Ecology and environmental niches

Where is *L. mono* found and why rockmelons?

L. mono occurs in natural environments, including fields where rockmelons are grown. It can also grow in food processing environments like produce packhouses and contaminate fruit that is processed and packed there.

L. mono prefers a cool and wet environment where there is decaying food or plant material which provides simple nutrients it needs for its growth.

In food processing factories like packhouses *L. mono* can establish itself in places ('niches') that provide water and simple nutrients. Examples of niches in food processing factories where *L. mono* can become established include pockets of water under peeling paint, food contact surfaces that are difficult to clean, hollow rollers on processing lines, pads that can absorb water, and damaged belts (e.g. rough surfaces, cracks) on processing lines.

In the growing environment it can become established in places where there is decaying vegetation, including compost heaps or mounds containing spent plant material (e.g. leaves, vines, damaged fruit), or where water stands for long periods of time.

It can also be introduced to the growing environment, and then the packhouse, from other sources during unusual weather events (e.g. during flooding that could carry contamination from animal faeces from nearby agricultural businesses).

Rockmelons in the field ready for harvest

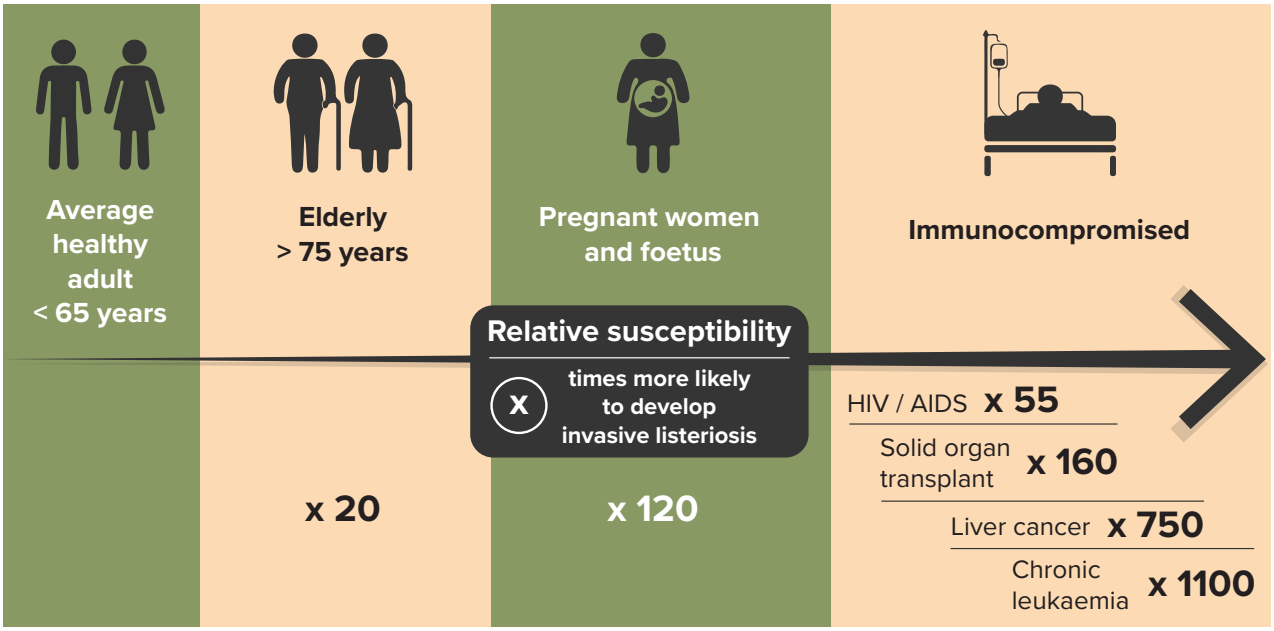
The consumer and susceptibility to infection

Who is at risk?

Anyone can get sick from *L. mono*, but not all people have the same susceptibility or symptoms. The illness caused by *L. mono* is called listeriosis and this can be mild (non-invasive, like a flu or gastro) or severe (invasive, potentially leading to infections of the blood, heart, brain or a fetus) and, while invasive listeriosis is rare, it causes death in 20 - 30% of cases.

L. mono generally only causes severe illness in the very young (< 6 months), the elderly, pregnant women, and people with weakened immune systems due to underlying medical conditions or treatments. These groups are much more likely to develop invasive listeriosis compared to the average healthy adult.

High numbers of *L. mono* are usually required to cause infections, even in susceptible individuals. For example, 10 million to 100 million cells (about 1 million cells per gram for a typical meal) would need to be ingested to have a high likelihood to cause an infection in pregnant women that might then harm the foetus. Although significantly lower numbers of *L. mono* cells can also cause illness, it is much less likely to occur. It is widely considered that a dose of 10,000 cells of *L. mono* (e.g. 100 cfu/g in a typical serving of food) *at the time of consumption*, represents a negligible risk of illness to consumers.



Relative susceptibility to listeriosis for susceptible individuals compared to healthy adults less than 65 years old

Growth potential of *L. mono* on whole rockmelons

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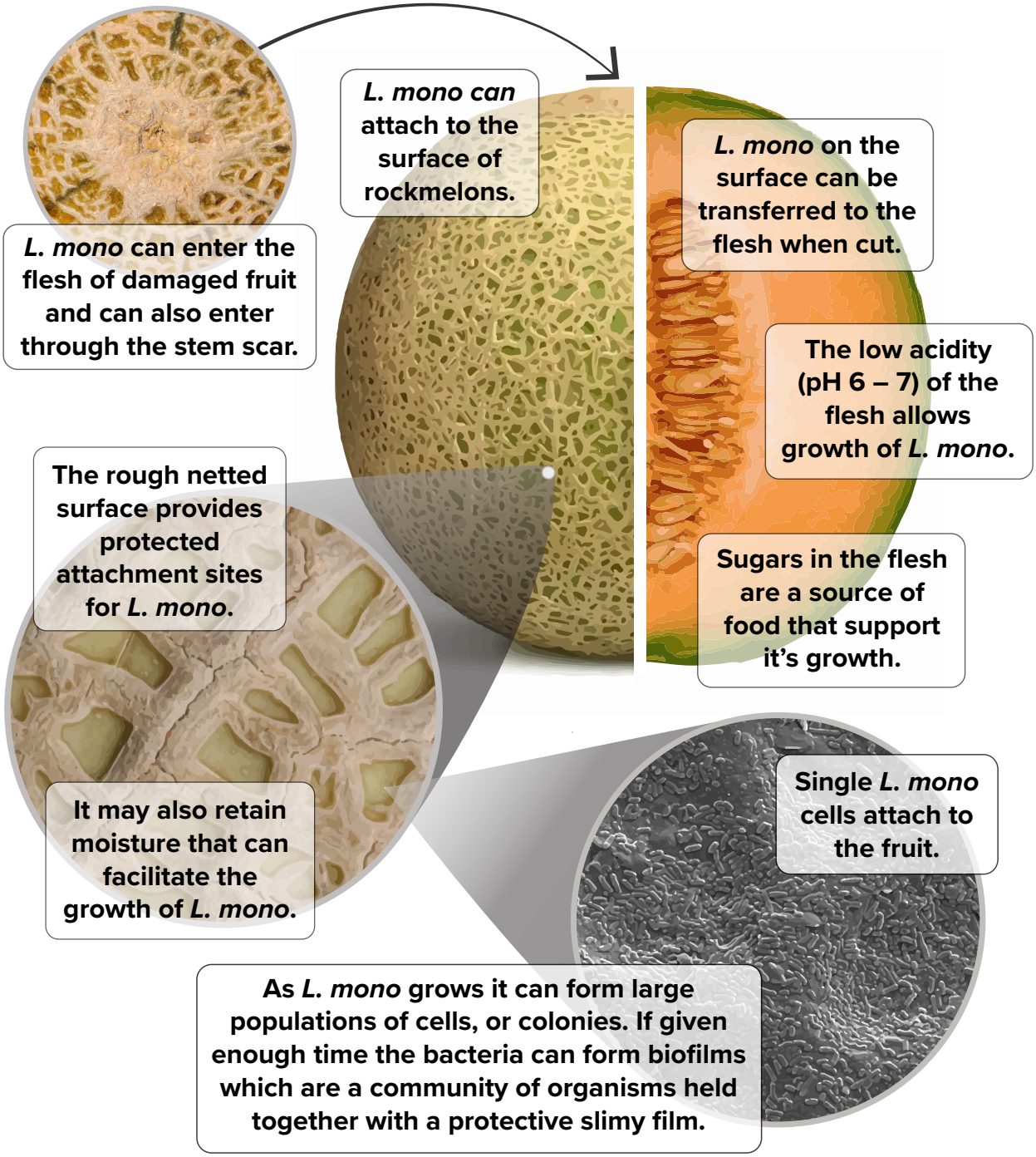
How and why does *L. mono* grow on whole rockmelons?

Rockmelons have particular characteristics that support the attachment, survival and growth of *L. mono*.

On most foods initial contamination levels with *L. mono* are low and the bacterium has to grow (reproduce) to reach numbers high enough to be likely to cause human illness when the food is eaten.



Food safety management

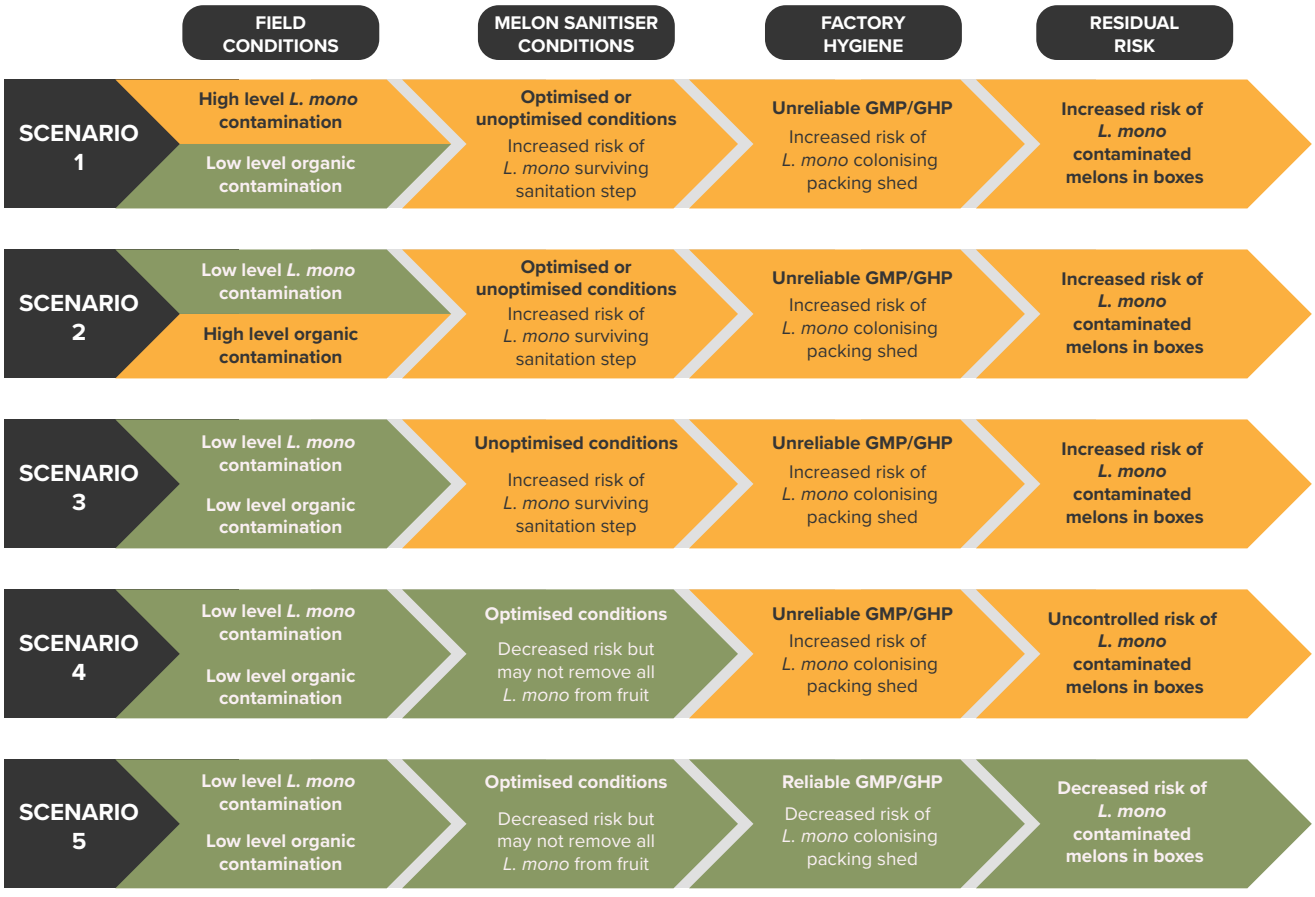
A whole-of-supply chain approach to food safety management

Analysis of the few documented previous outbreaks of listeriosis from rockmelons suggests there is a sequence or coincidence of events that leads to outbreaks.

Unusual weather events can lead to contamination of rockmelons in the field which can overwhelm even effective sanitisers and lead to colonisation of the packhouse and contamination of subsequently processed rockmelons. That contamination can be exacerbated if the surface of the fruit remains wet or becomes wet again (e.g. through condensation) and the fruit is not adequately cooled.

Even low level environmental contamination or accidental introduction of *L. mono* from activity in the packhouse can lead to colonisation if Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP) (including environmental monitoring) are not practiced consistently.

As such, minimising the risk of listeriosis from rockmelons requires a multi-faceted approach including monitoring and reacting to conditions in the field, factory hygiene, hygiene monitoring, assurance of control of critical processing operations (e.g. HACCP) and appropriate storage and transport conditions. If informed food safety management is not consistently undertaken at one or more of the points in the whole-of-supply chain approach there is a higher risk of contaminated rockmelons.



Simplified processing scenarios that demonstrate the importance of a whole-of-supply chain approach by growers and packhouses to reduce the risk of listeriosis from rockmelons

How the scoping review was conducted

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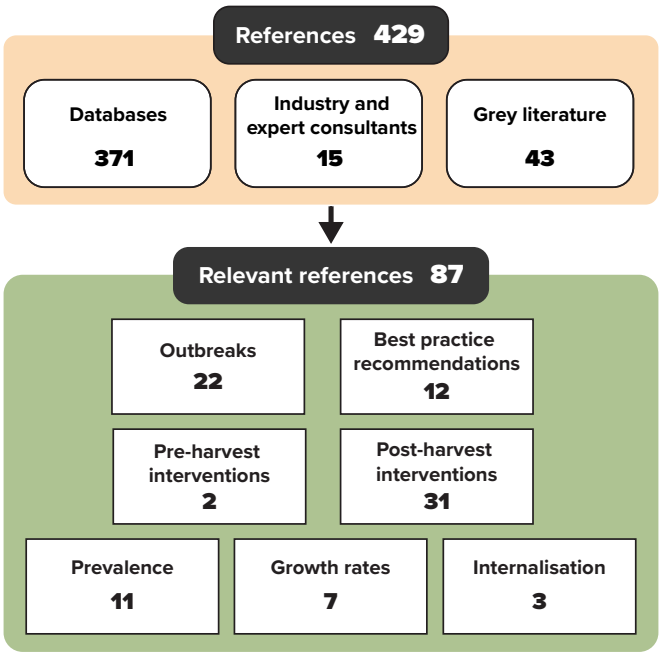
1. Visited farms and packhouses

Members of the research team visited several farms and packhouses in eastern Australia (NSW and Far North Queensland) and Western Australia (Carnarvon and Perth region) to better understand practices that affect rockmelon food safety. The team also used these visits to assess the relevance of rockmelon sanitation studies in an Australian context.

2. Reviewed the literature

To identify publications relevant to the project objectives, a series of keywords were developed and used to search scientific databases (Web of Science and Scopus), grey literature (including industry and regulatory websites), Google Scholar, and books. Experts and industry stakeholders were also formally invited to identify relevant literature. A total of 429 references were identified in the initial search but only 87 were relevant for the current study. These were reviewed, categorised and summarised by the research team (see figure below).



Overview of the literature review

3. Developed a draft

A draft report was developed and industry stakeholders were invited to provide additional information, insights and comments before the report was finalised.

Limitations

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- Some of the relevant literature may not have been identified in the search (for example, articles not published in English).
- Studies reporting data for the effects of interventions on other pathogens on whole rockmelons, and commercially available interventions not reported in the published literature, may not have been identified.
- As the purpose of the review was to describe the breadth of research, data quality was not systematically assessed but we have indicated where data may be unreliable.

Previous outbreaks of *L. mono* and *Salmonella* associated with melons

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1 **There have been nine previous outbreaks of *L. mono* and *Salmonella* associated with melons from 2010 to 2020.**

Twenty-two authoritative reports concerning nine outbreaks related to melons and *L. mono* or *Salmonella* from 2010 to 2020 in Australia, North America, or Europe were identified. These are summarised below.

2010

AUSTRALIA
L. mono

Melons and/or melons contained within fruit salads

9 cases: 9 hospitalisations, 2 deaths

See following page for review.

2011

USA
Salmonella panama

Rockmelons

20 cases: 3 hospitalisations

Not identified. Melons implicated in the outbreak were sourced from Guatemala.

2011

USA
L. mono

Rockmelons

147 cases: 143 hospitalisations, 33 deaths

See following page for review.

2012

USA
Salmonella typhimurium* and *Salmonella newport

Rockmelons

261 cases: 94 hospitalisations, 3 deaths

A variety of contributing factors initially from the production fields but spread through operations and practices within the packhouse as well as storage and transport.

2012

EUROPE
Salmonella newport

Watermelons

63 cases: 5 hospitalisations, 2 deaths

Not identified. Brazilian melons were implicated as the source. This outbreak potentially involved use of water contaminated by faecal residues of animals.

2016

AUSTRALIA
Salmonella hvittingfoss

Rockmelons

144 cases

Contributing factors identified: no monitoring of sanitiser, poor general hygiene.

2018

USA
Salmonella adelaide

Pre-cut rockmelons

77 cases: 36 hospitalisations

Not identified.

2018

AUSTRALIA
L. mono

Rockmelons

22 cases: 7 deaths, 1 miscarriage

See following page for review.

2019

USA
Salmonella carrau

Pre-cut rockmelons

137 cases: 38 hospitalisations

Not identified.

Previous outbreaks of *L. mono* and *Salmonella* associated with melons

Previous outbreaks of *L. mono* associated with whole rockmelons

46

2 Potential causes of *L. mono* outbreaks associated with rockmelons include changing environmental or packhouse conditions.

The literature suggests that outbreaks of listeriosis from rockmelons seem to be associated with a change in conditions in the field or packhouse that introduces and/or allows growth of *L. mono*.

If contaminated rockmelons from the field pass through or overwhelm the sanitising systems and there is no environmental monitoring, or if reliable cleaning regimes are not implemented, *L. mono* can colonise the packhouse unchecked and contaminate even 'clean' rockmelons.

The findings of the outbreak investigations are summarised below:

AUSTRALIA
L. mono

No data or observations are available to determine the cause of the contamination.

2010

AUSTRALIA
L. mono

- The consumers affected were pregnant or immunocompromised and predominantly elderly.
- One isolate of the outbreak strain was found in the implicated packhouse, and from one melon at the packhouse.
- Survey results of the product at retail suggest the melons were systematically contaminated over time with the same strain that caused the outbreak.

Investigators did not find a definitive cause for the outbreak but suggested:

- Hygiene and sanitation practices were on par with, or better than, most other rockmelon growing operations across Australia.
- Several factors combined and processing was not able to reduce a higher prevalence of *L. mono* on fruit.
- Extreme weather events (dust storm and heavy rainfall) are likely to have significantly increased the organic load and amount of *L. mono* prior to harvest.
- Environmental contamination from weather events may have led to contamination of the processing facility via contaminated fruit.

2011

U.S.A.
L. mono

- Five distinct strains of *L. mono* caused the outbreak.
- *L. mono* was found in wet areas of the packhouse and on rockmelons purchased at retail, but not in the growing environment or in the field.

Investigators concluded that the likely causes of contamination were:

- Introduction of *L. mono* into the packing facility from a truck used to transport discarded rockmelons.
- Pooled water on the floor of the packing facility.
- The packing facility and equipment were difficult to clean.
- Equipment used to wash and dry the melons during packing was designed for, and previously used for, another agricultural product.
- There was no pre-cooling step to remove field heat from the rockmelons before cold storage. As the melons cooled there may have been condensation that promoted the growth of *L. mono* on the surface of the melons.
- Sanitiser not used on melons.

2018

Potential causes of *L. mono* outbreaks associated with melons

Previous best practice recommendations provided to the melon industry

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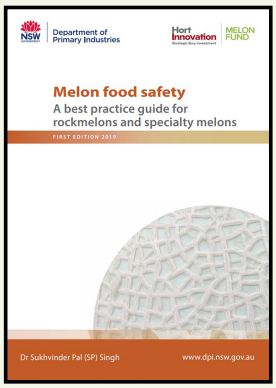
3 There are five existing best practice guides specific for rockmelon production.

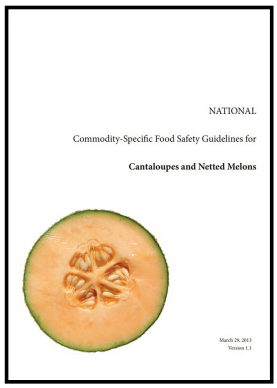
The review aimed to identify best practice recommendations that have previously been provided to the rockmelon industry and that consider best practices from primary production to when whole rockmelons leave the farm gate.

Five best practice guides specific for melon production were identified in the literature for food safety control during growing, harvesting and processing of melons (four from the US and one from Australia). These are shown below (and can be accessed via internet links when clicked/touched in the digital .pdf version of this guide). Seven documents from authoritative bodies providing recommendations were also identified.

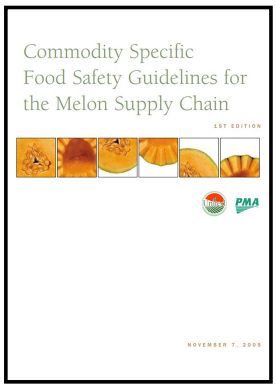
The most recent and relevant guide for Australia is the *Melon food safety: A best practice guide for rockmelons and specialty melons* published by the NSW Department of Primary Industries, and all stakeholders are encouraged to read and become familiar with that document.



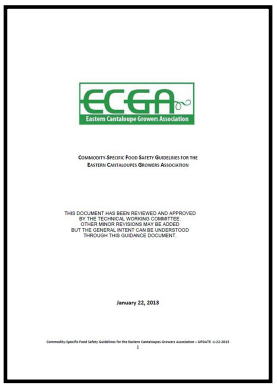
Melon food safety: A best practice guide for rockmelons and specialty melons (AUS, 2019)



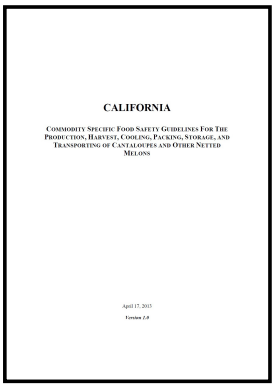
National Commodity-Specific Food Safety Guidelines for Cantaloupes and Netted Melons (USA, 2013)



Commodity Specific Food Safety Guidelines for the Melon Supply Chain (USA, 2005)



Commodity-Specific Food Safety Guidelines for the Eastern Cantaloupes Growers Association (USA, 2013)



California Commodity Specific Food Safety Guidelines for the Production, Harvest, Cooling, Packing, Storage and Transporting of Cantaloupes and other Netted Melons (USA, 2013)

Previous best practice recommendations provided to the melon industry

64

4 There are inconsistent recommendations for post-harvest sanitiser use.

The review identified different recommendations in the various guidelines for the sanitisation of whole melons.

US guidelines recommend that users following the manufacturer's instructions, however, NSW Department of Primary Industries recommends longer contact times than those proposed by manufacturers on product labels.

This difference is because the NSW Department of Primary Industries recommendations are based on scientific research that specifically assessed the reductions of pathogens on the surface of rockmelons.

It is important for growers and packhouse operators to understand that the manufacturer recommendations for the use of sanitisers may not be based on the assessment of their efficacy for reducing specific pathogens on their specific products, rather than being aimed at keeping wash water adequately sanitised.

Therefore, a different contact time than labelled by the manufacturer may be required to achieve the desired risk reduction in *L. mono* on whole melon surfaces.

A note on sanitisers

Sanitisers in fresh produce processing have historically been used to prevent processing wash water from becoming contaminated, however they are increasingly being used in spray systems to kill and remove pathogens from the surface of produce.

The 'bacterial kill' achieved by a sanitiser depends on factors such as the type of sanitiser, pH, temperature, the presence of organic matter, the commodity, and the target organism.

In routine operation, concentration and contact time with the fruit are fundamental to sanitiser efficacy.

Review of pre-harvest and post-harvest interventions for reducing the risk of *L. mono* on melons

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5 Chlorine, chlorine dioxide, peroxyacetic acid and ozone are currently used as sanitisers by the Australian melon industry.

The review of available research indicated that chlorine at a concentration of 100 or 200ppm for 2 minutes provided a 2 log reduction in *L. mono* on the surface of whole rockmelons. Similar log reductions in *L. mono* were reported for contact times of 2 minutes of 3 or 5ppm chlorine dioxide (aqueous) (2.4 – 2.9 reduction) and 80ppm peroxyacetic acid (1.4 reduction).

Of the currently used sanitisers, ozone in water at 3ppm for 2 minutes had the highest reported log reduction of *L. mono* (3 reduction), however, only a single study was identified.

There is a lack of published research specifically assessing reductions in *L. mono* on melon surfaces for all currently used sanitisers at industry relevant concentrations and contact times. In the absence of further evidence, the findings of the review support the current recommendations of the NSW DPI for the use of chlorine (100ppm), peroxyacetic acid (80ppm), and chlorine dioxide (aqueous) (5ppm) for 2 minutes.

The available research for the sanitisers currently used by the Australian industry is summarised in the figure below.

Chlorine

Concentration / Contact times	Number of studies	Log reduction
100 ppm / 2-5 min	1	2.0 - 5.0
200 ppm / 2-5 min	2	1.9 - 5.0
200 ppm / 5 min	2	0.9 - 1.9
200 ppm / 8 min	1	0.8
200 ppm / 10 min	1	> 3.1

Chlorine dioxide (aqueous)

Concentration / Contact times	Number of studies	Log reduction
3 ppm / 2-5 min	1	2.4 - 5.0
3 ppm / 8 min	1	-0.8
5 ppm / 2-5 min	2	0.8 - 5.0

Peroxyacetic acid

Concentration / Contact times	Number of studies	Log reduction
45 ppm / 5 min	1	3.0
80 ppm / 2-5 min	1	1.4 - 5.0
85 ppm / 5 min	1	3.0
100 ppm / 2 min	1	1.5 - 1.8
100 ppm / 5 min	1	4.5

Ozone (aqueous)

Concentration / Contact times	Number of studies	Log reduction
3 ppm / 2-5 min	1	3.0 - 5.0

Summary of evidence available for the efficacy of sanitisers currently used in the Australian rockmelon industry

Review of pre-harvest and post-harvest interventions for reducing the risk of *L. mono* on melons

6 **There are a range of alternative post-harvest interventions that have the potential to reduce the risk of *L. mono* on melons, but more research is required.**

There is a desire in the industry to identify interventions able to reliably achieve a > 3 log reduction of *L. mono* from the surface of whole rockmelons.

28 publications were identified that described the effectiveness of different post-harvest sanitisers/ interventions to reduce the risk of *L. mono* on whole melons.

While some showed promising results limitations in the research included:

- only being assessed in a laboratory setting
- interventions not applied to whole melons
- not assessing multiple pathogens
- not assessing the effect on quality and shelf life

Even those assessed at pilot scale (chlorine dioxide gas, hot water and some steam sanitisers) would still require significant scaling up and further validation of efficacy.

A summary list of the different sanitisers/interventions relevant to post-harvest processing is on the following page. Each sanitiser/intervention is detailed in the Technical Report. The table on the next page provides an indication of the scope of research and not recommendations for the use of any of these interventions.

Sanitiser/ intervention assessed	Example application in experiments	Range of Concentrations/ contact times assessed	Number of studies identified	Efficacy range for removal of *Listeria* spp. from rockmelon rind (Log reduction)	Page No.
Chlorine dioxide (Gas)	Melons exposed to gas in treatment chamber	0.5 - 10ppm 2 - 3min	2	1.2 - 3.3	87
Hydrogen peroxide	Applied as a wash or spray combined with water	2.5 - 5% 2 - 5min	5	1.8 - >3.2	92
Hot water	Wash	Water and 3% Hydrogen peroxide 20˚C/80	1	2.7 - >3.3	95
Steam - various	Dry, wet, superheated steam	Steam temps from 68˚C - 200˚C	6	3.3 - 5.4	95
Levulinic acid and sodium dodecyl sulfate	Applied as a combined solution	5.0%LVA with 2.0% or 2.5%SDS 8 - 10min	1	2.4 - >3.1	106
Lactic acid	Wash, or in combination with other sanitisers	2% 5min	1	2.5	109
Octenidine dihydrochloride	Wash	0.1 - 0.2% 5min	1	>3.6	111
Antimicrobial coatings	Coatings with various active ingredients	Varying concentrations and reductions are from after 24h of coating applications	4	0.6 - >5.0	114
Essential oil emulsions	Applied in research as produce washes or as additions to antimicrobial coatings	0.1% - 0.5% 1 - 2min	2	-0.5 - 2.9	120
X-ray	Can be applied to packaged items on a conveyor system.	0.1 - 2.0 kGy 1.6 - 32min	1	0.6 - 4.6	124
Ultraviolet-C	Conveyor and other systems incorporating UV-C lamps.	11KJ/m2 14min	1	1.0	127
Cold plasma	There are multiple ways of generating cold plasma. But it is a surface application, similar to UV light.	Cold plasma activated 7.8% hydrogen peroxide 30min	1	3.0	130
Lauroyl arginate ethyl	In research it has been applied as a wash for produce	200 - 2000ug/mL 5min	1	<1	133
Electrolysed water	As a wash using acidic and near neutral EO	100ppm free chlorine pH 2.5 - 6.2 5min	1	1.7 - 2.1	135

Reported efficacy of potential anti-listerial interventions not currently used in Australia

Potential sanitisers that can produce >3 log reductions

7 **Ozone, X-ray, octenidine dihydrochloride, hot water, superheated steam, and dry steam demonstrate relatively high effectiveness against *L. mono*.**

The review identified a number of interventions that can produce >3 log reductions in *L. mono* on the surface of whole rockmelons, including ozone, X-ray, octenidine dihydrochloride, hot water, superheated steam, and dry steam. A summary diagram is below.

It is important to note that not all of these interventions will be relevant for the Australian industry due to limited research, cost, practicality, and other considerations. A cost-benefit analysis was beyond the scope of this review, however, general indications of the potential benefits and limitations of these interventions are provided in the main report.

Lactic acid 2% 2.12 pH 1min + superheated steam 200˚C 20s	>5.4
Sequential application 35ml sodium chlorite (1.6%) + 35ml hydrochloric acid 6mM 1h	5.2
Octenidine dihydrochloride 0.1% + Chitosan 2% coating 24h	>5.0
Superheated steam 200˚C 30s	>5.0
Lactic acid 2% 2.12 pH 1min + superheated steam 150˚C 20s	4.7
X-ray 1.5 kGy 32min	>4.6
Cinnamon bark oil 2% + Alginate 1.0% coating 24h	>4.6
Cinnamon bark oil 2% + Soybean oil 0.5% + Alginate 1.0% coating 24h	>4.6
Peracetic acid 100ppm 5min	4.5
Lactic acid 2% 2.12 pH 1min + superheated steam 150˚C 20s	4.1
Lactic acid 2% 2.12 pH 1min + saturated steam 100˚C 20s	4.1
Lauric arginate 0.1% + EDTA 0.1% + cinnamon oil 1.0% + Chitosan 1.0% coating 24h	>4.1
Octenidine dihydrochloride 0.2% + Chitosan 2% coating 24h	>4.1
X-ray 2.0 kGy 40min	4.1
Vacuum/Steam/Vacuum 1.02min	4.1
Chlorine (hypochlorite) 1000ppm 2min	>4.0
Hydrogen peroxide 5% 2min	>4.0
Chitosan 1% coating 24h	4.0
Vapour heat 84˚C 4min	4.0
Superheated steam 150˚C 30s	4.0
Aerated steam 85˚C 4min	3.9
Lactic acid 2% 2.12 pH 1min + saturated steam 100˚C 20s	3.8
Wet steam 68˚C 3min	3.8
Saturated steam 100˚C 30s	3.6
Octenidine dihydrochloride 0.1% wash 5min	>3.6
Octenidine dihydrochloride 0.2% wash 5min	>3.6
Vacuum/Steam/Vacuum 50s	3.5
Lauric arginate 0.1% + EDTA 0.1% + Chitosan 1.0% coating 24h	3.4
3% Hydrogen peroxide at 80°C 5min	>3.3
Water at 80°C 5min	>3.3
Wet steam 68˚C 3min	3.3
Chlorine dioxide gas 10mg/L 3min	3.3
Lauric arginate 0.1% + EDTA 0.1% + cinnamon oil 0.5% + Chitosan 1.0% coating 24h	3.1

Interventions producing >3 log reductions in *Listeria* on rockmelon surfaces

Other anti-listerial interventions

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8 **There is limited evidence for interventions other than sanitisers to reduce *L. mono* on rockmelons.**

Only four studies were identified that specifically assess the reduction of *L. mono* on the surface of whole melons via interventions – apart from the sanitisation of melons – that can be implemented from processing through to transport.

The results of the studies are summarised in the graphic below.

PRE-HARVEST

In field injections / spray

One study reported the results of using in field stem scar injections of levulinic acid (LVA) with sodium dodecyl sulfate (SDS) followed by a spray of LVA with SDS over the entire melon surface immediately at harvest to prevent contamination through transport to the packing shed.

Log reductions of *L. mono* were reported, but the labor required would not be feasible in the industry.

Cultivar type

One study assessed the different effects of cultivar type on the growth of *L. mono* on whole rockmelons.

The type of cultivar made no difference to the proliferation of *L. mono* on the surface of whole melons during storage at different temperatures.

POST-HARVEST

Blue light emitting diodes

One study assessed the efficacy of blue light emitting diodes (LEDs) of 405nm and 460nm wavelengths against *L. mono* and *Salmonella* on rockmelon rinds. This was suggested as an intervention to be used during transport or in production lines.

1.5 - 36.30 hours were required for a 1 log reduction depending on the temperature and wavelength.

Sanitiser + forced air cooling

One study assessed the efficacy of the addition of aerosolised sanitisers during forced air cooling to reduce *L. mono* and *Salmonella* on the rind of melons.

Log reductions did not exceed 2 log and the variability in results was high.

Other pre- and post-harvest interventions

Risk assessment: Growth rates, prevalence, and internalisation of *L. mono* on/in whole rockmelons

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9 **Risk assessment is vital as no sanitisers completely eliminate *L. mono* from rockmelons.**

Risk assessment requires information about the prevalence and concentration of *L. mono* on whole rockmelons (across seasons, regions, and weather conditions) and the potential for growth of *L. mono* on rockmelons throughout the farm-to-fork process.

It is an essential process that should be undertaken on each farm or at least at a national level in order to identify risk management options.

Growth rates

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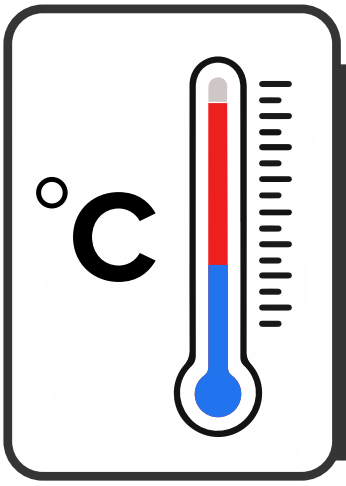
10 ***L. mono* grows on rockmelon rind and flesh at different rates, but more research is needed to understand factors that influence its growth.**

Seven studies were identified that reported growth rates (or generation times) for *L. mono* on the rind or flesh of rockmelons.

While there is limited information about the levels of *L. mono* on whole rockmelons at the time of consumption, research indicates that *L. mono* can grow on both the rind and flesh for different time and temperature combinations.

The reviewed data was more consistent for growth on the flesh than on the rind, which is an important consideration for risk assessment.

The figure below shows the estimated growth of a single *L. mono* cell on rockmelon rind or flesh for different times and temperatures. These are based on actual observations from controlled experiments by Danyluk and colleagues (2014) in the US using heavily netted rockmelons.



°C	1 HOUR	10 HOURS	20 HOURS
30	2 cells	~1700 cells	~50 million cells
20	No growth	~30 cells	~3100 cells
10	No growth	2 cells	~10 cells
4	No growth	No growth	No growth

Potential increase of *L. mono* from one cell on rockmelon at various times and temperatures

Prevalence

11 ***L. mono* has been detected at very low levels on rockmelons in post-harvest and retail environments.**

11 published studies were identified that assessed the prevalence of *L. mono* in and on whole rockmelons or in pre-harvest (in the field), post-harvest (in the packhouse or transport) or retail environments. The results, *excluding outbreak investigations*, are summarised below.

The review suggests that *L. mono* is generally present at very low levels in rockmelon production environments. Notably, data for these Australian production environments and whole melons are from the NSW region only. These results support anecdotal evidence that testing undertaken by Australian producers indicates a low prevalence of *L. mono*.

Pre-harvest	Post-harvest	Retail
4 studies	**4 studies**	**7 studies**
4 countries (USA, Korea, Mexico, Australia)	**2 countries** (USA, Australia)	**5 countries** (Korea, Canada, Germany, USA, Australia)
560 samples (Rockmelon, seed, plant leaf, irrigation water, soil)	**>363 samples** (rockmelons and various environmental surfaces)	**3293 samples** (rockmelons)
0 positive detections of *L. mono*	**3 positive detections** of *L. mono*	**1 positive detection** of *L. mono*

Summary of the reviewed studies that assessed the prevalence of *L. mono* in the rockmelon supply chain

Internalisation

12 ***L. mono* can potentially be introduced into the flesh of intact rockmelons (internalisation), but little is known about how this occurs.**

The US Food and Drug Administration have reported that pathogens can be internalised and survive in a variety of fruits.

The current review identified three studies that assessed the internalisation of *L. mono* into whole rockmelons. The key findings from these studies are summarised below.

It is important for the industry to understand the potential mechanisms and probability of internalisation of *L. mono* in rockmelons as current sanitisation methods do not inactivate internalised bacteria.

Future research should investigate the potential for internalisation of *L. mono* into whole rockmelons at different points in the rockmelon supply chain (e.g., field, packhouse, consumer handling).

Study one	Study two	Study three
Webb and colleagues, 2015	**Macarisin and colleagues, 2017**	**Estebane-Cuesta and colleagues, 2018**
More internalisation of *L. mono* occurred at the stem scar than at the rind. This was attributed to the stem scar being more porous than the rind.	Internalisation of *L. mono* occurred during dump tank washing and hydrocooling of melons regardless of the temperature difference between the water and the fruit, however it appeared to be aided by warmer fruit entering cooler water.	No *L. mono* was identified in the pulp of rockmelons for sale at retail, however internalisation of bacteria in melons was found to occur regularly as almost all of the samples (>89%) tested positive for *Staphylococcus* spp., *Clostridium* spp. or Enterobacteriaceae.

Summary of the studies that consider potential for internalisation of *L. mono* in rockmelons

Environmental testing for *Listeria*

End-product testing of rockmelons for *L. mono* is impractical for food safety management because, given the volume of production, even if only 1 in 1000 rockmelons were contaminated with *L. mono*, it could still lead to an outbreak from a large processor.

It would be more practical to conduct environmental testing for *Listeria* spp. in the packhouse, however staff training in sampling and disposal of potentially contaminated material is essential given the risk associated with culturing *Listeria* spp. in-house.

Environmental testing for *Listeria* spp. alone is unlikely to prevent an outbreak, and is only effective when 'Good Manufacturing Practices' and a HACCP program are in place and reliably implemented. Before undertaking environmental testing for *Listeria* spp., specialists and authoritative advice including that provided by NSW DPI and United Fresh Food Safety & Technology Council should be consulted.

A complete review of all environmental testing methods for *Listeria* spp. was beyond the scope of this project. As there was significant industry interest in more rapid and easy methods to screen for *Listeria* spp. in-house as an indicator of potential contamination by *L. mono*, technically easy and rapid methods are described in the table on the following page.

Rapid methods for assessing *Listeria* presence in the packhouse

The review revealed 5 'rapid' systems for environmental monitoring of *Listeria*. Notably, four of the methods that are commercially available (Hygiena InSite Listeria, CONTAM SWAB Listeria, Hyserve Listeria Swab and Listeria Transwab) all use an all-in-one swab in a tube containing sterile media that is selective for the growth of *Listeria* and that contains a 'chromogenic' compound (aesculin) that changes colour from clear to black when metabolised by *Listeria*. All need to be incubated at 37°C for up to 48 hours and provide qualitative ('presence/absence') results. The tubes/swabs must be sterilised before disposal.

The other method involves the use of Petrifilm, a simplified form of an agar plate. This method provides quantitative results and takes approximately the same amount of time as the swab methods, however it involves considerably more manual handling and specialised equipment and requires more expertise to interpret the results.

Brand name	What is it?	How is it used?	AOAC approved?
Hygiena™ InSite Listeria environmental swabs	An all-in-one environmental swab that includes a chromogenic enhanced aesculin medium to determine the presence of *Listeria* spp. via a colour change after incubation.	A swab is taken from the test site and placed into the culture tube so that it is immersed in the media. A change in color from yellow to brown or black after incubation at 37˚C for 24-48 hours is considered a presumptive positive result.	YES Certificate Number 04051
3M™ Petrifilm™ Environmental Listeria Plates	An environmental swab that is then added to a diluent, mixed, and then poured onto the 3M™ Petrifilm™ Environmental Listeria Plate which are thin films of premade agar set between protective films for easy use.	A swab is collected from the test site and added to a diluent, mixed and then poured onto the plate. The film plates are then incubated for 20-30 hours and colonies counted to determine the concentration in the sample. This method requires the use of a pipette, an incubator and an autoclave for sterilisation before disposal.	YES Certificate Number 030601
CONTAM SWAB Listeria	An all-in-one environmental swab that includes a chromogenic medium (aesculin) to determine the presence of *Listeria* spp. via a colour change after incubation.	A swab is taken from the test site and placed into the culture tube so that it is immersed in the media. A change in colour from yellow to black after incubation at 37˚C for 18-24 hours is considered a presumptive positive result.	NO
Hyserve Listeria Swab	An all-in-one environmental swab that includes an enhanced aesculin medium to determine the presence of *Listeria* spp. via a colour change after incubation.	A swab is taken from the test site and placed into the culture tube so that it is immersed in the media. A change in colour from light brown to black or dark brown after incubation at 37˚C for 24-48 hours is considered a presumptive positive result.	NO
Listeria Transwab ®	An all-in-one environmental swab that includes an enhanced aesculin medium to determine the presence of *Listeria* spp. via a colour change after incubation.	A swab is taken from the test site and placed into the culture tube so that it is immersed in the media. A change in colour from light brown to black or dark brown after incubation at 37˚C for 24-48 hours is considered a presumptive positive result.	NO

Overview of rapid methods for environmental testing for *L. mono*

Controlling the risk of listeriosis from Australian rockmelons will require a whole-of-supply chain approach

The rare outbreaks of listeriosis from rockmelons seem to be associated with a change in conditions in the field or the packhouse that introduces and/or concentrates the pathogen.

If contaminated melons from the field then pass through or overwhelm the sanitising systems and no environmental monitoring or sufficient cleaning regimes are implemented, *L. mono* can colonise the packhouse unchecked and contaminate even 'clean' melons.

As this scoping study suggests, the efficacy of most of the current sanitising systems for whole rockmelons may be limited. Even if those sanitising systems are optimised, *L. mono* may not be completely removed and may persist at low levels.

Therefore, it is important for all procedures prior to sanitising to reduce the likelihood of the pathogen entering the sanitising system. Moreover, following sanitising, hygiene procedures must strive to prevent recontamination of the fruit, and to reduce the potential for growth of the pathogen, and to prevent colonisation of the facilities by pathogens from the field or via other routes.

The scoping study has identified a range of potentially more effective sanitisers that warrant further research due to the potential they offer for improved risk reduction for both consumers and the industry. However, all will have limitations and, based on the review of available literature and expert opinion, their overall effectiveness on rockmelon food safety will depend on the implementation of a vigilant and whole-of-supply chain approach to food safety throughout the industry.