Identification and quantification of hazards and risks to human health in the vegetable industry

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TABLE OF CONTENTS

MEDIA SUMMARY......1

TECHNICAL SUMMARY......1

1. INTRODUCTION......4

2. DEVELOPMENT OF FOOD SAFETY GUIDES 4

2.1 SAFE VEGETABLE PRODUCTION - A MICROBIAL FOOD SAFETY GUIDE FOR THE AUSTRALIAN VEGETABLE INDUSTRY......4
2.1.1 Consultation process for developing the guide 4
2.1.2 Development of the guide 5
2.1.3 Format and publishing of the guide......5
2.2 SAFE PRODUCTION OF STRAWBERRIES - A GUIDE TO MINIMISE MICROBIAL FOOD SAFETY HAZARDS FOR THE VICTORIAN STRAWBERRY INDUSTRY 6
2.2.7 Consultation process to develop the guide 6
2.1.2 Poster preparation......6

3. VEGETABLE FARMGATE SURVEY 8

3.1 INTRODUCTION......8
3.2 METHODOLOGY......8

3.2.2 Vegetables surveyed......8
3.2.3 Farms......8
3.2.4 Sampling 8
3.2.5 Microbiological analysis......9

3.2.5.1 Listena......9
3.2.5.2 *Salmonella......9*
3.2.5.3 E. coli and faecal coliforms 9
3.2.5.4 Campylobacter......10
3.2.5.5 Total Aerobic Count (TAC) 10
3.2.6 Data analysis......10
3.3 RESULTS AND DISCUSSION 11
*3.3.1 Cultural practices used by the growers......*11
3.3.2 *Listeria......*15
3.3.3 Salmonella......16
3.3.4 *Campylobacter......17*
3.3.5 *E. coli 17*
3.3.6 Faecal coliforms 18
3.3.7 Total aerobic count (TAC)......18
3.4 DISCUSSION......**19**

4. EFFECTIVENESS OF CHLORINE TO REMOVE PATHOGENS FROM VEGETABLES 21

4.1. INTRODUCTION......21
4.2 MATERIALS AND METHODS 21
*4.2.1 Preparation of the E. coli suspension......*27
4.2.2 Preparation of the hypochlorite solutions......22
4.2.3 Preparation of the neutralising solution and its effect on E. coli.. ...... *22*
4.2.4 Effect of chlorine on E. coli in-vitro...... *22*

4.2.5 Effect of chlorine on E. coli inoculated on cos lettuce *22*
4.2.6 Effect of chlorine on E. coli inoculated on to broccoli florets.............. ....*23*
4.2.7 Dip temperature effects... ....*23*
4.2.8 Data Analysis.. ....*23*
4.3 RESULTS 24
4.3.1 Effect of the neutralising solution on E. coli...................................... ....*24*
4.3.2 Effect of chlorine on inactivation of E. coli in-vitro *24*
4.3.3 Effect of chlorine on inactivation of E. coli inoculated on cos lettuce. *24*
4.3.4 Effect of chlorine on inactivation of E. coli inoculated on broccoli florets ...*26*
4.3.5 Effect of temperature of chlorine solution on inactivation of E. coli inoculated on broccoli florets *27*
4.4 DISCUSSION 27

5. IRRIGATION WATER AND SOIL ANALYSES **29**

5.1 INTRODUCTION 29
5.2 IRRIGATION WATER.. 29
5.2.1 Methodology *29*
5.2.1.1 Water sources... ....*29*
5.2.1.2 Sampling and analysis.. ....*29*
5.2.2 Results and discussion *30*
5.3 SOIL.. 33
5.3.7 *Methodology*... ....*33*
5.3.2 Results and discussion *33*

6. TECHNOLOGY TRANSFER **37**

6.1 PUBLICATIONS RELATED TO THE PROJECT 38

7. ACKNOWLEDGEMENTS........................... .... **38**

PREFERENCES...**39**

APPENDIX 1. QUESTIONNAIRE FOR GROWERS INVOLVED IN THE SURVEY.. **41**

APPENDIX 2. MICROBIOLOGICAL HAZARDS IN THE VEGETABLE INDUSTRY -A REVIEW **42**

Media Summary

Until recently, food safety with respect to fresh produce was primarily concerned with pesticide residues. It was not commonly accepted that fresh produce could be associated with food borne diseases caused by microorganisms. However, outbreaks of food borne disease linked to the consumption of vegetables and fruit in developed countries is becoming more commonly reported. Consequently, the fresh produce industry has had to deal with a lot of new issues revolving around a new definition of food safety that includes human pathogens. With this have come a lot of challenges.

Uncertainty exists with regard to some of the technical aspects of on-farm food safety. There has been much confusion and many inconsistencies in the way systems have been implemented and audited because of a lack of information available on which to base these systems. This project originated to address these issues.

The major outcome has been the production of a national food safety guide that the vegetable industry can use as a reference tool. The guide covers all stages of vegetable production, highlights the risks involved for each input and makes recommendations to minimise those risks. Developed as a direct result of working hand in hand with an industry reference group, the guide will provide a practical point of reference on food safety. Subsequently, a similar guide was developed for the Victorian strawberry industry.

A farmgate survey of vegetables for human pathogens was carried out as part of this project. Whilst there are many studies published overseas looking at isolation of different human pathogens on whole fresh produce, this data cannot be found in Australia. We analysed around 200 vegetable samples from 35 farms in Victoria for a number of human pathogens. The vegetables chosen were salad types and include cos lettuce, salad mix, celery, cabbage and Dutch carrots. Overall incidence of pathogens found on the vegetable samples was low with one positive for *Salmonella victoria* and one for *Listeria monocy to genes.* Whilst it is preferable not to find such pathogens it is also encouraging that the numbers found were low, particularly when comparisons are made with overseas studies of this type.

Technical Summary

Until recently, food safety in fresh produce was primarily concerned with pesticide residues. It was not commonly accepted that fresh produce could be associated with food borne disease. However, outbreaks of food poisoning linked to the consumption of vegetables and fruit in developed countries is becoming more commonly reported. Consequently, the fresh produce industry has had to deal with a lot of new issues revolving around a new definition of food safety that includes human pathogens. With this have come a lot of challenges.

Uncertainty exists with regard to some of the technical aspects of on-farm food safety. There has been much confusion and many inconsistencies in the way systems have been implemented and audited because of a lack of information available on which to base these systems. This project originated to address these issues.

The major outcome has been the production of a national food safety guide that the vegetable industry can use as a reference tool. Subsequently, a similar guide was developed for the Victorian strawberry industry.

A desktop review and experimental work was undertaken to provide information on which to base the guide. This included challenge trials to study the effectiveness of chlorine as a postharvest wash, irrigation water analysis and soil analysis. A farmgate survey of vegetables for human pathogens was also carried out.

Whilst there are many studies published overseas looking at isolation of different human pathogens on whole fresh produce, this data cannot be found in Australia. We analysed around 200 vegetable samples from 35 farms in Victoria comprising of 5 different types of vegetables for *Salmonella* spp., *Listeria* spp.. *E.coli. Campylobacter* spp. and faecal coliforms. Overall incidence of pathogens found on the vegetables sampled was low with one positive (0.5%) for *Salmonella victoria* and one (0.5%) for *Listeria monocytogenes.* Whilst it is preferable not to find such pathogens it is also encouraging that the numbers found were low and also that the levels detected were very low. A few samples (3.8%) were found to be positive for *E. coli,* mostly at low and acceptable levels.

The effectiveness of calcium hypochlorite on inactivanon of *E coli* inoculated on fresh produce was investigated. Different times of exposure and concentrations of chlorine were studied. Dipping was not effective at eliminating *E. coli* populations although it significantly reduced the *E. coli* counts compared to inoculated, undipped lettuce. Dipping inoculated cos lettuce leaves into hypochlorite solutions containing 50 mg/L or greater free chlorine for times of 30 seconds or greater reduced *E. coli* cells by approximately 1.9 to 2.8 log_{10} colony forming units per gram (CFU/g) from an initial population of approximately 6.8 log_{10} CFU/g. Dipping lettuce in water alone reduced cell numbers by 1.7 log_{10} CFU/g. Dipping inoculated broccoli florets into hypochlorite solution reduced *E. coli* cells by approximately 1.7 to 2.5 log_{10} CFU/g, depending on the time and concentration of the free chlorine in the wash water. Dipping broccoli in water alone reduced cell numbers by 1.5 to 1.8 log_{10} CFU/g. Dipping broccoli florets for 2 minutes in a 100 mg/L free chlorine solution at temperatures between 4 and 25°C reduced *E. coli* cells by approximately 2.4 log_{10} CFU/g. No significant effect of temperature on the level of cell reduction was observed.

Water samples were collected from seven farms in three main growing areas of Victoria, Werribee, the Mornington Peninsula and East Gippsland. Different water sources were looked at which included bore. dam. river and lake water. All of the samples except for one fell within the current Australian water quality guidelines of 1000 faecal coliforms per 100 mL. We found bore water to have much lower levels of faecal streptococci, faecal coliforms and *E. coli* than dam, river or channel water. This is not that surprising since surface water could come from some distance and there may be less control over potential sources of contamination. Most bore water samples had levels of faecal coliforms of less than *2* most probable number per 100mL (MPN/100mL), with the highest level being 14. Channel/river water samples contained from less than 2 to 350 MPN/100mL. Dam water had mainly between 5 to 540 MPN/100mL, with 2 samples containing 920 MPN/100mL faecal coliforms.

To enable limits to be set for *E. coli* in the selection of new land section of the 'Safe Vegetable Production' guide, soil samples were collected from a number of farms in Victoria and one in Queensland. Of the 188 samples taken *E. coli* was present in 23 (12.2%). In the remaining 165 samples *E. coli* was not detected. Of the samples where *E. coli* was found, 15 had levels of less than 50 CFU/g, and 2 had less than 100 CFU/g. Consideration of this data resulted in a recommendation that soil on new land should contain less than 100 *E. coli* (CFU)/g.

1. Introduction

Until recently, food safety in fresh produce was primarily concerned with pesticide residues. It was not commonly accepted that fresh produce could be associated with food borne disease outbreaks. However, outbreaks of food bome disease linked to the consumption of vegetables and fruit in developed countries is becoming more common. The number of documented fresh produce-related outbreaks in the USA more than doubled from between 1973 and 1987 to the period 1988 to 1991 (Tauxe *et ah,* 1997). Bacterial diseases have been attributed to *E. coli, Salmonella, Listeria, Shigella, Bacillus, Clostridium* and *Campylobacter* (Beuchat. 1995. Fain, 1996; Little *et al.,* 1997). Viruses and parasites have also been linked to produce-related disease outbreaks.

Consequently the fresh produce industry has had to deal with a lot of new issues revolving around a changing definition of food safety. Many quality assurance systems have been introduced which encompass food safety to address the risks involved in production. However, with this have come a lot of challenges. Uncertainty exists with regard to some of the technical aspects of on-farm food safety. There has been much confusion and inconsistencies in the way systems have been implemented and audited because of a lack of information available on which to base these systems.

This project originated to address these issues. A review was carried out early in the project looking at the microbiological hazards in the vegetable industry. This was submitted as a draft in an earlier milestone (number 2) but has since been finalised (Appendix 2).

The major outcomes of this project have been the production of food safety guides that the vegetable and strawberry industries can use as reference tools. The guides cover all stages of vegetable production, highlights the risks involved for each input and makes recommendations to minimise those risks.

2. Development of food safety guides

2.1 Safe Vegetable Production - A microbial food safety guide for the Australian vegetable industry

2.1.1 Consultation process for developing the guide

In developing the guide it was decided very early on that we should work very closely with the vegetable industry; firstly to ensure that it would be practical to use and therefore readily adopted and secondly that the industry would have some ownership of it. We enlisted a group of growers who were motivated and interested in being involved, many of whom are members of the Horticulture Australia industry advisory committees. We had regular meetings with these growers from early development of the guide through to its completion and their input was invaluable.

As the guide was developed it was sent out to a broader audience for comment. It was circulated to around 40 people from the broader horticultural community. This included researchers, extension officers, the supermarkets, the Australia New Zealand Food Authority (ANZFA), auditors in the industry, Agriculture, Fisheries

and Forestry Australia (AFFA), EPA Victoria, Australian Horticulture Corporation (AHC) and consultants. Whilst not all persons responded, there were representatives from each sector of the community who did respond and the comments received were very constructive in developing the guide further. The appropriate changes were made to the guide and it was sent out for further comment but at this time few changes were suggested.

Finally, it was sent to the Australian Vegetable and Potato Growers Federation and Industry development officers (IDO) in each state for them to get feedback from some of their growers.

2.1.2 Development of the guide

A review was initially carried out to identify the microbiological hazards that may be associated with fresh vegetables (Appendix 2). This included looking at overseas studies on the incidence of pathogens in fresh produce and outbreaks associated with fresh produce. Limited Australian data that could be found was included.

Desktop searches were carried out and contacts made for all areas of production to identify any existing information that could be applied or drawn on. Whilst this information was difficult to find, fragmented and diverse, there were some areas where such information was available and this was included.

Experimental work was carried out to supplement this information and this is detailed in this report.

Finally, the knowledge and experience of the food safety group was drawn on for areas where information was not really available to make some of the recommendations.

2.1.3 Format and publishing of the guide

The guide was designed and published in a binder style to allow for the addition of new or revised information, as it becomes available. Its format is easy to use and read with sections covering each stage of vegetable production, starting with land selection through to transport after leaving the farm. It also includes a section on produce testing which has been one of the areas of great debate.

For each section the guide discusses the hazards involved with the particular input, for example irrigation water quality or hygiene of workers, and then states any facts that can be drawn on. This is followed by the industry recommendations to minimise the hazards. The guide is unique in that it is very specific and prescriptive and therefore provides answers that the growers, as well as auditors, have been asking for.

The guide was officially launched by Dr Jane Wilson at Hort 17, a horticultural field **event** held in Gatton, Queensland, in May this year. It has been submitted to HAL as
A separate publication (milestone number 5).

2.2 Safe Production of Strawberries - A guide to minimise microbial food safety hazards for the Victorian strawberry industry

Work on the Safe Production of Strawberries guide began in June 2001. This was added on to the project when additional funding was provided by the Victorian Strawberry Industry. The guide identifies the microbial food safety hazards that may exist during the primary production of strawberries. It does not include distribution at the retail level or handling and preparation in the food service sector or in the home. It is modelled on the Safe Vegetable Production guide since many areas such as irrigation water quality, hygienic handling, etc have common issues. As with the vegetable guide it is designed for operations to use in conjunction with a HACCP based Quality Assurance System.

The purpose of the guide is to describe:

- the sources of microorganisms that can cause foodbome disease in strawberries.
- the conditions that favour the growth and survival of these organisms in strawberries.
- recommendations that may prevent or minimise contamination during the growing, harvesting, packing and transporting of strawberries.

2.2.1 Consultation process to develop the guide

Members of the Victorian strawberry industry and the Strawberry Industry Development Officer were included in the development of the guide. A range of strawberry growers from small to large enterprises were interviewed and asked questions in regard to each stage of production. They were asked about the growing environment, the inputs, the equipment and facilities, the staff and potential hazards that they felt might exist.

The guide did not need to undergo the same extensive consultation process as for the vegetable guide, since many issues were resolved by that process and this didn't need to be repeated. The guide was edited by staff at NRE including those involved in strawberry research and members of the Victorian strawberry industry. A draft was presented to growers at a Victorian Strawberry Growers Association meeting in April 2002 for comment and it was extremely well received.

The guide has been published in a brochure style that the Victorian strawberry industry wants to add to The Victorian Strawberry Industry Resource Manual. It has been designed in a user-friendly format, with illustrative photographs throughout. It is submitted as a separate publication to this report.

The guide will be officially launched in August 2002 at the Victorian Strawberry Industry's annual general meeting.

2.1.2 Poster preparation

The second component of this work was to design a poster on food safety to reinforce training messages for workers in the Victorian strawberry industry. The poster can be displayed inside the strawberry packing shed and is pertinent to all workers on the strawberry farm. The strawberry HX) was involved in the discussions on the contentof the poster. The poster is composed of pictures (in this

case photographs) and simple statements reminding workers of important food safety considerations. The statements are printed in English as well as in Italian, Vietnamese and Cambodian, the native languages of many of the workers on the farms. The hazards described in the poster are as follows:

- always use the toilets provided
- always wash hands before starting work and after the toilet, eating or blowing your nose
- always handle fruit carefully
- cover sores, cuts with bright bandages and gloves if on hands
- if ill let your supervisor know
- keep work surfaces clean

The poster was then given to graphic designers to complete the artwork and the file has been given to the strawberry IDO for their use. It is submitted on disk as a separate appendix to this report.

3. Vegetable farmgate survey

3.1 Introduction

Whilst there are many studies published overseas looking at isolation of different human pathogens on whole fresh produce, this data is not readily available in Australia. Therefore, it was decided to undertake a study on Australian produce, as this is an unknown area here. Such a study provides an indication of how well farm practices are working to minimise food safety hazards. In addition, collection of samples from the farmgate provides information on practices on-farm as opposed to what might happen once the produce reaches distribution and marketing.

3.2 Methodology

3.2.2 Vegetables surveyed

Vegetables chosen for the survey were cos lettuce, cabbage, celery, salad mix and Dutch (baby) carrots. These were chosen on the basis **that** they are normally eaten without being cooked or can be eaten raw. In addition these crops are grown close to or in the ground and some (eg. Salad mix. lettuce, cabbage) have uneven, large surface areas where microorganisms may attach and be protected. All of these factors place these vegetables into a higher food safety risk category than those that are typically cooked before consumption or are grown off the ground.

3.2.3 Farms

Farms selected for the survey were in Wembee South. Bacchus Marsh, Keilor Oaklands Junction and the Mornington Peninsula (Boneo. Clyde, Pearcedale, Cranbourne, Somerville, Dangenong. Tyabb. Rosebud. Devon Meadows, Fiveways, Heatherton, and Keysborough). In total 35 farms were involved, with 8-10 farms chosen for each vegetable type. Farms provided 1, 2 or 3 different types of the selected vegetables.

Each grower was questioned on practices used on the farm. The questionnaire is shown in Appendix 1.

3.2.4 Sampling

Each farm was visited twice to collect samples over summer and autumn and in some cases a third visit was carried out in spring. At each collection two samples were analysed from each farm for each produce type.

Two boxes of celery or cos lettuce were collected from each farm, with each box being one sample. For celery, four stalks were removed from five bunches. The lowest 2cm of the stem and the leaves were removed and discarded and the remainder was chopped and mixed. 100g was taken as the sample. In the case of lettuce, the outer, damaged leaves were discarded and then four leaves were taken from five lettuce heads. 100g was taken as the sample.

One box of salad mix was collected and two 50g samples taken from the mix.

Ten bunches of Dutch carrots were collected, with five bunches making up one sample. Five or six carrots, depending on size, were removed from each bunch, chopped and mixed, and 100g was taken for the sample.

Eight cabbages were collected, with 4 making up one sample. A few of the older, outer leaves were removed and discarded and then the cabbages were quartered. A section from each quarter was cut off, with different parts of the cabbage selected from each quarter. Sections were selected in this way for each of the four cabbages and then chopped and mixed. 100g was taken for the sample.

Produce was stored overnight at 2-4°C prior to being analysed.

3.2.5 Microbiological analysis

Each sample was analysed for *Listeria* spp., *Salmonella* spp., *E. coli,* faecal coliforms and total aerobic counts for the summer and autumn collections. In addition the summer collection samples were analysed for *Campylobacter* spp.

The samples collected in spring were analysed for *Listeria* spp.. *Salmonella* spp., *E. coli* and *Campylobacter* spp.

3.2.5.1 *Listeria*

Samples were stomached for 2 minutes in 225 ml half Fraser broth (Oxoid) and plated onto Oxford agar (Oxoid). The plates were incubated at 37^{C}C for 48 hours. Following plating, the bags were incubated at 30°C for 24 hours. A second enrichment was carried out by transferring 0.1 ml tolO ml Fraser broth and incubating at 37°C for 48 hours. These samples were plated onto Oxford agar and if typical colonies were found the plates were sent to the Microbiological Diagnostic Unit, Melbourne for confirmation and identification. Positive and negative control organisms were taken through the same procedure. The organisms used were L. *innocua* 2305 and *Staphylococcus aureus* ATCC 25923.

3.2.5.2 *Salmonella*

Samples were stomached for 2 minutes with 250 ml *Wc* bacto peptone. They were then plated onto XLD agar and incubated for 24 hours at 37°C. After samples were plated the bags were incubated for 16-20 hours at 37°C, the samples were then transferred to mannitol selenite cystine broth (Oxoid) and incubated at 37°C for 24 hours. The enrichment broth was plated onto XLD *agar* and incubated as above. If typical colonies were found the plates were sent to the Microbiological Diagnostic Unit, Melbourne for confirmation and identification. Positive and negative control organisms were taken through the same procedure. The organisms used were *S. salford IMB* 1710 and *Citrobacter freundii* NCTC 9750.

3.2.5.3 E. coli and faecal coliforms

Samples were stomached for 2 minutes with 250 ml tryptone soya broth. They were then »ere plated onto 3M Petrifilm *E.* co/i/coliform count plates and incubated in a water bath at 44-44.5°C. The bags were incubated for 24 hours at 37°C for enrichment and plated and incubated in the same way.

Positive and negative control organisms were taken through the enrichment procedure. The organisms used were, *E. coli* NCTC 10418 and *Enterobacter aerogenes* NCTC 10006.

3.2.5.4 Campylobacter

Samples were sent to Silliker Microtech Pty Ltd, Melbourne for analysis. *Campylobacter* was determined according to the *Campylobacter* Method M48.

3.2.5.5 Total Aerobic Count (TAC)

Samples were stomached for 2 minutes with 250 ml 0.1% tryptone soya broth. Following serial dilutions, they were plated onto 3M Petrifilm Aerobic Count Plates and incubated at 26°C for 48 hours.

3.2.6 Data analysis

Total aerobic counts were $\log_{10}$ transformed to meet the assumption of constant variance for comparing the season by farm effects using ANOVA for each product. The least significant difference (LSD) was used to compare pairs of treatments. Regression analysis was used to compare products, farms and seasons and their interactions as not all farms were able to provide all 5 products.

The presence or absence of E. *coli* as measured after enrichment was analysed using a generalised linear model with a binomial distribution and logit link function to compare products, farms and seasons and their interactions.

All tests were conducted using 5% significance level.

3.3 Results and Discussion

3.3.1 Cultural practices used by the growers

Tables 1 - 5 show the growers responses to questions about on-farm practices related to manure use, irrigation and wash water.

Table 1 relates to the cultural practices used for growing cos lettuce. Practices varied between farms. Six farmers did not use manure; four did incorporate chicken manure during soil preparation. The manure was either partially composted or certified composted. Mostly the lettuce was field packed and the irrigation source varied. Six of the ten growers washed the lettuce with municipal or treated bore water.

Table 1. Practices used on-farm for cos lettuce production

Farm	Area	Is chicken manure used?	Type of manure used	When is manure applied?	Irrigation source	Where produce packed?	Type of water for washing
1	Werribee South	No	n/a	n/a	Dam from Melton weir via channels	Field	Municipal
2	Werribee South	No	n/a	n/a	Dam from Pikes ck via channels	Field	Not washed
3	Werribee South	No	n/a	n/a	As for 2	Field	Not washed
4	Werribee South	Yes	Partially composted	During soil cultivation	Dam from Melton weir via channels	Field	Not washed
5	Boneo	Yes	Partially composted	During soil cultivation	Mainly bore, some dam from run-off	Field	Rinsed with municipal
6	Clyde	No	n/a	n/a	Dam (rainfall) and bore	Shed	Treated bore
7	Pearcedale	Yes	Certified composted	During soil cultivation	Bore	Shed	Treated bore
8	Cranbourne	Yes	Certified composted	During soil cultivation	Dam from rainfall	Field	No
9	Somerville	No	n/a	n/a	Dam from rainfall and run-off	Field	Treated municipal
10	Dandenong	No	n/a	n/a	Dam from creek and rainfall	Shed	Treated municipal

Table 2 shows the on-farm practices used for cabbage production. Generally chicken manure was used during soil preparation and one grower used it after transplanting as a side dressing. The manure used ranged from uncomposted to certified composted. Mostly, cabbage was packed in the field and not washed or they were rinsed with municipal water.

Table 2. Practices used on-farm for cabbage production

Farm	Area	Is chicken manure used?	Type of manure used	When is manure applied?	Irrigation source	Where produce packed?	Type of water for washing
1	Werribee South	No	n/a	n/a	Dam from Melton weir via channels	Field	Not washed
3	Werribee South	No	n/a	n/a	Dam from Pikes ck via channels	Field	Not washed
5	Boneo	Yes	Partially composted	During soil cultivation	Mainly bore, some dam from run-off	Field	Rinsed with municipal
10	Dandenong	Yes	Certified composted	During soil cultivation	Dam from creek and rainfall	Field	Not washed
11	Werribee South	Yes	Partially composted	During soil cultivation	Channel and bore	Field	Not washed
12	Werribee South	Yes	Certified composted	During soil cultivation	Dam via channel	Field	Rinsed with municipal
13	Werribee South	Yes	Partially composted	During soil cultivation	Dam via channel	Field	Not washed
14	Boneo	Yes	Not composted	2-3 weeks after trans-planting	Bore	Field	No
15	Tyabb	Yes	Certified composted	During soil cultivation	Dam from rainfall	Shed	Rinsed with municipal
16	Clyde North	Yes	Partially composted	During soil cultivation	Dam from rainfall and runoff	Field	Not washed

Table 3 shows the practices for celery production. All growers used chicken manure during soil preparation and this varied from uncomposted to partially composted or certified compost. Celery was packed in the shed and washed in tanks in municipal or treated water.

Table 3. Practices used on-farm for celery production

Farm	Area	Is chicken manure used?	Type of manure used	When is manure applied?	Irrigation source	Where produce packed?	Type of water for washing
5	Boneo	Yes	Partially composted	During soil cultivation	Mainly bore, some dam from run-off	Shed	Pre washed, then hydro cooled in treated water
6	Clyde	Yes	Partially and certified	Partially during soil preparation and certified after trans-planting	Dam from rainfall and bore	Shed	Treated dam water
8	Cranbourne	Yes	Certified composted	During soil cultivation	Dam from . rainfall	Shed	Municipal
9	Somerville	Yes	Not composted	During soil cultivation	Dam from rainfall and runoff	Shed	Treated municipal
14	Boneo	Yes	Not composted	During soil cultivation	Bore	Shed	Bore water
17	Rosebud	Yes	Partially composted	During soil cultivation	Bore	Shed	Municipal
18	Clyde	Yes	Partially composted	During soil cultivation	Mainly dam, some bore	Shed	Municipal
19	Clyde North	Yes	Partially composted	During soil cultivation	Dam from run-off, creek and bore	Shed	Treated bore
20	Devon Meadows	Yes	Partially and not composted	During soil cultivation	Dam from run-off and rainfall, some bore	Shed	Washed twice in municipal

Table 4 shows on-farm practices for carrot production. Mostly growers did not use chicken manure except for two that used partially composted manure. Carrots were packed in the shed and washed in municipal or treated water. Two growers did an initial spray wash with dam water before the final wash.

Table 4. Practices used on-farm for carrot production

Farm	Area	Is chicken manure used?	Type of manure used	When is manure applied?	Irrigation source	Where produce packed?	Type of water for washing
15	Tyabb	No	n/a	n/a	Dam from rainfall	Shed	Treated mains
21	Pearce-dale	No	n/a	n/a	Dam from rainfall	Shed	Municipal
22	Devon Meadows	No	n/a	n/a	Dam from rainfall	Shed	Sprayed with dam then washed in treated dam water
23	Clyde	Yes	Partially composted	Straight after planting	Dam from rainfall and bore	Shed	Sprayed with dam then washed in treated mains
24	Somer-ville	No	**n/a**	**n/a**	Dam from catchment	Shed	Municipal
25	Devon Meadows	Yes	Partially composted	During soil cultivation	Dam from catchment	Shed	Municipal
26	Devon Meadows	No	n/a	n/a	Dam from rainfall	Shed	Municipal
27	Fiveways	No	n/a	n/a	Dam from rainfall	Shed	Municipal
28	Heatherton	No	n/a	n/a	Dam from rainfall	Shed	Municipal

Table 5 shows the on-farm practices used for the production of salad mix. Half of the growers used chicken manure for soil preparation either partially composted or certified compost. Most of the growers used municipal or treated municipal water to wash the salad. However, one used treated river water and one treated dam water, since municipal water was not available at the farms. Use of such waters in the final wash step needs to be carefully monitored to ensure that the quality is appropriate for this use.

Table 5. Practices used on-farm for salad mix production

Farm	Area	Is chicken manure used?	Type of manure used	When is manure applied?	Irrigation source	Where produce packed?	Type of water for washing
6	Clyde	No	n/a	n/a	Dam from rainfall and bore	Shed	Treated bore
7	Pearce-dale	Yes	Certified composted	During soil cultivation	Bore	Shed	Treated bore
18	Clyde	No	n/a	n/a	Mainly dam, some bore	Shed	Treated municipal
29	Pearce-dale	Yes	Fully composted and certified	During soil cultivation	Dam from rainfall	shed	Treated municipal
30	Keys-borough	Yes	Partially composted	During soil cultivation	Municipal	Shed	Treated municipal
31	Keys-borough	No	n/a	n/a	Municipal and run-off	Shed	Treated municipal
32	Bacchus Marsh	Yes	Partially composted	During soil cultivation	Dam from Pikes ck via channels	Shed	Treated river water
33	Oaklands Junction	No	n/a	n/a	Dam from Maribynong river	Shed	Treated dam water
34	Keilor	No	n/a	n/a	Maribynong river	Shed	Washed twice in municipal
35	Keilor	Yes	Fully composted	During soil cultivation	Maribynong river	Shed	Washed 3 times in municipal

3.3.2 *Listeria*

Twenty samples were analysed in spring, 94 in summer and 96 in autumn. No *Listeria* spp. were found by direct plating of samples, but 9 were found by enrichment of the samples (Table 6). One sample (0.5%) of the 210 analysed had L. *monocytogenes,* 7 (3.3%) had *L. seeligeri* and 1 (0.5%) had *L. welshimeri.* Most of these were detected in autumn, with two detected in late summer.

Listeria spp. are widespread in the environment and consequently there is considerable opportunity for fresh produce to become contaminated. *L. welshimeri* and *L seeligeri* are non-pathogenic strains of *Listeria* whereas *L. monocytogenes* is a human pathogen that can cause food poisoning with serious consequences in

some population groups such as the very young, the old, pregnant women and the Immuno compromised. In this case it was detected at a very low concentration and in this type of product that has a short shelf-life the potential risk is far less than foods, such as soft ripened cheese, that have a long refrigerated shelf-life. UK guidelines consider levels of <20 CFU/g to be satisfactory for fresh fruits and vegetables (Gilbert et al., 2000) although in Australia and the US there is a zero tolerance.

Table 6. *Listeria* spp. found in vegetable samples.

Farm	Collection date	Product	Spp.
32	4/3/02	Salad mix	*L. monocy to genes*
22	4/3/02	Dutch carrots	*L. seeligeri*
6	22/4/02	Cos lettuce	*L. seeligeri*
6	22/4/02	Cos lettuce	*L. seeligeri*
6	22/4/02	Celerv	*L. seeligeri*
23	22/4/02	Dutch carrots	*L. seeligeri*
31	20/5/02	Salad mix	*L. welshimeri*
34	20/5/02	Salad mix	*L. seeligeri*
34	20/5/02	Salad mix	*L. seeligeri*

3.3.3 Salmonella

Twenty samples were analysed in spring. 94 in summer and 96 in autumn. No *Salmonella* spp. were found by direct plating of samples, but one out of the 210 (0.5%) was found by enrichment. *S. victoria* was detected in a cos lettuce sample.

Salmonella is a notifiable organism as it is unacceptable to detect it in foods. The Australian standard is that it should be absent in 25g and this is the quantity normally used for analysis in commercial laboratories. We increased the chance of finding *Salmonella* as the sample size we took was 100g**.**

We investigated the cause of the *Salmonella* by visiting the grower and looking for possible sources of faecal contamination but could not find an obvious source. This grower also had a higher than acceptable level of *E. coli* in one of his lettuce samples (see section 3.3.5). The cos lettuce was packed in the field so this excluded the packing shed area. This particular grower did not use organic fertiliser and did not have stockpiles of manure on the farm. There were no obvious sources of animal contamination such as cattle grazing in adjacent areas. The irrigation water was from a weir via channels to the dam. We tested the dam water but found low levels of *E.coli* and faecal coliforms of 2 CFU/mL. This is well within the acceptable limits for irrigation water. Water quality does vary and it is still possible that this may have been a source of contamination. The lettuce from the paddock from which the sample was collected did not end up going to market, as there was a glut at the time

3.3.4 *Campylobacter*

Twenty samples were analysed in spring and 94 in summer for *Campylobacter* spp. None were detected and so we did not continue testing during the autumn since at low temperatures the bacterium does not grow and requires temperatures of 32° C for reasonable growth.

3.3.5 *E. coli*

Twenty samples were analysed in spring, 94 in summer and 96 in autumn. Eight samples (3.8%) had direct counts for *E. coli* (Table 7). Generally these were found at low levels but one sample had an unacceptably high level of 2.4×10^2 CFU/g. This was the same grower who had a positive for *Salmonella* and we visited him to discuss these results (see section 3.3.3). UK guidelines consider levels of *E. coli* of <20 CFU/g to be satisfactory and less than 100 to be acceptable for fresh fruits and vegetables (Gilbert et al., 2000).

Table 7. *E. coli* found in vegetable samples

Farm	Collection	Product	CFU/g
1	21/1/02	Cos lettuce	9.5
1	21/1/02	Cos lettuce	2.4×10^2
3	21/1/02	Cos lettuce	4.9
12	21/1/02	Cabbage	2.4
30	4/2/02	Salad mix	2.5
9	18/3/02	Cos lettuce	25
6	22/4/02	Cos lettuce	14
31	20/5/02	Salad mix	70

By enrichment of the samples a further 81 samples were found to be positive. It is not surprising that there would be residual levels of *E. coli* since manure is often used during soil preparation. Analysis of positive results for *E. coli* showed that there were differences between product type. The percentage of positive samples for each product was; cabbage 25.0%, celery 41.1%, carrots 47.2%, cos lettuce 55.3% and salad mix 56.8%. When farm differences were allowed for in the analysis, the differences between products were found to be greater (Table 8).

The number of positive samples for cabbage was found to be significantly lower than celery, lettuce and salad mix. This can perhaps be explained by the sample preparation, where the outer leaves were removed. Therefore, the part of the cabbage used for the sample had been protected from contamination. Salad mix and cos lettuce had the highest number of positive samples and they were not significantly different to each other but were significantly higher than the other vegetables. This might be expected since leafy type vegetables have large, uneven surface areas that microorganisms can more readily attach to. During growth these leafy type vegetables are also very open so may be more readily exposed to contaminants.

Table 8. Percentage of samples for each product found positive for *E. coli* after enrichment, adjusted for farm differences

Product	% positive *E. coli*
Cabbage	13.1 a*
Carrots	24.7 ab
Celery	40.7 be
Cos lettuce	64.8 d
Salad mix	81.5 de

*sharing the same letter are not significantly different (P = 0.05)

3.3.6 Faecal coliforms

Ninety-four samples were analysed in summer and 96 in autumn. Of these faecal coliforms were detected in 18 (11.3%) (Table 9). Generallv levels detected were low although one sample had an unacceptable level of 4.4 x 10" CFU/g. Low levels of faecal coliforms are acceptable.

Table 9. Faecal coliforms found in vegetable samples.

Farm	Collection	Product	CFU/g
11	21/1/02	Cabbase	4.4×10^2
5	4/2/02	Cabbage	9
5	4/2/02	Cabbage	17
5	4/2/02	Cos lettuce	5
6	4/2/02	Cos lettuce	7
6	4 2/02	Celery	2
6	4/2/02	Salad mix	5
30	4 2 02	Salad mix	25
30	4/2/02	Salad mix	7
7	18/2/02	Salad mix	2.5
15	18/2/02	Cabbage	32
32	4/3/02	Salad mix	2.5
8	4/3/02	Celery	12
10	18/3/02	Cos lettuce	2.4
16	18/3/02	Cabbage	15
5	22/4/02	Cos lettuce	14
18	22/4/02	Celery	12.5
31	20/5/02	Salad mix	10

3.3.7 Total aerobic count (TAC)

Ninety-four samples were analysed in summer and 96 in autumn. The mean TAC for each product is shown in Table 10a. Farm differences were greater than product or seasonal differences. Although some seasonal differences were seen for each product except carrots, for individual farms, there was no consistency of one season being different to the other. The only product where an overall significant difference (P=0.05) was observed between seasons was cos lettuce

where autumn values were generally higher than summer (Table 10b). Overall the TAC ranged from 10^5 to 10^7 CFU/g, with some higher values of 10^8, mainly for salad mix samples from the Keilor and Bacchus Marsh areas. These results tie together with the *E. coli* results in that lowest values were found for cabbage and highest for salad mix. This was probably for the same reasons of morphology and growing differences.

Table 10a. Total aerobic count (TAC)

Product	Mean TAC* CFU/g	TAC* range CFU/g
Cabbage	7.7×10^5	1.3×10^5-6.8×10^6
Carrots	2.8×10^6	6.8×10^5-1.0×10^7
Celery	8.5×10^5	1.1×10^5-4.9×10^7
Cos lettuce	1.1×10^6	1.2×10^5-2.3×10^7
Salad mix	3.9×10^6	1.8×10^5-1.5×10^8

*Back transformed from log values

Table 10b. Seasonal values for TAC

Product	Mean autumn logTAC CFU/g	Mean summer logTAC CFU/g	LSD* (P=0.05)
Cabbage	5.84 (6.9×10^5)	5.94 (8.7×10^5)	0.42
Carrots	6.54 (3.5×10^6)	6.30 (2.0×10^6)	0.56
Celery	5.86 (7.2×10^5)	5.97 (9.3×10^5)	0.61
Cos lettuce	6.29 (1.9×10^6)	5.81(6.4×10^5)	0.37
Salad mix	6.34 (2.2×10^6)	6.70 (5.0×10^6)	0.51

Numbers in brackets are back transformed
* LSD for comparing logTAC mean

3.4 Discussion

Overall incidence of pathogens found on the vegetables sampled was low with one positive (0.5%) for *Salmonella victoria* and one (0.5%) for *Listeria monocytogenes.* Whilst it is preferable to not find such pathogens it is also encouraging that the number of contaminated samples were low and also that the levels detected were very low. In other published surveys the incidence of *Salmonella* spp. and *L. monocytogenes* isolated have been much higher. For example, studies in Italy have shown that 68% of 120 random samples of lettuce and 72% of 89 random samples of fennel contained *Salmonella* (Ercolani, 1976). In the US, a survey of fresh salad vegetables revealed that 4 of 50 samples (7%) contained *Salmonella* spp. (Rude *et al.* 1984). These and other studies have usually been carried out at retail markets. In a UK study of commercially prepared salad mix, of the 60 samples tested, 7% were contaminated with *L. monocytogenes* (Sizmur and Walker, 1988). Other examples can be found in the literature review (Appendix 2).

In our study no *Campylobacter* spp. were detected. *Campylobacter jejuni* and C. *coli* are the most common cause of bacterial foodborne illness in the developed world (Park, 2002). In Australia, it has been the most common cause of

gastrointestinal disease in the last few years (Communicable Diseases Network - Australia New Zealand - National Notifiable Diseases Surveillance System, personal communication).

There has been one reported outbreak in Australia linked to salad/vegetables (Crerar *et al.* 1996). However, *Campylobacter* spp are very sensitive to environmental stress and have fastidious growth requirements. Although it is a common cause generally of foodborne illness, perhaps the reason we did not find it in our study is because of its environmental sensitivity. Perhaps this indicates that it is not a big problem in the horticultural industry.

A few samples (3.8%) were found to be positive for *E. coli,* mostly at low and acceptable levels. Normally, generic *E. coli* is detected by direct counts as an indicator organism for faecal contamination. We also looked for presence or absence by enriching the samples and found quite a large number to have *E. coli* present.

Whilst at this low level this is not a food safety issue, it is of interest to look at differences between product types. We found that lettuce and salad mix had a significantly higher incidence of *E. coli* than the other products and cabbage was significantly lower.

This shows that the leafy type products are at a higher risk of contamination. This may be due to biofilm formation, which has been observed in leafy vegetables (Morris and Nguyen-The, 1996). Biofilms are known to offer some protection to microorganisms within the biofilm. The TAC followed a similar trend in that salad mixes had some of the highest counts and cabbage some of the lowest.

4. Effectiveness of chlorine to remove pathogens from vegetables

4.1. Introduction

Hypochlorite dips are commonly used for washing fruits and vegetables after harvest, particularly in the fresh-cut industry. Washing reduces the total microbial load and in so doing may reduce spoilage and maintain quality, thereby increasing the shelf life. Another important reason to wash produce in sanitised water is to increase product safety. There is often a general assumption that washing in sanitised water will eliminate pathogenic organisms that may be present. However, little published data is available to support this.

Of the few studies that have been carried out, most have investigated the effect of chlorine on the inactivation of *Listeria monocytogenes* (Brackett, 1987; El-Kest and Marth, 1988; Beuchat and Brackett, 1990; Zhang and Farber, 1996). *In-vitro* testing of chlorine against *L. monocytogenes* has shown it to be an effective sanitiser (Zhang and Farber, 1996). Its effectiveness on vegetables has not been shown to be particularly good. Chlorine washing was found to reduce *Listeria monocytogenes* populations on lettuce and Brussels sprouts by only 2 logio CFU/g or less (Brackett, 1987; Beuchat and Brackett, 1990; Zhang and Farber, 1996). Furthermore, the initial reduction observed on lettuce was not evident when compared to controls after 15 days storage at 5°C (Beuchat and Brackett, 1990). Zhuang *et al.* (1995) looked at the effect of chlorine on *Salmonella montevideo* inoculated on tomatoes. Chlorine was found to reduce populations by around 1 logio CFU/g.

The presence of the coliform *E. coli* is often used as an indicator of faecal contamination and the possible presence of pathogens. There have been several human disease outbreaks overseas associated with fresh produce caused by enterotoxigenic and enterohaemorrhagic *E. coli* and other faecal organisms such as *Salmonella,* and *Campylobacter* (Beuchat, 1995; Little *et al.,* 1997). There is increasing pressure being placed on primary producers to ensure that produce is safe for human consumption. The introduction of HACCP based quality assurance programs and the emphasis on food safety has meant that chlorination is used more and more as a tool to satisfy HACCP requirements. The main aim of this study was to look at the effect of various chlorine concentrations and contact times on the fate of *E. coli* inoculated on fresh produce.

4.2 Materials and methods

4.2.1 Preparation of the E. coli suspension

A modified *E. coli* (strain TGI) was used for this study. The *E. coli* were cultured each week on luria-bertani broth (LB) agar (containing ljxl/ml ampicillin). For each experiment, one loopful of the culture was inoculated into a flask with LB (containing 1 ul/ml ampicillin) and incubated with shaking for 24h at 35°C. The concentration of this stock suspension was confirmed by making serial dilutions in peptone buffer containing 0.1% bacto peptone (Difco, Detroit, USA) in deionised water. These dilutions were plated onto Petrifilm *E. coli/cohform* count plates (3M

Australia, NSW) and incubated for 24h at 35°C. The stock suspension was stored at 4°C during this incubation time and the concentration checked at the same time as it was used.

4.2.2 Preparation of the hypochlorite solutions

Hypochlorite solutions were prepared using calcium hypochlorite (650g/kg available chlorine, Premium Quality Pool Products, NSW, Australia) and deionised water. Chlorine solutions (50 and 100 mg/L) were prepared and the pH adjusted to 6.0 - 6.5 by addition of 1% citric acid solution (Sigma, NSW, Australia). These solutions and a control solution of deionised water were cooled to 4°C. The concentrations of free chlorine were measured using a Hach DR/2000 meter and found to be 45-46 mg/L and 78-83 mg/L for the 50 and 100 mg/L solutions respectively.

4.2.3 Preparation of the neutralising solution and its effect on E. coli

Sodium thiosulphate solution (0.01M) was prepared by dissolving $Na_2S_2O_3$.5 H2O in deionised water. Its effect on the *E. coli* was investigated to ensure that the neutraliser did not reduce cell populations. A stock suspension of *E. coli* was prepared as above and the concentration determined. One ml of the bacterial suspension was added to 9 ml of the neutraliser in duplicate tubes and allowed to stand for 15 min. As a control, 1 ml of the suspension was added to peptone buffer (0.1%) in duplicate tubes and left for 15 min. After this time the viable populations were determined as above.

4.2.4 Effect of chlorine on E. coli in-vitro

The stock bacterial suspension was diluted by adding 3ml to 27ml of peptone buffer (0.1%) and the concentration of cells determined. Aliquots of this solution (2ml) were added to 18ml of each of 50 mg/L chlorine. 100 mg/L chlorine and deionised water, cooled to 4°C. This meant that the concentration added was diluted by a further 1 $\log_{10}$. At 30 seconds. 2 min and 5 min. 1ml was removed and added to 9ml of neutralising solution. Surviving populations were determined by making serial dilutions, plating onto 3M Petrifilm plates and incubating for 24 hours at 35°C. Three replicates of each treatment were carried out according to a randomised complete block design.

4.2.5 Effect of chlorine on E. coli inoculated on cos lettuce

Fresh cos lettuce *(Lactuca sativa)* was purchased from a local produce market and cooled overnight to 4^{C}C. The older and damaged, outer leaves were discarded and the leaves used were cut in half transversely to enable ease of dipping. An inoculation solution of the *E. coli* was prepared by dilution of the stock suspension in peptone buffer (0.1%) to make 2L and the concentration determined. Approximately 100g of the leaves were placed into the inoculation suspension for 1 min. They were removed, the excess solution shaken off and then dipped into 2L of either the 50 mg/L, 100 mg/L chlorine solutions or deionised water for 30s, 2 min or 5 min. Four replicates of each treatment were carried out and fresh solutions were used for each sample. The order of dipping was carried out according to a

randomised complete block design to allow for any variations which might occur during the course of the experiment, such as use of different parts of the lettuce. Following dipping, the samples were weighed into sterile bags and stomached for 2 min in 225ml of peptone buffer (0.1%). Serial dilutions in peptone buffer were prepared and plated as described above. This experiment was carried out 3 times, twice using a high inoculum level of approximately 7.3 logio CFU/ml and once using a lower inoculum level of 2.64 log$_{10}$ CFU/ml. For the second high inoculum experiment, 6L of chlorine or water dip was used for each sample to enable agitation via a magnetic stirrer, with three replicates of each treatment. The concentration of *E. coli* on inoculated but undipped lettuce was determined on two replicates that were included in the block design. The concentration of *E. coli* on fresh lettuce (not inoculated or dipped) was also determined in duplicate.

4.2.6 Effect of chlorine on E. coli inoculated on to broccoli florets

Fresh broccoli *(Brassica oleracea,* Botrytis Group) was purchased from a local produce market and cooled overnight to 4°C. It was then processed manually into florets. An inoculation solution was prepared as described for cos lettuce. Approximately 50g of florets were dipped in 2L as above according to a randomised complete block design and the concentration of surviving *E. coli* determined as above. This experiment was carried out twice, once using a high inoculum of 6.68 log$_{10}$ CFU/ml and once using a lower inoculum of 2.62 logio CFU/ml. During both experiments the broccoli was agitated during subsequent chlorine/water dipping using a magnetic stirrer. The concentration of *E. coli* on inoculated but undipped broccoli was determined on three replicates that were included in the block design. The concentration *E. coli* on fresh broccoli (not inoculated or dipped) was also determined on three replicates.

4.2.7 Dip temperature effects

Inactivation of *E. coli* inoculated onto broccoli florets was determined at 4, 8, 15, 20 and 25°C dip temperatures using 100 mg/L chlorine for 2 minutes. Fresh broccoli was purchased from a local produce market and cooled overnight to 4°C. It was then processed manually into florets. An inoculation solution was prepared as described and approximately 50g of florets were dipped. Four replicates of each treatment were carried out. The inoculum contained 7.16 log$_{10}$ CFU/ml. The order of dipping was carried out according to a randomised complete block design and the concentration of surviving *E. coli* determined as above. The concentration of *E. coli* on inoculated but undipped broccoli was determined on four replicates that were included in the block design. The concentration on fresh broccoli (not inoculated or dipped) was also determined in duplicate.

4.2.8 Data Analysis

Analysis of variance (GENSTAT 5.4.1) was performed on log$_{10}$ *E.coli* count (or logio *E coli* count +1) data to meet the assumption of constant variance. The low inoculum broccoli experiment was not analysed as only 7 experimental units had non-zero counts. The treatments were partitioned as a factorial structure of contact time x chlorine concentration plus an extra treatment (undipped inoculated lettuce

or broccoli), where appropriate, in a randomised complete block design. For the high inoculum broccoli experiment, linear effects of chlorine concentration x contact time were also tested. For the *in-vitro* chlorine experiment, only the deionised water treatments were included as all but one of the experimental units of the remaining treatments had zero counts. In the experiments where the undipped inoculated product were missing one or two replicates, the appropriate least significant difference (LSD) for comparing this treatment to the dip treatments was used. It was not presented in the tables for simplicity, as the LSD is only marginally larger than the one presented and the treatment effects are large in comparison. All tests were at the 5% significance level.

4.3 Results

4.3.1 Effect of the neutralising solution on E. coli

The sodium thiosulphate solution did not reduce *E. coli* cell populations. The concentration of the stock suspension was 8.3 $\log_{10}$ CFLVml. After 15 minutes the concentration of cells in the thiosulphate solution was 8.0 $\log_{10}$ CFLVml in comparison to 7.5 $\log_{10}$ CFU/ml for the peptone buffer. In subsequent experiments *E. coli* cell populations were assumed not to be affected by the neutralising solution, as the maximum exposure time of 5 minutes is less than the 15 minutes used in this experiment.

4.3.2 Effect of chlorine on inactivation of E. coli in-vitro

Chlorine (100 mg/L) for 30s or longer inactivated the 7.0 $\log_{10}$ CFU *E. coli* /ml (Table 11). Concentrations of 50 mg/L exposure for *2* minutes or longer were also effective. Exposure times of 30s and 50 mg/L concentrations eliminated the *E. coli* in two of the three replicates and reduced *E. coli* concentration in the third replicate by 6 $\log_{10}$ CFU/ml. Exposure to water alone did not significantly affect cell concentration.

Table 11. Effect of concentration of calcium hypochlorite solution (pH 6.0) on inactivation of *E. coli in-vitro*

Free chlorine (mg/L)	Contact time mean $\log_{10}$ CFU/ml			
	30s	2 min	5min	LSD (P=0.05)
0	6.91	6.79	7.00	0.27
50	0.40	ND	ND	
100	ND	ND	ND	

Concentration of *E. coli* suspension added to each dip: 8.0 $\log_{10}$ CFU/ml. This resulted in 7.0 $\log_{10}$ CFU/ml concentration exposed to the dips.
ND = not detected

4.3.3 Effect of chlorine on inactivation of E. coli inoculated on cos lettuce

The population of *E. coli* recovered from the cos lettuce following inoculation was 0.5 - 0.7 $\log_{10}$ counts less than that in the suspension (Tables 12-13). Dipping was not effective at eliminating *E. coli* populations although it significantly reduced the *E. coli*

counts compared to inoculated, undipped lettuce. When lettuce was inoculated with 7.3 $\log_{10}$ CFU *E. coli*/ml, lettuce dipped in solutions with 50 or 100 mg/L chlorine had significantly reduced *E. coli* counts compared to deionised water (Table 12). Dipping cos lettuce leaves into deionised water reduced *E. coli* cells by approximately 1.7 $\log_{10}$ CFU/g compared to inoculated, undipped lettuce.

Dipping lettuce leaves into a chlorine solution containing 50 mg/L or greater free chlorine for at least 30 seconds reduced *E. coli* cells by between 1.9 and 2.8 $\log_{10}$ CFU/g. Agitation of the lettuce during dipping did not appear to affect *E. coli* populations compared to not agitating the lettuce, since reduction of populations was similar in the two experiments.

At the lower inoculum concentration (2.7 $\log_{10}$CFU *E, coli*/ml*)*, lettuce dipped in solution with 100 mg/L chlorine had significantly reduced *E. coli* counts compared to deionised water (Table 13). *E. coli* cells were reduced by between 1.3 and 1.8 $\log_{10}$ CFU/g by dipping in 100 mg/L free chlorine for 30 seconds or longer (Table 13). *E. coli* were not detected on uninoculated, undipped lettuce.

Table 12. Survival of *E. coli* on cos lettuce leaves dipped in different concentrations of chlorine in solution (pH 6.0-6.5). Experiment 1 was without agitation of the lettuce in the dip whereas in experiment 2 the lettuce was agitated during dipping

Free chlorine (mg/L)	Contact time mean $\log_{10}$ CFU/g							
	Experiment 1				Experiment 2			
	30s	2 min	5 min	Mean	30s	2 min	5 min	Mean
0	5.30	5.24	5.22	5.25	5.21	4.75	5.07	5.01
50	4.60	4.61	4.30	4.50	4.63	4.30	4.28	4.40
100	4.40	4.19	4.31	4.30	4.65	4.50	4.34	4.50
No dip		6.97				6.55		
LSD (P=0.05)		0.38		0.22		0.70		0.40

Concentration of inoculating suspension: Experiment 1 = 7.32 $\log_{10}$ CFU/ml, Experiment 2 = 7.28 $\log_{10}$ CFU/ml. *E. coli* were not detected on uninoculated, undipped lettuce.

Table 13. Survival of *E. coli* on cos lettuce leaves dipped in different concentrations of chlorine solutions (pH 6.1-6.2)

Free chlorine (mg/L)	Contact time mean $\log_{10}$ CFU/g			
	30s	2 min	5 min	mean
0	0.73 ,	0.86	0.71	0.77
50	0.24	0.51	0.70	0.48
100	0.15	0.53	0.15	0.28
No dip		1.98		
LSD (P=0.05)		0.58		0.33

Concentration of inoculating suspension: 2.64 $\log_{10}$ CFU/ml.
E. coli were not detected on uninoculated, undipped lettuce.

4.3.4 Effect of chlorine on inactivation ofE. coli inoculated on broccoli florets

The population of *E. coli* recovered from the broccoli following inoculation was 1 $\log_{10}$ count lower than that in the suspension. Dipping was not effective at eliminating *E. coli* populations although it significantly reduced the *E. coli* counts compared to inoculated, undipped broccoli. Dipping inoculated broccoli florets into deionised water or chlorine reduced *E. coli* cells by between 1.7 and 2.5 $\log_{10}$ CFU/g (Table 14) compared to the undipped broccoli. There was no significant difference between water and chlorine after the 30s dip. For the 2 and 5 minute dip times, a significant linear decrease in *E. coli* was observed as the chlorine concentration was increased. There was no significant linear effect of contact time for the water dip and the 50 ppm chlorine dip. In the 100 ppm chlorine treatment there was a significant linear decrease as time was increased. When broccoli florets were inoculated with a suspension containing 2.62 $\log_{10}$ CFU/ml the numbers were reduced to the extent that only 7 experimental units had cells detected (Table 15). *E. coli* were not detected on uninoculated, undipped broccoli.

Table 14. Survival of *E. coli* on broccoli florets dipped in different concentrations of chlorine solutions (pH 6.0-6.3)

Free chlorine	Contact time		
(mg/L)	mean $\log_{10}$ CFU/g		
	30s	2 min	5 min
0	3.75	3.96	3.60
50	3.62	3.44	3.26
100	3.82	3.17	2.98
No dip		5.52	
LSD (P=0.05)		0.35	

Concentration of inoculating suspension: 6.68 $\log_{10}$ CFU/ml. *E. coli* were not detected on uninoculated, undipped broccoli.

Table 15. Survival of *E. coli* on broccoli florets dipped in different concentrations of chlorine solutions (pH 6.0)

Free chlorine	Contact time		
(mg/L)	mean CFU/g		
	30s	2 min	5 min
0	3.25	ND	0.75
50	1	ND	1
100	1	1	ND
No dip		85	

Concentration of inoculating suspension: 2.62 $\log_{10}$ CFU/ml.
E. coli were not detected on uninoculated, undipped broccoli.

4.3.5 Effect of temperature of chlorine solution on inactivation ofE. coli inoculated on broccoli florets

The population *of E. coli* found on the broccoli following inoculation was 1 logio count lower than that in the suspension (Table 16). Dipping the broccoli for 2 minutes in 100 mg/L chlorine solution significantly reduced *E. coli* cells by between 2.3 and 2.5 $\log_{10}$ CFU/g compared to inoculated undipped broccoli (Table 16). This is comparable to the reduction seen in the previous broccoli dipping experiment for 100 mg/L chlorine and 2 minutes dipping duration. No significant effect of dip temperature was observed. *E. coli* were not detected on uninoculated, undipped broccoli.

Table 16. Effect of temperature of chlorine solution (100 mg/L, pH 6.0) on inactivation of *E. coli* inoculated on broccoli florets. Broccoli was dipped for 2 minutes

Temperature (°C)	mean $\log_{10}$ CFU/g
4	3.85
8	3.71
15	3.92
20	3.94
25	3.93
No dip	6.19
LSD (P=0.05)	0.49

Concentration of inoculating suspension: 7.161og$_{10}$ CFU/ml.
E. coli were not detected on uninoculated, undipped broccoli.

4.4 Discussion

Calcium hypochlorite was found to be effective at inactivating high numbers of *E.coli* cells *in-vitro.* Exposure to 100 mg/L concentration for 30s completely eliminated 7.0 $\log_{10}$ CFU/ml *E. coli* cells. However, once the *E.coli* cells were inoculated onto broccoli or lettuce the effectiveness of chlorine was greatly decreased. Reductions of 1.7 to 2.8 $\log_{10}$ CFU/g were observed when broccoli or lettuce were inoculated with high concentrations *of E. coli.* However, water alone reduced numbers by 1.5 to 1.8 $\log_{10}$ CFU/g. This is comparable to results observed by Brackett (1987) who found that dipping Brussels sprouts in 200 mg/L chlorine for 10s reduced *Listeria monocytogenes* concentration by 2 $\log_{10}$ CFU/g and dipping in water reduced counts by 1 $\log_{10}$ CFU/g. It should be considered that the reductions seen in these experiments were under ideal conditions, where for each sample a fresh dipping solution was prepared using deionised water and the pH was optimally adjusted to maximise the hypochlorous acid concentration. Furthermore, the inoculum and dipping method employed represents recent contamination and would not allow for adherence of the *E. coli* to the produce surface or to biofilms. Consequently, the reducuction in viable *E. coli* cell numbers observed here may be higher than those achieved under commercial conditions.

Differences in the level of *E. coli* deactivation were observed between lettuce and broccoli. For lettuce, counts were significantly reduced by exposure to 50 mg/L chlorine for 30s compared to water. Longer exposure times or a higher concentration of chlorine did not have any significant additional benefit. Whereas for broccoli, a contact time x concentration interaction was found and in some combinations water was as effective as chlorine. This may be due to the difference in morphology. Broccoli is protected by a relatively thick waxy cuticle that repels water and possibly discourages bacteria from adhering to its surface. This may also prevent biofilm formation that has been observed in leafy vegetables (Morris and Nguyen-The, 1996). Biofilms are known to offer some protection to microorganisms within the biofilm. However, Seo and Frank (1999) found that *E. coli* 0157:H7 did not preferentially adhere to biofilms produced by *Pseudomonas fluorescens* on the leaf surface of lettuce. It is unlikely that the differences observed were because of differences in chlorine demand due to organic matter in the dipping solution, as for both lettuce and broccoli, the volume of dip compared to the amount of plant material was very large.

No significant differences were observed in the level of cell inactivation during a 2 minute dip in 100 mg/L chlorine solution at temperatures between 4 and 25°C. Longer dipping times may have shown differences between the temperatures. Chlorine efficiency *in vitro* increases with temperature up to the point before vaporisation occurs (Bovette *et ai,* 1993). Thus, *in vivo* effects may differ from those *in vitro.* Conversely. El-Kest and Marth (1988) found that chlorine was more effective in killing *L. monocytogenes* at 5°C than at 25° or 35^{C}C. Hoffman *et al.* (1981) found that lowering the pH of the chlorine solutions resulted in less stability of the available chlorine at 25^{C}C. In a study looking at the effect of temperature on coliform and enteric pathogens, 25°C was more effective than 5°C and the greatest differences occurred at pH values higher than 8.5 and at low concentrations of chlorine (El-Kest and Marth, 1988). It would seem then that the effectiveness of chlorine in killing pathogens at different temperatures is complex and a number of factors need to be considered.

These results show that chlorine reduces *E. coli* populations in water and on produce surfaces. However, it cannot be assumed that it will completely eliminate pathogens on produce. Chlorine washing should be used to complement a production system that utilises good agricultural practices and good handling practices for all stages of production and postharvest handling. It should not be used as a treatment to sanitise produce with the aim of eliminating potential human pathogens.

5. Irrigation water and soil analyses

5.1 Introduction

Current Australian water quality guidelines (Australian and New Zealand Environment and Conservation Council, 1992) recommend that irrigation water should contain not more than 1000 faecal coli forms/100mL. There was some suggestion that replacement guidelines might recommend less than 10 faecal coliforms/100mL for some crops. As there was no data available to suggest whether this was a reasonable recommendation or whether irrigation waters adhere with current guidelines, we carried out some sampling in different growing regions of Victoria.

To enable limits to be set for *E. coli* in 'the selection of new land' section of the 'Safe Vegetable Production' Guide, soil samples were collected from a number of farms in Victoria and one in Queensland.

5.2 Irrigation water

5.2.1 Methodology

5.2.1.1 Water sources

Water samples were collected from seven farms in three main growing areas of Victoria; Werribee, the Momington Peninsula and East Gippsland. Farms in East Gippsland were in Lindenow, Stratford and Boisdale and on the Momington Peninsula, in Somerville and Clyde.

Different water sources were looked at, these included bore water, dam, river and lake water.
Farm 1 had dam water made up of rain and bore water.
Farm 2 had dam water that consisted mainly of catchment from farm run-off with some bore water added.
Farm 3 had dam water distributed via channels from the Werribee river.
Farm 4 had dam water distributed via channels from the Werribee river, except for the autumn measurement when water was supplemented from the D1 drain run-off. This is catchment water from the surrounding area. This farm also had bore water.
Farm 5 accessed water directly from the channel that originated from Lake Glenmaggie.
Farm 6 pumped water direct from the river Avon and also had bore water
Farm 7 pumped water direct from the Mitchell river and also had bore water.

5.2.1.2 Sampling and analysis

Sampling was carried out during each season.

Around 200mL of water was collected into a sterile bottle for each sample. This was ransported under ice back to the laboratory and stored overnight at 4°C prior to analysis**.** Analysis was carried out within 24 hours as specified in the Australian standard AS 1095.4.1.1.

Samples were analysed for *E.coli,* faecal coliforms, faecal streptococci and total aerobic count.

E.coli, faecal coliforms and faecal streptococci were determined by the Most Probable
Number (MPN) methods:
AS 4276.8 -1995, Australian Standard®, Water microbiology, Method 8: Faecal streptococci – Estimation of most probable number (MPN)
AS 4276.6 – 1995, Australian Standard®, Water microbiology. Method 6: Thermotolerant coliforms and *Escherichia coli* –Estimation of most probable number
(MPN)
Total aerobic count was determined by performing serial dilutions and plating onto 3M Petrifilm aerobic count plates.

5.2.2 Results and discussion

All of the samples except for one fell within the current Australian water quality guidelines of 1000 faecal coliforms per 100 mL. The one sample that did not meet the guidelines was during one season where the only water available was from a drain containing run-off from the surrounding area.

We found bore water to have much lower levels of faecal streptococci, faecal coliforms and *E. coli* than dam. River or channel water (Tables 17-19). This is not that surprising since surface water could come from some distance and there may be less control over potential sources of contamination.

Most bore water samples had levels of faecal coliforms of less than 2 MPN/100mL, with the highest level being 14. Channel/river water samples contained from less than 2 to 350 MPN/100mL. Dam water had mainly between 5 to 540 MPN/100mL, with 2 samples containing 920 MPN/100mL faecal coliforms.

Generally the levels of faecal coliforms, faecal streptococci and *E. coli* varied between the different times the water was analysed, although for bore water the levels were very consistent.

It was encouraging to find that irrigation water from a wide range of sources generally fell well within the current guidelines.

Table 17. Dam water measurements taken over each season

Farm/source	Area	Season	TAC cfu/100ml	Faecal streptococci cfu/100ml	Faecal conforms cfu/100ml	*E. coli* cfu/100ml
Farm 1/dam 1	Peninsula	Spring	3.3×10^{6}	33	110	70
		Summer	6.4×10^{6}	49	240	240
		Autumn	1.8×10^{6}	23	110	7
		Winter	2.8×10^{7}	130	920	280
Farm 1/dam 2	Peninsula	Spring	1.7×10^{6}	49	33	33
		Summer	Not used			
		Autumn	5.5×10^{6}	110	350	350
		Winter	1.4×10^{7}	8	94	26
Farm 1/dam 3	Peninsula	Spring	1.1×10^{6}	79	17	17
		Summer	Not used			
		Autumn	7.8×10^{5}	<2	4	4
		Winter	1.2×10^{7}	13	220	220
Farm 1/dam4	Peninsula	Spring	8.0×10^{5}	9	220	140
		Summer	Not used			
		Autumn	1.1×10^{7}	540	350	130
		Winter	Not used			
Farm 1/dam 5	Peninsula	Spring	1.5×10^{7}	23	170	170
		Summer	3.8×10^{6}	220	540	220
		Autumn	7.4×10^{7}	350	920	170
		Winter	3.3×10^{7}	49	280	33
Farm 1/dam 6	Peninsula	Spring	1.2×10^{6}	8	5	5
		Summer	1.5×10^{6}	23	17	11
		Autumn	3.0×10^{6}	49	110	6
		Winter	1.3×10^{7}	2	34	34
Farm 2/dam1	Peninsula	Spring	4.1×10^{5}	14	130	34
		Summer	4.0×10^{5}	27	49	22
		Autumn	2.4×10^{6}	130	350	9
		Winter	1.4×10^{6}	170	350	34
Farm 2/dam 2	Peninsula	Spring	1.4×10^{6}	8	11	8
		Summer	2.2×10^{6}	22	350	170
		Autumn	4.0×10^{6}	27	280	26
		Winter	8.7×10^{6}	17	11	11
Farm 3/dam 1	Werribee	Spring	1.4×10^{5}	28	64	38
		Summer	6.6×10^{5}	13	34	8
		Autumn	2.2×10^{6}	12	11	11
		Winter	6.1×10^{5}	8	63	63
Farm 3/dam 2	Werribee	Spring	2.3×10^{5}	0	21	21
		Summer	2.9×10^{6}	49	220	21
		Autumn	5.8×10^{5}	Not used		
		Winter	7.9×10^{5}	<2	21	14
Farm 3/dam 3	Werribee	Spring	2.2×10^{5}	11	8	8
		Summer	4.4×10^{5}	17	63	5
		Autumn	1.4×10^{6}	31	9	4
		Winter	1.8×10^{7}	<2	5	5
Farm 4 dam 1	Werribee	Spring	2.2×10^{5}	<2	5	5
		Summer	1.2×10^{5}	8	33	5
From D1 drain run-off		Autumn	6.4×10^{6}	1600	1600	17
		Winter	3.9×10^{4}	2	9	4

Table 18. Channel and river water samples taken over each season

Farm/Source	Area	Season	TAC cfu/100ml	Faecal streptococci cfu/100ml	Faecal coliforms cfu 100/ml	E. coli cfu/100ml
Farm 4 /channel	Werribee	Spring	$6.5x10^{6}$	920	>490	>490
		Summer	$4.5x10^{6}$	540	49	49
		Autumn	not running			
		Winter	not running			
Farm 5/channel	Boisdale	Spring	$1.4x10^{4}$	17	49	14
		Summer	$1.6x10^{4}$	<2	<2	<2
		Autumn	$2.9x10^{4}$	<2	<2	<2
		Winter	$1.1x10^{5}$	8	22	22
Farm 6/river	Stratford	Spring	$5.0x10^{5}$	5	13	2
		Summer	$1.6x10^{5}$	33	110	110
		Autumn	$1.1x10^{5}$	8	23	23
		Winter	$5.2x10^{5}$	17	140	140
Farm 7/river	Lindenow	Spring	$8.8x10^{4}$	11	79	17
		Summer	$6.7x10^{4}$	23	350	240
		Autumn	$2.9x10^{4}$	13	33	33
		Winter	$3.1 x10^{5}$	4	170	130

Table 19. Bore water samples taken over each season

Source	Area	Season	TAC cfu/100ml	Faecal streptococci cfu/100ml	Faecal coliforms cfu/100ml	*E. col* cfu/100i
Farm 1	Peninsula	Spring	$2.6x10^{3}$	<2	<2	*<2*
		Summer	$7.3x10^{3}$	<2	<2	*<2*
		Autumn	$4.0 x10^{2}$	<2	9	*4*
		Winter	$9.8x10^{4}$	<2	2	*2*
Farm 4	Werribee	Spring	$2.4x10^{4}$	<2	<2	*<2*
		Summer	$3.0x10^{5}$	33	11	*2*
		Autumn	$2.3x10^{5}$	<2	<2	*<2*
		Winter	$2.9x10^{4}$	<2	<2	*<2*
Farm 6	Stratford	Spring	$1.0 x10^{2}$	<2	<2	*<2*
		Summer	$8.9x10^{4}$	<2	<2	*<2*
		Autumn	$3.0x10^{2}$	<2	<2	*<2*
		Winter	$8.2x10^{2}$	<2	4	*4*
Farm 7	Lindenow	Spring	$1.3x10^{5}$	<2	<2	*<2*
		Summer	$4.5x10^{4}$	<2	<2	*<2*
		Autumn	$6.8x10^{4}$	<2	<2	*<2*
		Winter	$2.6x10^{5}$	<2	14	*14*

5.3 Soil

5.3.1 Methodology

Samples (n=188) were collected from five different growing areas and seven farms. The areas were Werribee South (Table 20), Somerville, Mornington Peninsula (Table 21), Boisdale and Lindenow, East Gippsland (Table 22) and Queensland (Table 23).

Generally, soil samples were collected from three fields for each farm (A to C). For each field, eight separate samples were taken at random. In Queensland samples were collected from two fields for farm 1 and one for farm 2 (Table 23). In Somerville, there were 7 fields (A to G), with one of these fields being sampled twice (Table 21).

The soil was taken from a depth of 5-10cm, into a sterile container. Approximately 50g of soil was collected for each sample.

15-20 grams of soil was taken from each sample and placed into 100 ml 0.1% peptone water. The sample was then shaken on a rotary shaker at 150 rpm for 20 minutes and allowed to settle for 5 minutes. Serial dilutions were prepared in 0.1% peptone water. These were placed onto 3M *E.coli*/coliform plates and incubated at 37°C for 48 hours. As well as *E.coli,* the level of total coliforms were enumerated.

5.3.2 Results and discussion

Of the 188 samples taken *E. coli* was present in 23 (12.2%). In the remaining 165 samples *E. coli* was not detected. Of the samples where *E. coli* was found, 15 had levels of less than 50 CFU/g, and 2 had less than 100 CFU/g. The other 6 positive samples (3.2%) had considerably higher levels. These were all detected on one farm and four of these were from 1 block early in the season where there had been recent manure application (Table 21). The other two were also early in the season (14th March) and later measurements taken on 10th May showed no *E.coli* to be present. This is in agreement with some work carried out at the Institute where *E. coli* present in manure applied to soil were not detected eight weeks after the application (Robert Premier, pers. comm.).

The level of total coliforms varied as would be expected. They occur naturally in the environment and soil and are commonly isolated from fresh vegetables at levels up to 10^4 CFU/g. Therefore, the levels observed here, commonly 10^2 to 10^3, are within the expected range.

Based on these results, that generally show that soil has very low levels of *E. coli,* we recommend in the guide that soil on new land should contain less than 100 CFU/g. This value also takes into consideration that the land may have previously been used for applications that could introduce many different human pathogens and lower levels of *E. coli* as an indicator organism are a good safeguard and indication that levels of other potential pathogens would also be low.

Table 20. Soil measurements taken in Werribee during May 2000

Farm 1			**Farm 2**		
Sample	***E. coli***	**Total coliforms**	**Sample**	***E. coli***	**Total coliforms**
	CFU/g			**CFU/g**	
A1	ND	3.9×10^2	A1	ND	7.5×10^2
A2	ND	7	A2	ND	1.8×10^3
A3	ND	6.1×10^2	A3	ND	5.7×10^3
A4	ND	7	A4	ND	1.5×10^2
A5	ND	22	A5	8	32
A6	ND	8	A6	ND	2.9×10^3
A7	ND	ND	A7	ND	4.7×10^2
A8	ND	ND	A8	ND	8.1×10^2
B1	ND	7	B1	ND	2.9×10^2
B2	ND	45	B2	ND	24
B3	ND	7.3×10^2	B3	ND	2.1×10^3
B4	ND	73	B4	ND	86
B5	ND	1.2×10^3	B5	ND	1.5×10^2
B6	ND	2.9×10^2	B6	ND	5.0×10^2
B7	ND	2.5×10^3	B7	ND	1.2×10^2
B8	ND	75	B8	16	6.5×10^2
C1	ND	5.9×10^2	C1	ND	8.8×10^2
C2	ND	7	C2	ND	1.4×10^3
C3	ND	ND	C3	ND	5.8×10^2
C4	ND	15	C4	ND	6.3×10^3
C5	ND	76	C5	ND	6.0×10^2
C6	ND	8	C6	ND	45
C7	ND	72	C7	7	9.9×10^2
C8	ND	76	C8	ND	9.0×10^2

ND = not detected

Table 21. Soil measurements taken in Somerville, Mornington Peninsula during March and May 2000.

Date	Sample	*E. coli*	Total coliforms	Date	Sample	*E. coli*	Total coliforms
		CFU/g				CFU/g	
27th Mar	A1	ND	67	27th Mar	E1	ND	2.4×10^2
	A2	ND	1.5×10^3		E2	ND	ND
	A3	ND	2.6×10^3		E3	ND	3.0×10^2
	A4	ND	1.2×10^5		E4	ND	1.0×10^2
	A5	ND	1.3×10^3		E5	ND	4.8×10^2
	A6	ND	62		E6	ND	12
	A7	ND	7.6×10^3		E7	ND	61
	A8	ND	1.9×10^2		E8	ND	3.7×10^2
	B1	49	6.9×10^2		F1	ND	1.2×10^2
	B2	2.0×10^2	1.3×10^3		F2	ND	1.8×10^2
	B3	ND	8.9×10^2		F3	ND	79
	B4	49	3.9×10^2		F4	ND	49
	B5	31	88		F5	ND	1.8×10^2
	B6	9.7×10^2	2.9×10^3		F6	ND	1.0×10^2
	B7	1.9×10^3	3.5×10^3		F7	ND	74
	B8	1.2×10^2	2.8×10^3		F8	ND	82
	C1	60	7.9×10^3				
	C2	ND	5.5×10^2	14th Mar	G1	ND	1.6×10^3
	C3	ND	77		G2	ND	1.9×10^3
	C4	ND	2.2×10^2		G3	6.1	4.9×10^2
	C5	ND	1.2×10^2		G4	6.4	4.5×10^2
	C6	ND	59		G5	ND	1.3×10^2
	C7	ND	54		G6	8.1×10^4	8.5×10^4
	C8	ND	18		G7	ND	2.0×10^3
	D1	ND	2.3×10^3		G8	ND	3.1×10^2
	D2	ND	5.3×10^3		G9	5.9×10^4	5.9×10^4
	D3	ND	5.3×10^3		G10	ND	1.3×10^3
	D4	ND	5.2×10^3	10th May	G1	ND	4.2×10^2
	D5	ND	1.4×10^4		G2	ND	2.9×10^2
	D6	ND	4.5×10^3		G3	ND	1.1×10^3
	D7	ND	51		G4	ND	2.2×10^2
	D8	ND	3.7×10^2		G5	ND	1.4×10^3
					G6	ND	5.6×10^3
					G7	ND	73
					G8	ND	2.1×10^2
					G9	ND	6.9×10^2
					G10	ND	6.0×10^2

ND = not detected

Table 22. Soil measurements taken in East Gippsland during March and April 2000

Farm 1 (Boisdale)				Farm 2 (Lindenow)			
Date	Sample	*E. coli*	Total coliforms	Date	Sample	*E. coli*	Total coliforms
CFU/g				CFU/g			
7th March	A1	ND	1.1×10^4	12th April	A1	ND	3.1×10^2
	A2	ND	6.3×10^2		A2	ND	1.3×10^3
	A3	66	2.2×10^3		A3	ND	2.4×10^3
	A4	ND	1.3×10^3		A4	ND	6.5×10^2
	A5	ND	1.9×10^2		A5	ND	3.6×10^3
	A6	ND	5.7×10^3		A6	ND	1.4×10^4
	A7	27	4.0×10^3		A7	ND	1.9×10^2
	A8	ND	3.2×10^4		A8	ND	8.5×10^2
	B1	ND	5.7×10^4		B1	ND	4.8×10^3
	B2	ND	7.1×10^2		B2	ND	4.4×10^2
	B3	ND	8.1×10^3		B3	ND	8.8×10^3
	B4	ND	1.7×10^3		B4	ND	4.6×10^3
	B5	ND	4.3×10^2		B5	ND	2.8×10^3
	B6	ND	1.3×10^3		B6	ND	4.6×10^3
	B7	ND	1.9×10^4		B7	ND	2.3×10^3
	B8	ND	1.3×10^4		B8	ND	1.0×10^4
	C1	24	1.2×10^4		C1	ND	1.4×10^3
	C2	ND	7.1×10^3		C2	ND	3.3×10^3
	C3	ND	4.4×10^3		C3	7	8.6×10^3
	C4	ND	2.3×10^4		C4	20	4.6×10^3
	C5	ND	6.6×10^3		C5	13	5.5×10^2
	C6	6	9.9×10^3		C6	ND	1.4×10^3
	C7	6	1.1×10^4		C7	ND	3.2×10^3
	C8	ND	1.6×10^4		C8	ND	1.1×10^4

ND = not detected

Table 23. Soil measurements taken in Queensland during April 2000

Farm 1			Farm 2		
Sample	*E. coli*	Total coliforms	Sample	*E. coli*	Total coliforms
CFU/g			CFU/g		
A1	ND	56	A1	ND	6
A2	ND	1.1×10^2	A2	ND	11
A3	ND	1.1×10^2	A3	ND	11
A4	ND	11	A4	ND	ND
A5	ND	39	A5	ND	ND
A6	ND	5.8×10^2	A6	ND	ND
A7	ND	1.5×10^2	A7	ND	ND
A8	ND	7.7×10^2	A8	ND	ND
B1	ND	36			
B2	ND	1.3×10^3			
B3	ND	9.0×10^2			
B4	ND	61			
B5	ND	38			
B6	ND	6			
B7	ND	74			
B8	ND	3.3×10^3			

ND = not detected

6. Technology transfer

The guide was published and launched as described (section 2.1.3). The launch and availability of the food safety guide has been publicised widely. Flyers with details about the guide were given to attendees at the National lettuce conference held in May 2002. Press releases have been sent to the EDOs to advertise in their newsletters and articles have been published in the newsletters IHD links (no. 6, May 2002) and Vegetable matters (no. 4, April 2002) and the journals Fruit and Vegetable news (June 2002) and Good Fruit and Vegetables (July 2002).

The release was also sent to around 30 different newspapers. Some of these have used the article and it has generated enquires about the guide. Flyers were sent to Tasmanian Quality Assured to include in their satchel for an On-farm Food Safety conference held in July 2002.

The launch and articles published to date have led to many enquires from growers, auditors, education establishments and exporters requesting a copy of the guide.

Another project led by Swinburne Tafe resulted in a video for poor language skill workers to be produced based on the content of the guide. This is for growers to use to train their employees. The video and training manual package was launched in November 2001.

There have been a number of presentations at conferences and in printed format during this project. Results of work on the effectiveness of chlorine washing in particular has been published in industry journals as well as in the scientific community. A one-page leaflet on this work was distributed widely to individual growers via the IDOs in each state. Around 1000 copies were sent out.

6.1 Publications related to the project

Behrsing, J., Winkler, S., Franz, P. and Premier, R. (1999) Inactivation of *Escherichia coli* on vegetables by chlorine. Australasian Postharvest Horticulture Conference, 3-8 October, Waitangi, New Zealand, (poster)

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Appendix 1. Questionnaire for growers involved in the farmgate survey.

SURVEY OF CULTURAL PRACTICES FOR VEGETABLES

Grower ....

Crop...

1. Is chicken manure used for growing vegetables? Y N
2. What type of manure is used?

Partially composted Fully Composted Certified composted Uncomposted

3. When is manure applied? ....

4. What is the irrigation source? ....
5. What type of irrigation is used? ....
6. Is produce packed in the field or shed? ......
7. Is produce washed and what type of water is used?