RURAL INDUSTRIES RESEARCH
& DEVELOPMENT CORPORATION

A Quality Survey of

Australian Honeys

A report for the Rural Industries Research and Development Corporation

by WH Ward & KF Trueman
Queensland Department of Primary Industries

May 2001

RIRDC Publication No 01/049
RIRDC Project No DAQ-231A

ISBN 0 642582 7
ISSN 1440-6845

A Quality Survey of Australian Honeys
Publication no. 01/049
Project no. DAQ-231A

Researcher Contact Details
W H Ward
Animal Research Institute
Locked Bag No 4
MOOROOKA Q 4104

Phone: 07 3362 9446
Fax: 07 3362 9440
Email: wardw@dpi.qld.gov.au

K F Trueman
Animal Research Institute
Locked Bag No 4
MOOROOKA Q 4104

Phone: 07 3362 9484
Fax: 07 3362 9440
Email: Truemaf@dpi.qld.gov.au

RIRDC Contact Details
Rural Industries Research and Development Corporation
Level 1, AMA House
42 Macquarie Street
BARTON ACT 2600
PO Box 4776
KINGSTON ACT 2604

Phone: 02 6272 4539
Fax: 02 6272 5877
Email: rirdc@rirdc.gov.au
Website: http://www.rirdc.gov.au

Published in May 2001
Printed on environmentally friendly paper by Canprint

Foreword

The Rural Industries Research and Development Corporation (RIRDC) Research and Development (R&D) plan for the Honeybee Program 1996-2001 (page 18) identified some issues which could contribute to marketing honey and efficiency. The issues identified included "improved methods of extraction, storage and transport of honey …… to maximise retention of product quality and minimise the possibility of contamination". In order to meet these objectives data on the factors which influence product quality and purity were seen as areas for investigation.

The proposed Australian and New Zealand Food Authority Bill outlines some definitions of "unsafe" and "unsuitable" food. In this legislation one of the references relating to unsuitable food is one that "contains biological or chemical agent, or other matter or substance that is foreign to the nature of the food".

In line with other food industries Quality Assurance programs are to be implemented into the Honey Industry. Therefore, the aim of the present study was to gather some information on the quality of Australian honeys in terms of microbiological and chemical residue contamination, and to recommend some standard test procedures to ensure the suitability of honey as a food product for human consumption.

This project was funded from industry revenue which is matched by funds provided by the Federal Government.

This report, a new addition to RIRDC's diverse range of over 700 research publications, forms part of our Honeybee R&D program, which aims to improve the productivity and profitability of the Australian beekeeping industry.

Most of our publications are available for viewing, downloading or purchasing online through our website:

- downloads at www.rirdc.gov.au/reports/Index.htm
- purchases at www.rirdc.gov.au/eshop

Peter Core
Managing Director
Rural Industries Research and Development Corporation

Acknowledgments

The financial support provided by RIRDC is gratefully acknowledged.

There are a number of people whose technical skills in specialised areas have contributed to this work. Their help is acknowledged with thanks.

Mark Kelly, Animal Research Institute, Yeerongpilly assisted with chalkbrood identifications. Dennis Rogers prepared the media and diluents used in these investigations.

Special mention must be made of Gary Everingham for his dedicated technical support in this work.

The cooperation of Joe Pippia and Hugh Mawhinney and staff for performing the elemental and residue analyses is acknowledged with thanks.

Mr Graham Kleinschmidt offered considerable support, and his keen interest in our investigation is gratefully acknowledged.

Melissa Hastings, Amanda Lloyd and Glenda Loxton had the difficult task of typing and preparing this manuscript. We thank them for their patience and typing ability.

Finally this work would not have been possible without the help of Apiary Officers in each State and Territory of Australia who collected the honey samples for this work.

We are also grateful for the cooperation of all apiarists who agreed to provide their honey for screening in the survey.

Contents

Executive Summary

Objectives

- To survey the quality (chemical residues honeybee disease agents) of honey samples produced in all states and territories in Australia.
- To examine the honeys for microbial quality (bacteria, yeasts and moulds).
- To compare the results with those obtained in a previous report DAQ 202A Introduced Honeys – a Quality Survey.
- To research standard methodologies for assessing the microbial quality of honeys.
- To establish acceptable honey quality standards in terms of chemical residues bacteria yeasts and moulds.

Research

Sixty honey samples representing all states and territories of Australia were examined in this survey. Fifty two samples were collected from the honey drums of individual apiarists and 8 samples were purchased from retail outlets.

All samples were cultured for honeybee disease agents namely American foulbrood (AFB) and chalkbrood. All honeys were also examined for microbial flora (bacteria, yeasts and moulds) and for chemical residues.

Two separate diluent preparations were trialled to determine the most sensitive methods for assessing numbers of bacteria yeasts and moulds.

Results

- Eight or 13.3% of 60 Australian honey samples screened returned positive results for AFB spores and 7 or 11.1% cultured positive for Chalkbrood disease. These results compare favourably with those reported for International honeys which showed that 17 or 47.2% of 36 samples tested cultured positive for AFB and 5 or 12.5% of 40 International honeys were positive for chalkbrood disease agent.
- Fifty seven or 95.0% of 60 honey samples from Australian sources returned bacteria plate counts of <500 CFU's per gram honey. By comparison, only 31 or 79.5% honeys from International sources returned <500 CFU's per gram honey. However, 5 or 8.3% honeys returned counts of 71000 CFU's per gram honey.

- A diluent preparation of peptone water with 40% glucose recovered greater numbers of yeasts and moulds than peptone water. However diluent composition had little effect on recovery of bacterial colonies from honey. 41 or 68.3% Australian honeys returned <10 total CFU's yeasts and moulds combined. Comparisons with International Honeys using the more sensitive preparation, are not available.

- Low levels of Aluminium, Iron and Zinc were detected in most honeys, but were not considered of significance. However, unacceptable levels of lead were detected in2 honey samples. Immediate reaction was taken to determine the sources and reason for this contamination. Investigation revealed that honey which was left in extracting

equipment became contaminated from lead soldering. Coating the soldering with food grade paint is believed to reduce the risk of this contamination. Thorough cleaning of extracting equipment after use is also essential.

- All 60 honeys were found to be free of antimicrobial residues, organochlorines, organophosphorous type pesticides, synthetic pyrethroids at the levels of reporting (LOR). All honeys were also found to be free of fumagillin and phenol residues. These results compare favourably with those reported for International honeys. Six or 14.36% of International honeys returned detections of phenol and a detection of fumagillin, oxytracycline and sulphathiazole was reported in each of 3 or 7.1% of the International honeys.

Outcomes

- Recommendation on standard techniques for determining numbers of bacterial, yeasts and moulds in honey can now be made.
- It is recommended that honey should contain <1000 CFU's/gram bacteria and a total of<10 CFU's/gram yeasts and moulds combined to be acceptable as a food product. In this project the majority of honeys contained <500 CFU's/gram bacteria, and a total of <10 CFU's/gram yeasts and moulds. With good hygienic practices this is a standard that all Australian apiarists should aim to achieve.
- This work provides useful information for honey packers who now have scientific information to guide their overall HACCP plans.
- Only 8 (13.3%) of Australian honeys returned positive culture results for AFB disease agent compared to 47.2% of International honeys detected in a previous report DAQ 202A. This result appears to justify the AFB control programs which are in place in Australia.

General Introduction

Australia is the fourth largest exporter of honey worldwide, and Australian producers and packers are keen to maintain existing markets and find opportunities to expand domestic and international sales. The Australian Honeybee Industry is committed to producing a high quality, natural product free from residues and contaminants. Factors such as chemical residues, antibiotic residues and high microbial counts could have serious impacts on international and domestic trade. The establishment of acceptable quality standards for honey will reassure consumers of the purity of honey and provide a sound basis for promoting and increasing national and international markets.

A Quality Survey was conducted in 1995/1996 (Trueman, Ward and Mawhinney RIRDC Project DAW-202A) International Honeys – A Quality Survey. This project examined 42 honey samples representing 20 different countries for disease agents, microbial quality and chemical residues.

The results of this survey confirmed a high prevalence and wide distribution of honeybee disease agents especially American foulbrood (AFB). Thirty-six samples representing 19 countries were cultured for AFB and 17 (47.2%) honeys from 11 (57.9%) countries were positive for AFB spores.

Forty honeys from 19 countries were cultured for Chalkbrood, yielding 5 (12.5%) samples positive from 3 (15.8%) countries.

Forty honey samples representing 19 different countries were examined for counts of aerobic bacteria, yeasts and moulds. All samples met suggested Australian bacterial standards of less than 5000 colony forming units (CFU) per gram of honey. However, 7 honeys recorded greater than 1000 CFU which would be outside a more stringent recommendation for honey.

Two media were trailed for growing yeasts and moulds, with a malt extract yeast media containing 40% glucose showing greater sensitivity for detecting yeasts. Using this media 11 (28.2%) honeys from 9 (47.4%) countries yielded greater than 10 CFU per gram of honey. This standard has been recommended by some authors for honey, while the suggested Australian Standard is less than 100 CFU per gram. At this level 10 (26.6%) honeys from 8 (42.1%) countries would be seen as unacceptable.

The study indicated that further work was required to determine the most suitable media for maximum recovery of yeasts and moulds in honey. The results also showed that there was a need to determine an acceptable industry standard in terms of numbers of yeasts and moulds per gram of honey.

Forty two samples representing 20 different countries were examined for chemical residues including metals, pesticides, phenol and antimicrobial agents. Nineteen percent of the honeys were found to contain one or more residues at or above the level of detection. The most commonly detected residue was phenol, but at a low level (<0.52mg/kg). Other single detection's were Fumagillin, Oxytetracycline and Sulphathiazole.

The purpose of this present study is to survey the quality (honeybee disease agents, microbial quality and chemical residues) of honey samples produced and packed in each state and territory of Australia, and to compare these results with the International honeys already surveyed.

This study also aims to research methodologies for assessing the microbial quality of honey and to provide further information on which to establish acceptable standards for honey quality.

1. Detection of Honeybee Disease Agents in Australian Honeys

1.1 Introduction

Until recent years the Australian Honeybee Industry was protected from importation of disease agents by confiscation at ports of entry of all honey arriving from overseas destinations.

This practice ensured that any honeybee disease control programs already in place in this country were not jeopardised and helped prevent entry of disease agents which may not be present. However, since the diagnosis of Chalkbrood in Australia in 1993 some relaxation of import restrictions into this country has occurred.

American Foulbrood (AFB) disease is a cause of economic loss to the Beekeeping Industry in Australia and the Honeybee Industry is committed to reducing the prevalence of the disease in Australia. The decision to embark on a control program for this disease followed a cost benefit analysis compiled by Fraser *et al* (1995) and most States are heavily committed to improved detection and control programs for AFB disease. AFB disease can be produced in a hive by feeding larva bees honey infected with AFB spores (Sturvetant 1932). These *Paenibacillus larvae* subsp. *larvae* spores can remain infective for 35 years (Haeman 1961).

Chalkbrood was first diagnosed in Australia in Queensland in 1993. Subsequently this disease has been identified in New South Wales, Victoria and South Australia, Western Australia, Northern Territory and Tasmania.

The causative agent of chalkbrood is fungus *Ascosphaera apis* and spores of this fungus can remain viable in honey and bee products and equipment for at least 15 years (Bailey and Ball 1991a). Feeding infected honey to bees is one method of spreading infection (Warhurst and Goebel 1995).

This paper describes detection of AFB and chalkbrood spores in honeys originating from all States and Territories of Australia.

1.2 Materials and Methods

1.2.1 Materials

Honey Samples

Sixty honey samples originating from Queensland (QLD), New South Wales (NSW), Victoria (VIC), South Australia (SA), Western Australia (WA), Tasmania (TAS) and Northern Territory (NT) were examined in this survey. The honey samples were collected by Apiary Officers in each State and Territory between October 1997 and April 1998.

Fifty two (87%) of the sixty samples were from drums of representative individual apiaries and these samples were collected from drums destined for the honey packers. Eight (13%) honey samples were purchased from retail outlets in Queensland and were representative of honey packers in QLD, NSW, SA, VIC and WA.

The numbers of individual honey samples including those from retail outlets from any one State or Territory range from a maximum of 11 to a minimum of 6. The samples were identified by State followed by a numerical identification number. All honey samples were cultured for AFB and Chalkbrood disease agents.

The States of origin, numbers and percentages of individual honey samples examined in this paper are shown in Table 1.

Table 1. Honey samples collected from each State and Territory and number of and percentages of samples representative of each State.

	State of Origin	Number of Represented Honeys	Percentage of Representative Honeys
	QLD	10	17
	NSW	9	15
	VIC	11	18
	SA	9	15
	WA	8	13
	NT	6	10
	TAS	7	12
TOTAL	7	60	100

1.2.2. Methods

1.2.2.1. American Foulbrood

Culture procedure

Honey samples were cultured for the presence of *P. larvae* subsp. *larvae* according to the method described by Hornitzky and Clark (1991). The technique involved mixing 75mL of each honey sample with 75mL phosphate buffered saline (PBS pH 7.2) and centrifuging at 3000g for 45 minutes. The supernatant was discarded and the pellet was mixed in the small volume of remaining diluted honey. An aliquot of this mixture was heated in a water bath at 80^{o}C for 15 minutes. One bacteriological loop (approximately 10µl) of each inoculum was streaked onto bacteriological plates containing J medium (Hornitzky and Nicholls 1993). The plates were incubated at 35^{o}C in air containing 5% CO_2 for four days.

Bacterial identification

Colonies grown on J agar plates were classified as *P. larvae* subsp. *larvae* positive if the following criteria were met; colonies were grey/white, flat, had irregular edges, and measured 6mm in diameter. Gram stained smears revealed gram positive rods which were 0.5 – 0.6mµ wide and 1.5 – 6mµ long were catalase negative. The catalase test was performed by the method of Cowan and Steele (1974).

Grading of B. larvae subsp. larvae culture positive honey samples

The culture positive honey samples were scored according to the method described by Hornitzky and Clark (1991). J agar plates with 1 – 20 colonies of *P. larvae* subsp. *larvae* were scored as 1+, those with 21 – 50 colonies at 2+ and plates with more than 50 colonies were scored as 3+.

1.2.2.2. Chalkbrood

Each honey sample was cultured for the presence of *A. apis* using two separate culture media.

(i) Sabourauds Dextrose Agar (SDA) which consists of neopeptone, dextrose and bacto agar.

(ii) Yeast/glucose phosphate medium (YGP) which contains yeast extract, glucose, KH_2PO_4 and soluble starch (Bailey and Ball 1991b).

Antibiotics, Penicillin and Streptomycin were added to both media preparations.

The culture technique used was that described by Honitzky and Anderson (1993). One gram of each test honey sample was mixed with approximately 15mls molten culture medium which had been held in a water bath at 48^{o}C. Each honey sample was cultured in duplicate for each of the two media preparations. The mixtures of media and test honey were allowed to set in plastic petrie dishes and all plates were incubated for 10 days at 35^{o}C under conditions of 5% CO_2.

At the conclusion of the incubation period, plates were examined for the presence of fungal colonies which were morphologically similar to *A. apis*. *A apis* colonies are 5 – 7mm in diameter, are white to pale buff colour and have floccose, matted mycelia.

1.3 Results

1.3.1. American Foulbrood

The numbers of honey samples tested and the numbers and percentages of positive identifications and the numbers of States returning one or more culture positive results are shown in Table 2.

Table 2. Numbers and percentages of honeys, numbers and percentages of States yielding positive *P. larvae* subsp. *larvae* culture results.

	Number tested	Number positive	% Positive	Number negative	% Negative
Honey samples	60	8	13.3	52	86.7
States and territories of Australia	7	6	85.7	1	14.3

The numbers of honey samples origination from each State and Territory and the numbers and percentages of honeys returning positive results from each State are shown in Table 3.

Table 3. Numbers and percentages of honeys from each State and Territory yielding positive *P. larvae* subsp. *larvae* results.

	QLD		NSW		VIC		SA		WA		NT		TAS	
Honey samples	**No. tested**	**No. positive (%)**	**No. tested**	**No. positive (%)**	**No. tested**	**No. positive (%)**	**No. tested**	**No. positive (%)**	**No. tested**	**No. positive (%)**	**No. tested**	**No. positive (%)**	**No. tested**	**No. positive (%)**
60	10	2 (20.0)	9	2 (22.2)	11	1 (9.1)	9	1 (11.1)	8	1 (12.5)	6	0 (0)	7	1 (14.3)

All honey samples which cultured positive for *P. larvae* subsp. *larvae* were scored as either 1^+, 2^+ or 3^+.

Table 4 shows the numbers and percentages of samples returning 1^+, 2^+ and 3^+ results.

Table 4. Numbers and percentages of *P. larvae* subsp. *larvae* culture positive honey samples yielding 1^+, 2^+ and 3^+ scores.

		1^+ (%)	2^+(%)	3^+(%)
Culture positive honey samples	8	5 (62.5)	0 (0)	3 (37.5)

The eight individual honey samples and States of origin returning 1^+, 2^+ and 3^+ scores of *P. larvae* subsp. *larvae* are shown in Table 5.

Table 5. Details of 8 individual honey samples showing States or origin and honey identification returning 1^+, 2^+ and 3^+ scores of *P. larvae* subsp. *larvae*.

***P. larvae* subsp. *larvae* score**	**1^+**	**2^+**	**3^+**
P. larvae subsp. *larvae* positive honey samples	QLD - 1 NSW - 7 NSW - 8* SA - 9* WA - 8*		QLD - 4 VIC - 11* TAS - 1
TOTAL	5	0	3

*Sample purchased from retail outlet.

1.3.2. Chalkbrood

The number of honey samples tested, the numbers and percentages of positive identifications using either SDA or YGP medium are shown in Table 6.

Table 6. Numbers and percentages of honey samples tested and numbers and percentages of States yielding positive *A. apis* culture results.

	Number tested	**Number positive**	**% Positive**	**Number negative**	**% Negative**
Honey samples	60	7	11.7	53	88.7
States and territories of Australia	7	4	57.1	3	42.9

The numbers of honey samples originating from each State and Territory and the numbers and percentages of honeys returning positive results from each State are shown in Table 7.

Table 7. Numbers and percentages of honeys from each State and Territory yielding positive *A. apis* results.

	QLD		NSW		VIC		SA		WA		NT		TAS	
Honey samples	**No. tested**	**No. positive (%)**	**No. tested**	**No. positive (%)**	**No. tested**	**No. positive (%)**	**No. tested**	**No. positive (%)**	**No. tested**	**No. positive (%)**	**No. tested**	**No. positive (%)**	**No. tested**	**No. positive (%)**
60	10	0 (0)	9	1 (11.1)	11	2 (18.2)	9	3 (33.3)	9	1 (11.1)	6	1 (16.7)	7	0 (0)

Six honey samples cultured positive for *A. apis* on YGP medium but returned negative results using SDA medium.

Table 8 shows details of honey samples and States of origin yielding positive results with either SDA on YGP culture medium.

Table 8. State of origin and honey identification of samples yielding positive *A. apis* results from SDA and YGP media.

Honey Identification	Culture Media	
	SDA	**YGP**
NSW - 7	-	+
VIC - 1	-	+
VIC - 6	-	+
SA - 1	-	+
SA - 4	+	+
SA - 5	-	+
NT - 2	-	+

+ = *A. apis* isolated
- = No *A. apis* isolated

1.4. Discussion

1.4.1. American Foulbrood

AFB disease of honeybees was first detected in Australia last century (M Hornitzky *pers. com*) and since that time the disease has been diagnosed in all states of Australia. However, at the time of writing no AFB has been diagnosed in the NT.

The results of this survey indicate that the prevalence of AFB in Australian apiaries is fairly low as only 13.3% of honeys surveyed returned positive *P. larvae* subsp. *larvae* results. However as the sample size in this study was small, the findings may not be a true indication of the current AFB status in all states.

In a recent survey to detect the latent level of AFB infection amongst share holders of "Capilano Honey" (W. Ward unpublished data) 16 of 43 honey samples (37.2%) originating from Victorian apiarists returned positive *P. larvae* subsp. *larvae* culture results compared to 1 of 9 (11.1%) detected in this survey. Conversely the results obtained in this survey for

QLD honey samples showed that 2 of 10 samples cultured (20.0%) were contaminated with spores of *P. larvae* subsp. *larvae*. These results therefore are not indicative of the true prevalence of AFB in QLD. At the time of writing, with over 90% of the Queensland industry tested, the prevalence of AFB is 8.1%.

In spite of these discrepancies the authors believe that the AFB control programs presently operating in Australia will ensure that the prevalence of AFB in this country will decline. These results obtained in this survey have returned a considerably lower prevalence of AFB in Australia compared to the 47.2% of positive AFB samples detected in 36 honeys from international sources (F Trueman, W Ward, H Mawhinney 1996).

1.4.2. Chalkbrood

Although Chalkbrood disease has been known to occur in Europe, USA, Canada, New Zealand and some of Asia (Bailey and Ball 1991c, Seal 1957, Rose and Christensen 1984, Matheson 1993) it was not reported in Australia until 1993 (Hornitzky and Anderson 1993). Chalkbrood disease has now spread within Australia to NSW, VIC, SA, WA and NT and TAS.

Isolations of *A. apis* in this study were low as only 7 (11.7%) of 60 honey samples returned positive identifications of Chalkbrood disease. The results reported in this survey confirm the presence of this disease in NSW, VIC, NT, SA and WA. Although no *A. apis* was cultured from the 10 honeys originating from QLD Chalkbrood disease is known to be present in that State (Hornitzky and Anderson 1993).

A. apis can be grown on a variety of culture media most of which contain yeast extract or malt extract (Heath 1982) and on Sabourauds Dextrose Agar (SDA). SDA is a well recognised medium for the primary isolation of fungi from a variety of sources. This medium has been used extensively as a diagnostic tool at this Institute for many years, and was used for *A. apis* isolations when chalkbrood was first identified in Queensland. Anderson (1989) tested a number of media to determine the most sensitive technique for the culture of *A. apis*. He concluded that the yeast/glucose phosphate agar (YGP) used in this study was the most suitable media for detecting small inocula of *A. apis*. However, SDA was not included as a test medium in Anderson's study.

In this survey 6 of 7 isolations of *A. apis* were recorded with YGP medium when negative results were returned with SDA medium. The remaining positive diagnosis of *A. apis* was made with both YGP and SDA media.

In a previous report using international honey samples (Trueman, Ward and Mawhinney 1996) the YGP and SDA medium returned identical isolations of *A. apis* except for one honey which cultured positive using SDA and negative with YGP medium.

The YGP medium in this survey appeared superior to SDA. Since the fungal spores are assumed to be distributed evenly throughout any given honey sample, the discrepancies with the results using these media preparations are unable to be explained. The YGP medium was recommended by Hornitzky and Anderson 1993 for routine diagnostic use for isolation of *A. apis* and the present study confirms this choice of medium.

2. Microbiological Quality of Australian Honeys

2.1. Introduction

With the development of agar media, bacterial colony counts have become recognised methods of estimating microbial flora in food products. The aerobic plate count method has wide application in the field of food microbiology, and relies on the ability of a bacteriological medium to support the growth of the microflora in a particular food sample. This medium must produce visible bacterial colonies after a predetermined incubation time and temperature. The type of media and growth conditions vary according to the type of food, and the type of microorganisms likely to be present in that food.

Honey is a confectionary product with a high concentration of sugar and low water (a_w) content. Microflora which are capable of living and multiplying in this type of environment are termed osmophilic organisms.

The types of bacteria which have been isolated from ripened honey (other than those which are responsible for honeybee disease) are *Bacillus* sp and *Clostridia* sp (White 1992). Honey is a known source of *Clostridium botulinum* spores which has caused cases of infant botulism (Midura *et al* 1979).

Osmophilic yeasts are responsible for the fermentation of honey. Fermentation is caused by the action of the yeasts on the dextrose and levulose in the honey, producing alcohol and carbon dioxide and this process is heavily dependent on the moisture content of the honey (White 1992). Yeasts isolated from honey include *Zygosaccharmyces* spp and to a lesser extent *Saccharomycess* spp (White 1992).

Moulds can cause spoilage of high sugar products by the development of visible growth and by the production of a musty odour and taste (Lenovich and Konkel 1992). Moulds in this class of mycoflora include *Rhizopus, Penicillium* and *Aspergillus* genera.

Since all microorganisms present in a given food product may not multiply under the experimental conditions selected in a given survey, aerobic colony counts are an estimate only of the total microbial flora, and should not be treated as absolute values (Swanson *et al* 1992).

This paper describes microbiological examination of Australian honeys to determine their quality and suitability for human consumption. This work will provide a valuable guide for honey packers in this country who are introducing quality assurance programs into the honey industry. The scientific material reported in this survey could be utilised as part of an overall HACCP plan for the Australian honeybee industry.

2.2. Materials and Methods

2.2.1. Materials

Sixty honey samples originating from Queensland (QLD), New South Wales (NSW), Victoria (VIC), South Australia (SA), Western Australia (WA), Tasmania (TAS) and Northern Territory (NT) were examined in this survey. The honey samples were collected by Apiary Officers in each State and Territory between October 1997 and April 1998. Fifty-two (87%) of the 60 samples were representative of individual apiarist operations, and these samples were collected from drums destined for the honey packer. Eight (13%) of the honey samples were purchased from retail outlets in QLD and were representative of packers in QLD, NSW, SA, VIC and WA.

The number of individual honey samples including those from retail outlets from any one State ranged from a maximum of 11 to a minimum of 6.

The samples were identified by an abbreviation of each State followed by a numerical identification number.

The States of origin, numbers and percentages of individual honey samples collected for this study are shown in Table 9.

Table 9. State of origin, numbers and percentages of representative honey samples

	State of Origin	Number of Representative honeys	Percentage of representation honeys
	QLD	10	17
	NSW	9	15
	VIC	11	18
	SA	9	15
	WA	8	13
	NT	6	10
	TAS	7	12
Total	7	60	100

2.2.2. Methods

2.2.2.1. Bacteria

Preparation of honey dilutions and inoculation of plates

Enumeration of bacteria on each honey sample was assessed using Plate Count Agar (PCA) and 2 separate diluents

(a) 0.1% peptone water
(b) 0.1% peptone water + 40% glucose

Duplicate 10 gm samples of test honeys were diluted with media preparations (a) and (b) as described above. The honey samples were dissolved by mixing on a vortex mixer. The preparation of subsequent dilutions of test honey samples and inoculation and pouring of

plates were carried out as described in Food microbiology Australian Standard AS1766-2.1 (1991). After each honey dilution was prepared 5mls were added to the next 5mL dilution blank, and 1ml was transferred to each of the three bacteriological plastic petrie dishes. This procedure was repeated for each honey dilution, resulting in triplicate test plates for each dilution.

Approximately 15ml molten PCA which had been held in a water bath at 48°C was added to each petrie dish. A control plate containing molten PCA only was poured for each 1 litre volume of medium used. After all plates had been poured, inoculum and mecium were mixed, by five to and from movements, five circular clockwise movements, and five to and fro movements at right angles first set, followed by five figure of 8 movements.

Incubation

When the medium in all test and control plates had set, the plates were incubated at 30°C for 72 hours.

Counting of colonies

Bacterial colonies were counted as described by Swanson *et al* (1992) using a colony counter with magnification, illumination and a tally. Doubtful material was examined under a plate microscope, or by preparing smears for dark ground examination (DGE). All culture plates yielding 25 – 250 colonies were counted. For each dilution the average number of colonies on triplicate plates was calculated. This number was multiplied by the dilution factor to obtain colonies per mL of honey. When more than one dilution returned 25-250 colonies the number of colonies per mL of honey was calculated for each dilution and the arithmetic mean was taken as the final result.

If all plates yielded fewer than 25 colonies, the actual number of colonies at dilution 1:2 were counted, and the results were reported as 'estimated' (est).

When plates from all dilutions had no colonies, the final result was reported as <2 (est) i.e. <one times the lowest dilution.

If the number of colonies on each plate was greater than 250 colonies, the colonies in 4 representative 1cm squares were counted. The average number of colonies per square cm was multiplied by 56 to obtain the number of colonies per ml of honey ($56cm^2$ is the area of standard plastic petrie dishes used for these counts). This result was also reported as (est).

When the number of colonies on crowded plates exceeded 100 per square cm, the number of colonies per gram of honey was recorded as > plate area x 100 multiplied by highest dilution factor, and reported as (est).
To calculate the total numbers of colony forming units (CFU's) per gram of honey, the number of colonies per ml of honey was multiplied by 1.67 to account for the average volume increase observed when 10gms honey was diluted with 10mL diluent.

2.2.2.2. Yeasts and Moulds

Enumeration of yeasts and moulds on each honey sample was assessed using malt extract yeast extract 40% glucose (MY40G) agar, Pivnick and Gabis (1984), and 2 separate diluents

(a) 0.1% peptone water
(b) 0.1% peptone water + 40% glucose

The MY40G medium was prepared by combining malt extract powder, yeast extract and agar with distilled water. These ingredients were dissolved by steaming followed by the addition of AR glucose. This resulted in a medium which was of low water (a_w) content and high sugar concentration.

Preparation honey dilutions and inoculation of plates
The test honey samples were diluted as previously described for bacterial plate counts.

Inoculation and pouring of plates was carried out as described in Food Microbiology Australian Standard 1766-2.2 (1991) and was identical to that described for bacteria.

Incubation
Test and control plates were incubated at 30°C for 5 days.

Counting of colonies
Yeasts were identified as small white, mucoid colonies. Dark-ground examination (DGE) revealed spherical or ellipsoidal cells which were reproducing by budding. Fungal species formed large filamentous colonies with a cottony or powdery appearance. Vegetative mycelia around the edges of the colonies were seen when examined microscopically.

Colony counts for yeasts and moulds were carried out as previously described for bacteria. Separate counts and calculations were recorded for yeasts and for moulds, and a total value of CFU's mycoflora per gram of honey was computed for each honey sample.

2.3. Results

2.3.1. Bacterial Plate Counts

Bacterial plate counts for the 60 individual honeys ranged from <10 to >2000 colony forming units (CFU's) per gram honey irrespective of the diluent used to prepare the culture plates. Forty-six honey samples (76.7%) yielded less than 250 CFU's per gram honey while only 3 (5.0%) yielded >1000 CFU's per gram honey.

Table 10 shows numbers of percentages of honey samples with bacterial CFU's ranging from <250 to >1000.

Table 10 Numbers and percentages of honey samples returning bacterial colony counts of <250 to >1000 CFU's/gram honey

Bacterial CFU's per gram honey					
	<250	250-500	500-1000	>1000	Total
Number of honey samples and (%)	46 (76.7)	4 (6.7)	7 (11.7)	3 (5.0)	60 (100)

The state of origin, honey identifications and calculated numbers CFU's per gram honey for each of the 60 individual honey samples using 0.1% peptone water and 0.1% peptone water + 40% glucose diluents are listed in Table 11.

Table 11. **State of origin, honey identifications and total bacterial counts (CFU's/gram honey) for 60 individual samples using either 0.1% peptone water or 0.1% peptone water +40% glucose as diluent.**

State of Origin and Honey Identification		Diluents	
		0.1% peptone water	0.1% peptone water + 40% glucose
QLD	1	276	368
	2	14 (est)	8 (est)
	3	114	86
	4	12 (est)	24
	5	9 (est)	9 (est)
	6	35 (est)	17 (est)
	7	27 (est)	28 (est)
	8	5 (est)	7 (est)
	9	35 (est)	2 (est)
*	10	98	38 (est)
NSW	1	27 (est)	18 (est)
	2	1 (est)	2 (est)
	3	205	154
	4	13 (est)	4 (est)
	5	207	194
	6	2 (est)	12 (est)
	7	7 (est)	6 (est)
*	8	225	194
*	9	164	130
VIC	1	110	97
	2	204	152
	3	493	398
	4	31 (est)	21 (est)
	5	3 (est)	8 (est)
	6	20 (est)	5 (est)
	7	**758**	**678**
	8	**1492**	**1619**
*	9	187	223
*	10	120	116
*	11	**1535**	**1559**
SA	1	8 (est)	9 (est)
	2	**873**	**798**
	3	11 (est)	7 (est)
	4	387	228
	5	250	150
	6	138	90
	7	6 (est)	13 (est)
	8	5 (est)	15 (est)
*	9	**1125**	**958**
WA	1	12 (est)	7 (est)
	2	0 (est)	1 (est)
	3	11 (est)	1 (est)
	4	2 (est)	4 (est)
	5	8 (est)	3 (est)
	6	107	8 (est)
	7	3 (est)	2 (est)
*	8	453	283

State of Origin and Honey Identification		Diluents	
		0.1% peptone water	0.1% peptone water + 40% glucose
NT	1	32 (est)	58
	2	**1034**	**885**
	3	160	91
	4	292	331
	5	**714**	**2131**
	6	169	131
TAS	1	**837**	**678**
	2	**814**	**724**
	3	134	187
	4	86	31
	5	16 (est)	32 (est)
	6	**504**	493
	7	25	30

*R = Honey samples purchased from retail outlets

2.3.2 Yeasts and Moulds

Total CFU's for yeasts and moulds combined for 60 honey samples screened ranged from <2 to >73178 per gram honey, when 0.1% peptone water + 40% glucose was used as diluent. In contrast, when 0.1% peptone water was used as diluent the range of total CFU's for yeasts and mould was from <2 to 5534 CFU's per gram honey.

Table 12 shows number and percentage of honeys and number of states with representative honey samples returning >10 CFU's yeasts and moulds combined per gram honey using MY40G culture media and 0.1% peptone water and 0.1% peptone water + 40% glucose as honey diluents.

Table 12. Number and percentages of honey samples and States of Australia yielding >10 CFU's yeasts and moulds combined per gram honey using MY40G culture medium and 0.1% peptone water and 0.1% peptone water + 40% glucose as diluents.

	Diluents	
	0.1% peptone water	**0.1% peptone water + 40% glucose**
Number of honey samples and (%)	17 (28.3)	19 (31.7)
Number of States and (%)	6 (85.7)	6 (85.7)

The States of Origin, the identifications and calculated total values of CFU's of yeasts and moulds for 60 honey samples using MY40G culture medium and 0.1% peptone water and 0.1% peptone water + 40% glucose are shown in Table 13.

Table 13. States of origin, honey identifications and total values of yeasts and moulds combined using MY40G culture medium and 0.1% peptone water and 0.1% peptone water + 40% glucose.

State of Origin and Honey Identification		Diluent					
		0.1% peptone water			0.1% peptone water +40% glucose		
		Yeasts	Moulds	Total Yeasts and Moulds	Yeasts	Moulds	Total Yeasts and Moulds
QLD	1	<2	<2	<2	<2	<2	<2
	2	<2	<2	<2	<2	<2	<2
	3	4 est	4 est	8 est	**58**	2 est	**60**
	4	<2	<2	<2	<2	<2	<2
	5	<2	<2	<2	<2	<2	<2
	6	**4312**	<2	**4312**	**7504**	<2	**7504**
	7	<2	<2	<2	<2	<2	<2
	8	<2	<2	<2	<2	<2	<2
	9	<2	<2	<2	<2	<2	<2
	10	<2	<2	<2	<2	<2	<2
NSW	1	9 est	<2	9 est	7 est	1 est	8 est
	2	<2	<2	<2	<2	<2	<2
	3	<2	<2	<2	<2	<2	<2
	4	7 est	9 est	**16 est**	**19 est**	**19 est**	**38 est**
	5	<2	4 est	4 est	<2	2 est	2 est
	6	<2	6 est	6 est	<2	4 est	4 est
	7	<2	8 est	8 est	<2	**10 est**	**10 est**
	8	<2	2 est	2 est	<2	<2	<2
	9	<2	<2	<2	<2	<2	<2
VIC	1	<2	<2	<2	<2	<2	<2
	2	<2	<2	<2	<2	<2	<2
	3	<2	2 est	2 est	<2	<2	<2
	4	3 est	**36 est**	**39 est**	1 est	**31 est**	**32 est**
	5	<2	<2	<2	<2	<2	<2
	6	<2	<2	<2	<2	6 est	6 est
	7	**99**	**269**	**368**	**114**	**212**	**326**
	8	<2	<2	<2	<2	<2	<2
	9	<2	4 est	4 est	<2	7 est	7 est
	10	<2	<2	<2	<2	<2	<2
	11	<2	<2	<2	<2	<2	<2
NT	1	<2	<2	<2	<2	<2	<2
	2	**91**	4 est	**95**	**41 est**	<2	**41 est**
	3	**518**	<2	**518**	**1980**	2 est	**1982**
	4	**477**	2 est	**479**	**1293**	6 est	**1299**
	5	**5534**	<2	**5534**	**73178**	<2	**73178**
	6	<2	<2	<2	<2	<2	<2

State of Origin and Honey Identification		Diluent					
		0.1% peptone water			0.1% peptone water +40% glucose		
		Yeasts	Moulds	Total Yeasts and Moulds	Yeasts	Moulds	Total Yeasts and Moulds
WA	1	<2	<2	<2	<2	<2	<2
	2	<2	<2	<2	<2	<2	<2
	3	<2	<2	<2	<2	<2	<2
	4	1 est	1 est	2 est	2 est	<2	2 est
	5	<2	<2	<2	<2	<2	<2
	6	1 est	5 est	6 est	2 est	5 est	7 est
	7	<2	<2	<2	<2	<2	<2
	8	<2	<2	<2	<2	<2	<2
TAS	1	<2	<2	<2	<2	<2	<2
	2	<2	<2	<2	<2	<2	<2
	3	**35 est**	7 est	**42 est**	**141**	8 est	**149**
	4	**223**	8 est	**231**	**304**	6 est	**310**
	5	**37 est**	4 est	**41 est**	**304**	4 est	**34 est**
	6	4 est	1 est	**5 est**	**14 est**	4 est	**18 est**
	7	**163**	**239**	**402**	**319**	**702**	**1021**
SA	1	<2	3 est	3 est	<2	2 est	2 est
	2	<2	**121**	**121**	<2	**154**	**154**
	3	<2	<2	<2	<2	<2	<2
	4	<2	20 est	**20 est**	<2	**160**	**160**
	5	**20 est**	5 est	**25 est**	**17 est**	<2	**17 est**
	6	**466**	6 est	**452**	**358**	6 est	**364**
	7	<2	<2	<2	<2	3 est	3 est
	8	<2	<2	<2	<2	<2	<2
	9	<2	<2	<2	<2	<2	<2

2.4 Discussion

2.4.1. Bacteria

Interpretation of bacterial plate counts of honey is difficult due to the lack of published standards relating to acceptable numbers of bacterial flora in this food product. Lenovich and Konkel (1992) state that aerobic plate counts are generally less than 10,000 per gram for sugar confectionary products. Bonvehi and Jorda (1993) report that 10,000 CFU's per gram honey is the maximum number of bacterial colonies permitted in honey from Spain. Coopers Fine Foods in Australia have set a limit of 5000 CFU's per gram of honey (G Kleinschmidt pers. comm.) and Arnott's Biscuits, Australia require a standard of not greater than 10,000 CFU's/gram honey (L Dimitriou pers. comm.). Bonvehi and Jorda (1993) studied the microbiological quality of 72 samples of Spanish honey, and reported plate counts ranging from 80-1000 CFU's per gram of honey. These workers claimed that aerobic plate counts were unreliable due to the presence of spreading bacteria. To overcome this problem they used a membrane filter method to count honey samples heavily contaminated with spreading organisms.

Spreading bacteria were not found to cause major problems in the honey samples tested in the present survey, and no specialised techniques were required to obtain bacterial counts for the 60 samples screened.

The practise of testing samples in doubling dilutions from 1:2 to 1:8 is unusual when enumerating bacterial plate counts in food products. However, confectionary products, such as honey, which have a high concentration of sugar and low water content, do not harbour large numbers of bacterial flora. Consequently, if countable plates are to be obtained low dilutions are necessary (Swanson, Busta *et al* 1992).

The results of the present work have indicated that, for enumeration of bacteria, the 0.1% peptone water and 0.1% peptone water + 40% glucose diluents returned similar results, and that therefore low a_w content in diluent is not a critical factor when assessing honey for bacterial flora.

In a previous survey using 39 honeys from International sources (Ward, Trueman, Mawhinney 1996) 32 (82.1%) honey contained bacterial counts of <1000 CFU's per gram honey and 31 or 79.5% returned bacterial counts of <500 CFU's per gram honey. The authors at that time reported that 1000 CFU's per gram honey could be considered as a benchmark for good manufacturing practice. In the present study using honey from Australian sources 57 (95.0%) of the 60 samples screened returned bacterial plate counts of <1000 CFU's per gram honey and 50 or (83.3%) contained bacterial counts of <500 CFU's per gram honey. These results confirm that a standard of <1000 CFU's can be met by most samples tested, but that bacterial counts of <500 CFU's per gram honey may be a reasonable aim for QA programs for the Australian Honey Industry.

2.4.2. Yeasts and Moulds

The successful enumeration of yeasts and moulds from food products is dependent on the choice of the most suitable medium. This in turn is governed by the nature of the food product under investigation Mossel *et al* (1975) and Seiler (1986).

Honey is a food product which is high in sugar content and low in water content. Therefore, a medium with similar sugar and water concentrations is required for growth of the osmophilic mycoflora likely to be present in normal honey.

In a previous survey using honey from International sources (Trueman, Ward and Mawhinney) we found that MY40G medium (Pivnick & Gabis 1984) was satisfactory for enumerating yeasts and moulds in honey samples. However, we also suggested that further work may be necessary to determine the most sensitive medium or media and diluents for determining mycofloral quality of honey.

Baross and Lenovich (1992) suggested that successful enumeration of osmophilic yeasts can be dependent on choice of both culture media and diluents. The workers claim that inaccurate results may be obtained if a high a_w diluent or agar medium is used or if the agar medium has a reduced a_w but the diluent does not.

In the present survey we trialled MY40G medium using two separate diluents ie 0.1% peptone water and a low water high sugar diluent consisting of 0.1% peptone water + 40% glucose. Our results have shown that in most cases the use of low water, high sugar diluent resulted in recovery of higher numbers of total yeasts and moulds than the diluent consisting of 0.1% peptone water alone.

Published values for acceptable levels of mycoflora in honey are conflicting. Deak *et al* (1986) suggest that 10 CFU's yeasts or moulds per gram honey could be considered as an established base-line count. Lenovich and Konkel (1992) report that in confectionery products, yeast counts usually do not exceed 100 CFU's per gram and that mould counts are also rarely above 100 CFU's per gram. Coopers Fine Foods ' Australia have set a limit of <100 CFU's per gram honey for yeasts and moulds combined (G Kleinschmidt pers. comm.) and Amott's Biscuits, Australia also suggest a combined count of not greater than 100 CFU's per gram honey.

While overall counts of yeasts and moulds can be a useful indicator of the hygienic handling of honey, in certain circumstances the presence of specific yeasts may be unacceptable. Baross and Lenovich (1992) claim that counts of 10 CFU's per gram or fewer of yeasts in liquid sugars or syrups could be highly significant if the yeast is a spoilage type such as *Zygosaccharomyces rouxii.* These workers suggest that low counts of yeasts and moulds (10 or fewer CFU's per gram) may be indicative of inadequate processing. Such low levels of contamination by yeasts require identification to species, so that an effective course of action can be implemented.

Jermini *et al* (1987), studied 40 samples of sugar-rich food products including 4 honey samples. Over 50% of the food products returned *Zygosaccharomyces* species of yeast and Z. *rouxii* was isolated from all 4 of the honey samples. Farris *et al* (1985) examined 52

honey samples in Sardinia and found Z. *rouxii* to be present in high concentrations in some honeys. Poncini and Wimmer (1 985) cultured 7 honey samples from different locations in Fiji and isolated either *Zygosaccharomyces* sp or *Saccharomyces* spp of yeast from 4 of the 7 samples. A comprehensive study of the types of osmophilic yeasts and moulds in normal honey was conducted by Lochhead and Farrell (1931). They isolated 8 different species of yeasts including *Zygosaccharomyces Schizosaccharomyces and Tortila* spp. This study concluded that the predominating yeast in a sample is not necessarily the cause of fermentation. *However, Zygosaccharomyces* spp were found to be the most abundant yeast species encountered after fermentation.

Fermentation of honey by yeasts is dependent on the moisture content of the honey and on the concentration of yeast in any particular sample. Regardless of yeast count, a honey sample is unlikely to ferment if the moisture content is less than 17. 1 %. If a moisture content is of up to 18% the honey is still safe from fermentation provided the yeast count is less than 100 CFU's per gram of honey. However, if the moisture content is as high as 19% a yeast count of less than 10 CFU's per gram honey can cause fermentation, and a honey sample with a moisture content of over 20% is always in danger of fermentation (Lochhead 1993).

The results showed that high counts of yeasts and moulds were recovered from honey samples primarily from Northern Territory and Tasmania where temperature ranges are very different. However, the temperature range for growth yeasts and moulds is a broad one (5^0 to 35^0), with some species capable of growth above and below range (Mislivec *et al* 1984). Temperature differences between these two states could therefore not necessarily explain the high recorded values of total yeasts and moulds from these 2 states. Since air, dust and particularly moisture are important factors in microbial transmission, conditions of high humidity as experienced in Northern Territory and the cold, wet conditions often occurring in Tasmania may account for the high levels of contamination detected in honeys from these States. Honey produced in tropical areas of Australia such as Northern Territory generally has a high moisture content, and the authors feel that this is probably a major factor in the high yeast and mould values recorded from honeys from this Territory. It is highly probable that bees are unable to adequately cure the unextracted honey and undoubtly some uncapped honey is also extracted. Moisture is probably a significant factor in high rainfall areas of Tasmania (eg 1000 - >2000mm annually, Barnes–Keoghan meteorologist, Tasmania pers comm). Frequent frosts in late or mid-summer and the cold conditions occurring in that State indicated moisture may be continually present in beekeeping equipment producing favourable conditions for proliferation yeasts and moulds. Improved hygiene and the introduction of de-humidifiers in extraction houses are required in beekeeping areas where humidity is high and where high moisture content can affect the quality of honey.

The results of the present survey showed that 41 (68.3%) of 60 honeys returned <10 total CFU's yeasts and moulds combined per gram honey using the MY40G medium and diluent containing a high concentration of sugar, and that 48 or 80% of the 60 honeys contained <100 total CFU's per gram honey yeasts and moulds combined using the same medium and diluent. In a previous study using 39 honeys from International sources 28 (71.8%) returned combined total of yeasts and moulds of <10 CFU's/gram honey and 29 (74.4%) returned <1000 CFU's/gram honey yeasts and moulds combined. However, in that study a high water concentration diluent of 0.1% peptone water was used in the testing protocol. However, since the results of the present study showed that a diluent containing 0.1% peptone water and 40% glucose recovered more yeast and mould colonies than the 0.1% peptone water alone, more of the International honeys may have returned greater number of colonies than those reported above.

Eight percent of the 60 Australian honeys screened contained <100 CFU's yeasts and moulds combined. It is clear therefore that this value could be considered as a reasonable standard for honey in terms of yeast and mould colonies. However, as more than half of the Australian honeys (68.3%) returned <10 CFU's per gram of yeasts and moulds combined, this value may not be an unreasonable aim for the Honey Industry in this country.

3. Detection Of Chemical Residues In Australian Honeys

3.1. Introduction

Honey is promoted as a healthy natural product. In order to maintain this image, reassurance must be given that honey is free of any chemical residues or additives. This applies to both domestic supplies, and any honey that may be imported. The recent pesticide residue problems in the Australian beef industry illustrates how quickly international trade can be affected and non-tariff trade restrictions applied.

Australian apiarists do not use pesticides (organochlorines and organophosphates) as part of beekeeping husbandry. However, a range of such pesticides are used worldwide in agricultural areas, and have been associated with widespread loss of colonies. Bees collecting contaminated nectar usually die before returning to their hive, but the possibility of storing contaminated honey and pollen remains (Warhurst and Goebel 1995).

Heavy metals may contaminate honey particularly during storage. In Australia the use of galvanised steel drums for storage and transport has resulted in unacceptable levels of zinc in honey. Antibiotics continue to be used in the control of brood diseases, and if used inappropriately or at high levels will result in contaminated of honey (Goodman and Azoulas 1994).

As recently as 2 years ago, Phenol was commonly used by Australian apiarists to drive bees from the honey supers prior to extracting honey. Some contamination of honey was detected (Mawhinney 1993), and it was recommended that the practice discontinue.

This paper describes residue examination of 60 honeys representing all States and Territories of Australia.

3.2. Materials and Methods

3.2.1. Materials

3.2.1.1. Honey Samples

Sixty honey samples originating from Queensland(QLD), New South Wales (NSW), Victoria (VIC), South Australia (SA), Western Australia (WA), Tasmania (TAS) and Northern Territory (NT) were examined in this survey. The honey samples were collected by Apiary Officers in each State and Territory between October 1997 and April 1998.

Fifty two (87%) of the sixty samples were from drums of representative individual apiaries and these samples were collected from drums destined for the honey packers. Eight (13%) honey samples were purchased from retail outlets in Queensland and were representative of honey packers in QLD, NSW, SA, VIC and WA.

The numbers of individual honey samples including those from retail outlets from any one State or Territory range from a maximum of 11 to a minimum of 6. The samples were identified by State followed by a numerical identification number. All honey samples were cultured for AFB and Chalkbrood disease agents.

The States of origin, numbers and percentages of individual honey samples examined in this paper are shown in Table 14.

Table 14. Honey samples collected from each State and Territory and number of and percentages of samples representative of each State.

	State of Origin	Number of Represented Honeys	Percentage of Representative Honeys
	QLD	**10**	**17**
	NSW	**9**	**15**
	VIC	**11**	**18**
	SA	**9**	**15**
	WA	**8**	**13**
	NT	**6**	**10**
	TAS	**7**	**12**
TOTAL	**7**	**60**	**100**

All 60 honey samples were tested for the presence of antimicrobial residues and residues of fumagillin and phenol. All honey samples were also screened for 17 organochlorine type pesticides, 17 organophosphorous type pesticides and for 11 synthetic pyrethroid residues (see Appendix 1).

3.2.2. Methods

3.2.2.1. Antimicrobial Residues

Honey samples were screened for the presence of antimicrobial residues using the laboratory's five plate bioassay procedures. This procedure was developed in-house and is based on methodologies used by Canadian and Japanese laboratories. An inovative extraction followed by sample concentration allows quite low Limits of Detection (LODs) to be achieved. The bioassay permits the presumptive identification of sulphonamide, tetracycline, aminoglycoside, marcolide and beta lactam antimicrobials to at least class level. A series of validate confirmatory procedures based on HPLC techniques complement the screen and provide quantitative results.

The Level of Reporting (LOR) for antimicrobials is as follows:

Tetracyclines ..... 0.05 mg/Kg

Oxytetracycline ..... 0.05 mg/Kg

Chlortetracycline ..... 0.05 mg/Kg

β-lactams
- Amoxicillin ..... 0.3 mg/Kg
- Ampicillin ..... 0.02 mg/Kg
- Penicillin ..... 0.02 mg/Kg
- Cloxacillin ..... 0.05 mg/Kg

Aminoglycosides
- Streptomycin ..... 0.1 mg/Kg
- Dihydrostreptomycin ..... 0.1 mg/Kg
- Neomycin ..... 0.1 mg/Kg

Others
- Tilmicosin ..... 0.1 mg/Kg
- Tylosin ..... 0.2 mg/Kg
- Erythromycin ..... 0.2 mg/Kg
- Lincomycin ..... 0.2 mg/Kg

Sulphonamides ..... 0.05 mg/Kg

3.2.2.2. Pesticide Residues

Honey samples were analysed for a range of Organochlorine (OC), Organophosphorus type (OP) pesticides and Synthetic Pyrethroid (SP) residues. The procedure used solvent extraction, liquid-liquid partition and a Florisil clean-up prior to determination by Gas Chromatography using Electron Capture and Nitrogen Phosphorus detectors.

Limits of Quantitation:	OCs	0.02 mg/Kg
	OPs	0.05 mg/Kg
	SPs	0.05 mg/Kg

3.2.2.3. Elemental Residues

Honey samples were analysed for the following suite of elements: aluminium, arsenic, chromium nickel, cadmium, copper, iron, mercury, lead, selenium and zinc. The procedure involved a microwave assisted nitric acid digestion of the honey prior to determination by Inductively Coupled Plasm-Mass Spectrometry.

Limit of Quantitation: 0.01 mg/Kg

3.2.2.4. Fumagillin Residues

The honey samples were analysed for fumagillin residues using a simple extraction procedure prior to determination by High-Performance Liquid Chromatography using Ultraviolet Detection.

Limit of Quantitation: 0.05 mg/Kg

3.2.2.5. Phenol Residues

Honey samples were analysed for phenol residues using a solvent extraction procedure followed by determination using Gas Chromatography with a Flame Ionisation Detector.

Limit of Quantitation: 0.01 mg/Kg

3.3. Results

All 60 honeys were found to be free of antimicrobial residues, organochlorines, organophosphorus type pesticdes, synthetic pyrethroids at the levels of reporting (LOR). All honeys were also found to be free of fumagillin and phenol residues.

Low levels of Aluminium, Iron and Zinc were detected in most honeys, but were not considered of significance.

Two honeys returned unacceptable levels of Lead in excess of 0.5 mg/kg maximum allowable level. One honey from Northern Territory recorded 1.5 mg/kg and 1 from Victoria returned a level of 4.8 mg/kg.

Full results for elemental analyses are listed in Appendix 2.

3.4. Discussion

The results obtained in this survey for Organochlorine, Organophosphorus Type Pesticides fumagillin, phenol and antibiotic residues compare favourably with a previous study conducted using International Honeys (Trueman, Ward and Mawhinney 1996). In that survey 6 (14.3%) of 42 honeys contained levels of phenol ranging from 0.07 to 0.51 mg/kg. Fumagillin was detected in 1 (0.2%) honey at a level of 0.01 mg/kg and 0.08 mg/kg oxytetracline was recorded in 1 (0.2%) honey.

Antibiotics are often used for treatment of European foulbrood, and in some countries to mask American foulbrood. If used incorrectly residues can persist in honey for a considerable time (Goodman and Azuolas 1994). The results obtained in this survey for antibiotic residues are indicative of responsible use of oxytetracycline in Australia.

The levels of lead in 2 of the honeys screened in this report were of particular concern and immediate investigations were undertaken to determine the source of the lead contamination. Further honey samples and subsequent testing revealed that soldering points in extracting plants can result in seepage of lead into residual honey which may be left in that equipment. It is recommended that measures such as coating soldering points with food grade paint and frequent cleaning of extracting equipment be routinely adopted to ensure that hygienic practices are maintained in all individual beekeeping operation.

4. Conclusions and Recommendations

4.1. Conclusions

Thirty-two (53.3%) of 60 honeys from all States and Territories of Australia screened in this survey either contained honeybee disease agents (American Foulbrood, Chalkbrood) greated than recommended numbers of bacteris (<1000 CFU's), combined yeasts and moulds (<10 CFU's) or unacceptable chemical residues. These results compare favourably with those found in a previous survey of International honeys in which 76% of 42 samples screened contained disease agents, unacceptable levels of bacteria, yeasts, moulds and chemical residues.

Two honey samples screened in this survey were contaminated with lead, and further honey sampling from the offending apiaries was immediately undertaken. It was determined that soldering in honey extractors results in seepage of lead into residual honey left in extracting equipment.

Although the sample size in this work was small, the results presented provide an important quality benchmark for HACCP programs envisaged for the Australian Honey Industry.

It is concluded, that although fewer Australian honey samples contained honeybee disease agents and unacceptable levels of bacteria, yeast and mould contaminants compared to International samples, the excessive numbers of yeast and mould contaminants in some honeys and lead levels in two samples are of concern. The Australian Honeybee Industry has no cause for complacency if Quality Assurance programs are to succeed.

4.2. Recommendations

As a result of this survey the authors believe that a number of recommendations relating to Quality Assurance programs for the Honeybee Industry can be made.

They are as follows:-

- current AFB control programs should be maintained to ensure a continuing decline in the prevalence of AFB in Australia
- enumeration of bacteria should be performed as described in this report using 0.1%peptone water as honey diluent and standard plate count Agar as growth medium
- enumeration of yeasts and moulds should be performed as described using 0.1% peptone water + 40% glucose as diluent and MY40G as growth medium.
- the benchmark limits for Quality Assurance programs for honey in terms of CFU's bacteria and yeasts and moulds combined be –

    (a) <1000 CFU's per gram honey bacteria
    (b) <100 CFU's per gram honey for yeasts and moulds combined.

- to consider the more stringent values of
    (a) <500 CFU's per gram honey bacteria
    (b) <10 CFU's per gram honey for yeasts and moulds combined.
  as indicators of hygienic management practice.

- that industry be informed of the dangers of exposing honey to soldering in beekeeping equipment for a prolonged time.
- that judicious use of water for removing honey residues from difficult to clean areas of extracting equipment be established.
- that extraction of honey is carried out only when equipment is perfectly dry to control environments suited to proliferation of yeasts and moulds.
- that extracting sheds be constructed to food grade standards away from contamination from soil and dust.
- that honey processing plants install adequate air handling systems which minimise cross contamination of in-process honey and protect honey from dust.
- that de-humidifiers be installed in extracting sheds and processing plants to minimise uncontrolled moisture which provides opportunities for microbial growth.
- that all honey packers implement Hazard Analysis Critical Control Point (HACCP) programs to control the quality and safety of honey as a food product.

Appendix 1

The following suite of analytes was included in the analyses performed on the samples included in this report.

Organochlorine Type Pesticides

Aldrin
BHC
Chlordane
DDD
DDE
DDT
Dieldrin
Endosulfan
Endosulfan (alpha)
Endosulfan Sulphate
Endrin
HCB
Heptachlor
Heptachlor Epoxide
Lindane
Methoxychlor
Oxychlordane

Organophosphorus Type Pesticides

Bromophos ethyl
Carbophenothion
Chlorfenvinphos
Chlorpyrifos
Chlorpyrifos methyl
Coumphos
Diazinon
Dichlorvos
Ethion
Fenchlorphos
Fenitrothion
Fenthion
Malathion
Methidathion
Parathion
Pirimphos methyl
Trichlorphon

Synthetic Pyrethroids

Cyhalothrin
Cyfluthrin
Cypermetrin
Alpha Cypermethrin
Deltramethrin
Beta Cytluthrin
Lambda Cyhalothrin
Flumethrin
Fenvalerate
Esfenvalerate
Permethrin

Appendix 2

Heavy metal analysis of 60 Australian honeys

Lab No.	Sample No.	Metal concentration mg/kg										
		Al	As	Cd	Cr	Cu	Fe	Ni	Pb	Se	Zn	Hg
TMG584	QLD1	0.88	<0.01	<0.01	0.091	0.33	7.2	0.060	0.16	<0.01	7.8	<0.005
TMG585	QLD2	0.75	<0.01	0.023	0.064	0.12	1.6	0.073	0.076	<0.01	30.8	<0.005
TMG586	QLD3	1.8	<0.01	<0.01	0.075	0.33	3.9	0.12	0.068	<0.01	7.3	<0.005
TMG587	QLD4	2.0	<0.01	<0.01	0.097	0.51	4.7	<0.051	0.097	<0.01	3.0	<0.005
TMG588	QLD5	0.71	<0.01	<0.01	0.070	0.11	2.0	<0.05	0.049	<0.01	17.2	<0.005
TMG589	QLD6	0.23	<0.01	<0.01	0.074	0.053	1.3	<0.05	0.017	<0.01	0.90	<0.005
TMG590	QLD7	1.8	<0.01	<0.01	0.097	0.22	2.9	<0.05	0.039	<0.01	8.3	<0.005
TMG591	QLD8	2.2	<0.01	<0.01	0.058	0.14	2.8	<0.05	0.073	<0.01	8.0	<0.005
TMG592	QLD9	6.9	<0.01	<0.01	0.063	0.22	5.2	<0.05	0.040	<0.01	8.0	<0.005
TMG593	QLD10	11.6	<0.01	<0.01	0.051	0.18	3.5	<0.05	0.077	<0.01	16.2	<0.005
TMG561	NSW1	0.48	<0.01	<0.01	<0.03	0.092	0.81	<0.05	0.026	<0.01	1.1	<0.005
TMG562	NSW2	0.33	<0.01	<0.01	<0.03	0.081	1.0	<0.05	0.031	<0.01	0.51	<0.005
TMG563	NSW3	4.5	<0.01	<0.01	<0.03	0.15	3.7	<0.05	0.039	<0.01	23.8	<0.005
TMG564	NSW4	1.9	<0.01	<0.01	<0.03	0.11	0.80	<0.05	0.032	<0.01	1.0	<0.005
TMG565	NSW5	0.71	<0.01	<0.01	0.071	0.090	2.2	0.053	0.031	<0.01	1.3	<0.005
TMG566	NSW6	0.22	<0.01	<0.01	0.082	0.068	0.55	<0.05	0.014	<0.01	0.45	<0.005
TMG567	NSW7	0.55	<0.01	<0.01	0.052	0.15	1.5	<0.05	0.033	<0.01	0.77	<0.005
TMG568	NSW8	2.8	<0.01	<0.01	0.097	0.29	2.9	<0.05	0.13	<0.01	28.8	<0.005
TMG569	NSW9	5.5	<0.01	<0.01	0.083	0.42	5.7	<0.05	0.15	<0.01	24.3	<0.005
TMG601	VIC1	2.0	<0.01	<0.01	<0.03	1.3	3.8	<0.05	4.8	<0.01	2.5	<0.005
TMG602	VIC2	1.4	<0.01	<0.01	<0.03	0.18	2.0	0.070	0.042	<0.01	1.3	<0.005
TMG603	VIC3	1.9	<0.01	<0.01	<0.03	0.23	1.8	<0.05	0.058	<0.01	0.63	<0.005
TMG604	VIC4	1.9	<0.01	<0.01	<0.03	0.16	1.8	<0.05	0.030	<0.01	0.42	<0.005
TMG605	VIC5	0.73	<0.01	<0.01	<0.03	0.14	2.4	<0.05	0.039	<0.01	0.44	<0.005
TMG606	VIC6	1.7	<0.01	<0.01	<0.03	0.22	3.5	<0.05	0.028	<0.01	1.2	<0.005
TMG607	VIC7	2.0	<0.01	<0.01	<0.03	0.57	2.7	<0.05	0.13	<0.01	8.8	<0.005
TMG608	VIC8	1.4	<0.01	<0.01	0.059	0.14	1.8	0.054	0.057	<0.01	0.74	<0.005
TMG609	VIC9	1.6	<0.01	<0.01	0.10	0.20	6.1	0.060	0.37	<0.01	6.8	<0.005
TMG610	VIC10	4.8	<0.01	<0.01	0.071	0.12	3.7	0.056	0.082	<0.01	13.5	<0.005
TMG611	VIC11	0.68	<0.01	<0.01	0.064	0.099	3.2		0.077	<0.01	28.0	<0.005
TMG552	SA1	3.2	<0.01	<0.01	0.036	0.087	1.2	<0.05	0.015	<0.01	0.41	<0.005
TMG552rpt	SA1	3.0	<0.01	<0.01	<0.03	0.084	1.4	<0.05	0.018	<0.01	0.40	<0.005
TMG553	SA2	0.59	<0.01	<0.01	<0.03	0.27	1.2	<0.05	0.026	<0.01	0.57	<0.005
TMG554	SA3	0.66	<0.01	<0.01	<0.03	0.096	1.4	<0.05	0.097	<0.01	0.86	<0.005
TMG555	SA4	2.8	<0.01	<0.01	<0.03	0.24	3.8	<0.05	0.16	<0.01	9.6	<0.005
TMG556	SA5	0.24	<0.01	<0.01	0.034	0.12	1.2	<0.05	0.017	<0.01	0.49	<0.005
TMG557	SA6	1.8	<0.01	<0.01	<0.03	0.16	2.6	<0.05	0.080	<0.01	3.3	<0.005
TMG558	SA7	3.5	<0.01	<0.01	<0.03	0.13	1.4	<0.05	0.030	<0.01	0.53	<0.005
TMG559	SA8	<0.2	<0.01	<0.01	0.039	0.083	1.4	<0.05	0.062	<0.01	0.75	<0.005
TMG560	SA9	1.3	<0.01	<0.01	0.031	0.18	3.3	<0.05	0.11	<0.01	22.2	<0.005
TMG570	WA1	19.7	<0.01	<0.01	0.055	0.16	3.3	<0.05	0.38	<0.01	95.2	<0.005
TMG571	WA2	0.61	<0.01	<0.01	0.042	0.11	13.5	<0.05	0.067	<0.01	13.6	<0.005
TMG572	WA3	44.1	<0.01	<0.01	0.054	0.84	4.9	<0.05	0.050	<0.01	4.6	<0.005
TMG573	WA4	0.30	<0.01	<0.01	<0.03	0.047	0.92	<0.05	0.087	<0.01	3.5	<0.005
TMG574	WA5	0.40	<0.01	<0.01	<0.03	0.11	3.3	0.13	0.47	<0.01	33.1	<0.005
TMG575	WA6	39.6	<0.01	<0.01	<0.03	0.61	5.3	0.33	0.14	<0.01	7.0	<0.005
TMG576	WA7	0.47	<0.01	<0.01	<0.03	0.084	1.6	0.35	0.062	<0.01	5.4	<0.005
TMG577	WA8	4.1	<0.01	<0.01	<0.03	0.11	2.4	<0.05	0.024	<0.01	6.1	<0.005

Lab No.	Sample No.	Metal concentration mg/kg										
		Al	As	Cd	Cr	Cu	Fe	Ni	Pb	Se	Zn	Hg
TMG578	NT1	0.43	<0.01	<0.01	<0.03	0.23	0.89	<0.05	0.13	<0.01	0.86	<0.005
TMG579	NT2	1.0	<0.01	<0.01	<0.03	0.38	1.7	0.12	0.11	<0.01	3.2	<0.005
TMG580	NT3	5.1	<0.01	<0.01	0.082	0.23	3.1	0.052	0.017	<0.01	0.99	<0.005
TMG580rpt	NT3	4.4	<0.01	<0.01	0.077	0.21	2.9	<0.05	0.015	<0.01	0.95	<0.005
TMG581	NT4	1.8	<0.01	<0.01	0.068	0.31	9.6	0.066	0.053	<0.01	3.6	<0.005
TMG582	NT5	5.6	<0.01	<0.01	0.060	0.17	13.1	0.050	1.5	<0.01	7.8	<0.005
TMG583	NT6	2.5	<0.01	<0.01	0.080	0.27	3.4	<0.05	0.13	<0.01	4.2	<0.005
TMG594	TAS1	3.2	<0.01	<0.01	0.038	0.17	2.7	<0.05	0.036	<0.01	1.7	<0.005
TMG594rpt	TAS1rpt	2.8	<0.01	<0.01	0.043	0.16	2.2	<0.05	0.047	<0.01	1.6	<0.005
TMG595	TAS2	3.1	<0.01	<0.01	0.033	0.17	2.5	<0.05	0.037	<0.01	1.6	<0.005
TMG596	TAS3	1.3	<0.01	<0.01	0.052	0.16	2.6	0.050	0.011	<0.01	1.8	<0.005
TMG597	TAS4	1.0	<0.01	<0.01	0.065	0.13	2.7	<0.05	<0.01	<0.01	0.95	<0.005
TMG598	TAS5	0.82	<0.01	<0.01	0.058	0.12	2.5	<0.05	0.012	<0.01	0.89	<0.005
TMG599	TAS6	1.0	<0.01	<0.01	0.056	0.15	2.5	<0.05	0.016	<0.01	0.98	<0.005
TMG560	TAS7	0.77	<0.01	<0.01	0.042	0.12	2.4	<0.05	<0.001	<0.01	0.89	<0.005

5. References

Anderson DL (1989). Destruction of Viable Chalkbrood (*Ascosphaera apis*) in export honeys and on leafcutter bee (*Megachile rotunda*) nest material in New Zealand. A Research Report.

Bailey L and Ball BV (1991a). *Honey Bee Pathology* Academic Press, London p59 (Second edition)

Bailey L and Ball BV (1991b). *Honey Bee Pathology* Academic Press, London p36 (Second edition)

Bailey L and Ball BV (1991c). *Honey Bee Pathology* Academic Press, London p58 (Second edition)

Baross JA and Lenovich LM (1992). *Compendium of Methods for the Microbiological Examination of Foods* p 209 Third Edition edited by C Vanderzant and D F Splittstoesser. American Public Health Association.

Bonvehi JS and Jordá RE (1993). The microbiological quality of honey as determined by aerobic colony counts. *Journal of Food Protection* **56** (4):336-337.

Cowan ST and Steele KJ (1972). *Manual for the identification of medical bacteria.* Cambridge University Press, Cambridge, U K p 171 (Second edition revised by ST Cowan).

Deak, T, Tabajdi-Pinter V, Fabri I (1986). *Methods for the Mycological Examination of Food* Plerum Press, New York, USA pp 190-192 edited by King AD, Pitt JI, Beuchat LR and Cory Janet EL.

Farris GA, Fatichenti F, Deiana P and Forteleoni M (1985). *Apicoltore-Moderno* **76** (4):133:140.

Food Microbiology Australian Standard (1991) 1722-2,1.

Food Microbiology Australian Standard (1991) 1766-2.2.

Fraser K, Greenhalgh S and Hornitzky M (1995). A cost-benefit analysis of the eradication of American foulbrood in Australia. NSW Agriculture EMAI Camden NSW.

Goodman RD and Azoulas JK (1994). Oxytetracycline hydrochloride in Australian honey and the storage of Oxytetracycline hydrochloride lin beehives. Project report to Honeybee Research and Development Council. Institute for Horticultural Development, Victoria, Australia.

Haseman L (1961). How long can spores of American foulbrood live? *American Bee Journal* **101**:298-290.

Heath, LA (1985). Occurrence and distribution of Chalkbrood disease of honeybees. *Bee World* **66**:9-15.

Hornitzky MAZ and Anderson DL (1993). Honeybee Diseases. *In* Australia standard diagnostic techniques for animal diseases CSIRO Information Services, Victoria, Australia (Revision: Chalkbrood).

Hornitzky MAZ and Clark S (1991). Culture of *Bacillus larvae* from bulk honey samples for the detection of American foulbrood. *Journal of Apicultural Research* **30**(1):13-16.

Hornitzky MAZ and Nicholls PJ (1993). J medium is superior to sheep blood agar and brain heart infusion for the isolation of *Bacillus larvae* for honey samples. *Journal of Apicultural Research* **32**(11):51-52.

Jermini M F G, Geiges O and Schmidt-Lorenz W (1987). Detection, isolation and identification of osmotolerant yeast from high-sugar products. *Journal of Food Protection* **50** (6):468-472.

Lenovich L M and Konkel P J (1992). *Compendium of Methods for the Microbiological Examination of Foods* p 1010 (Third edition edited by C Vanderzant and D F Splittsoesser). American Public Health Association, Washington D C.

Lochhead A G (1933). Factors concerned with the fermentation of honey. *Zentralblatt Bakteriologie Parasitenkunde II Abt* **88**:296-302.

Lockhead A G and Farrell L (1931). The types of osmophilic yeasts found in normal honey and their relation to fermentation. *Canadian Journal Research* **5**:665-672.

Matheson A (1993). World bee health report. *Bee World* **74** (4):176-212.

Mawhinney H (1993). Phenol residue in honey. *Australian Bee Journal* **74** (3):15.

Mislivic, P.B., Beuchat, L.R. and Cousin, M.A. (1984). *Compendium of Methods for the Microbiological Examination of Foods* p239 (Third Edition edited by C. Vanderzard and D.F. Splittoesser). American Public Health Association, Washington .D.C.

Mossel D A A, Vega Clara L and Put Henrietta M C (1975). Further studies on the suitability of various media containing antibacterial antibiotics for the enumeration of mould in food and food environments. *Journal Applied Bacteriology* **39**:15-22.

Midura T F, Snowden S, Wood R M and Arnon S (1979). Isolation of *Clostridium botulinum* from honey. *Journal of Clinical Microbiology* **9** (2) 282-283.

Pivnick H and Gabis D A (1984). *Compendium of Methods for the Microbiological Examination of Foods* 2nd Ed ed M L Speck p 700 American Public Health Association, Washington D C.

Poncini L and Wimmer F L (1985). Characterisation of the Yeasts (*Blastomycetes*) in some Fijian honeys. *Apieacta* **20** (4):118-126.

Rose Joan B and Christensen Martha (1984). *Ascosphaera* species inciting Chalkbrood in North America and a Faxonomic Key. *Mycotaxon* **19**:41-45.

Seal D W A (1957). Chalkbrood disease of bees. *New Zealand Journal of Agriculture* **6**:562.

Seiler D A L (1986). Effect of diluent and medium water activity on recovery of yeasts from high sugar coatings and fillings. *Methods for Mycological Examination of Food* p 162 Edited by A D King Jr, J I Pitt, L R Beuchat and J E L Corry. Plenum Press, New York.

Sturterant A P (1932). Relation of commercial honey to the spread of American foulbrood. *Journal of Apicultural Research* **45** (5):257-265.

Swanson K M J, Busta, F F, Peterson E H and Johnson M G (1992). *Compendium of Methods for the Microbiological Examination by Foods* p 75 (Third edition edited by C Vanderzant and D F Splittsoesser). American Public Health Association, Washington D C.

Trueman K F, Ward W H and Mawhinney H (1996). *International Honeys* – a Quality Survey RIRDC Report DAQ 202A.

Ward, WH (1998). Survey to determine the latent level of American Foulbrood disease amongst share holders of "Capilano Honey" by culturing from bulk honey. Unpublished data.

Warhurst P and Goebel R 91995). *The Bee Book* p 172 DPI Publishing Services.

White J R (1992). *The Hive and the Honeybee* Dadant Publication, Illiinios p 901 (Revised edition edited by Joe M Graham).