Food Safety Risks Associated with Prawns Consumed in Australia

Seafood CRC Project: 2009/787
Prawn Market Access Defenders

John Sumner

September 2011
Foreword

In 2009 The Australian Council of Prawn Fisheries (ACPF) held a meeting in Brisbane to discuss and plan future research priorities; food safety was identified as a priority for the industry at this meeting.

Subsequently, the SARDI Food Safety group were engaged by the Australian Seafood Cooperative Research Centre (ASCRC) and the ACPF to undertake a project to identify food safety risks posed by potential hazards in prawns and if necessary, prioritise opportunities for reducing risk through targeted initiatives. This report presents the findings of the project.

The report scientifically evaluated the human health impact of chemical and microbial hazards associated with prawns. Risk ratings indicate a VERY LOW risk of human illness associated with the consumption of prawns produced domestically, imported prawns and exported prawns. This finding is consistent with the public health record which shows few reports of illness related to the consumption of prawns that have been handled appropriately.

The report was favourably peer reviewed by Dr Iddya Karunasagar (United Nations Food and Agricultural Organisation) and Dr Alan Reilly (Food Safety Authority of Ireland) (Appendix 1).

The scientific findings contained in this report may assist negotiations for improved trade access conditions into domestic and overseas markets, and risk commensurate testing requirements for retail outlets.

SARDI gratefully acknowledges Dr John Sumner for authorship of the report, members of the project steering group for valuable input and advice (Jayne Gallagher, Lynda Feazey, Dr Andrew Pointon, Dr Cath McLeod and Graeme Stewart) and the contributions of Dr Andreas Kiermeier and Jo Slade. We also thank the ASCRC and ACPF for their financial support.
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Important Notice

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## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABARE</td>
<td>Australian Bureau of Agricultural and Resource Economics</td>
</tr>
<tr>
<td>ADI</td>
<td>Acceptable Daily Intake</td>
</tr>
<tr>
<td>AHD</td>
<td>1-aminohydantoin (Nitrofurantoin)</td>
</tr>
<tr>
<td>AMOZ</td>
<td>3-amino-5-morpholinomethyl-1,3-oxazolidin (Furaltdone)</td>
</tr>
<tr>
<td>AOZ</td>
<td>3-amino-oxazolidinone (Furazolidone)</td>
</tr>
<tr>
<td>APFC</td>
<td>Australian Prawn Fisheries Council</td>
</tr>
<tr>
<td>AQIS</td>
<td>Australian Quarantine Inspection Service</td>
</tr>
<tr>
<td>ASCRC</td>
<td>Australian Seafood Cooperative Research Centre</td>
</tr>
<tr>
<td>CAP</td>
<td>Chloramphenical</td>
</tr>
<tr>
<td>CCP</td>
<td>Critical Control Point</td>
</tr>
<tr>
<td>CSIRO</td>
<td>Commonwealth Scientific and Industrial Research Organisation</td>
</tr>
<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration (US)</td>
</tr>
<tr>
<td>FSA</td>
<td>Food Science Australia</td>
</tr>
<tr>
<td>FSANZ</td>
<td>Food Standards Australia New Zealand</td>
</tr>
<tr>
<td>GHP</td>
<td>Good Hygiene Practices</td>
</tr>
<tr>
<td>GMP</td>
<td>Good Manufacturing Practices</td>
</tr>
<tr>
<td>HACCP</td>
<td>Hazard Analysis and Critical Control Point</td>
</tr>
<tr>
<td>HAV</td>
<td>Hepatitis A</td>
</tr>
<tr>
<td>ICMSF</td>
<td>International Commission on Microbiological Specifications for Foods</td>
</tr>
<tr>
<td>IQF</td>
<td>Individually quick frozen</td>
</tr>
<tr>
<td>JECFA</td>
<td>Joint Expert Committee on Food Additives (FAO/WHO)</td>
</tr>
<tr>
<td>ML</td>
<td>Maximum Level</td>
</tr>
<tr>
<td>MRL</td>
<td>Maximum Residue Limit</td>
</tr>
<tr>
<td>NACMCF</td>
<td>National Advisory Committee on Microbiological Criteria for Foods</td>
</tr>
<tr>
<td>NEPSS</td>
<td>National Enteric Pathogens Surveillance Scheme</td>
</tr>
<tr>
<td>NoV</td>
<td>Norovirus</td>
</tr>
<tr>
<td>NRVP</td>
<td>National Risk Validation Program</td>
</tr>
<tr>
<td>PFGE</td>
<td>Pulsed-Field Gel Electrophoresis</td>
</tr>
<tr>
<td>PTWI</td>
<td>Provisional Tolerable Weekly Intake</td>
</tr>
<tr>
<td>SARDI</td>
<td>South Australian Research and Development Institute</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>SEM</td>
<td>Semicarbazide (Nitrofurazone)</td>
</tr>
<tr>
<td>SSOP</td>
<td>Sanitation Standard Operating Procedure</td>
</tr>
<tr>
<td>TOR</td>
<td>Term of Reference</td>
</tr>
<tr>
<td>VBNC</td>
<td>Viable but non-culturable</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
Executive Summary

The Project

- The Australian Prawn Fisheries Council (APFC) and the Australian Seafood Cooperative Research Centre (CRC) commissioned SARDI to undertake a food safety risk rating of prawns consumed in Australia.

- The terms of references (TORs) for this study are to:
  1. Undertake a hazard identification for imported and domestic prawns
  2. Construct hazard sheets for each identified hazard
  3. Undertake a qualitative risk ranking and where data permit, a semi-quantitative ranking using Risk Ranger
  4. Write a draft report and circulate to reference group (including technical information as appendices)
  5. Prepare a final report
  6. Arrange a stakeholder workshop to discuss report.

- This report covers TORs 1-4 above and focuses on hazards in:
  - Prawns produced domestically
  - Imported prawns
  - Export prawns.

TOR 1: Hazard Identification

- Food safety hazards in prawns in international trade have been identified by interrogating the following data sources:
  - National Risk Validation Project
  - OzFoodNet food poisoning data
  - FSANZ recalls
  - European Union (EU) alerts for crustaceans
  - AQIS sampling program for imported prawns
  - EU Market Access Program

- For prawns caught/harvested in Australia:
  - The sole perceived hazard according to EU authorities is cadmium.
  - There have been no instances of illness caused by prawns produced by the domestic industry; consideration of risks from pathogenic bacteria in domestically-produced prawns did not pass the Hazard Identification stage of risk assessment.

- For imported prawns a range of chemical and microbiological hazards has been identified:
  - Nitrofurans
  - Sulphites
  - Chloramphenicol
  - Cadmium
- *Vibrio parahaemolyticus*
- *Vibrio cholerae*
- *Salmonella*
- Hepatitis A

**TOR 2: Construct Hazard Sheets for each Hazard**

- Hazard sheets have been constructed which, in the case of microbiological hazards, update those used for the National Seafood Risk Assessment of 2001 commissioned by Seafood Services Australia.
- The update was facilitated greatly by material available from the joint FAO-WHO expert panel on *Vibrios in Seafoods* and the FAO expert consultation on *The application of biosecurity measures to control Salmonella contamination in sustainable aquaculture*.

**TOR 3: Undertake Risk Ranking**

- Risk ratings were carried out for each of these perceived hazards using a qualitative tool based on one developed by CSIRO and where sufficient data were available, a semi-quantitative tool, Risk Ranger:

<table>
<thead>
<tr>
<th>Biological Hazards</th>
<th>Risk Assessment Carried Out</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vibrio parahaemolyticus</em></td>
<td>Qualitative</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>Qualitative</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>Qualitative</td>
</tr>
<tr>
<td>Hepatitis A*</td>
<td>None</td>
</tr>
</tbody>
</table>

*Chemical Hazards*

<table>
<thead>
<tr>
<th></th>
<th>Qualitative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphite</td>
<td></td>
</tr>
<tr>
<td>Cadmium</td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td></td>
</tr>
<tr>
<td>Nitrofurans</td>
<td></td>
</tr>
</tbody>
</table>

*There was insufficient information to undertake even a qualitative risk assessment*

- The estimated risk of illness caused by pathogens in imported prawns – less than one illness/annum - is in line with observed public health data.
- For several reasons, achieving a useful assessment of risk from ingesting chemicals contained in prawns is difficult:
  - There are no recorded cases of illness associated with the hazard:product pairing.
  - Chronic exposure over many years may be required for adverse reaction (e.g. cadmium intake and kidney disease).
  - Risk of exposure is considered dose-independent e.g. any dose of chloramphenicol and nitrofurans is considered by some authorities (EU) to be disease-causing at any dose.
The application of ‘zero tolerance’, with its implied ‘zero risk’, effectively precludes any application of probability to an adverse event occurring.

- The foregoing has led some authorities (e.g. EU) to set levels based on the ‘precautionary principle’, which is a concept diametrically opposed to risk assessment.

- As part of an EU Market Access Program, the South Australian Research and Development Institute (SARDI) undertook residue testing of prawns in conjunction with the Australian Prawn Farmers Association (APFA). All samples of prawns from six prawn farms had levels below the laboratory limit of detection for chloramphenicol (0.19µg/kg), AHD, AMOZ and AOZ (0.2µg/kg) and SEM (0.4µg/kg).

- Risk ratings were Very Low for sulphite, chloramphenicol, nitrofurans and cadmium in prawns.

### Risk ratings of microbiological hazards in prawns imported to Australia

<table>
<thead>
<tr>
<th>Hazard</th>
<th>Product</th>
<th>Qualitative Assessment</th>
<th>Semi-quantitative Assessment</th>
<th>Risk Rating</th>
<th>Estimated Illnesses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>V. cholerae</em></td>
<td>Raw prawns</td>
<td>Very low</td>
<td>28</td>
<td>1.7/decade</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prawns cooked at the plant and eaten without further heat treatment</td>
<td>Very low</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prawns cooked immediately before consumption</td>
<td>Very low</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em></td>
<td>Raw prawns</td>
<td>Very low</td>
<td>22</td>
<td>1.5/decade</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prawns cooked at the plant and eaten without further heat treatment</td>
<td>Very low</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prawns cooked immediately before consumption</td>
<td>Very low</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>Raw prawns</td>
<td>Very low</td>
<td>16</td>
<td>1.5/decade</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prawns cooked at the plant and eaten without further heat treatment</td>
<td>Very low</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prawns cooked immediately before consumption</td>
<td>Very low</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

### Conclusions

The present project ratings are in line with public health data linking prawns with illness. Despite being a huge commodity in international trade there are few reports of illness where handling standards are maintained according to those contained in the Food Standards Codes of importing countries.
Background

As indicated in Table 1, Australia typically produces around 20,000 t of prawns annually, of which around 25% is exported (based on ABARE statistics for the years 2005-2008). Domestic consumption is augmented by around 20,000 t of imported prawns, with Vietnam, Thailand and China accounting for around 75% of imports (Table 2). Japan is by far the largest single export market, though, as indicated in Table 3, exports fell significantly between 2005 and 2008.

The data presented in Tables 1-3 serve to inform the exposure Australian and overseas consumers face from consumption of prawns. Under TOR 1, these hazards are identified for prawns produced in and imported to Australia. This report aims to integrate exposure to specific hazards with each hazard’s characteristics to produce a risk ranking.

As such, this report will update previous risk rankings for microbial hazards published in the national seafood risk assessment (Seafood Services Australia, 2001) and in Sumner and Ross (2002). Two risk assessments relevant to the present study were made:

- Enterics (non-Vibrio) in cooked crustaceans
- Vibrios in molluscs and crustaceans

In the former, the researchers noted that there had been only two recorded outbreaks of food poisoning, both due to *Shigella* spp (Table 4). In these outbreaks imported Asian prawns were incriminated in the Dutch incident, while in the UK incident the importing country was not identified. The shigelloses were attributed to post-process contamination, possibly involving a food handler in the carrier state, or the use of contaminated water. No recorded outbreaks were found for other enteric pathogens such as *Salmonella*, *Escherichia coli*, *Campylobacter* or *Yersinia*.

In the 2001 risk assessment referred to above, cooked prawns were imported from Asian countries and comprised aquaculture prawns, almost exclusively *Penaeus monodon*. Using a semi-quantitative tool, Risk Ranger, a ranking of 31 was established for enterics in cooked prawns with a prediction of five illnesses per annum, almost all coming from vulnerable consumers (young, old, pregnant and immunocompromised).

It was noted that there had been two large outbreaks of *V. parahaemolyticus* gastroenteritis in Australia linked with consumption of prawns (Kraa, 1995). In 1990 an outbreak affecting more than 100 people, one of whom died, was linked to fresh, cooked prawns from Indonesia. In 1992 there were two outbreaks affecting more than 50 people linked to the same wholesale supplier of cooked prawns. The Risk Ranger ranking for *V. parahaemolyticus* in imported cooked prawns was 37, with six illnesses predicted per annum. Similar ranking and illnesses were predicted for vulnerable consumers consuming cooked imported prawns contaminated with *V. cholerae*.

It should be noted that, in the decade following the first risk assessment, the body of information has increased greatly. The FAO and WHO have undertaken a series of risk assessments on Vibrios in Seafoods, while FAO has examined measures to control *Salmonella* contamination in aquaculture products. These, and other sources, will be used in the present update of hazards and risks associated with Australian and imported prawns.
Table 1: Summary statistics Australia’s prawn industry 2005-2008 (after ABARE statistics)

<table>
<thead>
<tr>
<th></th>
<th>Volume (t)</th>
<th>Value ($,000)</th>
<th>Price ($/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exports</td>
<td>8744</td>
<td>6376</td>
<td>4916</td>
</tr>
<tr>
<td>Imports</td>
<td>23165</td>
<td>26016</td>
<td>18731</td>
</tr>
<tr>
<td>Production</td>
<td>20046</td>
<td>17490</td>
<td>19342</td>
</tr>
</tbody>
</table>

Table 2: Main sources of prawns imported into Australia (after ABARE statistics)

<table>
<thead>
<tr>
<th></th>
<th>Volume (t)</th>
<th>Value ($,000)</th>
<th>Price ($/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indonesia</td>
<td>1094</td>
<td>686</td>
<td>197</td>
</tr>
<tr>
<td>India</td>
<td>2459</td>
<td>2000</td>
<td>1084</td>
</tr>
<tr>
<td>China</td>
<td>4465</td>
<td>8469</td>
<td>5486</td>
</tr>
<tr>
<td>Thailand</td>
<td>6106</td>
<td>5503</td>
<td>4694</td>
</tr>
<tr>
<td>Vietnam</td>
<td>6855</td>
<td>7229</td>
<td>4856</td>
</tr>
<tr>
<td>Other</td>
<td>2132</td>
<td>2128</td>
<td>2414</td>
</tr>
</tbody>
</table>

Table 3: Main destinations for export of prawns from Australia 2005-2008 (after ABARE statistics)

<table>
<thead>
<tr>
<th></th>
<th>Volume (t)</th>
<th>Value ($,000)</th>
<th>Price ($/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>3116</td>
<td>2442</td>
<td>1792</td>
</tr>
<tr>
<td>China</td>
<td>1124</td>
<td>1101</td>
<td>529</td>
</tr>
<tr>
<td>Spain</td>
<td>1434</td>
<td>877</td>
<td>331</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>401</td>
<td>413</td>
<td>425</td>
</tr>
<tr>
<td>Vietnam</td>
<td>548</td>
<td>458</td>
<td>317</td>
</tr>
</tbody>
</table>

Table 4: Documented outbreaks of illness from cooked prawns with enteric pathogens

<table>
<thead>
<tr>
<th>Date</th>
<th>Country</th>
<th>Organism</th>
<th>Impact</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1983-4</td>
<td>Holland</td>
<td>Shigella flexneri</td>
<td>14 dead</td>
<td>van Spreckens (1985)</td>
</tr>
</tbody>
</table>
Terms of Reference

The terms of references (TORs) for this study are:

1. Undertake a hazard identification for imported and domestic prawns.
2. Construct hazard sheets for each identified hazard.
3. Undertake a qualitative risk ranking and where data permit, a semi-quantitative ranking using Risk Ranger.
4. Write draft report and circulate to reference group (including technical information as appendices).
5. Prepare a final report.
6. Arrange a stakeholder workshop to discuss report.

References


TOR 1: Hazard Identification – Australian Prawns in International Trade

Approach
A number of sources have been explored in identifying food safety hazards in prawns in international trade. The following data sources have been interrogated:

1. National Risk Validation Project
2. OzFoodNet food poisoning data
3. FSANZ recalls
4. European Union (EU) alerts for crustaceans
5. AQIS sampling program for imported prawns
6. SARDI EU market access program

Together, the data sources inform on hazards encountered in:
- Prawns produced domestically
- Imported prawns
- Prawns exported

National Risk Validation Project (NRVP) and OzFoodNet Food Poisoning Data

The NRVP and OzFoodNet data on ten outbreaks of food poisoning following consumption of prawns over the 21-year period 1990-2010 are presented in Table 5. During the period, more than 230 individuals became sufficiently ill to enter the medical system and become registered cases; one died.

Aetiological agents identified as hazards are, in order of prevalence:
- *V. parahaemolyticus*
- Hepatitis A
- *V. cholerae* non 01, non 139
- *S. typhi*
Table 5: Food poisoning outbreaks due to the consumption of prawns in Australia 1990-2010 (after NRVP and OzFoodNet)

<table>
<thead>
<tr>
<th>Source</th>
<th>Hazard</th>
<th>Country</th>
<th>Cases (deaths)</th>
<th>Year</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retail</td>
<td><em>V. parahaemolyticus</em></td>
<td>Indonesia</td>
<td>27</td>
<td>1990</td>
<td>NSW Health</td>
</tr>
<tr>
<td>Caterer</td>
<td><em>V. parahaemolyticus</em></td>
<td>Indonesia</td>
<td>100 (1)</td>
<td>1990</td>
<td>NSW Health; Kraa, 1995</td>
</tr>
<tr>
<td>Importer</td>
<td><em>V. parahaemolyticus</em></td>
<td>Indonesia</td>
<td>&gt;50</td>
<td>1992</td>
<td>Kraa, 1995</td>
</tr>
<tr>
<td>Eating est.</td>
<td><em>S. typhi</em></td>
<td>Thailand</td>
<td>4</td>
<td>1995-1996</td>
<td>NSW Health</td>
</tr>
<tr>
<td>Eating est.</td>
<td>Hepatitis A</td>
<td>Burma</td>
<td>23</td>
<td>1997</td>
<td>NSW Health</td>
</tr>
<tr>
<td>Eating est.</td>
<td><em>V. cholerae</em> non 01, non 139*</td>
<td>Not recorded</td>
<td>10</td>
<td>1999</td>
<td>OzFoodNet</td>
</tr>
<tr>
<td>Eating est.</td>
<td>Hepatitis A</td>
<td>Not recorded</td>
<td>2</td>
<td>2003</td>
<td>OzFoodNet</td>
</tr>
<tr>
<td>Eating est.</td>
<td>Unknown</td>
<td>Not recorded</td>
<td>2</td>
<td>2009</td>
<td>OzFoodNet</td>
</tr>
</tbody>
</table>

* Red claw crayfish
FSANZ Recalls

Over the period 2000-2011, FSANZ registered five recalls of prawn products (Table 6), from which may be added non-typhoidal serovars of *Salmonella* to the list of hazards.

<table>
<thead>
<tr>
<th>Year</th>
<th>Product</th>
<th>Format</th>
<th>Hazard</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td><em>P. monodon</em></td>
<td>Cooked, peeled</td>
<td><em>V. cholerae</em></td>
</tr>
<tr>
<td>2004</td>
<td><em>P. monodon</em></td>
<td>Cooked</td>
<td><em>S. infantis</em></td>
</tr>
<tr>
<td>2005</td>
<td><em>P. vannamei</em></td>
<td>Cooked</td>
<td>Microbial contamination</td>
</tr>
<tr>
<td>2007</td>
<td><em>P. monodon</em></td>
<td>Cooked</td>
<td>Metal</td>
</tr>
<tr>
<td>2007</td>
<td><em>P. monodon</em></td>
<td>Raw, peeled</td>
<td>Labelling (labelled as cooked)</td>
</tr>
</tbody>
</table>

EU Alerts for Crustaceans

Hazards in crustaceans registered by the EU rapid alert system are listed in Table 7. Over the period 1980-2010 (though preponderantly 1998-2010) the majority of alerts have been for chemicals perceived as hazards: nitrofurans, sulphite, chloramphenicol and cadmium, with a minority being due to vibrios and *Salmonella*.

Countries responsible for triggering alerts from crustaceans imported to the EU are listed in Table 8. The majority of alerts stem from Asian imports, reflecting the prevalence of Asian prawns in international trade. Alerts from French product are overwhelmingly linked with trade in crabs while those from Australian prawns are linked with cadmium.

An appraisal of microbiological hazards prompting alerts was undertaken over the period 1995-2010. Over this time there were 230 alerts, of which 200 occurred during 2000-2005 (Table 9); 209/230 were linked with prawns and the remainder with crabs and lobsters (Table 10). It cannot be determined why alerts have decreased so markedly post-2005.

Among microbiological hazards causing alerts, the vast majority were due to *V. parahaemolyticus* and *V. cholerae* (Table 11), with Malaysian, Indian, Bangladeshi and Thai product being implicated on 50, 31, 24 and 22 occasions, respectively (Table 12).

<table>
<thead>
<tr>
<th>Hazard</th>
<th>Number of alerts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrofurans</td>
<td>377</td>
</tr>
<tr>
<td>Sulphite</td>
<td>295</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>163</td>
</tr>
<tr>
<td>Cadmium</td>
<td>133</td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em></td>
<td>112</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>66</td>
</tr>
<tr>
<td><em>V. cholerae</em></td>
<td>50</td>
</tr>
<tr>
<td>Other</td>
<td>299</td>
</tr>
<tr>
<td>Total</td>
<td>1495</td>
</tr>
</tbody>
</table>
### Table 8: Country of origin of prawns triggering alerts in the EU (1980-2010)

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of Alerts</th>
<th>Main Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>215</td>
<td>Nitrofurans</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>161</td>
<td>Nitrofurans</td>
</tr>
<tr>
<td>China</td>
<td>135</td>
<td>Chloramphenicol, nitrofurans</td>
</tr>
<tr>
<td>Vietnam</td>
<td>123</td>
<td>Chloramphenicol, nitrofurans, vibrios</td>
</tr>
<tr>
<td>France</td>
<td>120</td>
<td>Sulphite, cadmium (crabs)</td>
</tr>
<tr>
<td>Thailand</td>
<td>87</td>
<td>Nitrofurans</td>
</tr>
<tr>
<td>Malaysia</td>
<td>71</td>
<td><em>V. parahaemolyticus</em></td>
</tr>
<tr>
<td>Indonesia</td>
<td>65</td>
<td>Nitrofurans</td>
</tr>
<tr>
<td>Brazil</td>
<td>62</td>
<td>Sulphite</td>
</tr>
<tr>
<td>Australia</td>
<td>39</td>
<td>Cadmium</td>
</tr>
<tr>
<td>Ecuador</td>
<td>39</td>
<td>Vibrios</td>
</tr>
<tr>
<td>Other</td>
<td>378</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1495</strong></td>
<td></td>
</tr>
</tbody>
</table>

### Table 9: EU alerts due to microbiological hazards (1995-2010)

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of Alerts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>1</td>
</tr>
<tr>
<td>1996</td>
<td>0</td>
</tr>
<tr>
<td>1997</td>
<td>3</td>
</tr>
<tr>
<td>1998</td>
<td>10</td>
</tr>
<tr>
<td>1999</td>
<td>20</td>
</tr>
<tr>
<td>2000</td>
<td>33</td>
</tr>
<tr>
<td>2001</td>
<td>40</td>
</tr>
<tr>
<td>2002</td>
<td>35</td>
</tr>
<tr>
<td>2003</td>
<td>17</td>
</tr>
<tr>
<td>2004</td>
<td>37</td>
</tr>
<tr>
<td>2005</td>
<td>18</td>
</tr>
<tr>
<td>2006</td>
<td>4</td>
</tr>
<tr>
<td>2007</td>
<td>2</td>
</tr>
<tr>
<td>2008</td>
<td>8</td>
</tr>
<tr>
<td>2009</td>
<td>1</td>
</tr>
<tr>
<td>2010</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>230</strong></td>
</tr>
</tbody>
</table>
Table 10: EU alerts according to crustacean products

<table>
<thead>
<tr>
<th>Product</th>
<th>Number of Alerts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crab</td>
<td>6</td>
</tr>
<tr>
<td>Crayfish</td>
<td>15</td>
</tr>
<tr>
<td>Shrimp</td>
<td>209</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>230</strong></td>
</tr>
</tbody>
</table>

Table 11: Microbiological hazards triggering EU alerts from crustaceans

<table>
<thead>
<tr>
<th>Hazard</th>
<th>Number of Alerts</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. cereus</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>6</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Clostridium</em></td>
<td>1</td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em></td>
<td>137</td>
</tr>
<tr>
<td><em>V. cholerae</em></td>
<td>98</td>
</tr>
<tr>
<td>Vibrios</td>
<td>8</td>
</tr>
<tr>
<td><em>V. alginolyticus</em></td>
<td>5</td>
</tr>
<tr>
<td><em>V. vulnificus</em></td>
<td>4</td>
</tr>
<tr>
<td><em>V. fluvialis</em></td>
<td>1</td>
</tr>
<tr>
<td><em>V. mimicus</em></td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>263</strong></td>
</tr>
</tbody>
</table>

Table 12: Countries of origin causing EU alerts due to microbiological hazards

<table>
<thead>
<tr>
<th>Country of origin</th>
<th>Number of Alerts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaysia</td>
<td>50</td>
</tr>
<tr>
<td>India</td>
<td>31</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>24</td>
</tr>
<tr>
<td>Thailand</td>
<td>22</td>
</tr>
<tr>
<td>China</td>
<td>19</td>
</tr>
<tr>
<td>Vietnam</td>
<td>18</td>
</tr>
<tr>
<td>Indonesia</td>
<td>14</td>
</tr>
<tr>
<td>Ecuador</td>
<td>13</td>
</tr>
<tr>
<td>Brazil</td>
<td>10</td>
</tr>
<tr>
<td>Others</td>
<td>29</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>230</strong></td>
</tr>
</tbody>
</table>

**AQIS Sampling Program for Imported Prawns**

Over the six year period 2005-2010, AQIS arranged for 5,247 tests on consignments of imported prawns, of which 94 (1.79%) failed acceptance criteria (Table 13).
Table 13: Sampling frequency and rejection rate for prawns imported into Australia (2005-2010)

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Tests</th>
<th>Failed Tests</th>
<th>Percentage Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>450</td>
<td>8</td>
<td>1.78</td>
</tr>
<tr>
<td>2006</td>
<td>1577</td>
<td>49</td>
<td>3.11</td>
</tr>
<tr>
<td>2007</td>
<td>1570</td>
<td>33</td>
<td>2.10</td>
</tr>
<tr>
<td>2008</td>
<td>434</td>
<td>1</td>
<td>0.23</td>
</tr>
<tr>
<td>2009</td>
<td>530</td>
<td>2</td>
<td>0.38</td>
</tr>
<tr>
<td>2010</td>
<td>686</td>
<td>1</td>
<td>0.15</td>
</tr>
<tr>
<td>Total</td>
<td>5247</td>
<td>94</td>
<td>1.79</td>
</tr>
</tbody>
</table>

The vast majority of positive tests (82.7%) were in 2006 and 2007, when consignments from China and India were responsible for 72.3% of all positive tests (Table 14).

Hazards which caused rejection are listed in Table 15 from which it can be seen that chemical hazards comprise 84% of rejections, with nitrofurans accounting for 78.5% of chemical rejections; China and India together supplied 87% of consignments rejected for failing chemical criteria.

Microbiological rejections were due to presence of *V. cholerae* (13/93) and for high Standard Plate Count (2/94).

Table 14: Country of origin of rejected consignments (2005-2010)

<table>
<thead>
<tr>
<th>Year</th>
<th>Vietnam</th>
<th>Indonesia</th>
<th>Thailand</th>
<th>Singapore</th>
<th>China</th>
<th>India</th>
<th>Malaysia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>2006</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>29</td>
<td>17</td>
<td>0</td>
<td>49</td>
</tr>
<tr>
<td>2007</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>12</td>
<td>10</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>2008</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2009</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2010</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>3</td>
<td>12</td>
<td>1</td>
<td>42</td>
<td>27</td>
<td>4</td>
<td>94</td>
</tr>
</tbody>
</table>

Table 15: Country of origin and cause of rejection of prawns imported into Australia (2005-2010)

<table>
<thead>
<tr>
<th>Vietnam</th>
<th>Thailand</th>
<th>Singapore</th>
<th>China</th>
<th>India</th>
<th>Indonesia</th>
<th>Malaysia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cloramphenicol</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Fluoroquinones</td>
<td></td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Nitrofurans</td>
<td></td>
<td></td>
<td>36</td>
<td>26</td>
<td></td>
<td></td>
<td>62</td>
</tr>
<tr>
<td>Standard Plate Count</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sulphur dioxide</td>
<td>3</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>12</td>
<td>1</td>
<td>42</td>
<td>27</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>
Hazard Identification Summary

For consumers of Australian prawns the sole perceived hazard according to EU authorities is cadmium. By contrast, according to perceptions of risk by AQIS (on advice from FSANZ), imported prawns present a wide range of chemical and microbiological hazards: nitrofurans, sulphite, chloramphenicol, cadmium, *V. parahaemolyticus*, *V. cholerae*, *Salmonella* and Hepatitis A.

It should be noted that:

(i) Human illnesses are generally caused by strains of *V. parahaemolyticus* that are either tdh+ or rarely, trh+ and strains possessing these attributes constitute a very small proportion, if at all, of the natural population associated with the environment and seafood.

(ii) Public health risk should be equated with the natural presence of this organism and with the large number of import alerts that are triggered without further testing to determine whether the isolates are, in fact, toxigenic.

This factor is developed in Section 3 of the Hazard Sheet for *V. parahaemolyticus*.

References


**TOR 2: Hazard Sheets for Identified Hazards**

**Vibrio cholerae**

1. **Hazard Identification**

Outbreaks of cholera have been associated with consumption of seafood including oysters, crabs and prawns (Oliver & Kaper, 1997). In the early 1990s, there was a cholera pandemic in South and Central America, most outbreaks occurring in Peru, where there were >400 000 cases and an estimated 4,000 deaths (Wolfè, 1992). The pandemic was not associated with consumption of seafood produced commercially, but to contaminated water supplies used in the preparation of a popular fermented seafood dish, ceviche.

Warm-water prawn is an important commodity in international trade with the vast majority of production originating from developing countries (FAO, 1999), making it a very important commodity for these countries.

The global prawn trade has responded to the major HACCP initiatives of the United States of America (Seafood HACCP Regulation) and of the European Union (concept of ‘own checks’ and critical control points) as prerequisites for maintaining trade. Many importing countries, including Australia, operate microbiological monitoring systems at ports of entry.

1.1 **V. cholerae serovars of concern**

According to the WHO definition, choleragenic *V. cholerae* O1 and O139 are the only causative agents of cholera. Other serogroups (serovars) are generally termed non-O1, non-O139 strains, are generally non-choleragenic, usually cause a milder form of gastroenteritis than O1 and O139, and are normally associated with sporadic cases and small outbreaks rather than with epidemics and pandemics (Kaper et al. 1995; Borroto, 1997; Desmarchelier, 1997).

The O1 serovar has three antigenic forms: Inaba, Ogawa and Hikojima, and can be classified into two biotypes, Classical and El Tor, based on their phenotypic characteristics (Kaper et al. 1995). Recent studies have shown that the Classical biotype strains are rarely isolated from any part of the world (Sack et al. 2003). The choleragenic El Tor biotype strains of *V. cholerae* are grouped in four major clonal groups which seem to reflect broad demographic and epidemiological associations (Wachsmuth et al. 1994):

(i) The seventh pandemic
(ii) The U.S. Gulf Coast
(iii) Australia
(iv) Latin America (difficult to distinguish from the seventh pandemic strain and produces a very similar PFGE pattern),

The most important virulence factor associated with *V. cholerae* O1 and O139 is the cholera toxin. The *ctx* genes (*ctxA* and *ctxB*) encoding the production of the cholera toxin have been sequenced and this has enabled development of DNA probes and polymerase chain reaction (PCR) methods for detection of this gene, enabling specific detection of choleragenic *V. cholerae* from seafood and water.
In addition to cholera toxin, choleragenic strains of *V. cholerae* possess the ability to adhere to, and colonise, the small intestine (colonisation factor), which has been attributed, *inter alia*, to a toxin-co-regulated pilus (TCP).

Though *ctx*-positive non-O1, non-O139 strains have been found, these strains often lack the full set of virulence genes found in epidemic strains. Although *ctx*-positive non-O1, non-O139 serovars of *V. cholerae* have been implicated in cholera-like disease, only sporadic cases have been reported (Dalsgaard *et al.* 2001).

### 2. Exposure Assessment

#### 2.1 Prevalence of *V. cholerae* O1 and O139 in prawns and water

The primary source of *V. cholerae* O1 and O139 is faeces of persons infected with the organism, which reaches water supplies most often through sewage. The organism can survive in water for long periods with an average time for a 1-log decline in cell number (t$_{90}$) between 2-4 days (Feachem *et al.* 1981) both in fresh- and seawater.

In Australia, *V. cholerae* O1 was isolated intermittently over a 22-month period from river water that was used as an auxiliary town water supply and was implicated in a case of cholera in 1977 (Rogers *et al.* 1977). However, *V. cholerae* O1 and O139 are confined to fresh water and estuarine environments and there are no reports of the presence of these organisms in offshore environments.

In the aquatic environment, there is a strong association between levels of zooplankton and incidence of *V. cholerae* (Huq *et al.* 1983), with adhesion to chitin a major influence on its ecology (Nalin *et al.* 1979). It has also been reported to attach to the hindgut of crabs (Huq *et al.* 1996) and it is noted that the hindgut of crustaceans is an extension of the exoskeleton and is lined with chitin.

Dalsgaard *et al.* (1995a) found that *V. cholerae* O1 was present in 2% (2/107) of water, sediment and prawn samples collected from a major prawn culture area in South-east Asia. However, subsequent testing of the isolates indicated absence of the *ctx* genes in both of the O1 strains (Dalsgaard *et al.* 1995b). Data from India showed the presence of *V. cholerae* O1 in 0.2% of raw prawn (Ministry of Agriculture, India, personal communication, 2001). However, the choleragenic status of these prawn-associated strains is unknown. Prawn imported into Europe in early 2005 tested positive for *V. cholerae*; but the subsequent detailed analysis indicated that they were non-toxigenic strains.

Crustaceans, molluscs and finfish prepared in a variety of forms have been vectors for the transmission of *V. cholerae*. There is one outbreak linked to the consumption of raw prawn in the United States of America in 1986, where the source was domestic (Lowry *et al.* 1989). Another outbreak in Japan in 1978 was associated with lobsters imported from Indonesia (IASR, 1998). There was one other cholera outbreak linked to the consumption of raw prawns, in the Philippines in 1962, though, since the source of prawns is not known, it is not possible to assess whether *V. cholerae* O1 was naturally present or there as a result of cross-contamination after harvest (Joseph *et al.* 1965). The shellfish most often associated with cholera cases are molluscan shellfish (oysters) and crabs. While oysters are consumed raw in many countries, crabs are generally cooked, though even after boiling crabs for up to 10 minutes or steaming for up to 30 minutes, *V. cholerae* O1 may still retain viability (Blake *et al.* 1980).
2.2 Occurrence of choleragenic V. cholerae O1 and O139

In wild-caught prawns there is no evidence to show that marine prawn caught by trawling in offshore waters with salinities around 30 ppt harbour choleragenic V. cholerae O1 and O139, these organisms occurring in waters with salinities between 0.2 and 20 ppt (Colwell & Spira, 1992). Studies conducted on freshly harvested marine prawn in India, Sri Lanka and Thailand indicate absence of choleragenic V. cholerae (Suseela et al., 1988; Iyer et al. 1988; Fonseka, 1990; Karunasagar et al. 1990, 1992; Rattagool et al. 1990; Dalsgaard et al. 1995b).

In contrast to wild-caught, prawn aquaculture activities are generally in coastal areas and the water source is often estuarine, allowing the introduction of V. cholerae O1 or O139 in cholera-endemic areas. However, studies conducted in several Asian countries indicate absence of choleragenic V. cholerae in prawn from aquaculture ponds (Reilly & Twiddy, 1992; Nayyar Ahmad et al. 1995; Bhaskar et al. 1998; Otta et al. 1999; Shetty, 1999; Darshan, 2000; Dalsgaard et al. 1995b; Gopal et al. 2005).

2.3 Growth and survival characteristics

The physicochemical factors limiting the growth of V. cholerae O1 have been summarised by ICMSF (1996). The optimum temperature for growth is 37°C with a range of 10 to 43°C. The pH optimum for growth is 7.6 and V. cholerae can grow in a pH range of 5.0 to 9.6. The water activity optimum for growth is 0.984 and growth can occur between 0.970 and 0.998. V. cholerae can grow in a salt range of 0.1–4.0% sodium chloride (NaCl), with an optimum for growth of 0.5% NaCl.

2.4 Death or inactivation

V. cholerae O1 is highly sensitive to acidic environments and is killed within minutes in gastric juice with pH <2.4. Therefore, normochlorohydric individuals are less susceptible to cholera, provided the food matrix does not protect the organisms. V. cholerae O1 is also highly sensitive to desiccation, indicating the need to use well-dried containers in product handling to minimise the transmission of cholera. This organism is heat sensitive, with a D-value of 2.65 minutes at 60°C (ICMSF, 1996).

Most studies indicate that, while decline occurs at refrigeration temperatures, a proportion of the bacterial population remains viable.

2.5 Harvest, post-harvest handling and transport

Exposure assessment involves estimation of the likelihood of ingesting choleragenic V. cholerae O1 and O139 by eating prawns contaminated with these organisms, and the numbers of the organisms consumed. Since most of the world's prawn production and processing occurs in developing countries in Asia and Latin America, where cholera may be endemic, there can be multiple modes of contamination. Therefore, a process model for exposure assessment involves the possibility of contamination and of changes in population during pre-harvest, harvest, post-harvest handling, retail and at household level during preparation for consumption (Figure 1).
Marine prawn harvested by trawling are separated from the by-catch by manual sorting and then iced on board. Ice is generally produced using potable water in coastal ice plants and taken on board in insulated containers. During on-board handling, contamination with *V. cholerae* is possible if the person handling prawn and ice is a carrier of *V. cholerae* O1, or if the ice has become contaminated with choleragenic *V. cholerae*. However, the use of potable water – and in many cases the implementation of a HACCP system (as required for products exported to many countries e.g. the United States of America, the European Union and Australia) in the production and handling of the ice – minimises the opportunity for faecal contamination of ice.

In cholera-endemic areas, asymptomatic carriers play an important role in transmission of the pathogen. In fact, for water stored in households, contamination through the hand contact of carriers has been observed as a route for transmission of cholera (Deb *et al.* 1986). Thus, contamination via the hands of prawn handlers is a possible route. However, where personal hygiene and other hygienic conditions are controlled by the implementation of GHPs and a HACCP system in prawn processing, the likelihood of faecal contamination of prawn via fingers becomes very low.
In the case of cultured prawns, faecal contamination may occur during harvest and handling before the prawns are washed and chilled. The likelihood and level of such contamination is unknown, although the implementation of GHPs and HACCP along the chain should mean that this is low. Control points include dipping or washing in water, icing and packing in plastic crates for transport by truck to the processing plant. The process of dipping or washing may reduce the level of \textit{V. cholerae} in prawns, as shown by Dinesh (1991), who demonstrated that a one-log reduction in counts of \textit{V. cholerae} was brought about when whole prawns spiked with the organism were dipped or washed in tap water.

### 2.6 Processing and cooking

Post-harvest contamination with choleragenic \textit{V. cholerae} will be influenced by its concentration plus time-temperature during handling, processing and storage. Time-temperature distributions and the effects on densities of choleragenic \textit{V. cholerae} are presented in Table 16. The study of Kolvin and Roberts (1982) indicates that \textit{V. cholerae} O1 does not multiply in raw prawns. Further, the temperature of iced prawns during transport would be $<10^\circ C$, at which temperature \textit{V. cholerae} O1 does not multiply.

Product intended for export is processed in facilities, including prawn trawlers, which meet sanitary requirements for GHPs, GMPs and HACCP, where it is peeled manually or by machine, then washed, graded, processed (e.g. heading, gutting) and in some cases cooked, before being packed for freezing.

Cooking is undertaken for several reasons, most important of which are customer specifications or the prevention of melanosis (black spot formation), which can occur in the head during chilled storage. In Australia, Winkel (1997) studied the effect of cooking on black spot formation and organoleptic quality of \textit{Penaeus monodon} prawns. Winkel established that a core temperature of 75°C was sufficient to cook the flesh, but that prevention of black spot required a core temperature of 85°C. The time required to reach a 75°C core temperature was related to the size of prawn: almost four minutes for ‘large’ (50-65 g) prawns and 1.5 minutes for ‘small’ (25-30 g) prawns.

As part of an Australian code of practice for farmed prawns, Sumner (1997) observed prawn processing at six processing plants. Operators lowered each batch into ‘boiling’ water (ca 98°C) in a proportion of around 5:1 (water:prawns), a procedure which lowered the water temperature to around 92°C. The source of heat – usually a gas-fired ring – was then maximised and the water quickly brought to ‘boiling’ (ca 98°C), at which time the operator activated the timing device for the process. Since overcooking results in poor organoleptic quality and weight loss, cooking time is important. Depending on size, prawn were cooked for between 0.5 minute (‘small’) and 1.0 minute (‘large’), then immediately plunged into an ice-water slurry to bring an end to cooking.
Table 16: Time-temperature relations and their effect on concentration of choleragenic *V. cholerae* during processing of wild caught and aquaculture warm water prawns.

<table>
<thead>
<tr>
<th>Processing Step</th>
<th>Temp Range (°C)</th>
<th>Time</th>
<th>Concentration change (log)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HARVEST TIME PRE-ICING</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aquaculture prawn</td>
<td>15-35</td>
<td>0-1h</td>
<td>No effect</td>
<td>(a)</td>
</tr>
<tr>
<td>Wild caught prawn</td>
<td>10-30</td>
<td>0-3h</td>
<td>0-1 log increase</td>
<td>(b)</td>
</tr>
<tr>
<td><strong>WASHING, ICING</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aquaculture prawn</td>
<td>0-7</td>
<td>1-4h</td>
<td>1 log reduction</td>
<td>(c)</td>
</tr>
<tr>
<td>Wild caught prawn</td>
<td>0-30</td>
<td>1-4h</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ICING</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Icing during transport (including on board fishing vessel for wild caught prawn) to processor</td>
<td>0-7</td>
<td>2-16h (cultured)</td>
<td>2-3 log reduction</td>
<td>(d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-48h (wild)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>WATER USE AT PROCESSING</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature during processing before freezing</td>
<td>4-10</td>
<td>1-3h</td>
<td>No effect</td>
<td>(a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(b)</td>
</tr>
<tr>
<td><strong>TEMPERATURE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature during processing before freezing</td>
<td>4-10</td>
<td>2-8h</td>
<td>No effect</td>
<td>(a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(b)</td>
</tr>
<tr>
<td><strong>COOKING</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cooking at processing plant</td>
<td>&gt;90</td>
<td>0.5-1.0 min</td>
<td>&gt;6 log reduction</td>
<td>(e)</td>
</tr>
<tr>
<td>(holding time at &gt;90°C)</td>
<td></td>
<td></td>
<td></td>
<td>(f)</td>
</tr>
<tr>
<td><strong>FREEZING</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freezing of cooked and raw products, storage, and shipment time</td>
<td>-12-20</td>
<td>15-60 d</td>
<td>2-6 log reduction</td>
<td>(g)</td>
</tr>
</tbody>
</table>

Source: (a) Industry data for time, temperature (personal communication M/S Sterling Seafoods, Mangalore, India, 2002) (b) Kolvin and Roberts (1982) for multiplication; (c) Dinesh, 1991; (d) Karunasagar (personal communication, Karunasagar, India, 2002); (e) Based on industry data on total plate count (Pers. Comm. M/S Sterling Foods Mangalore, India) (f) In prawn homogenate $D_{82.2}=0.28$ (Hinton and Grodner, 1985); (g) INFOFISH (Pers. Comm) for shipment time, Reilly and Hackney (1985); Nascumento et al. (1998) for survival in frozen prawn

Since contamination with *V. cholerae* is likely to be external, the site of greatest microbiological concern for prawns is the carapace. From the foregoing, it is clear that the site of microbiological concern receives a highly lethal heat treatment e.g. $D_{82} = 0.28$ min in prawn homogenate (Hinton & Grodner, 1985). Thus, with at least 60 seconds at >90°C, the lethality is greater than 6 log units.

Post-cooking processing involves rapid chilling (ice slurry) prior to freezing. On board processing involves plate freezing while on-shore factories usually produce individually quick frozen (IQF) prawns. The opportunity for post-cooking re-contamination (from water, ice or handlers) of prawn is minimised by GHPs and SSOPs.

It is generally accepted that freezing reduces the concentration of contaminating vibrios, as does frozen storage. According to industry sources in India (M/S Sterling Seafoods, personal communication, 2002), the time interval between packing and the item reaching port-of-entry is usually > 30 days, with INFOFISH data indicating a range of 15 to 56 days.
The foregoing traces a processing continuum in which, at various stages, there is progressive inactivation of *V. cholerae*, particularly when product is held under refrigeration (iced or frozen), of the order of 5–6 log units. Cooking leads to additional inactivation of the order of 6 log units. Further inactivation during freezing and frozen storage provides an explanation for the lack of any documented involvement of internationally traded prawn in outbreaks of cholera in prawn importing countries.

As part of a risk assessment on *V. cholerae* in warm-water prawns, FAO/WHO obtained port-of-entry testing data from Japan, USA and Denmark. Of the more than 20,000 tests, over the period 1995-2000, two samples (in 1995) tested positive for choleragenic *V. cholerae*.

The ability of modern hygienic standards by prawn exporters was amply demonstrated during the Peruvian cholera epidemic in 1991. DePaola *et al.* (1993) showed that, while choleragenic *V. cholerae* O1 was present in all five samples of raw seafood collected from street vendors in Lima and Callao, it could be isolated from only one out of 1,011 samples of seafood destined for export.

### 2.7 Distribution and retail

Since the product is stored under frozen or refrigerated conditions, the retail market in importing countries provides little opportunity for contamination or multiplication of *V. cholerae* in prawns. This is supported by epidemiological data from countries such as Japan and the United States of America, where prawn consumption is high (estimated annual servings 8.5\(^8\) and 12.5\(^8\), respectively) and reported numbers of domestically acquired cholera cases are absent or very low (Table 17). There are no reports of either outbreaks or sporadic cases of cholera associated with imported prawn.

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>311</td>
<td>39</td>
<td>89</td>
<td>60</td>
<td>40</td>
<td>34</td>
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<td>USA</td>
<td>19</td>
<td>3</td>
<td>4</td>
<td>15</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Spain</td>
<td>6</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>France</td>
<td>5</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Netherlands</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>UK</td>
<td>10</td>
<td>13</td>
<td>6</td>
<td>18</td>
<td>-</td>
<td>33</td>
</tr>
<tr>
<td>Canada</td>
<td>7</td>
<td>2</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>6(4i)</td>
<td>4(1i)</td>
<td>14</td>
<td>71(38i)</td>
<td>18(11i)</td>
<td>9(3i)</td>
</tr>
<tr>
<td>Germany</td>
<td>1(1i)</td>
<td>-</td>
<td>2i</td>
<td>5(5i)</td>
<td>3(3i)</td>
<td>2(2i)</td>
</tr>
</tbody>
</table>

1\(^1\): imported cases (WHO 2001)

2\(^2\): 1 case in Guam and 1 case in Saipan reported by the CDC

3\(^3\): From 1997 Hong Kong Special Administrative Region of China

4\(^4\): Not in top ten importing countries but considered in the risk assessment due to the availability of relevant data
2.8 Consumption

As a generalisation, in importing countries, cold chain systems linking distribution, home and food service prevent temperature abuse. A survey by Audits International (2000) indicated that only 1.5% of home refrigerators operated warmer than 10°C, the minimum temperature for growth of *V. cholerae*.

Where data are not available on consumption patterns, the mean serving size may be assumed to be 275 g/meal, though, in reality, the edible portion will be <275 g after the carapace and cephalothorax have been removed prior to consumption. This assumption was made on the basis of an average serving of prawn consisting of 10 small prawns, described as weighing between 25 and 30 g or five large prawns, described as weighing 50–65 g (Winkel, 1997).

In international trade, prawns are marketed frozen in both the raw and cooked forms. In the cooked form, prawns are consumed without further heat treatment. Typically, raw prawns are cooked before consumption, though, with the growing popularity of sushi and sashimi, a proportion is eaten without further heat treatment; this proportion might be expected to be higher in Japan than in the other countries.

3. Hazard Characterisation

*V. cholerae* O1 and O139 cause the illness known as cholera, which in its severe form, *cholera gravis*, is an illness characterised by the passage of voluminous stools leading to dehydration. If untreated, the resulting dehydration can lead to hypovolemic shock and the death of the patient within 18 hours to several days of onset of symptoms, or sooner in extreme cases (Bennish, 1994). The case-fatality rate in untreated cases may reach 30-50%. However, treatment is straightforward and, if applied appropriately, the case-fatality rate is less that 1% (WHO, 2004).

*V. cholerae* is sensitive to acid and therefore must successfully pass the acid barrier of the stomach in order to cause infection. Choleragenic *V. cholerae* are known to have several genetic factors related to virulence. In order to establish and multiply in the human small intestine, the organism requires one or more adherence factors that enable them to attach to the microvilli or intestinal epithelial cells (Kaper et al. 1995). The *ctx* operon is, however, the primary factor associated with choleragenicity because it codes for cholera toxin (CT), which is made up of an A and B subunit and is responsible for the symptoms of cholera. These include the disruption of ion transport, with the subsequent loss of water and electrolytes, leading to severe diarrhoea.

This toxin is secreted by the choleragenic *V. cholerae* O1 and O139 strains. The O1 serogroup can be classified into three main sub-groups: Ogawa, Inaba and Hikojima. Strains may be subclassified into two biotypes: Classical and El Tor (Kaper et al. 1995). Genetic studies have shown that the *V. cholerae* O139 choleragenic strain has evolved from an El Tor biotype (Faruque et al. 2003).

3.1 Characteristics of the host

The host immune system is the critical defence mechanism against cholera. However, infection with cholera can result in a range of responses, from severe and life threatening diarrhoea to mild or non-clinical infections. In endemic areas, for example, only a minority (20–40%) of infections with *V. cholerae* O1 El Tor results in any illness (Bart et al. 1970; Shahid et al. 1984).
Prior immunological experience is an important factor and reports of much higher attack rates in children compared with adults in cholera-endemic areas support this (Glass et al. 1982). Another factor may be differences in gastric acidity. As gastric acid is an important defence mechanism against cholera, low acid production can lead to increased susceptibility (Nalin et al. 1978; Van Loon et al. 1990).

Recurrent infections of cholera are rare and volunteer studies have shown that clinical cholera infection confers 90 to 100% protection against subsequent re-challenge with choleragenic *V. cholerae* (Cash et al. 1974; Levine et al. 1979, 1981; Levine, 1980). In addition, epidemiological studies in endemic areas have indicated that immunity follows an initial natural cholera infection (Glass et al. 1982).

Pregnant women appear to experience a more severe form of the disease than non-pregnant women. In addition, foetal loss is high, with one report indicating a foetal death rate of 50% among women in their third trimester of pregnancy who developed severe dehydration from cholera (Hirschhorn et al. 1969).

A number of demographic and socioeconomic factors, such as age, gender, nutritional status, social status, economic status and travel abroad, all play a role in susceptibility to choleragenic *V. cholerae*.

Infection is known to be more severe in individuals suffering from malnutrition, with Hypochlorhydria associated with malnutrition, B12 deficiency and gastritis predisposing to the development of cholera. However, under nutrition does not seem to be associated with increased risk (Richardson, 1994).

In cholera-endemic areas, children 2–15 years are considered most susceptible to cholera when this group experiences initial infection (Glass et al. 1982). The symptoms of first infections are severe, but rarely are people hospitalised a second time for the disease, suggesting that immunity is long lasting and protective against severe illness. Breastfeeding appears to be an important factor in reducing susceptibility to cholera among infants and young children. One study indicated 70% reduction in the risk of severe cholera among breast-fed children (Clemens et al. 1990). In cholera-endemic areas, women of childbearing age (15–35) are commonly infected. In developed countries where hygienic standards are high, all age groups are equally susceptible (Kaper et al. 1995). Most cases in countries where high hygienic standards exist are imported cases, in that exposure to *V. cholerae* occurred while travelling in another country.

Among host susceptibility factors, the association between cholera and blood group is notable. Barua and Paguio (1977) and Chaudhuri and De (1977) noted that the incidence of cholera in patients with blood group A was lower than that in the general population, while incidence in those with blood type O was significantly higher. The likelihood of *V. cholerae* infection progressing to the severe form, cholera gravis, appears to be related to the individual’s ABO blood group (Levine et al. 1979). Thus, individuals with blood group O are more likely to exhibit severe diarrhoea. In terms of genetic factors, there is a hypothesis that those heterozygous for the cystic fibrosis allele are apparently less susceptible to severe cases of cholera (Rodman & Zamudio, 1991).

### 3.2 Characteristics of the food matrix

While choleragenic *V. cholerae* O1 ingested with food is likely to be protected from gastric acid, human volunteer studies have produced mixed results. In one study, human volunteers ingested $10^6$ *V. cholerae* O1 El Tor with 2 g of sodium bicarbonate (NaHCO$_3$) in 300 mL
water or with a meal of fish, rice, milk and custard (Levine et al. 1981). Volunteers who ingested *V. cholerae* with water alone did not become infected, but those who ingested the organism in a meal had cholera of similar severity and attack rate to those who had buffered gastric acidity with NaHCO$_3$ (Levine et al. 1981). By contrast, experiments by Cash et al. (1974) showed different results (see below).

Choleragenic *V. cholerae* appear to be relatively sensitive to both low pH and to dehydration. The pH sensitivity of *V. cholerae* is illustrated by the epidemiological data of St Louis et al. (1990), who observed that, in an epidemic in Guinea, West Africa, the cholera patients were more likely to have eaten left-over peanut sauce (pH 6.0) but less likely to have eaten tomato sauce (acid). This was further confirmed by laboratory studies in which *V. cholerae* multiplied rapidly in peanut sauce but not in more acidic tomato sauce.

*V. cholerae* O1 are extremely sensitive to an acidic environment (Dalsgaard et al. 1997). In gastric juice with pH <2.4, *V. cholerae* O1 were inactivated rapidly (Nalin et al. 1978; Levine et al. 1984). Since *V. cholerae* O1 are transmitted via the oral route only, the organisms must pass through the gastric acid environment of the stomach to colonise the intestine. In normochlorhydric adult volunteers, doses of up to $10^{11}$ pathogenic *V. cholerae* O1 given without buffer or food did not reliably cause illness, whereas doses of $10^4$–$10^8$ organisms given with 2 g of NaHCO$_3$ resulted in diarrhoea in 90% of individuals (Cash et al. 1974). The characteristics of illness in individuals with $10^6$ organisms given with 2 g of NaHCO$_3$ were similar to that of cholera. In another volunteer study, doses of $10^5$, $10^4$ and $10^3$ organisms resulted in a 60% attack rate, although the diarrhoeal illness at the two lower doses was milder and appeared to have longer incubation periods (Levine et al. 1981).

Because of the nature of most foods associated with the unintended consumption of *V. cholerae*, pH and water activity are probably not relevant in modelling survival of *V. cholerae* in raw seafood. However, these parameters may be relevant in modelling the growth of *V. cholerae* in other foods as a result of cross-contamination.

### 3.3 Public health outcomes

When illness occurs, *V. cholerae* O1 and O139 cause mild to severe gastrointestinal illness and may bring about patient dehydration leading to death. Common symptoms include profuse watery diarrhoea, anorexia and abdominal discomfort. In *cholera gravis*, the rate of diarrhoea may quickly reach 500–1000 mL/h, leading rapidly to tachycardia, hypotension, and vascular collapse due to dehydration (Kaper et al. 1995). About 20% of those who are infected develop acute, watery diarrhoea and 10 to 20% of these individuals go on to develop severe watery diarrhoea with vomiting (WHO, 2004).

### 3.4 Number of cholera cases reported to WHO by prawn importing countries

Cholera is one of the diseases requiring notification to WHO according to the International Health Regulation. It is worth noting that the United Kingdom (Adak et al. 2002) and the United States of America (Mead et al. 1999) estimate that 50% of all cholera cases are reported, which is high compared with the level of reporting of some other gastrointestinal illnesses, such as non-typhoidal salmonellosis and campylobacteriosis, for which actual cases are estimated to be 38 times more than reported cases (Mead et al. 1999).

In the available documentation, none of the cholera cases reported has been associated with consumption of imported prawns. Except for a few, all cholera cases in the United States of America and European countries were overseas-acquired. The United States of America has
an endemic focus of *V. cholerae* in Gulf Coast waters and has experienced sporadic cases and small clusters of cholera related to the domestic consumption of contaminated seafood from those waters (Blake *et al.* 1980; Blake *et al.* 1983). Japan has reported an increasing number of apparent domestically-acquired cholera cases (IASR, 1998). The reason(s) for this increase is unknown, cases typically being sporadic with no known aetiology.

In Australia, data from the National Enteric Pathogens Surveillance Scheme (NEPSS) indicates a small number of reported annual illnesses from *V. cholerae*; over the period 2006-2009 there were a total of 17 reports, of which 13 were overseas-acquired.

### 3.5 Dose-response relationship

Dose-response relationships can be developed from epidemiological investigations of outbreaks and sporadic case series, human feeding trials or animal models of a particular pathogen and related (surrogate) pathogens. In this instance, human feeding trial data were available for *V. cholerae* and were used in the development of the dose-response curve.

There are numerous studies and references in the literature to the infectious dose of choleragenic *V. cholerae*. The most commonly reported infectious dose is approximately $10^6$ organisms or more (Levine *et al.* 1981; Tauxe *et al.* 1994; Health Canada, 2001; FDA, 2003). While, as indicated above, there are numerous other factors that influence whether or not a person becomes ill after ingestion of choleragenic *V. cholerae*, this estimate was used in both the qualitative and semi-quantitative risk characterisations described later. A number of human volunteer studies are available for choleragenic *V. cholerae*. Although these are between 15 and 30 years old, they are the best data available in terms of providing an insight into the dose response of the organism. These data are used as a basis to develop a dose-response model, as described below.

Human volunteer data are available for the Classical and El Tor biotypes and Inaba and Ogawa serogroups of *V. cholerae* O1. Cash *et al.* (1974) studied Classical Inaba and Ogawa strains, while Levine *et al.* (1988) and Black *et al.* (1987) studied El Tor Inaba and Ogawa strains. The results from these studies are shown in the dose-response curve presented in Figure 2. As noted above, volunteer data were also available for *V. cholerae* O139.

Many choleragenic *V. cholerae* O1 and O139 infections result in the serious condition called *cholera gravis*, which can be life threatening. There are no specific sequellae associated with the severe form of illness other than the risk of death.

While illness due to choleragenic *V. cholerae* O1 and O139 is observed to occur in families, it is thought that a common source of primary infection, rather than secondary transmission, is the more likely mode of transmission (Glass & Black, 1992). While there is anecdotal indication that direct person-to-person transmission may occur, it has never been demonstrated by rigorous scientific study (Mintz *et al.* 1994).

The probability of death as the result of choleragenic *V. cholerae* O1 and O139 is dependent on the public health infrastructure of the locality where the case of cholera is acquired. The cornerstone in cholera therapy is rapid oral rehydration. Administration of antibiotics may shorten the duration of diseases (Bennish, 1994). If adequate rehydration is not provided, mortalities range between 20 and 50%. However, in most affected developing countries, mortality rates are less than 5% where oral electrolyte solutions are available (Glass and Black, 1992).
3.6 Dose-response model

In this assessment, a dose-response curve can be obtained by fitting the approximate Beta-Poisson model to the data available from several volunteer studies (Cash et al. 1974; Levine et al. 1981, 1988).

\[ \text{Pr(ill)} = 1 - (1 + \text{dose})^{\alpha \beta} \]

Firstly, in the study by Cash et al. (1974), volunteers were exposed to a range of doses of the Classical biotype of \textit{V. cholerae} O1 in a food matrix (beef broth). The same organism was given also to human volunteers together with an acid-neutralising solution. A dose-response model was developed by FAO-WHO (2005) using both of these data sets, and resulted in a dose-response curve with higher attack rates at lower doses in volunteers given the organism with an acid-neutralising solution compared with a food matrix (Figure 2). The study of Cash \textit{et al.} (1974) was comprehensive as it examined a range of \textit{V. cholerae} doses administered both with a food matrix and with an acid-neutralising solution. However, as recent studies have shown that the Classical biotype strains are rarely isolated from any part of the world (Sack \textit{et al.} 2003) these data were not considered to be the most appropriate for developing a dose-response model relevant to current exposure to choleragenic \textit{V. cholerae}.

The studies of Levine \textit{et al.} (1981, 1988) focused on the El Tor biotype of choleragenic \textit{V. cholerae} and exposed volunteers to \textit{V. cholerae} in a food matrix, an acid-neutralising solution and water. In contrast to the results of Cash \textit{et al.} (1974), described above, there is evidence provided by Levine \textit{et al.} (1981) that there is no significant food matrix effect, and that dose-response curves obtained from human volunteer studies where \textit{V. cholerae} doses administered with acid-neutralising solutions adequately model the consumption of \textit{V. cholerae} with food. In their study, they found that for an El Tor \textit{V. cholerae} given to human volunteers at a dose of $10^6$ organisms, a similar response was observed whether the dose was administered with an acid-neutralising solution or with a standard meal of fish, rice, custard and skim milk (Levine \textit{et al.} 1981).

The conflicting evidence provided by Levine \textit{et al.} (1981) compared with Cash \textit{et al.} (1974) adds to the uncertainty of dose-response curve for \textit{V. cholerae}. Whether this reflects a difference in the two biotypes or not is not known. However, it is acknowledged that the effect of the food matrix on the dose response when consuming pathogenic vibrios is an important area for future research and represents a critical data gap for this risk assessment. All of these data and the resulting dose-response curves are included in Figure 2. As the study of Levine \textit{et al.} (1988) looked at a range of doses ($10^6$, $10^8$, $10^{10}$), these data were used for the development of a dose-response curve for the El Tor biotype.

Figure 2 essentially shows the maximum likelihood fit of the Beta-Poisson model to the available feeding trial data. A human volunteer study with \textit{V. cholerae} O139 reported similar infectious doses as described for the O1 serotype (Cohen \textit{et al.} 1999).
Figure 2: Beta-poisson dose-response curves for different strains of *V. cholerae*

KEY: □ = Classical with food matrix (Cash *et al.* 1974); – – – = fit to Classical with food matrix; □ = El Tor with antacid (Levine *et al.* 1988); – – – – = fit to El Tor with antacid; ▲ = Classical Inaba with antacid (Cash *et al.* 1974); – – – – – = fit to Classical Inaba with antacid; ◊ = miscellaneous El Tor strains tested; △ = Classical Ogawa (Cash *et al.* 1974); ○ = El Tor with bicarbonate (Levine *et al.* 1981); ◂ = El Tor with food (Levine *et al.* 1981); X = El Tor with water (Levine *et al.* 1981).

4. Summary in the Australian Context

- Data compiled by the National Enteric Pathogens Surveillance Scheme (NEPSS) indicates that cholera is almost unknown in Australia and of the cases which are reported, almost all are overseas-acquired.
- In testing of imported prawns, AQIS occasionally isolated *V. cholerae* from a 25 g sample; no further testing is done to determine whether the isolate is toxigenic.
- There have been no cases of cholera in Australia linked with consumption of prawns

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**Vibrio parahaemolyticus**

1. **Hazard Identification**

*Vibrio parahaemolyticus* is a marine micro-organism occurring in estuarine waters throughout the world. The organism was first identified as a foodborne pathogen in Japan in the 1950s (Fujino *et al.* 1953). By the late 1960s and early 1970s, *V. parahaemolyticus* was recognised as a cause of diarrhoeal disease worldwide, although most common in Asia and the United States. Vibrios concentrate in the gut of filter-feeding molluscan shellfish such as oysters, clams and mussels where they multiply and cohere. Although thorough cooking destroys these organisms, oysters are often eaten raw and, at least in the United States, are the most common food associated with *Vibrio* infection (Hlady, 1997).

In tropical and temperate regions, disease-causing species of *Vibrio* occur naturally in marine, coastal and estuarine (brackish) environments and Desmarchelier (2003) reports that, in Australia, pathogenic vibrios can be isolated from freshwater reaches of estuaries. The occurrence of these bacteria does not correlate with numbers of faecal coliforms and depuration of shellfish may not reduce their numbers. There is a positive correlation between water temperature and both the number of human pathogenic vibrios isolated and the number of reported infections, a correlation particularly marked for *V. parahaemolyticus* and *V. vulnificus*.

This hazard sheet relies heavily on information updated by the joint FAO-WHO work on Vibrios in seafoods (FAO-WHO, 2011).

1.1 **Human incidence**

In Asia, *V. parahaemolyticus* is a common cause of foodborne disease. In general, the outbreaks are small in scale, involving fewer than 10 cases, but occur frequently. Prior to 1994, the incidence of *V. parahaemolyticus* infections in Japan had been declining, however, in 1994-95 there were 1,280 reports of infection due to the organism (Anon., 1999). During this period, *V. parahaemolyticus* food poisonings outnumbered those of *Salmonella*. For both years, the majority of the cases occurred in the summer, with the largest number appearing in August. From 1996-1998, there were 496 outbreaks, 1,710 incidents and 24,373 cases of *V. parahaemolyticus* reported. The number of *V. parahaemolyticus* food poisoning cases doubled in 1998 as compared with 1997 and again exceeded the number of *Salmonella* cases (Anon., 1999). As in 1994-1995, outbreaks were more prevalent in the summer with a peak in August and with few outbreaks during winter months. Boiled crabs caused one large-scale outbreak, involving 691 cases. The increased incidence in Japan during 1997-1998 has been attributed to an increased incidence of serovar O3:K6.

During 1997 and 1998 there were more than 700 cases of illness due to *V. parahaemolyticus* in the United States, the majority of which were associated with the consumption of raw oysters. In two of the 1998 outbreaks a serotype of *V. parahaemolyticus*, O3:K6, reported previously only in Asia, emerged as a principal cause of illness for the first time. Subsequent studies on these strains have revealed their pandemic spread. It was suggested that warmer than usual water temperatures were responsible for the outbreaks.

In Europe few data exist on the incidence of *V. parahaemolyticus* infections, one reason being that such infections are not notifiable. However the current knowledge of the incidence in Europe has been summarised in *Opinion of the Scientific Committee on Veterinary Measures relating to Public Health on Vibrio vulnificus and Vibrio parahaemolyticus in raw*
and undercooked seafood issued by the European Commission (European Commission, 2001).

In Australia, the reported incidence is low, probably reflecting the consumption of seafood which is, with the exception of oysters, usually eaten fully cooked. Gastroenteritis caused by this organism is almost exclusively associated with seafood consumed raw or inadequately cooked or contaminated after cooking. In the USA, illness is most commonly associated with crabs, oysters, shrimp and lobster (Twedt, 1989; Oliver & Kaper, 2007). In Australia, outbreaks have been associated with prawns and oysters (Kraa, 1995).

There have been no reports of its presence in Australian prawns except for one of 63 samples tested in New Zealand (Lake et al. 2003); no information is provided on whether the isolate was toxigenic.

The incidence of V. parahaemolyticus in prawns in various countries, some of which are due to imports is presented in Table 18.

<table>
<thead>
<tr>
<th>Country</th>
<th>(% positive, number of samples)</th>
<th>Biotype information</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK</td>
<td>Retail cooked prawns and shrimps (0/148)</td>
<td>None detected</td>
<td>Greenwood et al. 1985</td>
</tr>
<tr>
<td>India</td>
<td>Crustaceans (79.3%), Fish and shrimps from coastal waters (60%)</td>
<td>Not reported</td>
<td>Lall et al. 1979</td>
</tr>
<tr>
<td>China</td>
<td>Prawns (25%)</td>
<td>Not reported</td>
<td>Qadri &amp; Zuberi 1977</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>Prawns (4/50, 8%)</td>
<td>2.5% KP+</td>
<td>Yam et al. 2000</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>Prawns</td>
<td>32/g</td>
<td>Chan et al. 1989</td>
</tr>
<tr>
<td>Japan (imports)</td>
<td>Raw prawns (26.3%, 21/80)</td>
<td>Tdh positive by PCR. Isolates mostly O3:K6</td>
<td>Hara-Kudo et al. 2003</td>
</tr>
<tr>
<td>Mexico</td>
<td>Prawns (27.6%)</td>
<td>Not reported</td>
<td>Vitela et al. 1993</td>
</tr>
<tr>
<td>Taiwan</td>
<td>Raw prawns (25%, 10/40)</td>
<td>Not reported</td>
<td>Wong et al. 1995</td>
</tr>
<tr>
<td>Taiwan (imports)</td>
<td>Prawns (Thailand) (75.8%, 47/62)</td>
<td>Isolates did not contain tdh or trh gene</td>
<td>Wong et al. 1999</td>
</tr>
</tbody>
</table>

1.2 Foods implicated

V. parahaemolyticus occurs in a variety of fish and shellfish including clams, shrimp, lobster, crayfish, scallops and crabs as well as oysters. Although oysters are the most common food associated with Vibrio infection in some countries (Hlady, 1997), there have been reports of V. parahaemolyticus infections associated with the other types of seafood:

- Outbreaks of V. parahaemolyticus gastroenteritis aboard two Caribbean cruise ships were reported in 1974 and 1975 (Lawrence et al. 1979). The outbreaks were most likely caused by contamination of cooked seafood by seawater from the ships’ seawater fire systems.
• In 1972, an estimated 50% of 1,200 persons who attended a shrimp feast in Louisiana in the United States became ill with *V. parahaemolyticus* gastroenteritis (Barker & Gangarosa, 1974) and samples of uncooked shrimp tested positive for the organism.

• Three outbreaks occurred in Maryland in the United States in 1971 (Dadisman *et al*. 1972). Steamed crabs were implicated in two of the outbreaks after cross-contamination with live crabs. The third outbreak was associated with crabmeat that had become contaminated before and during canning.

• A case-controlled study of sporadic *Vibrio* infections in two coastal areas of Louisiana and Texas in the United States conducted from 1992-93, in which crayfish consumption was reported by 50% (5/10) of the persons affected with *V. parahaemolyticus* infection (Bean *et al*. 1998).

• An outbreak in Louisiana in 1978 comprising 1133 cases was linked with raw shrimp shipped in wooden boxes, which were boiled, returned to the same boxes and transported unrefrigerated prior to consumption 7-8h later (Morbidity & Mortality Reports, 1978; also cited by Oliver & Kaper, 2007).

• More recently, sampling studies in the Adriatic Sea demonstrated the presence of *V. parahaemolyticus* in fish, mussels and clams (Baffone *et al*. 2000) and in mussels from the North-western coast of Spain. *V. parahaemolyticus* was isolated from 8% of samples (European Commission, 2001).

• There have been two large outbreaks of *V. parahaemolyticus* gastroenteritis in Australia linked with consumption of prawns. In 1990 an outbreak affecting more than 100 people, one of whom died, was linked to chilled, cooked prawns from Indonesia. In 1992 there were two outbreaks affecting more than 50 people linked to the same wholesale supplier of cooked prawns (Kraa, 1995).

2. Exposure Assessment

A process model for exposure assessment involves the possibility of contamination and of changes in population during harvest, processing, retail and at household level during preparation for consumption (Figure 3).

Prevalence of *V. parahaemolyticus* is associated with the presence of particulates, zooplankton and other chitin sources (Kaneko & Colwell, 1978; NACMCF, 1992; Venkateswaran *et al*. 1990). Several studies have shown that *Vibrio* spp. are capable of surviving and multiplying within certain protozoa such as *Amoeba* (Barker & Brown, 1994). It has also been reported that *V. parahaemolyticus* ‘over-winters’ in the sediment and is absent from the water column and oysters during the winter months (Joseph *et al*. 1983; Kaysner *et al*. 1989; United States Department of Health and Human Services Food and Drug Administration, 1995). Under extreme environmental conditions *V. parahaemolyticus*, may enter a ‘viable but non-culturable’ (VBN) phase in marine waters and could be missed by traditional cultural methods (Bates *et al*. 2000; Colwell *et al*. 1985; Oliver, 1995; Xu *et al*. 1982).

Until relatively recently, there have been no studies on the existence of pathogenic strains of *V. parahaemolyticus*. In the summer of 2001-02, Lewis *et al*. (2002) undertook a pilot study of prevalence of total and pathogenic *V. parahaemolyticus* from leases in NSW, SA and Tasmania (Table 5). The organism was isolated from 16/20 (80%) of oysters from NSW, 6/10 (60%) from Tasmania and 2/10 (20%) from SA. The study by Lewis *et al*. (2002), based on
only 40 samples of oysters from three states, was not regarded as definitive in the quantitative sense and a longitudinal study over an annual cycle was recommended. However, the study did isolate pathogenic strains, a finding of qualitative importance, especially for those areas where water temperatures are high for several consecutive months.

Such a study was undertaken in the summer of 2006-7 when Madigan et al. (2007) investigated South Australian oysters for presence of pathogenic vibrios. In 25 samples, each of twelve oysters, *V. parahaemolyticus* was isolated from four, of which three were trh+ and none was tdh+. Interestingly, while sucrose-negative vibrios (a category which contains *V. parahaemolyticus*) were relatively high (10^3-10^4/g) during warmer months, *V. parahaemolyticus* was isolated only after oyster samples were pre-enriched and molecular techniques were employed. When samples were enumerated the researchers considered pathogenic *V. parahaemolyticus* was present at below the limit of detection (<10/g) in oyster meat. It should be noted that some sucrose-negative vibrios (e.g. *V. harveyi*) do not cause human infections and, even among sucrose negative *V. parahaemolyticus*, only a very small proportion are tdh+ and hence pathogenic. Therefore presence of a sucrose-negative *Vibrio* count per se, should not be overstated in terms of presence of pathogenic strains.

More recently, in response to international activity regarding allowable concentrations of *V. parahaemolyticus* in seafood, a brief survey was undertaken of oysters grown in New South Wales, South Australia and Tasmania. *V. parahaemolyticus* was detected in 25/31 samples, generally at low concentrations in SA and Tasmanian oysters and ranging to 75 MPN/g in NSW oysters, where temperature and salinity were more favourable for the organism. In the 25 samples positive for the organisms, the *tdh* gene was recovered from two and *trh* from one sample, at low concentration (Madigan & May, 2010).

The three studies (above) indicate that, while *V. parahaemolyticus* is present in Australian waters, the ratio of pathogenic strains appears low.

### 2.1 Harvest, post-harvest handling and transport

Marine prawns harvested by trawling are separated from the by-catch by manual sorting and then iced on board. Ice is generally produced using potable water in coastal ice plants and taken on board in insulated containers. It is likely that *V. parahaemolyticus* will be present at low prevalence and low concentration after on-board processing.

In the case of cultured prawns, contamination with *V. parahaemolyticus* may occur during harvest and handling before the prawns are washed and chilled. The implementation of GHPs and HACCP along the chain should mean that this is low. Control points include dipping or washing in water, icing and packing in plastic crates for transport by truck to the processing plant.

### 2.2 Processing and cooking

Prawns intended for export are processed in facilities, including prawn trawlers, which meet sanitary requirements for GHPs, GMPs and HACCP, where they are peeled manually or by machine, then washed, graded, processed (e.g. heading, gutting) and, in some cases cooked, before being packed for freezing.

Cooking is undertaken for several reasons, most important of which are customer specifications or the prevention of melanosis (black spot formation), which can occur in the head during chilled storage. In Australia, Winkel (1997) studied the effect of cooking on
black spot formation and organoleptic quality of *Penaeus monodon*. Winkel established that a core temperature of 75°C was sufficient to cook the flesh, but that prevention of black spot required a core temperature of 85°C. The time required to reach a 75°C core temperature was related to the size of prawn: almost four minutes for ‘large’ (50–65 g) prawns and 1.5 minutes for ‘small’ (25–30 g) prawns.

As part of an Australian code of practice for farmed prawns, Sumner (1997) observed prawn processing at six processing plants. Operators lowered each batch into ‘boiling’ water (ca 98°C) in a proportion of around 5:1 (water:prawn), a procedure which lowered the water temperature to around 92°C. The source of heat – usually a gas-fired ring – was then maximised and the water quickly brought to ‘boiling’ (ca 98°C), at which time the operator activated the timing device for the process. Since overcooking results in poor organoleptic quality and weight loss, cooking time is important. Depending on size, prawns were cooked for between 0.5 minute (‘small’) and 1.0 minute (‘large’), then immediately plunged into an ice-water slurry to bring an end to cooking.

Post-cooking processing involves rapid chilling (ice slurry) prior to freezing. On board, product is plate-frozen in the carton while on-shore factories usually produce individually quick frozen (IQF) prawns. The opportunity for post-cooking re-contamination (from water, ice or handlers) of prawn is minimised by GHPs and SSOPs.

It is generally accepted that freezing reduces the concentration of contaminating vibrios, as does frozen storage. According to industry sources in India (M/S Sterling Seafoods, personal communication, 2002), the time interval between packing and the item reaching port-of-entry is usually > 30 days, with INFOFISH data indicating a range of 15 to 56 days.

### 2.3 Distribution and retail

Since the product is stored under frozen or refrigerated conditions, the retail market in importing countries provides little opportunity for contamination or multiplication of *V. parahaemolyticus* in prawns.

### 2.4 Consumption

In importing countries, cold chain systems linking distribution, home and food service prevent temperature abuse. There is no information on prawn consumption in Australia or in countries to which Australian product is exported. An assumption may be made that the mean serving size is 275 g/meal, though, in reality, the edible portion will be <275 g after the carapace and cephalothorax have been removed prior to consumption. This assumption was made on the basis of an average serving of prawn consisting of 10 small prawns, described as weighing between 25 and 30 g (average 27.5 g) or five large prawns, described as weighing 50–65 g (Winkel, 1997).

In international trade, prawns are marketed frozen in both the raw and cooked forms. In the cooked form, prawns are consumed without further heat treatment. Typically, raw prawns are cooked before consumption, though, with the growing popularity of sushi and sashimi, a proportion is eaten without further heat treatment; this proportion might be expected to be higher in Japan than in the other countries.
Figure 3: Production-consumption pathway for exposure assessment of *V. parahaemolyticus* in warm-water prawns

- **HARVEST**
  - Coastal areas
  - wild caught or from aquaculture ponds
  - *V. parahaemolyticus*/g

- **POST HARVEST HANDLING AND TRANSPORT**
  - Prawns washed and iced
  - *V. parahaemolyticus*/g

- **PROCESSING**
  - Prawns washed, peeled, graded, packed and frozen
  - *V. parahaemolyticus*/g

- **COOKING**
  - Graded prawns cooked, cooled, packed, frozen
  - *V. parahaemolyticus*/g

- **DISTRIBUTION AND RETAIL**
  - Frozen prawns in international transport, wholesale storage, supermarkets and restaurants
  - *V. parahaemolyticus*/g

- **CONSUMPTION**
  - Prawns thawed, prepared and eaten

- **No. of *V. parahaemolyticus*/g ingested**

- *V. parahaemolyticus*/g in ice
- *V. parahaemolyticus*/ml in water
- *V. parahaemolyticus*/ml in water
- Time and temperature of frozen storage
- Time and temperature of frozen storage
2.5 Growth and survival characteristics

*V. parahaemolyticus* is a mildly halophilic, mesophilic micro-organism and its general growth characteristics are shown in Table 19 (ICMSF, 1996). Warmer temperatures and moderate salinity favour the survival and growth of *V. parahaemolyticus* (Covert & Woodburne, 1972; Jackson, 1974; Nair et al. 1980; Zhu et al. 1992). A correlation exists between *V. parahaemolyticus* infection and environmental temperatures with most of the shellfish-borne illnesses caused by this organism occurring in the warmer months. This has been observed in the United States, Asia and Europe (Daniels et al. 2000; Geneste et al. 2000).

Although outbreaks of foodborne disease associated with *V. parahaemolyticus* are less commonly reported in Europe, there have also been a number of studies that indicate the importance of temperature in the survival and growth of *Vibrio*. In a two year study undertaken in Italy on seawater and molluscs from the Adriatic Sea it was found that *Vibrio* strains were most prevalent during the summer months (Croci et al. 2001). In another study conducted in Norwegian waters *V. parahaemolyticus* was only detected in July and August (Gjerde & Bøe, 1981).

In France, hydrobiological monitoring carried out near nuclear power plants built on the seashore, showed that the most spectacular effect was on the density of vibrios. The levels were 100 times higher after the construction of the nuclear power plant than before, and vibrios were found at a level of $10^5$/L in its surrounds. Also, the annual decline in *Vibrio* densities during the colder months of the year ‘overwinter’ no longer occurred (Gregoire et al. 1993).

| Table 19: Growth characteristics of *Vibrio parahaemolyticus* (ICMSF, 1996) |
|------------------|-----------------|-----------------|
| **Temperature (°C)** | **Optimum** | **Range** |
| pH | 7.8 – 8.6 | 4.8 – 11 |
| NaCl (%) | 3 | 0.5 - 10 |
| Water activity ($a_w$) | 0.981 | 0.940 – 0.996 |
| Atmosphere | Aerobic | Aerobic - anaerobic |

2.6 Growth rate

Growth of *V. parahaemolyticus* can be rapid, for example, doubling times of 27 minutes have been reported in crabmeat at both 20 and 30°C (Liston, 1974). Growth rates in a range of seafoods and tryptic soy broth with 2.5% salt (NaCl) have been recorded and summarised (ICMSF, 1996). These data indicate that moderate populations of $10^3-10^3$ organisms/g on seafood can increase to $>10^5$ organisms/g in two to three hours at ambient temperatures of between 20 and 35°C (ICMSF, 1996).

Miles et al. (1997) modelled the growth rate of *V. parahaemolyticus* based on studies of four strains at different temperatures and water activity. For each combination of temperature and water activity, bacterial growth was modelled using the Gompertz function, a sigmoid growth curve with a growth rate (slope) monotonically increasing to a maximum before falling to zero as the bacterial population reaches a steady state. The maximal rate of growth (μₘₐₓ) is the most relevant summary of the fit because the growth rate approaches its maximum rapidly.
and does not decline significantly until steady-state is reached. The model parameters describe the range of temperature and water activity over which growth can occur. The authors validated their model by comparing predicted growth with observed rates in eight other studies of growth in broth systems obtained from the literature.

### 2.7 Death and inactivation

Although the ecology of *V. parahaemolyticus* has been studied (Joseph *et al.* 1983; Kaneko & Colwell, 1978), little is known about the growth and survival of *V. parahaemolyticus* in prawns. By contrast, post harvest growth of *V. vulnificus* in oysters has been studied extensively (Cook, 1994; 1997) as have the effectiveness of various mitigation strategies for reducing *V. vulnificus* (Cook & Ruple, 1992; Eyles & Davey, 1984; Motes & DePaola, 1996; Richards, 1988; Son & Fleet, 1980). These include depuration, relaying, refrigerated storage and mild heat treatment.

### 3. Hazard Characterisation

Dose-response relationships can be developed from epidemiological investigations of outbreaks and sporadic case series, human feeding trials or animal models of *V. parahaemolyticus* and related (surrogate) pathogens. In Japan, for example, human trials showed an increase in the number of illnesses with increasing numbers of pathogenic *V. parahaemolyticus*. Different dose-response models have been compared for the purpose of extrapolating risk of illness estimated on the basis of human feeding trials at high levels of exposure to the lower levels of exposure associated with consumption of raw oysters (Anon. 2005). The human feeding trials were conducted under conditions of concurrent antacid administration.

#### 3.1 Description of the pathogen, host and food matrix factors and how these influence the disease outcome.

Infection by *V. parahaemolyticus* is characterised by an acute gastroenteritis usually within 4-30 hours of exposure. While most cases of *V. parahaemolyticus* infections are resolved without medical intervention, on rare occasions infection can lead to septicaemia and death.

The virulence of *V. parahaemolyticus* appears to be largely attributable to thermostable direct haemolysin (*tdh*⁺) (Miyamoto, *et al.* 1969). Strains of *V. parahaemolyticus* expressing this toxin lyse red blood cells on Wagatsuma agar and are also called Kanagawa positive (KP⁺); *tdh*⁺ and KP⁺ both indicate the presence of the toxin that is coded for by *tdh*⁺. The *tdh*⁺ allele is seldom found in environmental isolates of *V. parahaemolyticus*, but is frequently found in clinical isolates. Another genetic factor that may play a role in the virulence of *V. parahaemolyticus* is *trh*⁺ (*trh1* or *trh2*), an allele that codes for the TDH-related haemolysin (Honda *et al.* 1988; Shirai *et al.* 1990; Kinushita *et al.* 1992).

#### 3.2 Characteristics of the host

The immune system of the host responds to *Vibrio* spp infection to maintain health. The immunocompromised are at special risk for both infection and for more severe sequelae. In Japan cases of *V. parahaemolyticus* bacteraemia have been reported among patients who were all immunosuppressed, especially with leukaemia and cirrhosis (Ng *et al.* 1999).
While there are no known measures of physiological status relating to susceptibility to *V. parahaemolyticus* illness, analysis of epidemiological data indicate that pre-existing illnesses may predispose individuals with gastrointestinal illness to proceed to septicaemia (Anon., 2005).

In two epidemiological studies (Hlady, 1997; Klontz, 1990), *V. parahaemolyticus* accounted for 77/339 reported *Vibrio* infections, of which 68 reported gastroenteritis and nine had septicaemia; 29 persons were hospitalised for gastroenteritis with no deaths reported, while eight were hospitalised for septicaemia, of whom four died. Patients with septicaemia had underlying illness including, but not limited to, cancer, liver disease, alcoholism and *Diabetes mellitus* (Hlady, 1997; Klontz, 1990).

Hlady and Klontz (1996) reported that, of patients with infections, 25% had pre-existing liver disease or alcoholism. These included 75% of the septicaemia patients and 4% of the gastroenteritis patients. Of the remaining septicaemia patients, nine reported having a history of at least one of the following: malignancy, renal disease, peptic ulcer disease, gastrointestinal surgery, diabetes, antacid medication and pernicious anaemia. Among the gastroenteritis patients, 74% had none of the above pre-existing medical conditions or had insufficient information to classify.

There are no known human genetic factors that appear to be related to the susceptibility of individuals to *V. parahaemolyticus* illness.

### 3.3 Characteristics of the food matrix

*Vibrio* spp. appear to be relatively sensitive to both low pH and dehydration. Because of the nature of most foods associated with the unintended consumption of *Vibrio* spp., pH and water activity are probably not relevant in modelling survival of *Vibrio* spp. in raw seafood. However these parameters may be relevant in modelling the growth of *Vibrio* spp. in other foods as the result of cross contamination.

### 3.4 Public health outcomes

Gastroenteritis due to *V. parahaemolyticus* infection is usually a self-limiting illness of moderate severity and short duration (Barker, 1974; Barker & Gangarosa, 1974; Levine *et al.* 1993). However, severe cases requiring hospitalisation have been reported. Symptoms include explosive watery diarrhoea, nausea, vomiting, abdominal cramps and less frequently, headache, fever and chills. On rare occasions, septicaemia, an illness characterised by fever or hypotension and the isolation of the micro-organism from the blood, can occur. In these cases, subsequent symptoms can include swollen, painful extremities with haemorrhagic bullae (Hlady, 1997; Klontz, 1990). Duration of illness can range from two hours to 10 days (Barker & Gangarosa, 1974).

An outbreak is defined as the occurrence of two or more cases of a similar illness resulting from the ingestion of a common food. The incubation period ranges from 12-96 hours with a median of approximately 15-24 hours. Although *V. parahaemolyticus* outbreaks are less frequent in occurrence, sporadic cases are not infrequent.

According to statistics maintained by the National Enteric Pathogens Surveillance Scheme (NEPPS) there are occasional reports of illness caused by *V. parahaemolyticus*. Over the four-year period, 2006-2009, there were 34 reports of which 15 were acquired overseas.
3.5 Dose-response relationship

Human volunteer studies are available to estimate the probability of illness given exposure. Sanyal and Sen (1974), Takikawa (1958) and Aiso & Fujiwara (1963) have conducted dose-response investigations. In the United States, approximately 5% of culture-confirmed cases of \textit{V. parahaemolyticus} progress to septicaemia (Angulo & Evans, 1999). No reports were identified describing secondary or tertiary transmissions of illnesses caused by \textit{V. parahaemolyticus}. Based on United States statistics, around twenty percent of patients who are septicaemic with \textit{V. parahaemolyticus} die (Angulo & Evans, 1999).

3.6 Dose-response model

Figure 4, taken from the US risk assessment on \textit{V. parahaemolyticus} (Anon., 2005) shows the maximum likelihood fit of the Beta-Poisson to the available feeding trial data. Due to the small number of subjects exposed during these studies there is considerable uncertainty about the best estimate of the dose-response.

![Beta-Poisson dose-response curve for Vibrio parahaemolyticus](image)

4. Summary in the Australian Context

- Not surprisingly, given the high temperature of Australian waters, \textit{V. parahaemolyticus} is a natural component of the microbiota.
- Recently, pathogenic strains have been isolated from oysters.
- Illness caused by \textit{V. parahaemolyticus} is rare; over the four-year period 2006-09, there were 34 reports of illness of which 15 were overseas-acquired.
- This reflects the low concentration of the pathogen.
References


Covert, D. and Woodburne, M. 1972. Relationships of temperature and sodium chloride concentration to the survival of Vibrio parahaemolyticus in broth and fish homogenate.
Applied Microbiology, 23:321-325.


Madigan, T. and May, D. (2010) Pilot study to investigate occurrence of pathogenic strains of...
Vibrio parahaemolyticus in oysters from Tasmania, South Australia and NSW. South Australian Research and Development Institute, Adelaide, SA.


National Enteric Pathogens Surveillance Scheme (NEPSS). Microbiological Diagnostic Unit, University of Melbourne.


**Salmonella**

1. Hazard Identification

Historically, prawns in international trade have been subjected to stringent import regulations. In 1974 the United States Food and Drug Administration began the procedure of blocklisting, described as ‘an effective action used against severe or chronic violations or violators’. The practice was aimed at Asian prawn imports, which were tested for presence of filth, decomposition and *Salmonella*, detection of which resulted in automatic detention of the shipment and the placement of the supplier on an enhanced testing regime. The contamination profile of prawns imported to the USA was determined by Gecan *et al.* (1994) in which, *inter alia*, *Salmonella* was detected in 8.1% of samples. The European Union similarly imposed bans on seafood imports including, in 1997, one on imports of prawns from Bangladesh because of perceived public health risk.

Over recent decades there has been a shift in the type of prawn in international trade, from exclusively marine in 1970s to largely warm-water, farmed prawns, from tropical countries. Prawn farming involves exposure during growing and harvesting to animal and human waste, which might be expected to introduce enteric and other pathogenic bacteria (*Salmonella*, *Escherichia coli*, *Campylobacter*, *Yersinia* and *Shigella*).

Perhaps surprisingly, given the caution of major importing countries and blocs, there have been few documented outbreaks of food poisoning from prawns in international trade (Table 20). In the shigelloses outbreaks cited below, imported Asian prawns were incriminated in the Dutch incident and the importing country was not identified in the UK incident. The shigelloses indicate post-process contamination, possibly involving a food handler in the carrier state, or the use of contaminated water.

<table>
<thead>
<tr>
<th>Date</th>
<th>Country</th>
<th>Organism</th>
<th>Impact</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1983-4</td>
<td>Holland</td>
<td><em>Shigella flexneri</em></td>
<td>14 dead</td>
<td>van Spreekens (1985)</td>
</tr>
</tbody>
</table>

In 2010 an FAO working group gathered information on prawn-associated salmonellosis from major importers (Anon. 2010). Unfortunately data were aggregated so that prawns were subsumed with larger categories. However, as can be seen from Table 21, it is unlikely that there is a strong linkage between prawns and salmonellosis.

<table>
<thead>
<tr>
<th>Date</th>
<th>Country</th>
<th>Food Vehicle</th>
<th># Salmonellosis/# Outbreaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998-2002</td>
<td>USA</td>
<td>Crustaceans, shellfish, molluses and products</td>
<td>2/75</td>
</tr>
<tr>
<td>2007</td>
<td>EU</td>
<td>Shellfish</td>
<td>2/151</td>
</tr>
</tbody>
</table>
2. Exposure Assessment

2.1 Occurrence in the aquatic environment and in product

In Australia, prawns are consumed from several sources:

- Estuarine prawns cooked on board the vessel
- Aquaculture prawns, raw and cooked
- Marine prawns, raw and cooked on board the vessel
- Imported prawns, raw and cooked from tropical, mainly Asian, countries.

There has been very little published work on the microbiology of prawns caught and processed in Australia (Norhana et al. 2010). Chinivasagam et al. (1997) reviewed the process hygiene of prawns caught off Brisbane and cooked on board. In several cases post-process contamination was established in the form of coliforms (some of which were faecal coliforms) and *S. aureus*. In some cases Total Viable Counts approximated or exceeded 1 million/g indicating poor chilling aboard the vessel. Cooking and chilling aboard small vessels is a difficult task, which may be exacerbated by poor water quality, especially in estuaries.

*Salmonella* enters seafoods from the aquatic systems in two different patterns according to the temperature characteristics of the area. In temperate waters, such as the United States and the United Kingdom, prevalence of *Salmonella* on prawns was 7% and 8%, respectively (Brands et al. 2005; Martinez-Urtaza et al. 2004; Wilson & Moore, 1996).

By contrast, in tropical areas, *Salmonella* incidence in seafood can reach up to 20%, as it has been reported for areas of Asia and Africa (Hatha & Lakshmanaperumalsamy, 1997; Heinitz et al. 2000). In Vietnam, an incidence of 18% of positive samples for *Salmonella* has been reported for shellfish product (Van et al. 2007), while in India, presence of *Salmonella* was found in 24.3% of different seafood products investigated (Rakesh Kumar et al. 2008). In Table 22 are presented prevalence of *Salmonella* in environments and product in Asian countries.

Despite that a significant proportion of prawns are caught in warm, northern waters of Australia, they are cooked and frozen on board the vessel and together with aquaculture prawns cooked in processing plants, they are most unlikely to be contaminated with *Salmonella*.

**Table 22: *Salmonella* in environment and products in Asian countries**

<table>
<thead>
<tr>
<th>Country (n)</th>
<th>Sample</th>
<th>Positive (%)</th>
<th>Serovars</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia (1234)</td>
<td>Prawns</td>
<td>1.6</td>
<td>Weltevreden, Paratyphi B, Abaetetuba</td>
<td>Koonse et al. 2005</td>
</tr>
<tr>
<td></td>
<td>Pond water</td>
<td>3.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Source water</td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vietnam (50)</td>
<td>Shellfish</td>
<td>18.0</td>
<td>-</td>
<td>Van et al. 2007</td>
</tr>
<tr>
<td>India (443)</td>
<td>Prawns</td>
<td>29</td>
<td>Weltevreden, Rissen, Typhimurium</td>
<td>Kumar et al. 2008</td>
</tr>
</tbody>
</table>
In temperate and tropical regions presence of *Salmonella* in the environment is linked with rainfall events, particularly after the first heavy rains associated with monsoonal events (Baudart et al. 2000; Brands et al. 2005; Hatha & Lakshmanaperumalsamy, 1997; Martinez-Urtaza et al. & Field, 1991; Venkateswaran et al. 1989). Water temperature has been linked with survival of *Salmonella* in the environment, with cold waters reducing survival and warm waters, together with high levels of organic matter, increasing survival.

In Table 23 is presented data for *Salmonella* prevalence in prawns imported to the USA over a 10-year period (Gecan et al. 1994; Heinitz et al. 2000).

**Table 23: Prevalence of *Salmonella* in prawns imported to USA**

<table>
<thead>
<tr>
<th>Country</th>
<th>Product</th>
<th>Prevalence (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA (1989-90)</td>
<td>Raw prawns</td>
<td>17/211 (8.1)</td>
<td>Gecan et al. (1994)</td>
</tr>
<tr>
<td>USA (1990-98)</td>
<td>Raw crustaceans (imported)</td>
<td>365/3946 (8.5)</td>
<td>Heinitz et al. (2000)</td>
</tr>
<tr>
<td>USA (1990-98)</td>
<td>Raw crustaceans (domestic)</td>
<td>5/129 (3.9)</td>
<td>Heinitz et al. (2000)</td>
</tr>
</tbody>
</table>

Asai et al. (2008) surveyed prevalence of *Salmonella* in seafoods imported to Japan. Of 212 samples, five (2.4%) were positive by PCR and 2/212 (0.9%) also yielded positive cultures, both *S. Weltevreden*.

Serovar matching is sometimes used to imply linkage between a specific food product and human illness. In 1981 Sumner noted that, in the UK and Australia, while *S. Weltevreden* was the serovar most commonly isolated from imported prawns, it was rarely associated with salmonellosis in these countries. However, this serovar is prevalent in Asian environments. In Thailand, Bangtrakulnonth et al. (2004) determined its frequency in salmonelloses and in a range of foods: frozen chicken (19.9%), frozen seafood (26.3%), frozen duck (12.0%), water (14.5%). It is also a commonly-isolated serovar in raw seafood products from other Asian countries, having been isolated from products in India (Shabarinath et al. 2007; Rakesh Kumar et al. 2008) and in aquaculture prawns from other Asian countries (Reilly & Twiddy, 1992; Koonse et al. 2005).

Over the period 2006-09, *S. Weltevreden* accounted for around 1% of salmonelloses in Australia, with a significant proportion being acquired overseas, particularly in visitors to Bali (NEPSS data).

### 2.2 Growth and survival

*Salmonella* grows over a wide range of temperature, pH and water activity. It is a mesophile with a minimum growth temperature (7°C) and has little salt tolerance (Table 24).

**Table 24: Growth conditions for *Salmonella* (after ICMSF, 1996)**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Minimum</th>
<th>Optimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>7</td>
<td>35-43</td>
<td>46</td>
</tr>
<tr>
<td>pH</td>
<td>3.8</td>
<td>7-7.5</td>
<td>9.5</td>
</tr>
<tr>
<td>aw</td>
<td>0.94</td>
<td>0.99</td>
<td>&gt;0.99</td>
</tr>
</tbody>
</table>
3. Hazard Characterisation

*Salmonella* is a Gram-negative, facultative anaerobe. There are two species: *S. enterica* and *S. bongori*, with strains that cause human illness included mainly in the subspecies *S. enterica* subsp. *enterica*.

*Salmonella* are excreted in the faeces of animals and humans and is a component of the natural aquatic microbiota in aquatic environments in tropical regions (Reilly & Twiddy, 1992). The pathogen is transmitted to humans via water or food contaminated with faeces, or from infected food-handlers.

Many *Salmonella* serovars are motile and contain flagellar (‘H’) and somatic (‘O’) antigens. Approximately 2,500 serovars have been described of which, globally, *S. Enteritidis* and *S. Typhimurium* are the main serovars involved in human infection (Greig & Ravel, 2009). In the Asian region, *S. Weltrevreden* is a common cause of salmonellosis (Galanis *et al.* 2006), as is *S. Typhi* in Africa and South-East Asia.

There are two clinical manifestations of *Salmonella*:

- *Salmonella* Typhi/Paratyphi causing enteric fever, with an incubation period ranging from 7-28 days. Symptoms include malaise, headache, fever, cough, nausea, vomiting, constipation, abdominal pain, chills, rose spots, bloody stools.
- Non-typhoid *Salmonella*, causing gastroenteritis following 8-72 hours incubation. Symptoms include abdominal pain, diarrhoea, chills, fever, nausea, vomiting and malaise. The disease is generally self-limiting and resolves in one to three days. Invasion of the gastrointestinal tract followed by bacteraemia may occur. *S. Dublin* has a 15% mortality rate when septicaemic in the elderly, and *S. Enteritidis* has an approximately 3.6% mortality rate in hospital/nursing home outbreaks, with the elderly being particularly affected.

Reactive arthritis and Reiter's syndrome have also been reported to occur, generally after three weeks. Reactive arthritis may occur with a frequency of about 2% of culture-proven cases. Septic arthritis, subsequent or coincident with septicaemia, also occurs and can be difficult to treat.

3.1 Pathogenicity and host factors

All age groups are susceptible, but symptoms are most severe in the elderly, infants and the infirm. AIDS patients suffer salmonellosis frequently (estimated 20-fold more than the general population) and suffer from recurrent episodes. Individuals with underlying disease such as sickle-cell anaemia, liver and gall bladder disease, and immune deficiency are more prone to septicaemia. Hypochlorhydria and achlorhydria increases susceptibility to infection and the severity of disease.

The organism has several pathogenicity islands - *Salmonella* pathogenicity islands (SPI). At present 12 different SPI have been described which contain genes influencing attachment, invasiveness, production of toxins and survival/growth in the host (Hensel, 2004). Other factors that affect the ability of the pathogen to cause disease include the presence of cytotoxins and diarrhoeagenic enterotoxins. The enterotoxin is released into the lumen of the intestine and results in the loss of intestinal fluids (D’Aoust, 1991).
3.2 Food matrix

While gastric acidity is an important defence, *Salmonella* can survive the gastric barrier in foods of high fat content or in foods with high buffering capacity e.g. chocolates, cheese.

3.3 Dose-response

There have been a number of human feeding trials performed using six different serotypes where, as a generalisation, there were usually no illnesses at doses less than $10^6$. By contrast, outbreak investigations have shown that dramatically fewer cells can cause infection (Table 25).

Table 25: Salmonelloses produced by serovars at low dosage (after D’Aoust, 1991)

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>Serovar</th>
<th>Infectious dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate</td>
<td><em>S.</em> Eastbourne</td>
<td>100</td>
</tr>
<tr>
<td>Chocolate</td>
<td><em>S.</em> Napoli</td>
<td>10-100</td>
</tr>
<tr>
<td>Chocolate</td>
<td><em>S.</em> Typhimurium</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Cheese</td>
<td><em>S.</em> Heidelberg</td>
<td>100</td>
</tr>
<tr>
<td>Cheese</td>
<td><em>S.</em> Typhimurium</td>
<td>1-10</td>
</tr>
<tr>
<td>Hamburger</td>
<td><em>S.</em> Newport</td>
<td>10-100</td>
</tr>
</tbody>
</table>

It should be emphasized that prawns are not a high-fat food.

4. Summary in the Australian Context

- For more than three decades countries importing prawns from tropical countries have searched, and occasionally found, *Salmonella* in uncooked prawns.
- By contrast, globally, there have been few outbreaks of salmonellosis associated with prawns (raw and cooked), despite the enormous volume of product in international trade.
- There have been no reported salmonelloses in Australia from consumption of prawns.

References


Baudart, J., Lemarch, K., Brisabois, A. and Lebaron, P. 2000. Diversity of *Salmonella* strains isolated from the aquatic environment as determined by serotyping and amplification of the


National Enteric Pathogens Surveillance Scheme (NEPSS). Microbiological Diagnostic Unit, University of Melbourne.


Hepatitis A (HAV)

1. Hazard Identification

Hepatitis A is an enteric virus which multiplies in the gut of the host, is excreted in the faeces and contaminates foods or surfaces, from where they are ingested either directly (from food) or indirectly, when the hands pick up the virus from the surface. Thus enteric viruses are transmitted via the faecal-oral route.

In Australia enteric viruses have been implicated in a large number of outbreaks, mainly caused by Norovirus (NoV), but also by HAV (Table 26).

Table 26: Selected incidents of foodborne disease in Australia caused by enteroviruses

<table>
<thead>
<tr>
<th>Year</th>
<th>Vehicle</th>
<th>Causative Organism</th>
<th>Cases (deaths)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1978</td>
<td>Oysters</td>
<td>Norovirus</td>
<td>&gt;2,000</td>
<td>Murphy <em>et al.</em> (1979)</td>
</tr>
<tr>
<td>1989</td>
<td>Oysters</td>
<td>Norovirus</td>
<td>1,200</td>
<td>Kraa (1990a, b)</td>
</tr>
<tr>
<td>1991</td>
<td>Orange juice</td>
<td>Norovirus</td>
<td>&gt;4,000</td>
<td>Lester <em>et al.</em> (1991)</td>
</tr>
<tr>
<td>1996</td>
<td>Oysters</td>
<td>Norovirus</td>
<td>93</td>
<td>Stafford <em>et al.</em> (1997)</td>
</tr>
</tbody>
</table>

From Table 26 it can be seen that enteric viruses have been involved mainly with shellfish consumption, shellfish being able to concentrate virus particles from the watercourse because of their filter-feeding habit. The orange juice outbreak was traced to contamination of the potable water source to the factory.

In a review of viral outbreaks caused by consumption of fresh produce, Seymour and Appleton (2001) state that NoV has been implicated in outbreaks from washed salads, frozen raspberries, coleslaw, green salads, fresh cut fruits and potato salad and HAV in outbreaks from iceberg lettuce, strawberries, diced tomatoes and salads.

In 2009 in Victoria there were two outbreaks of Hepatitis A from consumption of semi-dried tomatoes, probably imported from Turkey, where Hepatitis A is endemic in certain rural regions (Anon. 2009).

In 1997 a cluster of Hepatitis A infections were linked with a Sydney restaurant and epidemiological investigation suggested that prawns imported from Burma were the likely cause (Anon. 1997). It is not stated whether prawns were imported cooked, or whether they were cooked and served in the restaurant. No further investigation was undertaken and no conclusion could be reached on whether the contamination occurred during processing in Burma or handling at the restaurant.

2. Exposure Assessment

Viral hazards associated with consumption of seafood were the topic of a number of reviews (Mosley, 1967; Goldfield, 1976; Gerba & Goyal, 1978; Richards, 1985; CDC, 1990; DeLeon & Gerba, 1990; Sobsey *et al.* 1991; Fleet *et al.* 2000; Lees, 2000), the latter two reviewing viral contamination of Australian oysters in the context of the Wallis Lake outbreak of 1997.
In summary, the main conclusions of these reviews were that:

- Viruses causing disease in fish are not pathogenic to humans.
- Food contaminated with human waste containing viruses that are infective via the faecal-oral route, is a cause of foodborne disease.
- Seafood can be contaminated with enteric viruses through exposure to raw or treated sewage and during processing and preparation by contaminated water supplies and infected food handlers.
- Finfish and crustaceans are not usually associated with the spread of viral foodborne disease unless contaminated by food handlers.
- Consumption of both raw and cooked molluscan bivalves (shellfish) is a well-documented cause of viral foodborne disease.
- Virus particles can remain detectable for several months under certain conditions in seawater and in food.
- Shellfish depuration techniques do not totally eliminate viral particles.
- Infectious doses are presumed to be low e.g. 10-100 virus particles.
- Human enteric viruses do not replicate in seafood products so that time and temperature of storage/handling are not risk factors.
- Viruses are resistant to moderate heat and pH conditions.

3. Hazard Characterisation

Hepatitis A is classified within the Hepatovirus genus of the Picornaviridae family. HAV has a single molecule of RNA surrounded by a small (27 nm diameter), non-enveloped, protein capsid.

Hepatitis A is usually a mild illness characterised by sudden onset of fever, malaise, nausea, anorexia and abdominal discomfort, followed by jaundice, perhaps up to four weeks after exposure. The infectious dose is unknown but presumably is similar to other RNA enteric viruses (10-100 virus particles). HAV is excreted in faeces of infected people and can infect susceptible individuals when they consume contaminated water or foods. Water, shellfish and salads are the most frequent sources. Contamination of foods by infected workers in food processing plants and restaurants is common. The virus has not been isolated from any food directly associated with an outbreak. Because of the long incubation period, the suspected food is often no longer available for analysis (Sobsey et al. 1991; FDA, 1999). Shellfish have been associated worldwide with a large number of hepatitis outbreaks (Tang et al. 1991; Xu et al. 1992; Leoni et al. 1998).

3.1 Illness caused

The incubation period for Hepatitis A, which varies from two to six weeks (mean four weeks) is dependent upon the number of infectious particles consumed. Infection with very few particles results in longer incubation periods. The period of communicability extends from early in the incubation period to about a week after the development of jaundice. The greatest danger of spreading the disease to others occurs during the middle of the incubation period, well before the first presentation of symptoms. Many infections with HAV do not result in clinical disease, especially in children. When disease does occur, it is usually mild and recovery is complete in one to two weeks. Occasionally, the symptoms are severe and
convalescence can take several months. Patients suffer from feeling chronically tired during convalescence and their inability to work can cause financial loss. Less than 0.4% of reported cases in the US are fatal, usually occurring in the elderly (Sobsey et al. 1991; FDA, 1999).

### 3.2 Unique host susceptibility factors

All people who ingest the virus and are immunologically unprotected are susceptible to infection (Sobsey et al. 1991; FDA, 1999). Hepatitis A infection in children is normally subclinical, while in adults overt hepatitis develops in the majority of individuals (Hollinger & Ticehurst, 1990). Infection results in long-term immunity as older individuals are more likely to have HAV antibodies than younger individuals. In one study of 245 blood donors, 57% of those aged 30-49 years were antibody positive, compared with only 30% of those aged 18-29 years. Immunity appears to be protective as repeat attacks are rare (Boyd & Marr, 1980). Other host factors affecting the severity of hepatitis infection are poorly characterised. Immune impairment is less significant than for other foodborne pathogens. Existing liver damage (e.g. cirrhosis) may be significant (Cliver, 1989).

### 4. Summary in the Australian Context

- The NSW report of this enteric virus in prawns (Anon. 1997) is the only one in the literature, as reviewed by Todd and co-workers at Michigan State University in a series of publications (so far numbering 11) under the general heading ‘Outbreaks where food workers have been implicated in the spread of foodborne disease’.

- Given its unique quality, the lack of confirmation that prawns were the infecting agent in the NSW outbreak, and of any other data it is not possible to estimate the risk of this hazard:product pairing. Note this in contrast to the Hepatitis A outbreak in oysters from Wallis Lakes in NSW, where there were sufficient data for Sumner and Ross (2002) to produce a risk rating using Risk Ranger.

### References


FDA. 1999. Bad Bug Book (Foodborne Pathogenic Microorganisms and Natural Toxins).


**Chloramphenicol**

1. **Hazard Identification**

In 2001 the European Union instituted a ban on seafood, mainly shrimp, containing the broad-spectrum antibiotic, chloramphenical (CAP). Some countries destroyed batches of shrimp containing CAP. The legislative framework for the ban, Council Regulation EEC No. 2377/90, was implemented to establish maximum residue limits (MRLs) of veterinary medicinal products in foodstuffs of animal origin.

Chloramphenicol is active against a range of Gram-positive and negative bacteria and is prescribed for topical application, especially in eye drops as treatment for bacterial conjunctivitis (Lancaster *et al.* 1998).

A side-effect of CAP application treatment is aplastic anaemia, an effect rare and generally fatal, though usually occurring weeks or months after treatment has stopped. Treatment is also associated with bone marrow suppression.

In 2001, EU authorities imposed a ‘zero tolerance’ criterion for presence of chloramphenicol which, with the capacity of modern analytical equipment yielded positives in the range µg/kg (parts/million) to µg/t (parts/trillion). In the absence of information on a maximum allowable level (from the toxicological viewpoints) EU authorities adopted the precautionary principle.

The assertion was that CAP was being used on prawns as an illicit veterinary chemical. This assertion has been challenged by Hanekamp *et al.* (2003) on the grounds that CAP is:

- A natural chemical, produced by *Streptomyces* spp, a ubiquitous soil bacterium.
- A commonly-used antibiotic, particularly in Asian countries, where it is widely available for over-the-counter purchase.

Its occurrence in aquaculture shrimp from Asian countries may therefore be from natural and/or community sources.

2. **Exposure Assessment**

As part of an EU Market Access Program, the South Australian Research and Development Institute (SARDI) undertook residue testing of prawns in conjunction with the Australian Prawn Farmers Association (APFA). Homan and Padula (2008) found that all samples of prawns from six prawn farms had chloramphenicol levels below the laboratory limit of detection (0.19µg/kg).

Over the period 2005-2010, testing of imported prawns by AQIS resulted in five samples positive for chloramphenicol from 269 samples tested, a prevalence of 1.9% (Table 27).

<table>
<thead>
<tr>
<th>Country of origin</th>
<th>Number of positive samples</th>
<th>Concentration (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vietnam</td>
<td>2</td>
<td>1.1, 0.089</td>
</tr>
<tr>
<td>Thailand</td>
<td>2</td>
<td>13, 8.7</td>
</tr>
<tr>
<td>Singapore</td>
<td>1</td>
<td>1.2</td>
</tr>
</tbody>
</table>

The concentration used by AQIS for rejection appears to be either <0.3µg/kg or <0.1µg/kg, though it is noted that one sample was rejected at 0.089µg/kg.
An exposure level to consumers from chloramphenicol in prawns was measured by Janssen et al. (2001) as 0.00000017 mg/kgbw/day.

3. Hazard Characterisation

According to Hanekamp et al. (2003), the Joint FAO/WHO Expert Committee on Food Additives (JECFA) estimated aplastic anaemia incidence in the order of 1.5 cases per million people per year, of which about 15% was associated with drug treatment; CAP was not a major contributor. These data gave an overall incidence of therapeutic CAP-associated aplastic anaemia in humans of less than one case per 10 million per year.

Regarding epidemiological data derived from the ophthalmic use of CAP, systemic exposure to this form of treatment was not associated with the induction of aplastic anaemia. There seems to be no evidence that low-level exposure to CAP, either as a result of ophthalmic use or of residues in animal food, is related to aplastic anaemia.

Hanekamp et al. (2003) estimate the difference between exposure levels in shrimp and those encountered in medication as 150,000,000-735,000,000.

4. Risk Characterisation

Janssen et al. (2001) estimated the risk of contracting cancer as a result of consuming shrimp containing CAP, where concentrations ranged between 1 and 10 µg/kg (ppb) concluding that the worst-case risk was a 1.1,000,000 added cancer risk in the human population.

References


Sulphite

1. Hazard Identification

Sulphur dioxide (220) and sodium and potassium sulphites (221, 222, 223, 224, 228) are permitted additives to a range of foods in the Australian Food Standards Code. Foods to which it is permitted to add these anti-browning preservative agents, include alcoholic beverages, cheeses, various fruit and vegetable products, crustacea, flour products, biscuits cakes and pastries.

In the case of crustacea sulphite has long been used to control the development of black spot (melanosis) in raw prawns and lobsters. Although alternatives such as 4-hexylresorcinol are available, sulphite remains the agent of choice for controlling black spot.

Levels of sulphur dioxide permitted in crustacea are prescribed in the Food Standards Code and are 30 µg/kg in cooked crustaceans and 100 µg/kg in uncooked crustaceans. Where the addition of sulphur dioxide and sodium and potassium sulphites is permitted it must be declared on the food label.

2. Exposure Assessment

As part of a longitudinal study of the marine prawn supply chain in Australia Thomas et al. (2003) measured sulphite levels in prawns caught in South Australia. Of twenty whole prawn samples measured immediately after dipping in metabisulphite on board the vessel, two had sulphite levels >100 mg/kg (107 and 140 mg/kg).

Over the period 2005-2010, testing of imported prawns by AQIS resulted in four samples exceeding the allowable limit. Of 341 samples tested, four (1.2%) exceeded the limit (Table 28).

<table>
<thead>
<tr>
<th>Country of origin</th>
<th>Date</th>
<th>Product</th>
<th>Concentration (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>9/2/06</td>
<td>Raw</td>
<td>491</td>
</tr>
<tr>
<td>Vietnam</td>
<td>27/4/06</td>
<td>Raw</td>
<td>42*</td>
</tr>
<tr>
<td>Vietnam</td>
<td>27/4/06</td>
<td>Cooked</td>
<td>78</td>
</tr>
<tr>
<td>Vietnam</td>
<td>8/5/06</td>
<td>Raw</td>
<td>115</td>
</tr>
</tbody>
</table>

* It is not clear why this sample failed; the FSC allows up to 100 mg/kg in raw prawns

It is not known whether whole prawns were tested, or only edible portions of prawn meat, since the carapace typically contains a much higher level. Hardisson et al. (2002) found that the sulphite level of prawn meat in Spain and Venezuela varied from 13 to 546 µg/kg (mean 115 µg/kg) while that of carapaces varied from 81 to 8256 µg/kg (mean 2428 µg/kg). Some samples exceeded the maximum level permitted under European legislation (Directive 95/2/CE) of 150 µg/kg.

3. Hazard Characterisation

A small proportion of the human population, estimated at 14-16% of children and 10-12% of adults (Asthma Foundation of Victoria), is hypersensitive when exposed to sulphur dioxide or
sulphites. For this group, symptoms include asthma, rhinitis (inflammation of the nasal passages often associated with discharge), rhinoconjunctivitis, urticaria (hives), angio-oedema (swelling of the tongue and throat), headache, gastrointestinal distress and anaphylaxis. In challenge studies, reactions in some individuals have been associated with elevated levels of immunoglobulin E (IgE) indicating a classic atopic allergic reaction, while in others reactions have occurred without an associated rise in IgE or positive skin prick test indicating that more than one mechanism is involved in causing symptoms amongst sensitive individuals (WHO, 1999).

Irritation of the airways by SO$_2$ gas is one possible mechanism (The Australasian Society of Clinical Immunology and Allergy, accessed 27/7/11). The dose of sulphur dioxide and sulphites that cause reactions varies according the medical condition of the individual, their medication and the food type ingested. In one individual, who had suffered severe asthmatic symptoms on exposure to sulphites in dried apricots and sulphited salad, a few sips of wine containing sulphites at a concentration of 92 mg/kg caused a fatal anaphylactic reaction (WHO, 1999). The reporting of adverse reactions to fresh fruit and vegetables, led to a ban on the use of sulphites on these foods, except potatoes and grapes, in the US in 1986 (WHO, 1999).

No reports of cases of illness associated with consumption of prawns could be found in the public health literature in Australia (Communicable Diseases Intelligence; State Health Bulletins).

References


Nitrofurans

1. Hazard Identification

Nitrofurans are synthetic, broad-spectrum antibacterial drugs used as feed additives for treatment of gastrointestinal infections in pigs, cattle and poultry. They are also growth promoters in poultry and prawns when fed at sub-therapeutic levels. Four nitrofurans are used in feeds, each of which has a metabolite which appears in food products:

<table>
<thead>
<tr>
<th>Nitrofuran Feed Additive</th>
<th>Metabolite (Marker) in Prawns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furazolidone</td>
<td>AOZ (3-amino-oxazolidinone)</td>
</tr>
<tr>
<td>Furaltadone</td>
<td>AMOZ (3-amino-5-morpholinomethyl-1,3-oxazolidin)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>AHD (1-aminohydantoin)</td>
</tr>
<tr>
<td>Nitrofurazone</td>
<td>SEM (semicarbazide)</td>
</tr>
</tbody>
</table>

Because nitrofurans have been linked with genotoxicity, Australia and the EU prohibited the use of nitrofurans in feeds in 1992 and 1995, respectively.

In 2003 the Australian Prawn Farmers Association provided evidence to FSANZ that imported prawns contained nitrofuran residues and nitrofuran metabolites are included in AQIS’ import testing program.

Recently, it has become known that one metabolite (SEM) occurs naturally in the freshwater crustacean, *Macrobrachium rosenbergii* (Poucke et al. 2011). The discovery came about when it was noted that import testing of *M. rosenbergii* in Belgium consistently yielded positive SEM results, with the implication that nitrofurans were being used. The researchers showed that SEM was synthesised by the prawn and deposited in the carapace, but not in the meat; positive tests had been carried out on whole prawns.

2. Exposure Assessment

In 2007, as part of an EU Market Access Program, the South Australian Research and Development Institute (SARDI) undertook residue testing of prawns in conjunction with the Australian Prawn Farmers Association (APFA). All samples of prawns from six prawn farms had AHD, AMOZ and AOZ levels below the laboratory limit of detection (0.2 µg/kg) and SEM levels were also below the limit of detection of 0.4 µg/kg (Homan & Padula, 2008).

Over the period 2005-2010, testing of imported prawns by AQIS resulted in 62 samples positive for nitrofuran metabolites from 1,741 samples tested, a prevalence of 3.6%; Chinese prawns tested positive on 36 occasions and Indian prawns on 26 occasions.

The designation of positives according metabolite is presented in Table 29 from which it can be seen that the four marker metabolites were detected in approximately similar proportions (18 described as ‘nitrofurans’ were probably misnamed). Concentrations of positive samples varied from ‘not detected’ (which would benefit from clarification) to 8.2 µg/kg ‘nitrofuran’.

Exposure of prawn consumers to AOZ was determined by FSANZ (2005). The mean exposure ranged from 0.0009-0.0019 µg/kgbw/day and that for ‘high-end’ consumers from 0.003-0.0064 µg/kgbw/day.
3. Hazard Characterisation

In 1993, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that:

- Furazolidone (AOZ) was a genotoxic carcinogen, based on increased incidence of malignant tumours in mice and rats
- Nitrofurazone (SEM) produced tumours (benign) in rats and mice

No acceptable daily intake (ADI) for furazolidone or nitrofurazone was advocated.

4. Risk Characterisation

When FSANZ compared the upper bound for consumption by ‘high-end’ prawn consumers with doses shown to cause tumours in laboratory animals there was a 4,000,000-times difference; for ‘average’ consumption of prawns the difference was 12,000,000-times.

FANZ concluded that the food safety risk from consumption of prawns containing nitrofuran residues was very low.

Table 29: Rejection of prawns imported to Australia for presence of nitrofuran residues

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Number of positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHD</td>
<td>11</td>
</tr>
<tr>
<td>AMOZ</td>
<td>11</td>
</tr>
<tr>
<td>AOZ</td>
<td>12</td>
</tr>
<tr>
<td>SEM</td>
<td>10</td>
</tr>
<tr>
<td>‘Nitrofurans’</td>
<td>18</td>
</tr>
</tbody>
</table>

Hanekamp et al. (2003) has noted that SEM is found in several sources other than animal feeds: plastic gaskets for glass jars, plant and animal matrices which had been dried, food samples treated with hypochlorite. These sources are in addition to the finding of Poucke et al. (2011) that SEM is a natural component of the carapace of at least one prawn in international trade.

References


Cadmium

1. Hazard Identification

In 1972, the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JEFCA) established a Provisional Tolerable Weekly Intake (PTWI) for cadmium of 400-500 μg per person, approximately 7-8 μg/kg per body weight per week (bw/w) and 60-70 μg per day for a 60 kg person.

In 1993, JEFCA introduced a safety factor, advising a reduction in the PTWI to 7 μg/kg bw/w, a level confirmed in 1995 by the European Commission and reconfirmed by JEFCA in 2003.

Cadmium can enter cells and bind with ligands; it is not easily cleared by the body and has a long residence time (half-life 10-30 years) in organs such as liver, kidney and the intestine. Cadmium has been associated with disorders of the kidneys, bones and nervous system, and is also identified as a carcinogen.

The EU set maximum levels (MLs) for foodstuffs, including seafood, based on the results of a dietary exposure assessment and the opinions of JEFCA (Table 30). Full details of the scientific underpinning is contained in a risk assessment carried out by EFSA (EFSA. 2009).

<table>
<thead>
<tr>
<th>Product</th>
<th>ML (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle meat of fish, excluding certain species listed below</td>
<td>0.05</td>
</tr>
<tr>
<td>Bonito (<em>Sarda sarda</em>)</td>
<td>0.10</td>
</tr>
<tr>
<td>Common two-banded seabream (<em>Diplodus vulgaris</em>)</td>
<td></td>
</tr>
<tr>
<td>Eel (<em>Anguilla anguilla</em>)</td>
<td></td>
</tr>
<tr>
<td>Grey mullet (<em>Mugil labrosus labrosus</em>)</td>
<td></td>
</tr>
<tr>
<td>Horse mackerel or scad (<em>Trachurus spp</em>)</td>
<td></td>
</tr>
<tr>
<td>Louvar or luvar (<em>Luvurus imperialis</em>)</td>
<td></td>
</tr>
<tr>
<td>Mackerel (<em>Scomber spp</em>)</td>
<td></td>
</tr>
<tr>
<td>Sardine (<em>Sardina pilchardus</em>)</td>
<td></td>
</tr>
<tr>
<td>Sardinops (<em>Sardinops spp</em>)</td>
<td></td>
</tr>
<tr>
<td>Tuna (<em>Thunnus spp</em>, <em>Euthynnus spp</em>, <em>Katsuwonus pelamis</em>)</td>
<td></td>
</tr>
<tr>
<td>Wedge sole (<em>Dicologoglossa cuneata</em>)</td>
<td></td>
</tr>
<tr>
<td>Muscle meat of bullet tuna (<em>Auxis spp</em>)</td>
<td>0.20</td>
</tr>
<tr>
<td>Muscle meat of anchovy (<em>Engraulis spp</em>)</td>
<td>0.30</td>
</tr>
<tr>
<td>Swordfish (<em>Xiphias gladius</em>)</td>
<td></td>
</tr>
<tr>
<td>Crustaceans, excluding brown meat of crab and excluding head and thorax meat of lobster and similar large crustaceans (<em>Nephropidae</em> and <em>Palinuridae</em>)</td>
<td>0.50</td>
</tr>
<tr>
<td>Bivalve molluscs</td>
<td>1.0</td>
</tr>
<tr>
<td>Cephalopods (without viscera)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Cadmium is a perceived hazard in the European Union and affects only exports to that market.
2. Exposure Assessment

The issue in the Australian context is that, within a lot of production of prawns, a proportion may contain cadmium in excess of the ML (0.5 mg/kg).

Over the period 2001-2011 the EU, via its Rapid Alert system, has rejected 133 consignments of crustaceans on the basis of the ML.

Among the rejections were 39 consignments of Australian frozen prawns with cadmium levels up to 2 mg/kg, reflecting the bioaccumulation of the metal by prawns in certain Australian waters.

Based on ABARE data, the volume of prawns exported from Australia to Europe has diminished significantly in the aftermath of the rejections (Table 31).

A detailed listing of cadmium levels in Australian prawns is presented in Anon. (2007).

<table>
<thead>
<tr>
<th>Table 31: Exports of prawns from Australia to the EU (2005-2009)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Export volume (t) to EU</td>
</tr>
<tr>
<td>Total exports (t)</td>
</tr>
<tr>
<td>Proportion (%) exported to EU</td>
</tr>
<tr>
<td>Value ($,000)</td>
</tr>
<tr>
<td>Total exports ($,000)</td>
</tr>
<tr>
<td>Proportion (%) exported to EU</td>
</tr>
</tbody>
</table>

3. Hazard Characterisation

Food represents the major source of cadmium exposure, with moderate smoking another major source. Toxicity in the kidney is progressive and requires many years of accumulation with a concentration in the renal cortex of 200 mg/kg at age 50 accepted as a critical concentration at which kidney damage may be manifested in susceptible individuals.

Detailed expositions of cadmium toxicity are contained in Anon. (2007) and EFSA (2009).

4. Risk Characterisation

In 2007, the Australian government (Anon. 2007) made a submission to the European Commission to review the ML for crustaceans citing, as evidence for its deletion that:

- The Codex Alimentarius Commission had set no ML for cadmium in crustaceans, on the basis that crustaceans represent a minor exposure to cadmium.
- While median levels for prawns in Australia’s export trade were 0.11 mg/kg (range 0.01-0.15 mg/kg) the distribution is highly skewed and a proportion could not meet the ML criterion at the 95th percentile.
- Australian prawns do not contribute significantly to the cadmium ingested by European consumers.
References
Anonymous. 2007. Australian government submission to the European Commission to review the cadmium maximum limit in crustaceans. Canberra.
TOR 3: Risk Assessment of Identified Hazards

Three types of risk assessments can potentially be used to provide an estimate of illness associated with consumptions of prawns:

- Qualitative risk assessment
- Semi-quantitative risk assessment
- Quantitative risk assessment

Choosing which type of assessment to carry out is influenced primarily by the requirements of the risk manager and secondarily by the availability of data which will inform the assessment. Unless there are extremely pressing reasons for undertaking it, a full quantitative risk assessment is not done. Such assessments are expensive and time-consuming, for example the USA risk assessment of *E. coli* O157 in hamburgers.

In this study a qualitative tool will be used where data are scarce and a semi-quantitative tool (Risk Ranger) where data allow. As well, where quantitative risk assessments have been done their outputs will be integrated into the study.

### 3.1 Qualitative Risk Assessment Tool

A qualitative framework for the rating of risk has been used based on premises published by the International Commission on Microbiological Specifications of Foods (ICMSF, 2002) and by Food Science Australia (FSA, 2000). The ICMSF formulated descriptors for severity of illnesses caused by various pathogens and these are used in conjunction with a matrix of factors assembled by FSA (2000) for use in risk profiling.

Taken together, the present qualitative matrix is based on criteria for:

**Severity**

The severity of the identified hazards was classified according to the International Commission of the Microbiological Specifications of Food (ICMSF 2002) with level of severity defined as follows:

IA. Severe hazard for general population; life threatening or substantial chronic sequelae or long duration.

IB. Severe hazard for restricted populations; life threatening or substantial chronic sequelae or long duration.

II. High hazard; incapacitating but not life threatening, sequelae rare, moderate duration.

III. Moderate; not usually life threatening, no sequelae, normally short duration, symptoms are self limiting, can be severe discomfort.

**Occurrence of illness**

This is classified as low, medium or high based on the hazard’s involvement as recorded in public health statistics.

**Growth**

An indication is given of whether growth of the pathogen in the product is required to cause
The production, processing or handling of food may increase, decrease or not affect the concentration of the hazard.

**Consumer terminal step**
This element considers whether a consumer terminal step, such as cooking, is applied to the product. Cooking by the consumer will, for most biological hazards, reduce the subsequent risk of disease.

**Epidemiology**
Consideration is given as to whether the hazard-commodity combination has been recorded as a cause of food poisoning.

### 3.2 Semi-Quantitative Risk Assessment Tool - Risk Ranger
In 2000, Huss *et al.* published a framework which proved useful for making a qualitative risk assessment. There were six criteria:

- Bad safety record
- No CCP for the hazard
- Possibility of contamination or recontamination
- Abusive handling possible
- Growth of pathogens can occur
- No terminal heating step

The authors provided examples of how the tool could be used for a range of seafood products and the framework was the basis for development of a semi-quantitative tool, Risk Ranger (Ross & Sumner, 2002), which was used for the national seafood risk assessment in Australia (Sumner & Ross, 2002).

Risk Ranger incorporates all factors that affect the risk from a hazard in a particular commodity including:

- Severity of the hazard and susceptibility of the population of interest
- Likelihood of a disease-causing dose of the hazard being present in a meal
- Number of meals consumed by a population of interest in a given period of time

A number of factors affect each of the above.
Disease severity is affected by:

a) Intrinsic features of the pathogen/toxin
b) Susceptibility of the consumer.

Exposure to the food will depend on how much is consumed by the population of interest, how frequently they consume the food and the size of the population exposed.

Probability of exposure to an infectious dose will depend on:

a) Serving size
b) Probability of contamination in the raw product
c) Initial level of contamination
d) Probability of contamination at subsequent stages in the catching-processing-distribution chain
e) Changes in the level of the hazard during the chain, including concentration or dilution, growth or inactivation of hazard.

Risk Ranger has a ‘shopfront’ with a list of boxes into which are entered information using the computer’s mouse. In total, there are eleven questions and a mathematical model then converts each answer to a numerical value or ‘weighting’ - the weightings are detailed in the paper by Ross and Sumner (2002). Some of the weightings are arbitrary, while others are based on known mathematical relationships e.g. from days to weeks, or years. To help make responses as objective as possible and to maintain transparency of the model, descriptions are provided and many of the weighting factors are specified. As well, in some cases, if the options provided don’t accurately reflect the situation being modelled, a numerical value can be entered using ‘Other’.

Behind the shopfront is the model, developed in Microsoft Excel software, using standard mathematical and logical functions. The list box macro tool is used to automate much of the conversion from qualitative inputs to quantities for calculations. For each selection made from the range of options, the software converts that selection into a numerical value.

**Outputs**

Risk Ranger combines the factors in Questions 1-11 including some logical tests to generate two estimates of risk:

- Risk Ranking – a score between 0-100
- Predicted annual illnesses in the population selected

Full details of the logic and equations leading to the risk estimates are shown in the paper by Ross and Sumner (2002).

**Risk ranking**

The Risk Ranking value is scaled logarithmically between 0 and 100. The former is equated to a probability of foodborne illness of less than, or equal to, one case per 10 billion people (greater than current global population) per 100 years. At the upper limit (Risk Ranking=100), every member of the population eats a meal that contains a lethal dose of the hazard every day. A Risk Ranking change of 6 corresponds to a 10-fold difference in the
absolute risk. Thus an increase in Risk Ranking from 36 to 48 means that the risk increased 100-times.

**Predicted annual illness**
Risk Ranger estimates the total number of illnesses in the population selected at Question 5. Obviously, the higher the risk ranking, the greater the proportion of the population will become ill. The absolute numbers of illnesses, however will depend on the population size.

### 3.3 Identified Hazards

Based on TOR 1, the following hazards have been identified.

<table>
<thead>
<tr>
<th>Biological Hazards</th>
<th>Risk Assessment Carried Out</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vibrio parahaemolyticus</em></td>
<td>Qualitative Semi-quantitative</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>Qualitative Semi-quantitative</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>Qualitative Semi-quantitative</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>None</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemical Hazards</th>
<th>Qualitative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphite</td>
<td>Qualitative</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Qualitative</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Qualitative</td>
</tr>
<tr>
<td>Nitrofurans</td>
<td>Qualitative</td>
</tr>
</tbody>
</table>

Qualitative risk assessments were carried out on all identified hazards, with the exception of Hepatitis A in imported prawns, for which there were insufficient data.

Where there were sufficient data, a semi-quantitative risk assessment was undertaken e.g. on *Vibrio* spp and *Salmonella* in prawns. Because of significant data gaps, it was only possible to undertake qualitative risk assessments for chemical hazards.

### References


TOR 4: Risk Assessment of Hazard: Product Pairings

4.1 Risk Assessment: *Vibrio cholerae*

Two scenarios are considered:

1. Raw prawns are eaten raw, as sashimi
2. Raw prawns are stored, cooked and consumed in accordance with the Food Standards Code either in the home or in the food service sector

For each scenario a qualitative and semi-quantitative risk analysis is made.

Data inputs to Risk Ranger are based on data accumulated by the joint FAO-WHO panel which worked on ‘Vibrios in seafood’ (Anon. 2005).

4.1.1 Qualitative assessment of the risk of contracting cholera from imported warm-water prawns

The following assumptions are made:

1. 90% of servings are consumed cooked and 10% consumed raw
2. Imports of prawns are around 20,000,000 kg/annum
3. An average serving size is 200 g
4. Total number of serves is 100,000,000/annum, of which 10 million are consumed raw and 90 million cooked.
5. The population is 23 million

AQIS import testing has detected *V. cholerae* in 13/498 (2.6%) of samples of cooked, imported prawns over the period 2005-11, there was no further analysis to determine whether any isolate was toxigenic.

However, the likelihood of being exposed to an infectious dose of choleraigenic *V. cholerae* through the consumption of imported warm-water prawns is extremely low, based on the data presented in the hazard sheet, which indicates only two isolations of choleraigenic *V. cholerae* in more than 20,000 port-of-entry analyses of imported warm water prawns.

A matrix embracing responses to the above qualitative criteria is presented (Table 31) from which it can be seen that, in qualitative terms, the risk of contracting cholera from consumption of warm-water prawns, whether eaten raw or cooked, is very low.
Table 31: Microbiological hazard risk rating for choleragenic *V. cholerae* in prawns imported to Australia (after ICMSF 2002 and FSA 2000)

<table>
<thead>
<tr>
<th>Product</th>
<th>Identified hazard</th>
<th>Severity¹</th>
<th>Occurrence of illness²</th>
<th>Growth in product required to cause disease</th>
<th>Prodⁿ/process/handling hazard³</th>
<th>Consumer terminal step⁴</th>
<th>Epidemiological link</th>
<th>Risk Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw prawns</td>
<td>Choleragenic <em>V. cholerae</em></td>
<td>II</td>
<td>Very low</td>
<td>Yes</td>
<td>↓ Inactivation during washing, icing, freezing</td>
<td>No</td>
<td>No</td>
<td>Very low</td>
</tr>
<tr>
<td>Prawns cooked at the plant and eaten without further heat treatment</td>
<td>Choleragenic <em>V. cholerae</em></td>
<td>II</td>
<td>Very low</td>
<td>Yes</td>
<td>↓ Inactivation during washing, icing, cooking (optional), freezing</td>
<td>No</td>
<td>No</td>
<td>Very low</td>
</tr>
<tr>
<td>Prawns cooked immediately before consumption</td>
<td>Choleragenic <em>V. cholerae</em></td>
<td>II</td>
<td>Very low</td>
<td>Yes</td>
<td>↓ Inactivation during washing, icing, (optional), freezing, thawing and cooking</td>
<td>Yes</td>
<td>No</td>
<td>Very low</td>
</tr>
</tbody>
</table>

¹ Severity level II refers to the severity of the identified hazard as classified according to the International Commission of the Microbiological Specifications of Food (ICMSF 2002). Level II: High hazard; incapacitating but not life threatening; sequelae rare; moderate duration.

² Very low occurrence of illness can, for the purposes of this risk assessment, be described as an average of less than 1 case per 10 million population per year based on the data that was available over a 6 year period (see hazard sheet).

³ As the level of inactivation for processing is in the range of a 5-6 log reduction and the initial contamination level less than 1 in 25g, the likelihood of having choleragenic *V. cholerae* in the prawns after cooking was considered to be very low.

⁴ Cooking of prawns also brings about a 5 - 6 log reduction in the level of *V. cholerae*, thus the likelihood of having choleragenic *V. cholerae* in the prawns after processing was considered to be very low.
4.1.2 Semi-quantitative risk of contracting cholera from imported warm-water prawns

**Question 1. Hazard Severity**
Risk Ranger alternative: ‘MODERATE hazard - requires medical intervention in most cases’ was chosen as the response to Question 1.

**Question 2. How susceptible is the population of interest**
For the current assessment: ‘GENERAL - all members of the population’ was selected.

**Questions 3 and 4: Frequency of consumption and proportion consuming**
The following assumptions are made:
1. 90% of servings are consumed cooked and 10% consumed raw
2. Imports of prawns are around 20,000,000 kg/annum
3. An average serving size is 200 g
4. Total number of serves is 100,000,000/annum, of which 10 million are consumed raw and 90 million cooked
5. The population is 23 million
6. 25% of the population consumed prawns raw twice/annum (a few times per year)
7. 25% of population consumed cooked prawns once a month

**Question 5. Size of consuming population**
Population is 23,000,000.

**Question 6. Probability of contamination of raw product per serving**
Dalsgaard et al. (1995a) found that *V. cholerae* O1 was present in 2% (2/107) of water, sediment and prawns samples collected from a major prawn culture area in South-east Asia though testing of isolates indicated absence of the *ctx* genes in both the O1 strains (Dalsgaard et al. 1995b). Data from India showed the presence of *V. cholerae* O1 in 0.2% of raw prawns (Ministry of Agriculture, India). However, the choleragenic status of these prawn-associated strains is unknown. Data submitted to FAO/WHO from Argentina (personal communication M. Costagliola, 2001) indicate the absence of *V. cholerae* O1 and O139 in 400 prawn and 15 water samples examined. Based on this information probability of contamination of incoming prawns was considered RARE (0.1%). While not stated, implicit at Question 6 is the need to estimate a concentration of *V. cholerae* on incoming prawns. In the absence of any information a premise used by other researchers which, at its simplest, states that, if the prevalence is low, the concentration is also likely to be low, has been used. While some risk assessments (Bemrah et al. 1999) have noted that pathogens are probably heterogeneously distributed in some foods, all to date have assumed that pathogens present in foods are distributed homogeneously. This is clearly a simplification.

In the present study, a concentration of 10 cfu/g was used (the limit of detection) equivalent to 2000 cfu/serve. This concentration is used to answer question 10 (increase to infectious dose) for prawns consumed raw.
Question 7. Effect of Processing
Data in the hazard sheets for *V. cholerae* illustrate the effect of processing on inactivation of *V. cholerae* for which a >5 log inactivation is documented during washing, icing and freezing. For Question 7 the alternative: USUALLY ELIMINATES is selected. Based on the information presented in the hazard sheet this is an extremely conservative estimation.

If prawns are cooked during processing the lethality of the temperature:time regimes used in the industry are greatly in excess of 6 log units which leads to selection that the hazard is RELIABLY ELIMINATED at this stage.

Question 8. Recontamination
Given the description of processing in the international trade (see hazard sheet), recontamination was considered not to occur.

Question 9. Effectiveness of Post-processing Controls
The cold chain in international trade (frozen and chilled) is well established and *V. cholerae* has a temperature minimum for growth of 10°C (see hazard sheet) and the Risk Ranger alternative: WELL CONTROLLED (no increase in population) has been selected.

Question 10. Increase in the post-processing contamination level that would cause infection or intoxication to the average consumer.
From consideration of dose response (See hazard sheet) and the data of Levine et al. (1981), Tauxe et al. (1994), Health Canada (2001) and FDA/CFSAN (2003), a dose of 1 million (10^6) *V. cholerae* was selected. In a serving of 200 g such an ID_{50} is equivalent to a concentration approximately 3000 cfu/g of prawns at the point of consumption. To answer question 10 it is necessary to divide the level at Q6 from the level for an infective dose. In this case:

\[ Q10 = 10^6 \]
\[ Q6 = 10^3 \text{ (200g serve with concentration 10 cfu/g = 2000 g/serve)} \]

The difference is approximately 10^3 and this value is used at Question 10 in connection with consumption of raw prawns.

Question 11. Effect of preparation before eating
Where prawns are cooked it is likely that this will result in complete elimination of *V cholerae*. The organism is not heat tolerant and the location of the site of microbiological concern as the carapace means that heat treatment will be immediate. Thus any form of cooking (steaming, boiling or barbecuing) will result in complete elimination.

Where prawns are eaten raw there is no effect on the hazard.

4.1.3 Risk ratings, predicted illnesses and reality check
In Table 32 are presented risk ratings and predicted illnesses based on consumption of raw and cooked imported prawns.

For prawns consumed raw, risk of illness has a rating of 28 with 1.7 illnesses every decade. In assessments of paired seafood hazard:products pairings in Australia, Sumner and Ross (2002) found that Risk Ranger ratings <30 were not equated with any reports of illness.
For prawns consumed cooked, the hazard is reliably eliminated during cooking either at the plant level (Risk Ranger Question 7: Reliably eliminates) or during meal preparation (Risk Ranger Question 11: Reliably eliminates). This leads to Risk Ranger rating of 0 and prediction of no illnesses.

Thus, a semi-quantitative assessment of the likelihood of contracting cholera from consumption of warm-water prawns by consumers in major importing countries is of the order of two cases/decade.

It should be noted that the joint FAO-WHO expert panel undertook a quantitative risk assessment of consumption of warm-water prawns by major importing countries. Median risk of contracting cholera from consuming prawns raw varied from two cases/century in several European countries to four cases/decade in the USA.

Based on the above, the likelihood of contracting cholera from consumption of raw imported prawns is extremely low.

**Table 32: Estimation of risk of cholera associated with consumption of imported warm-water prawns in Australia**

<table>
<thead>
<tr>
<th>Risk criteria</th>
<th>Prawns consumed raw</th>
<th>Prawns consumed cooked</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose and severity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hazard severity</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>Susceptibility</td>
<td>General – all population</td>
<td>General – all population</td>
</tr>
<tr>
<td><strong>Probability of exposure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of consumption</td>
<td>Few times a year</td>
<td>Monthly</td>
</tr>
<tr>
<td>Proportion consuming</td>
<td>Some (25%)</td>
<td>Some (25%)</td>
</tr>
<tr>
<td>Size of population</td>
<td>23 million</td>
<td>23 million</td>
</tr>
<tr>
<td><strong>Probability of contamination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probability of raw product contaminated</td>
<td>0.1% (10 cfu/g)</td>
<td>0.1% (10 cfu/g)</td>
</tr>
<tr>
<td>Effect of processing</td>
<td>2-log inactivation</td>
<td>2-log inactivation</td>
</tr>
<tr>
<td>Possibility of recontamination</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Post-process control</td>
<td>Well controlled</td>
<td>Well controlled</td>
</tr>
<tr>
<td>Increase to infective dose</td>
<td>1000x</td>
<td>1000x</td>
</tr>
<tr>
<td>Further cooking before heating</td>
<td>No effect</td>
<td>Reliably eliminates hazard</td>
</tr>
<tr>
<td>Predicted illnesses/decade</td>
<td>1.7</td>
<td>0</td>
</tr>
<tr>
<td>Risk ranking (0-100)</td>
<td>28</td>
<td>0</td>
</tr>
</tbody>
</table>

**References**


4.2 Risk Assessment: *Vibrio parahaemolyticus*

Two scenarios are considered:

1. Raw prawns are eaten raw, as sashimi
2. Raw prawns are stored, cooked and consumed in accordance with the Food Standards Code either in the home or in the food service sector

For each scenario a qualitative and semi-quantitative risk analysis is made.

Data inputs to Risk Ranger are based on data accumulated by the joint FAO-WHO panel which worked on ‘Vibrios in seafood’ (Anon. 2011).

4.2.1 Qualitative assessment of the risk of illness from *V. parahaemolyticus* in imported warm-water prawns

The following assumptions are made:

1. 90% of servings are consumed cooked and 10% consumed raw
2. Imports of prawns are around 20,000,000 kg/annum
3. An average serving size is 200 g
4. Total number of serves is 100,000,000/annum, of which 10 million are consumed raw and 90 million cooked.
5. The Australian population is 23 million

Based on information presented in hazard sheets for reduction of *V. cholerae* and *V. parahaemolyticus* populations during processing, the likelihood of being exposed to an infectious dose of toxigenic *V. parahaemolyticus* through the consumption of imported warm-water prawns is extremely low.

A matrix embracing responses to the above qualitative criteria is presented (Table 33) from which it can be seen that, in qualitative terms, the risk of contracting illness from toxigenic *V. parahaemolyticus* from consumption of warm-water prawns, whether eaten raw or cooked, is very low.

While there were two instances (1990, 1992) where prawns imported from Indonesia were implicated in food poisoning outbreaks in NSW (Kraa, 1995) it is understood that temperature abuse at the retail and caterer levels was the cause.
Table 33: Microbiological hazard risk rating for toxigenic *V. parahaemolyticus* (Vp) in prawns imported to Australia (after ICMSF 2002, FSA 2000)

<table>
<thead>
<tr>
<th>Product</th>
<th>Identified hazard</th>
<th>Severity$^1$</th>
<th>Occurrence of illness$^2$</th>
<th>Growth in product required to cause disease</th>
<th>Prod$^3$/process/handling hazard$^3$</th>
<th>Consumer terminal step$^4$</th>
<th>Epidemiological link$^5$</th>
<th>Risk Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw prawns</td>
<td>Toxigenic Vp</td>
<td>III</td>
<td>Very low</td>
<td>Yes</td>
<td>↓ Inactivation during washing, icing, freezing</td>
<td>No</td>
<td>No</td>
<td>Very low</td>
</tr>
<tr>
<td>Prawns cooked at the plant and eaten without further heat treatment</td>
<td>Toxigenic Vp</td>
<td>III</td>
<td>Very low</td>
<td>Yes</td>
<td>↓ Inactivation during washing, icing, cooking (optional), freezing</td>
<td>No</td>
<td>Yes</td>
<td>Very low</td>
</tr>
<tr>
<td>Prawns cooked immediately before consumption</td>
<td>Toxigenic Vp</td>
<td>III</td>
<td>Very low</td>
<td>Yes</td>
<td>↓ Inactivation during washing, icing, (optional), freezing, thawing and cooking</td>
<td>Yes</td>
<td>No</td>
<td>Very low</td>
</tr>
</tbody>
</table>

1 Sevèrity level III refers to the severity of the identified hazard as classified according to the International Commission of the Microbiological Specifications of Food (ICMSF 2002). Level III: Moderate; not usually life threatening, no sequelae, normally short duration, symptoms are self limiting, can be severe discomfort.

2 Very low occurrence of illness can is based on a lack of cases of illness for almost two decades.

3 As the level of inactivation for processing is in the range of a 5-6 log reduction and the initial contamination level less than 10/g, the likelihood of having toxigenic *V. parahaemolyticus* in prawns after processing was considered to be very low.

4 Cooking of prawns also brings about a 5-6 log reduction in the level of *V. parahaemolyticus*, thus the likelihood of having toxigenic *V. parahaemolyticus* in prawns after processing was considered to be very low.

5 Two outbreaks of illness in which *V. parahaemolyticus* was implicated are described in the Hazard Sheet for *V. parahaemolyticus* of this report (Kraa, 1995).
4.2.2 Semi-quantitative risk of contracting *V. parahaemolyticus* illness from imported warm-water prawns

**Question 1. Hazard Severity**
Risk Ranger alternative: ‘Mild hazard – sometimes requires medical intervention’ was chosen as the response to Question 1.

**Question 2. How susceptible is the population of interest**
For the current assessment: ‘GENERAL - all members of the population’ was selected.

**Questions 3 and 4: Frequency of consumption and proportion consuming**
The following assumptions are made:

1. 90% of servings are consumed cooked and 10% consumed raw
2. Imports of prawns are around 20,000,000 kg/annum
3. An average serving size is 200 g
4. Total number of serves is 100,000,000/annum, of which 10 million are consumed raw and 90 million cooked
5. The population is 23 million
6. 25% of the population consumed prawns raw twice/annum (a few times per year)
7. 25% of population consumed cooked prawns once a month

**Question 5. Size of consuming population**
Population is 23,000,000.

**Question 6. Probability of contamination of raw product per serving**
There have been two outbreaks of food poisoning due to the presence of *V. parahemolyticus* in imported, cooked prawns (Kraa, 1995). Based on this information probability of contamination of incoming prawns was considered RARE (0.1%).

While not stated, implicit at Question 6 is the need to estimate a concentration of *V. parahaemolyticus* on incoming prawns. In the absence of any information a premise used by other researchers which, at its simplest, states that, if the prevalence is low, the concentration is also likely to be low, has been used. While some risk assessments (Bemrah *et al.* 1999) have noted that pathogens are probably heterogeneously distributed in some foods, all to date have assumed that pathogens present in foods are distributed homogeneously. This is a clearly a simplification.

In the present study, a concentration of 10 cfu/g was used (the limit of detection) equivalent to 2000 cfu/serve. This concentration is used to answer Question 10 (increase to infectious dose) for prawns consumed raw.

**Question 7. Effect of Processing**
Data in the hazard sheets for Gram-negative bacteria illustrate the effect of processing on inactivation of vibrios for which a >5 log inactivation is documented during washing, icing and freezing; a similar level of inactivation is assumed for *V. parahaemolyticus*. For
Question 7 the alternative: USUALLY ELIMINATES is selected. Based on the information presented in the hazard sheet this is an extremely conservative estimation.

If prawns are cooked during processing the lethality of the temperature:time regimes used in the industry are greatly in excess of 6 log units which leads to selection that the hazard is RELIABLY ELIMINATED at this stage.

**Question 8. Recontamination**

Given the description of processing in the international trade (see hazard sheet), recontamination was considered not to occur.

**Question 9. Effectiveness of Post-processing Controls**

The cold chain in international trade (frozen and chilled) is well established and *V. parahaemolyticus* has a temperature minimum for growth of 5°C (see hazard sheet) and the Risk Ranger alternative: WELL CONTROLLED (no increase in population) has been selected.

**Question 10. Increase in the post-processing contamination level that would cause infection or intoxication to the average consumer.**

The USA risk assessment on *V. parahaemolyticus* (FDA, CFSAN, 2005) indicates that a dose of 1,000,000 cells corresponded to a probability of disease around 10%. In line with this dose level, oysters in the United States are permitted a maximum of 10,000 *V. parahaemolyticus*/g – equivalent to 1,000,000 cells in a 100 g serving of flesh.

To answer question 10 it is necessary to divide the level at Q6 from the level for an infective dose. In this case:

\[
Q10 = 10^6
\]

\[
Q6 = 10^3 \text{ (200 g serve with concentration 10 cfu/g = 2000 g/serve)}
\]

The difference is approximately \(10^3\) and this value is used at Question 10 in connection with consumption of raw prawns.

**Question 11. Effect of preparation before eating**

Where prawns are cooked it is likely that this will result in complete elimination of *V. parahaemolyticus*. The organism is not heat tolerant and the location of the site of microbiological concern as the carapace means that heat treatment will be immediate. Thus any form of cooking (steaming, boiling or barbecuing) will result in complete elimination.

Where prawns are eaten raw there is no effect on the hazard.

**4.2.3 Risk ratings, predicted illnesses and reality check**

In Table 34 are presented risk ratings and predicted illnesses based on consumption of raw and cooked imported prawns.

For prawns consumed raw, the risk of illness has a rating of 22 with 1.5 illnesses every decade. In assessments of paired seafood hazard:products pairings in Australia. Sumner and Ross (2002) found that Risk Ranger ratings <30 were not equated with any reports of illness.
For prawns consumed cooked, the hazard is reliably eliminated during cooking either at the plant level (Risk Ranger Question 7: Reliably eliminates) or during meal preparation (Risk Ranger Question 11: Reliably eliminates). This leads to Risk Ranger rating of 0 and prediction of no illnesses.

Thus, a semi-quantitative assessment of the likelihood of contracting illness from *V. parahaemolyticus* following consumption of warm-water prawns by consumers in major importing countries is of the order of 1-2 cases/decade.

### Table 34: Estimation of risk associated with consumption of imported warm-water prawns in Australia

<table>
<thead>
<tr>
<th>Risk criteria</th>
<th>Prawns consumed raw</th>
<th>Prawns consumed cooked</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose and severity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hazard severity</td>
<td>Mild</td>
<td>Mild</td>
</tr>
<tr>
<td>Susceptibility</td>
<td>General – all population</td>
<td>General – all population</td>
</tr>
<tr>
<td><strong>Probability of exposure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of consumption</td>
<td>Few times a year</td>
<td>Monthly</td>
</tr>
<tr>
<td>Proportion consuming</td>
<td>Some (25%)</td>
<td>Some (25%)</td>
</tr>
<tr>
<td>Size of population</td>
<td>23 million</td>
<td>23 million</td>
</tr>
<tr>
<td><strong>Probability of contamination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probability of raw product contaminated</td>
<td>0.1% (10 cfu/g)</td>
<td>0.1% (10 cfu/g)</td>
</tr>
<tr>
<td>Effect of processing</td>
<td>2-log inactivation</td>
<td>2-log inactivation</td>
</tr>
<tr>
<td>Possibility of recontamination</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Post-process control</td>
<td>Well controlled</td>
<td>Well controlled</td>
</tr>
<tr>
<td>Increase to infective dose</td>
<td>1,000x</td>
<td>1,000x</td>
</tr>
<tr>
<td>Further cooking before heating</td>
<td>No effect</td>
<td>Reliably eliminates hazard</td>
</tr>
<tr>
<td>Predicted illnesses/decade</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>Risk ranking (0-100)</td>
<td>22</td>
<td>0</td>
</tr>
</tbody>
</table>

### References


4.3 Risk Assessment: *Salmonella*

Two scenarios are considered:

1. Raw prawns are eaten raw, as sashimi
2. Raw prawns are stored, cooked and consumed in accordance with the Food Standards Code either in the home or in the food service sector

For each scenario a qualitative and semi-quantitative risk analysis is made.

Data inputs to Risk Ranger are informed by the FAO export workshop on the application of biosecurity measures to control *Salmonella* contamination in sustainable aquaculture (Anon. 2010).

4.3.1 Qualitative assessment of the risk of illness from *Salmonella* in imported warm-water prawns

The following assumptions are made:

1. 90% of servings are consumed cooked and 10% consumed raw
2. Imports of prawns are around 20,000,000 kg/annum
3. An average serving size is 200 g
4. Total number of serves is 100,000,000/annum, of which 10 million are consumed raw and 90 million cooked.
5. The Australian population is 23 million

Based on information gathered by the joint FAO-WHO panel on reduction of Gram-negative populations during processing, the likelihood of being exposed to an infectious dose of *Salmonella* through the consumption of imported warm-water prawns is extremely low.

There has been one recall (2004) of cooked prawns (FSANZ recall data).

Testing of imported prawns by AQIS did not isolate *Salmonella* from 473 samples of cooked, imported prawns.

A matrix embracing responses to the above qualitative criteria is presented (Table 35) from which it can be seen that, in qualitative terms, the risk of contracting salmonellosis from consumption of warm-water prawns, whether eaten raw or cooked, is very low.
Table 35: Microbiological hazard risk rating for *Salmonella* in prawns imported to Australia (after ICMSF 2002, FSA 2000)

<table>
<thead>
<tr>
<th>Product</th>
<th>Identified hazard</th>
<th>Severity(^1)</th>
<th>Occurrence of illness(^2)</th>
<th>Growth in product required to cause disease</th>
<th>Prod(^4)/process/handling hazard(^3)</th>
<th>Consumer terminal step(^4)</th>
<th>Epidemiological link</th>
<th>Risk Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw prawns</td>
<td><em>Salmonella</em></td>
<td>II</td>
<td>Very low</td>
<td>Yes</td>
<td>↓ Inactivation during washing, icing, freezing</td>
<td>No</td>
<td>No</td>
<td>Very low</td>
</tr>
<tr>
<td>Prawns cooked at the plant and eaten without further heat treatment</td>
<td><em>Salmonella</em></td>
<td>II</td>
<td>Very low</td>
<td>Yes</td>
<td>↓ Inactivation during washing, icing, cooking (optional), freezing</td>
<td>No</td>
<td>No</td>
<td>Very low</td>
</tr>
<tr>
<td>Prawns cooked immediately before consumption</td>
<td><em>Salmonella</em></td>
<td>II</td>
<td>Very low</td>
<td>Yes</td>
<td>↓ Inactivation during washing, icing, (optional), freezing, thawing and cooking</td>
<td>Yes</td>
<td>No</td>
<td>Very low</td>
</tr>
</tbody>
</table>

\(^1\) Severity level II refers to the severity of the identified hazard as classified according to the International Commission of the Microbiological Specifications of Food (ICMSF 2002). Level II: High hazard; incapacitating but not life threatening, sequelae rare, moderate duration.

\(^2\) Very low occurrence of illness can is based on a lack of cases of illness for almost two decades.

\(^3\) As the level of inactivation for processing is in the range of a 5-6 log reduction and the initial contamination level less than 10/g, the likelihood of having *Salmonella* in prawns after processing was considered to be very low.

\(^4\) Cooking of prawns also brings about a 5-6 log reduction in the level of *Salmonella*, thus the likelihood of having *Salmonella* in prawns after processing was considered to be very low.
4.3.2 Semi-quantitative risk of contracting *Salmonella* illness from imported warm-water prawns

**Question 1. Hazard Severity**
Risk Ranger alternative: ‘Mild hazard – sometimes requires medical intervention’ was chosen as the response to Question 1.

**Question 2. How susceptible is the population of interest**
For the current assessment: ‘GENERAL - all members of the population’ was selected.

**Questions 3 and 4: Frequency of consumption and proportion consuming**
The following assumptions are made:

1. 90% of servings are consumed cooked and 10% consumed raw
2. Imports of prawns are around 20,000,000 kg/annum
3. An average serving size is 200 g
4. Total number of serves is 100,000,000/annum, of which 10 million are consumed raw and 90 million cooked
5. The population is 23 million
6. 25% of the population consumed prawns raw twice/annum (a few times per year)
7. 25% of population consumed cooked prawns once a month

**Question 5. Size of consuming population**
Population is 23,000,000.

**Question 6. Probability of contamination of raw product per serving**
Apart from one recall of imported prawns, *Salmonella* has not been isolated from almost 500 samples of imported, cooked prawns by AQIS testing. Based on this information, the probability of contamination of incoming prawns was considered RARE (0.1%).

While not stated, implicit at Question 6 is the need to estimate a concentration of *Salmonella* on incoming prawns. In the absence of any information a premise used by other researchers which, at its simplest, states that, if the prevalence is low, the concentration is also likely to be low, has been used. While some risk assessments (Bemrah et al. 1999) have noted that pathogens are probably heterogeneously distributed in some foods, all to date have assumed that pathogens present in foods are distributed homogeneously. This is a clearly a simplification.

In the present study, a concentration of 1 cfu/g was used, equivalent to 200 cfu/serve. This concentration is used to answer Question 10 (increase to infectious dose) for prawns consumed raw.

**Question 7. Effect of Processing**
Data in the hazard sheets for *Salmonella* illustrate the effect of processing on inactivation of Gram-negatives, for which a >5 log inactivation is documented during washing, icing and
freezing. For Question 7 the alternative: USUALLY ELIMINATES is selected. Based on the information presented in the hazard sheet this is an extremely conservative estimation.

If prawns are cooked during processing the lethality of the temperature:time regimes used in the industry are greatly in excess of 6 log units which leads to selection that the hazard is RELIABLY ELIMINATED at this stage.

**Question 8. Recontamination**
Given the description of processing in the international trade (see hazard sheet), recontamination was considered not to occur.

**Question 9. Effectiveness of Post-processing Controls**
The cold chain in international trade (frozen and chilled) is well established and *Salmonella* has a temperature minimum for growth of 7°C (see hazard sheet) and the Risk Ranger alternative: WELL CONTROLLED (no increase in population) has been selected.

**Question 10. Increase in the post-processing contamination level that would cause infection or intoxication to the average consumer.**
It is difficult to establish a dose response model for *Salmonella* because:

- There are more than 2,000 serovars with widely differing pathogenicity
- Some foods (with high fat) facilitate passage of the pathogen through the strongly-acidic conditions of the stomach
- Consumers vary in their immune status

Several dose response curves have been developed over recent years for various nontyphoidal *Salmonella*. In general, an ingested dose around $10^4$ cells has little probability of causing disease, while $10^6$ cells are likely to cause infection in 80% of consumers.

For the purpose of the present assessment an infective dose of $10^6$ cells was used.

To answer question 10 it is necessary to divide the level at Q6 from the level for an infective dose. In this case:

$$Q10 = 10^6$$

$$Q6 = 10^2 \ (200 \text{ g serve with concentration } 1 \text{ cfu/g } = 200 \text{ g/serve})$$

The difference is approximately $10^4$ and this value is used at Question 10 in connection with consumption of raw prawns.

**Question 11. Effect of preparation before eating**
Where prawns are cooked it is likely that this will result in complete elimination of *Salmonella*. The organism is not heat tolerant and the location of the site of microbiological concern as the carapace means that heat treatment will be immediate. Thus any form of cooking (steaming, boiling or barbecuing) will result in complete elimination.

Where prawns are eaten raw there is no effect on the hazard.
4.3.3 Risk ratings, predicted illnesses and reality check

In Table 36 are presented risk ratings and predicted illnesses based on consumption of raw and cooked imported prawns.

For prawns consumed raw, risk of illness has a rating of 16 with 1.5 illnesses every century. In assessments of paired seafood hazard:products pairings in Australia. Sumner and Ross (2002) found that Risk Ranger ratings <30 were not equated with any reports of illness.

For prawns consumed cooked the hazard is reliably eliminated during cooking either at the plant level (Risk Ranger Question 7: Reliably eliminates) or during meal preparation (Risk Ranger Question 11: Reliably eliminates). This leads to Risk Ranger rating of 0 and prediction of no illnesses.

Thus, a semi-quantitative assessment of the likelihood of contracting illness from *Salmonella* following consumption of warm-water prawns by consumers in major importing countries is of the order of 1-2 cases/century.

Table 36: Estimation of risk of contracting salmonellosis following consumption of imported warm-water prawns in Australia

<table>
<thead>
<tr>
<th>Risk criteria</th>
<th>Prawns consumed raw</th>
<th>Prawns consumed cooked</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose and severity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hazard severity</td>
<td>Mild</td>
<td>Mild</td>
</tr>
<tr>
<td>Susceptibility</td>
<td>General – all population</td>
<td>General – all population</td>
</tr>
<tr>
<td><strong>Probability of exposure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of consumption</td>
<td>Few times a year</td>
<td>Monthly</td>
</tr>
<tr>
<td>Proportion consuming</td>
<td>Some (25%)</td>
<td>Some (25%)</td>
</tr>
<tr>
<td>Size of population</td>
<td>23 million</td>
<td>23 million</td>
</tr>
<tr>
<td><strong>Probability of contamination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probability of raw product contaminated</td>
<td>0.1% (1 cfu/g)</td>
<td>0.1% (1 cfu/g)</td>
</tr>
<tr>
<td>Effect of processing</td>
<td>2-log inactivation</td>
<td>2-log inactivation</td>
</tr>
<tr>
<td>Possibility of recontamination</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Post-process control</td>
<td>Well controlled</td>
<td>Well controlled</td>
</tr>
<tr>
<td>Increase to infective dose</td>
<td>10,000x</td>
<td>10,000x</td>
</tr>
<tr>
<td>Further cooking before heating</td>
<td>No effect</td>
<td>Reliably eliminates hazard</td>
</tr>
<tr>
<td>Predicted illnesses/decade</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>Risk ranking (0-100)</td>
<td>16</td>
<td>0</td>
</tr>
</tbody>
</table>

References


4.4 Risk Assessment of Chemical Hazards

For several reasons, achieving a useful assessment of risk from ingesting chemicals contained in prawns is difficult because:

- There are no recorded cases of illness associated with the hazard:product pairing.
- Chronic exposure over many years may be required for adverse reaction (e.g. cadmium intake and kidney disease).
- Risk of exposure are considered dose-independent e.g. any dose of chloramphenicol and nitrofurans is considered by some authorities (EU) to be disease-causing at any dose
- The application of ‘zero tolerance’, with its implied ‘zero risk’, effectively precludes any application of probability to an adverse event occurring

The basis for the responses to the questions posed in the qualitative matrix is contained in the hazard sheets.

<table>
<thead>
<tr>
<th>Product/hazard</th>
<th>Sulphur dioxide in prawns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severity</td>
<td>Range from nasal discharge to asthma and anaphylactic shock</td>
</tr>
<tr>
<td>Occurrence of illness</td>
<td>No reports from prawn consumption</td>
</tr>
<tr>
<td>Increase in concentration in prawns required to cause illness?</td>
<td>No</td>
</tr>
<tr>
<td>Impact of processing, handling</td>
<td>None</td>
</tr>
<tr>
<td>Consumer terminal step?</td>
<td>Cooking reduces concentration</td>
</tr>
<tr>
<td>Epidemiological link?</td>
<td>None</td>
</tr>
<tr>
<td>Assessed risk</td>
<td>Low</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Product/hazard</th>
<th>Chloramphenicol in prawns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severity</td>
<td>Aplastic anaemia</td>
</tr>
<tr>
<td>Occurrence of illness</td>
<td>Rare but sometimes fatal</td>
</tr>
<tr>
<td>Chronic exposure required to cause illness?</td>
<td>Yes – 150,000,000-735,000,000x intake from level in prawns</td>
</tr>
<tr>
<td>Impact of processing, handling</td>
<td>None</td>
</tr>
<tr>
<td>Consumer terminal step?</td>
<td>None</td>
</tr>
<tr>
<td>Epidemiological link?</td>
<td>None</td>
</tr>
<tr>
<td>Assessed risk</td>
<td>Extremely low</td>
</tr>
<tr>
<td>Product/hazard</td>
<td>Nitrofurans in prawns</td>
</tr>
<tr>
<td>---------------------------------------------------</td>
<td>-----------------------------------------------------------</td>
</tr>
<tr>
<td>Severity</td>
<td>Genotoxicity</td>
</tr>
<tr>
<td>Occurrence of illness</td>
<td>No cases reported</td>
</tr>
<tr>
<td>Chronic exposure required to cause illness?</td>
<td>Yes – 4,000,000-12,000,000x intake from prawns</td>
</tr>
<tr>
<td>Impact of processing, handling</td>
<td>None</td>
</tr>
<tr>
<td>Consumer terminal step?</td>
<td>No</td>
</tr>
<tr>
<td>Epidemiological link?</td>
<td>Not in Australia</td>
</tr>
<tr>
<td>Assessed risk</td>
<td>Very low (FZANZ risk assessment)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Product/hazard</th>
<th>Cadmium in Prawns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severity</td>
<td>Disorders of kidneys, liver, intestine</td>
</tr>
<tr>
<td>Occurrence of illness</td>
<td>Low</td>
</tr>
<tr>
<td>Chronic exposure required to cause illness?</td>
<td>Yes</td>
</tr>
<tr>
<td>Impact of processing, handling</td>
<td>None</td>
</tr>
<tr>
<td>Consumer terminal step?</td>
<td>None</td>
</tr>
<tr>
<td>Epidemiological link?</td>
<td>None</td>
</tr>
<tr>
<td>Assessed risk</td>
<td>Low</td>
</tr>
</tbody>
</table>
Appendix 1: Review Report on ‘Food Safety Risks Associated with Prawns Consumed in Australia’

Prepared jointly by:

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and

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The document ‘Food safety risks associated with prawns consumed in Australia’ has been well prepared taking into consideration available scientific evidence on microbiological and chemical hazards, epidemiological data from Australia and other prawn importing and consuming regions, other national and international risk assessments. Data source and scientific references have been indicated. The challenges involved in performing risk assessments for chemicals have been correctly pointed out.

Detailed comments on the three TORs are given below:

1. Hazard identification: This has been based on reported cases of illness during two decades, as well as perceived hazards causing recalls or import alerts. This section provides good coverage for both actual hazards involved in illnesses as well as perceived ones.

2. Hazard sheets for identified hazards:

   (a) Vibrio cholerae: This is largely based on FAO/WHO risk assessment (2005) and after this assessment was completed, there has been no new data that would warrant reconsideration of the conclusions. Further, National Enteric Pathogen Surveillance Scheme data has been used to identify the hazard in Australian context. Many alerts in import testing systems arise without considering toxigenicity of isolates found and the hazard sheet recognises this.

   (b) Vibrio parahaemolyticus: The hazard sheet takes into consideration global reports of incidence of V. parahaemolyticus in prawns and also the Australian data. We recommend that upfront, in section 1. Hazard identification, it would be useful to state that human illnesses are generally caused by strains that are either tdh+ or rarely, trh+ and strains possessing these attributes constitute a very small proportion, if at all of the natural population associated with the environment and seafood. This point comes later in section 3.2. This would be helpful to understand the public health risk in the right perspective, particularly in the context of natural presence of this organism, and large number of import alerts that are triggered without testing for toxigenicity/pathogenicity.

   Response: Agreed and the text has been amended in TOR 1: Hazard Identification

   (c) In Section 1.2, it will be useful to give some additional information about a large outbreak (1,133 cases) linked to shrimp that occurred in Port Allen, Louisiana, in US in 1978 (Morb. Mortality Weekly Rep 27: 345-346, 1978). In this situation, raw shrimp shipped in wooden boxes were boiled, returned back to the same boxes and transported unrefrigerated and consumed after 7-8 hr (Oliver and Kaper, 2007).
Response: Agreed and the text has been amended in Section 1.2 of the Hazard Sheet for *V. parahaemolyticus*

Section 2, last para, it will be useful to clarify regarding sucrose negative vibrios. The parenthesis suggests that these contain pathogenic strains. We suggest deleting the parenthesis or changing it as ‘a category that includes *V. parahaemolyticus*’ because there are sucrose negative vibrios (e.g. *V. harveyi*) that do not cause human infections and even among sucrose negative *V. parahaemolyticus*, only a very small proportion are tdh+ and hence pathogenic. Therefore presence of sucrose negative *Vibrio* count does not mean much in terms of presence of pathogenic strains.

Response: Agreed and the text has been amended in Section 2 of the Hazard Sheet for *V. parahaemolyticus*

In Summary (Section 4), we think it would be useful to state that though pathogenic strains have been detected in oysters, illnesses are rare, possibly because, when present they are in very low numbers as indicated in last para of section 2.

Response: Agreed and the text has been amended in Section 2 of the Hazard Sheet for *V. parahaemolyticus*

(d) *Salmonella*: The hazard sheet has been written well. In Section 3.3 Dose response, the second sentence needs to be qualified. Low cell numbers causing outbreaks is related to food matrix (mostly high fat foods) and there are no reports of low cell numbers associated with seafood causing outbreaks. As noted in FAO/WHO risk assessment for salmonella in broiler chicken and eggs (FAO/WHO, 2002), in one outbreak related to consumption of scallop with egg yolk, 6.3 log cells resulted in 56% attack rate. Also it will be useful to point out that prawns are not considered high fat foods.

Response: Agreed and the text has been amended in Section 3.3 of the Hazard sheet for *Salmonella*

(e) Hepatitis A virus: The hazard sheet has been well written with appropriate references.

(f) Chloramphenicol and nitrofurans: We would agree with the hazard sheet. The levels of residues found in seafood including prawns are extremely low and pose little or no risk to consumer health. The issue with these compounds has been that they have been used as indicators of misuse of antibiotics that have been banned in food producing animals.

(g) Sulphite: Agree with the hazard sheet

(h) Cadmium: Agree with the hazard sheet

3. **Risk assessment of identified hazards**: *V. cholerae* risk assessment is in line with the FAO/WHO risk assessment and the use of Risk Ranger provides a useful semi-quantitative output. *V. parahaemolyticus* assessment page 69, there is some mix-up with *Salmonella*. These need to be corrected.

Response: Agreed and the typos have been amended.

*Salmonella* assessment is also in agreement with epidemiological data and logical. The qualitative risk assessment for chemical hazards is quite reasonable and shows that risks are extremely low.
4. **Conclusions**: The Draft Report adequately covers the TORs. The hazards have been identified based on both global as well as Australian data and well referenced. We agree that Risk Ranger is a useful tool to place the risk in a semi-quantitative context and provide a ranking for further action by risk managers. The risk ratings obtained in this study are realistic for the hazard:product pairing.