



## UvA-DARE (Digital Academic Repository)

### Persistence and survival of pathogens in dry foods and dry food processing environments

Beuchat, L.; Komitopoulou, E.; Betts, R.; Beckers, H.; Bourdichon, F.; Joosten, H.; Fanning, S.; ter Kuile, B.

**Publication date**

2011

**Document Version**

Final published version

[Link to publication](#)

**Citation for published version (APA):**

Beuchat, L., Komitopoulou, E., Betts, R., Beckers, H., Bourdichon, F., Joosten, H., Fanning, S., & ter Kuile, B. (2011). *Persistence and survival of pathogens in dry foods and dry food processing environments*. (ILSI Europe report series). ILSI Europe.  
<http://www.ilsil.org/Europe/Documents/Persistence%20and%20survival%20report.pdf>

**General rights**

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

**Disclaimer/Complaints regulations**

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

# PERSISTENCE AND SURVIVAL OF PATHOGENS IN DRY FOODS AND DRY FOOD PROCESSING ENVIRONMENTS

---

REPORT

## **About ILSI / ILSI Europe**

Founded in 1978, the International Life Sciences Institute (ILSI) is a nonprofit, worldwide foundation that seeks to improve the well-being of the general public through the advancement of science. Its goal is to further the understanding of scientific issues relating to nutrition, food safety, toxicology, risk assessment, and the environment. ILSI is recognised around the world for the quality of the research it supports, the global conferences and workshops it sponsors, the educational projects it initiates, and the publications it produces. ILSI is affiliated with the World Health Organization (WHO) as a non-governmental organisation and has special consultative status with the Food and Agricultural Organization (FAO) of the United Nations. By bringing together scientists from academia, government, industry, and the public sector, ILSI fosters a balanced approach to solving health and environmental problems of common global concern. Headquartered in Washington, DC, ILSI accomplishes this work through its worldwide network of branches, the ILSI Health and Environmental Sciences Institute (HESI) and its Research Foundation. Branches currently operate within Argentina, Brazil, Europe, India, Japan, Korea, Mexico, North Africa & Gulf Region, North America, North Andean, South Africa, South Andean, Southeast Asia Region, as well as a Focal Point in China.

ILSI Europe was established in 1986 to identify and evaluate scientific issues related to the above topics through symposia, workshops, expert groups, and resulting publications. The aim is to advance the understanding and resolution of scientific issues in these areas. ILSI Europe is funded primarily by its industry members.

This publication is made possible by support of the ILSI Europe Task Force on Emerging Microbiological Issues, which is under the umbrella of the Board of Directors of ILSI Europe. ILSI policy mandates that the ILSI and ILSI branch Boards of Directors must be composed of at least 50% public sector scientists; the remaining directors represent ILSI's member companies. Listed hereunder are the ILSI Europe Board of Directors and the ILSI Europe Task Force on Emerging Microbiological Issues industry members.

### *ILSI Europe Board of Directors*

#### *Non-industry members*

Prof. A. Boobis, Imperial College of London (UK)  
Prof. P. Calder, University of Southampton (UK)  
Prof. G. Eisenbrand, University of Kaiserslautern (DE)  
Prof. A. Grynberg, Université Paris Sud – INRA (FR)  
Prof. em. G. Pascal, National Institute for Agricultural Research – INRA (FR)  
Prof. G. Rechkemmer, Max Rubner-Institut – Federal Research Institute of Nutrition and Food (DE)  
Dr. J. Schlundt, National Food Institute (DK)  
Prof. V. Tutelyan, National Nutrition Institute (RU)  
Prof. G. Varela-Moreiras, University San Pablo-CEU of Madrid (ES)

#### *Industry members*

Mr. C. Davis, Kraft Foods (CH)  
Mr. R. Fletcher, Kellogg Europe (IE)  
Dr. M. Knowles, Coca-Cola Europe (BE)  
Dr. G. Kozianowski, Südzucker/BENEOL Group (DE)  
Dr. G. Meijer, Unilever (NL)  
Prof. J. O'Brien, Nestlé (CH)  
Prof. C. Shortt, McNeil Nutritionals (UK)  
Dr. J. Stowell, Danisco (UK)  
Dr. G. Thompson, Danone (FR)  
Dr. P. Weber, DSM (CH)

### *ILSI Europe Emerging Microbiological Issues Task Force industry members*

Barilla G. & R. Fratelli  
Danone  
H J Heinz  
Institut Mérieux  
Kraft Foods  
Mars  
Nestlé  
Royal Friesland Campina  
Unilever

**PERSISTENCE AND SURVIVAL OF PATHOGENS  
IN DRY FOODS AND DRY FOOD PROCESSING  
ENVIRONMENTS**

*By Larry Beuchat, Evangelia Komitopoulou,  
Roy Betts, Harry Beckers, François Bourdichon,  
Han Joosten, Seamus Fanning, Benno ter Kuile*

---

REPORT OF AN ILSI EUROPE EXPERT GROUP  
COMMISSIONED BY THE ILSI EUROPE EMERGING MICROBIOLOGICAL ISSUES TASK FORCE

NOVEMBER 2011

© 2011 ILSI Europe

This publication may be reproduced for non-commercial use as is, and in its entirety, without further permission from ILSI Europe. Partial reproduction and commercial use are prohibited without ILSI Europe's prior written permission.

"A Global Partnership for a Safer, Healthier World ®", the International Life Sciences Institute (ILSI) logo image of concentric circles, the word mark "International Life Sciences Institute", as well as the acronym "ILSI" are trademarks of the International Life Sciences Institute and licensed for use by ILSI Europe. The use of trade names and commercial sources in this document is for purposes of identification only and does not imply endorsement by ILSI Europe. In addition, the opinions expressed herein and the conclusions of this publication are those of the authors and do not necessarily represent the views of ILSI Europe nor those of its member companies.

For more information about ILSI Europe, please contact

ILSI Europe a.i.s.b.l.  
Avenue E. Mounier 83, Box 6  
B-1200 Brussels  
Belgium  
Phone: (+32) 2 771 00 14  
Fax: (+32) 2 762 00 44  
E-mail: [info@ilsieurope.be](mailto:info@ilsieurope.be)  
[www.ilsieurope.eu](http://www.ilsieurope.eu)

*Printed in Belgium*

D/2011/10.996/29

ISBN 9789078637325

# CONTENTS

1. INTRODUCTION	4
2. PATHOGENS AND TOXINS IN LOW-MOISTURE FOODS AND PRODUCTION ENVIRONMENTS	6
2.1 <i>Bacillus</i> species	6
2.2 <i>Clostridium botulinum</i>	6
2.3 <i>Clostridium perfringens</i>	6
2.4 <i>Cronobacter</i> species	8
2.5 Verotoxigenic <i>Escherichia coli</i> (VTEC)	8
2.6 <i>Salmonella</i>	8
2.7 <i>Staphylococcus aureus</i>	8
2.8 Enteric viral pathogens	9
2.9 Mycotoxigenic moulds	9
2.10 Other pathogens not yet associated with dry foods as vehicles of foodborne disease	10
3. SOURCES AND ROUTES OF ENTRY INTO PRODUCTS	11
3.1 Raw materials and ingredients	11
3.2 Air	12
3.3 Water	12
3.4 Contact material	13
3.5 Personnel	13
3.6 Pests	13
4. PERSISTENCE IN DRY ENVIRONMENTS	14
4.1 Survival and persistence in dry foods and dry food processing plants	14
4.2 Persistence in biofilm	18
4.3 Consideration of spores	20
5. OUTBREAKS, ALERTS AND RECALLS ASSOCIATED WITH DRY FOODS	21
5.1 Outbreaks associated with dry foods	21
5.2 Pathogen Alerts for dry foods in the European Union	26
5.3 Recalls and market withdrawal of dry foods containing pathogens in the United States	27
6. POTENTIAL CONTROL MEASURES	28
6.1 Initial reduction of contamination	28
6.2 Prevention of recontamination	28
7. VERIFICATION	33
7.1 Microbial distribution in dry foods	33
7.2 Environmental monitoring	33
7.3 Utilisation of indicator microorganisms	34
7.4 Raw materials	34
7.5 Analytical methods	34
8. SUMMARY AND CLOSING COMMENTS	36
9. REFERENCES	37
10. ACRONYMS	47

Authors: Larry Beuchat, University of Georgia (US), Evangelia Komitopoulou, Leatherhead Food Research (UK), Roy Betts, Campden BRI (UK), Harry Beckers, Consultant (NL), François Bourdichon, Nestlé (CH), Han Joosten, Nestlé (CH) Seamus Fanning, University College Dublin (IE), Benno ter Kuile, Food and Consumer Product Safety Authority (NL)  
 Scientific Reviewers: Robert L. Buchanan, University of Maryland (US), Marcel Zwietering (NL)  
 Report Series Editor: Kevin Yates (UK)  
 Coordinator: Pratima Rao Jasti, ILSI Europe (BE)

# 1. INTRODUCTION

**L**ow-moisture foods and food ingredients, i.e., those appearing to be dry or that have been subjected to a drying process, represent important nutritional constituents of human diets. Some of these foods are naturally low in moisture, such as cereals, honey and nuts, whereas others are produced from high-moisture foods that were deliberately submitted to drying (e.g., egg and milk powders). The addition of large amounts of salt or sugar can also be regarded as a 'drying' process by reducing the amount of water available for microbial growth.

Drying (removal of water) has been used since ancient times to preserve food. Although many pathogens and spoilage microorganisms can survive the drying process, this preservation technology is very effective because microbial growth will cease if water is no longer available for biological reactions. The water activity ( $a_w$ ) necessary to prevent growth of microorganisms, i.e., to inhibit physiological activities necessary for cell division, is 0.60 or less. If more water is available, some species of xerophilic spoilage moulds and osmophilic yeasts can grow at  $a_w$  0.60 – 0.70; however, the minimum  $a_w$  for mycotoxin production by moulds is 0.80 with the majority not producing mycotoxins below  $a_w$  0.85 (Cousin *et al.*, 2005). The minimum  $a_w$  for growth of most bacteria is 0.87, although halophilic bacteria can grow at  $a_w$  as low as 0.75. Among the pathogenic microorganisms, *Staphylococcus aureus* is particularly well-adapted to reduced-moisture environments. Under optimal conditions it can grow at  $a_w$  as low as 0.83 but in most foods the minimum is  $a_w$  0.85 (ICMSF, 1996). With this exception aside, in the context of this monograph, all foods and food ingredients that have an  $a_w$  that prevents the growth of bacterial foodborne pathogens, i.e., with an  $a_w$  of 0.85 or lower, are considered. These foods and ingredients are referred to as having low moisture or low  $a_w$ .

A wide range of products falls in this category: animal feeds such as fishmeal and pet foods, cereals, chocolate, cocoa powder, dried fruits and vegetables, egg powder, fermented dry sausage, flour, meal and grits, herbs, spices and condiments, honey, hydrolysed vegetable protein powder, meat powders, dried meat, milk powder, pasta, peanut butter, peanuts and tree nuts, powdered infant formula, rice and other grains, and seeds (e.g., sesame, melon, pumpkin, linseed). Although low-moisture foods have some clear advantages with respect to food safety, there are nevertheless some major concerns:

- Many microorganisms, including pathogens, are able to survive drying processes. Once in a dried state, metabolism is greatly reduced, i.e., there is no growth but vegetative cells and spores may remain viable for several months or even years. They can often persist longer in low-moisture foods and in dry food processing environments than in high-moisture foods and wet environments.
- It is often difficult or even impossible to eliminate pathogens from foods with low moisture by processes such as application of mild heat treatment (e.g., pasteurisation) or high hydrostatic pressure that work very well for high-moisture foods.
- Food processing environments, in which dried foods are handled, must be maintained at low humidity and kept dry, and this can give rise to problems in cleaning and sanitising, which are usually 'wet' procedures.
- Finally, it is of concern that consumers sometimes wrongly believe that low-moisture foods are sterile, which may lead to dangerous practices such as keeping reconstituted infant formula at ambient temperature for prolonged periods, thereby creating growth opportunities for pathogens such as *Bacillus cereus* and *Cronobacter* species.

Microorganisms are much more heat-resistant in low- $a_w$  environments than at  $a_w$  levels supporting growth. It is difficult to predict the extent of this increase, and it does vary with the type of solute present, but an extreme example is that temperatures in excess of 100°C for a few minutes are necessary to reduce *Salmonella* in chocolate by 1 log CFU/g (Barrile and Cone, 1970; Davies et al., 1990; Goepfert and Biggie, 1968). A less extreme case would be that of survival of *Salmonella* during concentration and drying of milk. Dry ingredients such as sugar and salt can be the sources of microorganisms in foods preserved or seasoned by their addition.

Prevention of cross-contamination of high-moisture foods with pathogens or spoilage microorganisms from low-moisture foods that are microbiologically stable should be a goal of Good Manufacturing Practices (GMPs) and Hazard Analysis Critical Control Point (HACCP) systems. To minimise potential contamination of foods with high  $a_w$ , dried spices and herbs, dried egg and milk powders and other dry ingredients should be kept separate from other foods and food ingredients that will not be cooked. Upon rehydration of low-moisture foods or ingredients containing microorganisms, growth may occur. These foods should be used within a short time after rehydration or stored, either refrigerated or frozen, for a limited time before consumption. Otherwise, the risk of such foods causing infection or intoxication can markedly increase.

Because microorganisms may survive during drying processes or persist in low-moisture foods and dry food processing environments, it is imperative that Good Hygiene Practices (GHPs), GMPs and HACCP systems, with specific attention to preventing survival and persistence of foodborne pathogens, be implemented and effectively maintained on a continuous basis (see Section 7, Verification). With regard to assessing risks of contamination of products in dry food processing plants, routine sampling for pathogens that may be present on surfaces where dust can accumulate is valuable in providing information on their potential presence in the finished products.

This report summarises information on the survival of foodborne pathogens in low-moisture foods ( $a_w < 0.85$ ) and in dry food processing environments. Pathogens that have been known to cause outbreaks of infections or intoxications associated with consumption of low-moisture foods, as well as those not yet implicated in outbreaks, are discussed.



## 2. PATHOGENS AND TOXINS IN LOW-MOISTURE FOODS AND PRODUCTION ENVIRONMENTS

Vegetative bacterial cells, along with bacterial and fungal spores, may survive in foods and food ingredients with  $a_w < 0.85$ , as well as in dry production environments, for long periods. On rehydration, survivors may present a foodborne disease hazard. Characteristics of pathogens that have been associated with, or documented to have caused, outbreaks of foodborne diseases as a result of consumption of low- $a_w$  foods are summarised in Table 1. The following text is intended to provide further insights relevant to these pathogens.

### 2.1 *Bacillus species*

Some strains of *Bacillus cereus* and, very rarely, *Bacillus subtilis* and *Bacillus licheniformis* can produce one or two types of toxins. Heat stable emetic toxin (cereulide) is produced by *B. cereus* in starchy foods, e.g., quiche, cakes and pasta salad, but especially in cooked rice. Diarrhoeagenic toxin is produced only during growth in the gastrointestinal (GI) tract. *Bacillus cereus* spores survive in dry foods such as rice cereal (Jaquette and Beuchat, 1998) and in dry food processing environments for long periods of time, and can germinate and grow in reconstituted (rehydrated) products that are not properly processed or stored. The reader is referred to Blackburn and McClure (2009) and Granum (2007) for additional information on *Bacillus* species.

### 2.2 *Clostridium botulinum*

Several *Clostridium* species are pathogenic but only *C. botulinum* and *C. perfringens* (and rare strains of *C. butyricum* and *C. baratii*) are associated with foodborne intoxications. Honey consumption by infants may give rise to infant botulism, a toxico-infection, whereby low numbers of spores germinate in the GI tract and produce toxin. Isolates of *C. botulinum* cultured from honey ( $a_w < 0.60$ ) and linked to cases of infant botulism in the United States appear to reflect the same types found in the local soil (Barash et al., 2005). A case of infant botulism was associated with the consumption of reconstituted infant formula milk powder (Brett et al., 2005). It was suggested in another study, however, that the unopened brand of formula implicated in this case was not the source of transmission of spores to the infant (Johnson et al., 2005). See Gibbs (2009) and Johnson (2007) for reviews of *C. botulinum*.

### 2.3 *Clostridium perfringens*

Spores of *C. perfringens* can be found in soils and in the intestinal tracts of vertebrates. They survive well in dust and on surfaces and are resistant to routine cooking temperatures. Sporulation of large numbers of vegetative cells of *C. perfringens* in the GI tract can result in the production of an enterotoxin and severe diarrhoea, cramps and flatulence. Spores of *C. perfringens* have been found in powdered infant formula and also in dried herbs and spices, including black pepper which, if added to cooked meat dishes, may give rise to an infective dose if the food is temperature-abused during cooling or holding. See Gibbs (2009) and McClane (2007) for reviews of *C. perfringens*.

Table 1. Characteristics of bacterial pathogens associated with, or documented to have caused, outbreaks of illness associated with consumption of low- $a_w$  foods

Pathogen	Aerobic/anaerobic	Physiological features associated with heat resistance	Relevance to dry foods	Minimum $a_w$ for growth and toxin formation	Toxin formation or invasion of pathogen	Reference
<i>Bacillus cereus</i>	Facultative anaerobe	Spores: $D_{95^\circ\text{C}}$ 1.2–36 min; z-value 7.9–9.9°C	Spores can survive for very long periods	Growth and toxin formation: 0.92–0.93	Toxin formation or toxico-infection	Schraft and Griffiths, 2005
<i>Campylobacter</i> species	Microaerophilic	$D_{55^\circ\text{C}}$ 0.6–2.3 min; z-value 3.5–8°C	Does not survive in dry foods	0.98 (cells die rapidly at $a_w < 0.97$ )	Toxico-infection	ICMSF, 1996; Kusumaningrum et al., 2003; McClure and Blackburn, 2002
<i>Clostridium botulinum</i>	Anaerobe	Psychrotrophic spores: $D_{100^\circ\text{C}} < 0.1$ min; z-value 7–10°C Mesophilic spores: $D_{121^\circ\text{C}}$ 0.21 min; z-value 10°C	Spores survive in dusty and dry environments	Psychrotrophic: 0.97 Mesophilic: 0.93	Toxin formation	Silva and Gibbs, 2010
<i>Clostridium perfringens</i>	Anaerobic	Spores: $D_{95^\circ\text{C}}$ 17.6–63 min	Spores are capable of survival in dry environments	0.93 for growth	Toxico-infection: toxin produced during sporulation in GI tract	Labbe and Jejuna, 2006
<i>Cronobacter</i> species	Facultative anaerobe	$D_{60^\circ\text{C}}$ 2.5 min; z-value 5.82°C	Ability to survive in dry foods – up to 2 years in powdered infant formula	Survival at 0.2; minimum for growth not known	Pathogen invasion; possible toxin formation	Breeuwer et al., 2003; Edelson-Mammel et al., 2005; Gurtler and Beuchat, 2007
<i>Escherichia coli</i> O157:H7	Facultative anaerobe	$D_{63^\circ\text{C}}$ 0.5 min; z-value 6°C	Ability to survive in dry foods, e.g., dry fermented meats	0.95 for growth	Toxico-infection	ICMSF, 1996; Meng et al., 1994
<i>Listeria monocytogenes</i>	Facultative anaerobe	$D_{60^\circ\text{C}}$ 1.6–16.7 min in food substrates; 70°C for 2 min is the UK government approved heat treatment for elimination of <i>Listeria</i>	Ability to survive in dry foods ( $a_w$ 0.83), e.g., dry fermented meats, and peanut butter ( $a_w$ 0.33)	0.90–0.93 for growth	Pathogen invasion	ICMSF, 1996; Montville and Matthews, 2008; Kenney and Beuchat, 2004
<i>Salmonella</i>	Facultative anaerobe	$D_{60^\circ\text{C}}$ 0.1–10 min; z-value 4–5°C; heat resistance is greatly increased in low- $a_w$ and high-fat foods	Survives for weeks, months or years in low-moisture foods (up to $a_w$ 0.30)	0.94 for growth	Toxico-infection	Bell and Kyriakides, 2009b; ICMSF, 1996
<i>Staphylococcus aureus</i>	Facultative anaerobe	$D_{60^\circ\text{C}}$ 1–2.5 min in phosphate buffer; z-value 8–10°C	Can survive for months in dry foods	0.83–0.85 for growth (0.85 in most foods); 0.87 for toxin formation	Toxin formation	ICMSF, 1996

## 2.4 *Cronobacter* species

*Cronobacter* spp. (formerly *Enterobacter sakazakii*) are opportunistic pathogens for vulnerable neonates, with infants becoming infected following the consumption of contaminated reconstituted powdered infant formula, a food that is most often based on dried milk powder. *Cronobacter* has been detected in several dry food processing facilities, on food contact surfaces and in retail bakeries. There is evidence for strains persisting in some of these environments. Because *Cronobacter*, like other *Enterobacteriaceae*, can survive the spray-drying process (Arku *et al.*, 2008; Forsythe *et al.*, 2009), control in the production environment can be achieved through a combination of several measures including pasteurisation prior to concentration and spray-drying and control of the microbial ecology of the manufacturing facility (see Section 6 for further discussion). Guidelines for reconstituting, storing and handling powdered infant formula have been prepared by WHO/FAO (2007). See Forsythe *et al.* (2009) and Pagotto *et al.* (2007) for additional information on the behaviour of *Cronobacter*.

## 2.5 Verotoxigenic *Escherichia coli* (VTEC)

While verotoxigenic *Escherichia coli* (VTEC) strains have been shown to survive in moist environments on farms, survival is markedly reduced when the bacterium is exposed to dry conditions. Most cases of infection arise following the consumption of under-cooked beef products, or even following exposure to farmyard environments. Rather unusual is the finding that cookie dough has served as a source of the bacterium (ProMed Mail, 2009a). This food product was intended for baking but was eaten raw and 76 individuals were reported ill. It was suspected that flour contained the causative organism. Cases of human infections also have been associated with consumption of salami, semi-dried fermented sausage and cured meat products. In fermented dry sausage, VTEC can survive for at least 8 weeks (2-log reduction at 4°C), and Deng *et al.* (1998) reported that *E. coli* O157:H7 survived in several foods with low  $a_w$ . See Bell and Kyriakides (2009b) and Meng *et al.* (2007) for overviews of pathogenic *E. coli*.

## 2.6 *Salmonella*

Salmonellae are readily destroyed by heat pasteurisation of foods at high  $a_w$ . As the  $a_w$  is reduced by addition of solutes or by removal of water, heat resistance increases markedly. In foods such as chocolate, several seconds at 105°C may be required to reduce *Salmonella* counts by 1 log cfu/g. There is a high probability of infections at doses of  $> 10^5$  cells but in foods containing high levels of fat and/or protein, such as chocolate, salami and cheddar cheese, infection can result from ingesting as few as  $<10 - 100$  cells (Teunis *et al.*, 2010). There have been several large outbreaks of salmonellosis following the consumption of contaminated chocolate in Europe, Canada and the United States, with *Salmonella* being recovered from the incriminated chocolate many months after the outbreaks. However, outbreaks of salmonellosis are generally caused by inadequate control of cooking temperatures, cross-contamination after cooking, slow rates of cooling and poor refrigeration. Often implicated in outbreaks are improperly cleaned mass or domestic catering facilities and involve raw milk, poultry, meat or eggs but also fresh produce and dry foods as sources of the pathogen. See Bell and Kyriakides (2009a) and D'Aoust and Maurer (2007) for overviews of *Salmonella*. For reviews focused on control of *Salmonella* in low-moisture foods and their processing environments, see Chen *et al.* (2009a, 2009b), Podolak *et al.* (2010) and Scott *et al.* (2009).

## 2.7 *Staphylococcus aureus*

Staphylococcal intoxications are of minor importance compared with the number of cases and severity of illnesses linked to *Salmonella*, *Campylobacter* and VTEC. However, despite the low number of cases of foodborne staphylococcal intoxications, *S. aureus* is particularly relevant to dried foods due to its tolerance of low  $a_w$ . *Staphylococcus aureus* is salt tolerant and can grow aerobically at  $a_w$  0.83

but in most foods the minimum is  $a_w$  0.85 (anaerobically only at  $a_w \geq 0.90$ ), although toxin formation has been recorded only at  $a_w \geq 0.87$  (anaerobically only at  $a_w \geq 0.92$ ) (ICMSF, 1996). Salted and cured food products (defined as semi-dry), including ham, hard cheese and salami, and especially in foods where fermentation or drying (such as pasta) have been delayed or in 'natural fermentations' where starter cultures are not used, are at risk of staphylococcal growth and toxin production. *Staphylococcus aureus* cannot grow at temperatures  $<10^\circ\text{C}$ , so control is best effected by adequate refrigeration. See Adams (2009) and Seo and Bohach (2007) for overviews of *S. aureus*.

## 2.8 Enteric viral pathogens

Viruses are increasingly being recognised as important aetiological agents in outbreaks of foodborne illness in humans. Enteric viruses are a group of viruses that enter the body through the GI tract and are shed in faeces. These viruses are important because they can enter the food chain via the faecal-oral route. Three major groups of enteric viruses are recognised: viruses such as norovirus and rotavirus that are responsible for gastroenteritis; viruses such as hepatitis A virus (HAV) and hepatitis E virus that enter the body through the GI tract but replicate and cause disease in the liver; and viruses such as poliovirus, echovirus and coxsackievirus that replicate in the GI tract but cause illness only after they migrate to other organs. Norovirus and hepatitis A virus are the most commonly recognised viral agents linked to foodborne illness in humans.

Norovirus can spread by person-to-person contact, projectile vomiting or the faecal-oral route, thereby infecting persons sharing high-density living environments such as school classrooms, military bases and cruise ships. Infectious doses are very low, so foods are likely to be vehicles, but there are no documented cases of norovirus infections implicating dried foods.

In the case of HAV, two localised outbreaks of foodborne infection implicating semi-dried tomatoes involved some 200 people in Australia (ProMed mail, 2009b). The extended incubation period, up to 2 months, makes detection, diagnosis and identification of the original source of the virus difficult. Poor hygiene in production plants was the likely reason for contamination. Other outbreaks also believed to be associated with consumption of semi-dried tomatoes have occurred in The Netherlands and France (Pettrignani *et al.*, 2010). Enteric viruses, including hepatitis virus, poliovirus and coronavirus, are generally associated with fish, shellfish or animal products, and are often spread by cross-contamination from infected individuals. Several of these viruses have been shown to adhere strongly to food contact surfaces and to fresh produce. See D'Souza *et al.* (2007), Duizer and Koopmans (2009), ILSI (2002; 2009) and Mattison *et al.* (2009) for descriptions of enteric viral pathogens.

## 2.9 Mycotoxigenic moulds

Mycotoxins such as aflatoxins, ochratoxins and fumonisins have been detected in a range of dry products such as maize, rice, spices, coffee, cocoa, peanuts, tree nuts, seeds and dried fruits (Cousin *et al.*, 2005; Sinha and Bhatnager, 1998). Damaged, mould-infested peanuts stored in humid environments, for example, pose a serious risk to consumers, with heat treatment not always effective in destroying aflatoxins. Carry over of mycotoxins from raw commodities to dry products and fermented beverages is a public health concern.

## **2.10 Other pathogens not yet associated with dry foods as vehicles of foodborne disease**

### **2.10.1 *Listeria monocytogenes***

*Listeria monocytogenes* is capable of growing in substrates containing up to 10% (w/v) salt and at refrigeration temperatures. The pathogen has been detected in several types of food, including dried, smoked sausages (salami, chorizo, salpicão, alheiras), cold-smoked fish and cheeses, particularly soft cheeses with a high pH and artisanal cheeses made without starter cultures. *Listeria monocytogenes* is reported to survive well for at least 24 weeks at 20°C in peanut butter and chocolate-peanut butter spread at  $a_w$  0.33 and 0.65 (Kenney and Beuchat, 2004). Keto-Timonen *et al.* (2007) identified persistent strains of *L. monocytogenes* in food processing plants that are probable sources of contamination for food products. The main sources and vehicles of entry into food processing facilities are the raw materials. See Bell and Kyriakides (2009c) and Swaminathan *et al.* (2007) for overviews of *L. monocytogenes*.

### **2.10.2 Protozoan parasites**

Oocysts of several protozoan pathogens survive well in moist environments and in water, some requiring maturation in the environment before becoming infective for primary or intermediate hosts. Cysts of some free-living amoebae (including *Acanthamoeba* and *Naegleria* species) have been detected in deserts and are therefore capable of surviving in dry environments. Other protozoa, such as *Cryptosporidium parvum*, are killed by drying at moderate temperatures. Transmission between humans has been traced to handling foods. The oocytes are highly resistant to chlorine, although ozonation and boiling water are effective control measures. The infective dose for many protozoan pathogens is low. See Smith and Evans (2009) and Ortega (2007) for information on infective doses and overviews.

### 3. SOURCES AND ROUTES OF ENTRY INTO PRODUCTS

**F**ood producers and manufacturers of low- $a_w$  foods and food ingredients need to consider the ways consumers will use their products when assessing safety risks.

Should the product be considered Ready-to-Eat (RTE), i.e., not to be cooked before eating? The definition of RTE is given in European Commission Regulation 2073/2005 as 'food intended by the producer or manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level microorganisms of concern.' However, producers must also consider what the consumer will actually do with the product and not just what they assume consumers will do. There are products on the market that producers intend to be 'cooked' by the consumer before consumption, when in fact consumers may eat some or all of the product without cooking it. When food producers design a cooking process to be used by the consumer, they must consider that pathogens in dry environments often have a higher heat resistance than when in 'wet' environments; therefore, the cooking method, that the producer intends the consumer to use, may need consideration and validation to ensure it will inactivate the numbers and types of pathogens that may be found within the product.

#### 3.1 Raw materials and ingredients

Raw materials and ingredients may comprise a very wide range of items from primary agricultural products coming directly from the field to highly processed materials. It is therefore important that producers assess the risk that ingredients may contain pathogens. Once that risk is known, actions should be taken to control it during production of the final product. (For further discussion, see Section 6.)

Assessment of risks can be achieved by asking a series of questions:

- 1) Will the ingredient be put into a product that will receive an antimicrobial process before it leaves the production environment? Has that process been validated? This step on its own should produce a safe product.
- 2) Will the ingredient be used in a way that it will not receive an antimicrobial process before it leaves the production environment? In this case, points (3) or (5) or both should be used to ensure a safe product.
- 3) Has the ingredient been processed in a manner that is effective to eliminate pathogens before it is used by the food producer? Has the process been validated to show this? This type of ingredient could be used in products noted in points (1) or (2).
- 4) Is the process used by the producer sufficient to reduce the pathogen risk in the end-product to an acceptable level and has it been validated? This is used in products noted in point (1).
- 5) Is the instruction given to the consumer on cooking before eating sufficient to reduce the pathogen risk to an acceptable level, and is the consumer likely to follow exactly that cooking process? Have the instructions to consumers been validated for effectiveness at reducing pathogens? This point carries the most risk as there is little control over what the consumer actually does with the product. Resorting to this point should only be done after very careful consideration and assessment of risk.

### 3.2 Air

Within a production environment, air may make contact with the product on many occasions and this will introduce a risk that any microorganisms present in the air may enter the product. Factors potentially influencing the risk of air introducing pathogens into the product should be considered:

- 1) Is the production floor effectively a closed area and are there any access points directly to an external environment, e.g., doors, windows, fans, skylights, duct-ways and drains? Access to external environments introduces a risk that microorganisms from external sources may have easy access to the production area. These sources may also facilitate access to pests (insects, birds, rodents, etc.) that could further increase the risk of product contamination.
- 2) Is air used to convey product or material within the production area? Is the air filtered in any way and where does it originate? Is the level of filtration effective for removing foreign bodies or microorganisms?
- 3) Is any part of production under positive pressure? How is this managed and where is the source of the air used to maintain positive pressure? Is the incoming air filtered and is the level of filtration effective for removing foreign bodies or microorganisms? If the factory operates a zoning system (see Section 6.2.2.1), it should be ensured that air always flows from the highest hygiene zone to the lowest, with no chance of backflows that could introduce contamination into areas of highest cleanliness. The high hygiene zone must therefore always be under the highest positive pressure.
- 4) Are there any negative pressures within the production area? These will tend to pull air into the area and can introduce contamination. Check for negative pressure areas and, unless specifically required for any specific reason, try to eliminate them or ensure that the incoming air does not introduce significant numbers of microorganisms.
- 5) Has suitable consideration been given to protecting open product from airborne contamination, e.g., covers over exposed parts of a production line? Use of covers can provide a degree of protection from airborne contamination and additionally from airborne foreign bodies dropping onto/into product. However, covers can also inhibit good cleaning by making access to product contact areas difficult.

For further discussion of the importance of control of air quality and flow in dry food processing environments, see Section 6.2.2.1.

### 3.3 Water

Water may be found in many parts of a food production plant and can be used in many ways. In plants producing low- $a_w$  products, it is usual to try to reduce considerably, or eliminate, water usage in many areas. In production areas manufacturing dry materials, much of the microbiological control is centred on keeping these areas as dry as possible and thus preventing microbial growth. Allowing water to access such areas creates a potential for microorganisms that reside in a dormant state to begin to grow. Growth can be rapid and high numbers can be reached if the area is warm. This can provide a source of contamination of the final product. Points to consider:

- Unless required for a specific use within production, limit the presence of water to an absolute minimum.
- If it is used, water should, as far as is possible, be contained within a specific area, e.g., hand washing should be well segregated from the processing area and waste water should be drained well away from production. Washing of production tools and utensils should be in well-segregated areas with good drainage. Drainage water should not enter production areas and the tools should be dried before being taken back into these areas.
- Non-potable water should never enter production areas. Any indication that non-potable water has entered production should result in a full risk assessment of the implications of this issue. Non-potable water will include water draining from washing systems, leaking rain water, etc.
- Water used in 'contained systems' that enter production (e.g., completely enclosed cooling systems) should be assessed for risk and the potential for leakage should be considered. Such systems are ideally operated with potable water.

### **3.4 Contact material**

Food contact surfaces should be pathogen free. Such surfaces include pipe-work, hoppers, conveying systems, elevators, dispensing systems and tools such as scrapers. There should be a regular cleaning schedule for such equipment. There can be issues with the use of 'wet' cleaning in areas handling dried materials; indeed, wet cleaning may introduce or increase risks of contamination, so dry cleaning methods should be used. Tools and items that can be removed from production areas may be wet cleaned as long as they are fully dried before they are brought back into dry production areas. Aside from concerns about cross-contamination of foods, dry or wet, with pathogens upon contact with surfaces, migration of chemicals, e.g., plasticisers and printing inks, is also a safety concern.

### **3.5 Personnel**

Food production operatives (personnel) can be a source of incoming pathogens into food production areas. Producers of low-moisture foods and food ingredients should control the entry of staff into production areas and suitable procedures to reduce the risk of product contamination should be adopted. Points to consider:

- Clothing and footwear worn in production areas should be designed to protect the product from contamination by the staff. If personal protective equipment is required, this too should not be a potential source of product contamination. Clothing should be regularly laundered before being worn by staff entering production areas. Consideration should be given to having footwear that is only worn within production areas and is regularly cleaned.
- Staff entering production must always wash their hands using warm water and soap or alcohol based disinfectants.
- Health screening of staff for potentially pathogenic microorganisms must be considered.
- A notification system to enable staff to report suspect foodborne illness and visits to other countries must be considered.
- A policy to prevent members of staff, who have contracted foodborne illness, from entering food production areas until the infection is passed, should be in place.

### **3.6 Pests**

Pests such as insects, birds and rodents introduce a risk since they are likely carriers of pathogens that could contaminate food products. The ingress of pests of any sort into a food warehouse, dry storage and production areas introduces a risk that pathogens will also enter and contaminate finished products. The only way to minimise this risk is for producers to operate a full pest control programme. This may involve placing screens over openings into production areas and the positioning of baits and traps around the production site. Such pest management programmes are often operated by specialised pest elimination companies. Baits and traps should be regularly checked to ensure pest problems are not beginning to occur (an increase in the number of pests caught would indicate this). Traps should be regularly emptied to prevent the risk of build up of contamination in these areas.



## 4. PERSISTENCE IN DRY ENVIRONMENTS

**W**ater is an important factor that contributes to microbial deterioration of foods and to the persistence of microorganisms in manufacturing environments. Water activity, i.e., the ratio of vapour pressure of water in a food to that in pure water, is a numerical value that provides an indication of the extent by which microbial growth can be limited or prevented, and values differ depending on the type of microorganism under consideration. Gram-negative bacteria have the highest minimum  $a_w$  for growth, being  $> 0.93$  in the case of *Enterobacteriaceae*, with even higher values required for pseudomonads.

Pathogenic and spoilage microorganisms require nutrients, time and a suitable  $a_w$ , pH and temperature in order to grow. Little can be done about the availability of nutrients and time, given the large number of environmental niches in food processing facilities; therefore, methods for controlling microbial growth usually focus on  $a_w$  and temperature. During the production of dried foods, the control of moisture, and consequently the  $a_w$ , is key to controlling microbial growth. Dry cleaning, including the use of vacuum cleaners with integrated High-Efficiency Particulate Air (HEPA) filters, is generally regarded as a useful approach.

### 4.1 Survival and persistence in dry foods and dry food processing plants

Microorganisms cannot grow in the absence of water. Nonetheless, vegetative cells of certain genera of bacteria can survive for long periods in low-moisture foods and ingredients. Examples include the survival of *Salmonella* in chocolate, egg powder, nuts and nut butters and animal feeds, and *Cronobacter* in milk powder and powdered infant formula milk. Additional examples of survival of foodborne bacterial pathogens that have been associated with low-moisture food matrices, and their corresponding survival characteristics, are given in Table 2. Some of these pathogens can also be isolated from environmental samples taken from the same dry processing plants (Kornacki, 2006). This feature suggests that transmission could have occurred from the environment to the food matrix. Some pathogens have persistent strains with unique DNA fingerprint profiles when analysed by molecular sub-typing methods such as Pulsed-Field Gel Electrophoresis (PFGE). As an example, a study by Morita *et al.* (2006), investigating the survival of *Salmonella* in an oil-meal plant, recovered 15 strains of *Salmonella* Anatum, 14 of which had the same DNA fingerprint. Environmental samples from processing floors, process conveyors, dust in the air and rodents in the processing plant were analysed for the presence of *Salmonella* over a 5-month period. Four serotypes of the pathogen were common to three distinct areas of the plant (receiving, manufacturing and storage). The rate of detection in rodents was 46.4%. Shoes and gloves of workers in the manufacturing area had prevalence rates of 100 and 90%, respectively. PFGE analysis showed that three serotypes isolated from the processing floor, work shoes, brooms, rodents and dust were of the same origin, suggesting cross-contamination and persistence in the manufacturing area.

Stocki *et al.* (2007) studied colonisation of *Salmonella* on egg conveyor belts to determine if the red dry and rough (rdar) morphotype, a conserved phenotype associated with aggregation and long-term survival, contributed to persistence. Higher numbers of *Salmonella* remained on a hemp-plastic belt than on a vinyl belt after washing and disinfection. The rdar morphotype was involved in colonizing belts but was not essential for persistence.

Table 2. Survival of foodborne pathogens in dry foods

Pathogen	Food	Survival	Reference
<i>Cronobacter</i> species (formerly <i>Enterobacter sakazakii</i> )	Dried liquid infant formula	Inoculated with six capsulated and four non-capsulated strains, dried at 20 – 25°C, stored for up to 30 months at 20 – 25°C; encapsulated strains more resistant to drying and during storage; 2 strains (encapsulated) were recovered after 30 months.	Caubilla-Barron and Forsythe, 2007
	Infant cereal	Survival in infant cereals (rice, barley, oatmeal, and mixed grain; $a_w$ 0.30 – 0.83) for 24 wk at 4, 21, and 30°C; increases in $a_w$ or temperature accelerated the rate of death; survival was not affected by cereal composition	Lin and Beuchat, 2007
	Powdered infant formula	Survived for 687 days at 20 – 22°C ( $a_w$ 0.14 – 0.27; initial population, ca. 6 log cfu/g); 2.4-log reduction in 5 months, 1.0 log reduction during subsequent 19 months.	Edelson-Mammel <i>et al.</i> , 2005
		Survived in four milk-based and two soy-based formulas held at 4, 21, and 30°C for 12 months; reductions were greater at $a_w$ 0.43 – 0.50 than at 0.25 – 0.30; rate of inactivation was not markedly affected by composition of formula.	Gurtler and Beuchat, 2007
		Reduction of 1 – 2 log cfu/g during storage for 90 days at 30°C.	Dancer <i>et al.</i> , 2009
Skim milk powder	Survived spray drying (inlet, 160°C; outlet 90°C); recovered from dried milk (initially, 1.57 – 2.05 log cfu/g) stored for 12 wk at 18 – 20°C.	Arku <i>et al.</i> , 2008	
<i>Escherichia coli</i> O157:H7	Alfalfa seeds (for sprout production)	Survived in seeds (5.1 – 6.2% moisture; initial population, 3.04 log cfu/g) stored at 5°C for at least 54 wk, and 25 and 37°C for 38 wk but not 54 wk.	Taormina and Beuchat, 1999
	Apple powder, buttermilk powder, Cheddar cheese, seasoning, powdered chicken, sour cream powder	Survived in products ( $a_w$ 0.16 – 0.37, pH 4.07 – 6.49) stored for 19 wk at 5, 21, and 37°C; inactivation was enhanced by low pH and by increase in storage temperature.	Deng <i>et al.</i> , 1998
	Beef jerky	Beef was treated with acidic marinades, dried at 60°C for 10 h to $a_w$ 0.55 – 0.66, and stored for 60 days at 25°C; survival depended on acid adaptation of cells and marinade treatment	Calicioglu <i>et al.</i> , 2002
	Beef powder	Rate of inactivation during storage for 8 wk was enhanced at $a_w$ 0.28 – 0.41 compared to $a_w$ 0.68, at 25°C compared to 5°C, and in powder containing 20% NaCl compared to 0.5 or 3% NaCl; acid adaptation or shock did not affect retention of viability	Ryu <i>et al.</i> , 1999
	Infant cereal	Death was enhanced at reduced pH (4.0 vs. 8.0) and $a_w$ (0.35 vs. 0.73) and as storage time (up to 24 wk) and temperature (5, 25, 35, and 45°C) increased	Deng <i>et al.</i> , 1998
	Sausage (dry)	Reduction of 1 log cfu/g during drying fermented sausage for 18 – 21 days at 12.8°C; additional reduction of 1 log cfu/g during subsequent storage for 8 wk at 4°C	Glass <i>et al.</i> , 1992

Salmonella	Almonds	Kernels containing 7.1 – 8.0 log cfu/g were stored for 171 and 550 days at -20, 4, 23, and 35°C; no significant reductions in number after 550 days at -20 and 4°C; reductions of 0.18 and 0.30 log cfu/month on kernels held at 23°C for 171 and 550 days, respectively	Uesugi <i>et al.</i> , 2006
	Beef jerky	Beef was treated with acidic marinades, then dried for 10 h to $a_w$ 0.55 – 0.71; exposure to marinades resulted in reduced tolerance to drying and subsequent storage for 60 days at 25°C	Calicioglu <i>et al.</i> , 2003
	Cake mix, skim milk powder, onion soup mix, gelatin-based dessert	Inactivation at 25°C for 25 days was minimal at $a_w$ 0.00 – 0.22; survival decreased with increased $a_w$ up to 0.53 (4 – 5 log cfu/g reduction in 25 days) and pH (cake mix 6.8 vS. dessert, 3.1); at $a_w$ of foods as purchased, 2 log cfu/g reduction in cake mix ( $a_w$ 0.32), skim milk ( $a_w$ 0.22), onion soup mix ( $a_w$ 0.14) and dessert ( $a_w$ 0.42) within 10, 9, >27, and 2 wk, respectively	Christian and Stewart, 1973
	Chocolate	Survived 19 months; MPN values from composite samples were 4.3 – 24 cells/100 g (initial number not known)	Hockin <i>et al.</i> , 1989
		Initially at 100 cfu/g, decreased in milk chocolate to 14 MPN/100 g after storage for 15 months at room temperature	Barrile and Cone, 1970
		Survived in milk chocolate and bitter chocolate for 15 – 18 months at room temperature	Rieshel and Schenkel, 1971
		Survival in milk chocolate and bitter chocolate for 6 months at room temperature	Tamminga <i>et al.</i> , 1975
		Initially at 5.2 log MPN/100 g, decreased in milk chocolate ( $a_w$ 0.38) to 0.89 – 1.11 log MPN/100 g in 9 months	Tamminga <i>et al.</i> , 1976
	Dried milk products	Survived in naturally contaminated products for 10 months	Ray <i>et al.</i> , 1971
	Egg powder	Reduction of 1.6 – 2.8 log cfu/g in 8 wk at 13°C ( $a_w$ 0.29 – 0.37); rate of inactivation more rapid at 37°C than at 13°C and was influenced by the type of powder	Jung and Beuchat, 1999
Halva	Initial population of 3.87 log cfu/g ( $a_w$ 0.18) decreased to 2.20 – 2.76 log cfu/g at 6°C and 2.15 – 2.70 log cfu/g at 18 – 20°C after storage for 8 months; survival was better in vacuum-packaged halva than in air-sealed halva	Kotzekidou, 1998	
Paprika powder	Multiple serotypes survived for more than 8 months	Lehmacher <i>et al.</i> , 1995	
Pasta	Initial populations of 430 – 930 and 1.5 – 24 cells/100 g (MPN - Most Probable Number) of pasta (12% moisture) decreased to 0.4 – 23 and <0.3 – 1.5 cells/100 g, respectively, during storage at room temperature for 360 days	Rayman <i>et al.</i> , 1979	

	Peanut butter, peanut spread	Order of retention of viability in products ( $a_w$ 0.20 – 0.33) stored for 24 wk at 5 and 21°C was peanut butter spreads > traditional (regular) and reduced-sugar, low-sodium peanut butters > natural peanut butter; 6 of 7 products initially containing 1.51 log cfu/g were positive after 24 wk at 5°C; at 21°C, 6 of 7 products initially containing 5.68 cfu/g were positive after 24 wk at 5°C; 6 of 7 products at 21°C were positive	Burnett <i>et al.</i> , 2000
	Pecans	In-shell pecans (5.8 log cfu/g) and nutmeats (6.2 log cfu/g) were stored for up to 78 and 52 wk, respectively, at -20, 4, 21, and 37°C; no significant reduction on in-shell pecans and slight reduction on nutmeats at -20 and 4°C; 2.5 – 3.3 log cfu/g reduction on in-shell nuts and nutmeats stored at 21 and 37°C	Beuchat and Mann, 2010
	Potato slices, carrot slices	Potato and carrot slices were dried at 60°C for 6 h; carrots were then heated at 80°C; reductions of 0.81 log cfu/g of potatoes and 1.7 – 2.6 log cfu/g of carrots during storage for 30 days at 25°C	DiPersio <i>et al.</i> , 2005a, 2005b
	Skim milk powder, cocoa powder	Rates of inactivation at $a_w$ 0.43, 0.52, and 0.75 at 25°C for 14 wk were serotype dependent; survival was markedly greater in milk powder than in cocoa powder and at $a_w$ 0.43 and 0.52 compared to $a_w$ 0.75	Juven <i>et al.</i> , 1984
<i>Bacillus cereus</i>	Infant cereal	Survival of vegetative cells in infant rice cereal stored at 5, 25, 35, and 45°C for 36 wk was not affected by $a_w$ (0.27 – 0.78) or pH (5.6 and 6.7); death of spores at 45°C for up to 48 wk was enhanced at $a_w$ 0.78 but unaffected by pH; loss of viability at 5, 25, and 35°C was unaffected by $a_w$	Jaquette and Beuchat, 1998
<i>Listeria monocytogenes</i>	Peanut butter, chocolate peanut butter spread	Initial population of 4.42 log cfu/g of peanut butter at $a_w$ 0.33 and 0.65 decreased to 0.62 log cfu/g in 24 wk and 0.48 log cfu/g in 8 wk, respectively, at 20°C; initial population of 3.37 log cfu/g of a chocolate and peanut butter spread at $a_w$ 0.33 and 0.65 decreased to 0.90 log cfu/g in 16 wk and 0.95 log cfu/g in 4 wk, respectively, at 20°C	Kenney and Beuchat, 2004
<i>Staphylococcus aureus</i>	Cake mix, skim milk powder, onion soup mix, gelatin-based dessert	Inactivation at 25°C for 27 days was minimal at $a_w$ 0.00 – 0.22 but increased as $a_w$ increased to 0.53; survival better in vacuum vs. air and in food with higher pH (cake mix, 6.8 vs. dessert, 3.1); at $a_w$ of food as purchased, 2 log cfu/g reduction in cake mix ( $a_w$ 0.32), skim milk ( $a_w$ 0.22), onion soup mix ( $a_w$ 0.14), and dessert ( $a_w$ 0.42) within 27, 18, > 27, and 1 wk, respectively	Christian and Stewart, 1973
	Pasta	Initial populations of approximately 7 and 8 log cfu/g of pasta and egg pasta, respectively, decreased to approximately 1 – 2 and 3 – 4 log cfu/g in 90 days at room temperature; counts decreased to <100 cells/g after storage for 180 days	Rayman <i>et al.</i> , 1979

In another study, Proudly *et al.* (2008) subtyped 200 *E. sakazakii* (*Cronobacter* spp.) isolates recovered from a powdered infant formula factory. The majority (70%) of isolates were clonally identical, demonstrating the persistence of a resident strain in the processing environment. Mullane *et al.* (2007) monitored powdered infant formula and its processing environment for *E. sakazakii* for a period of 1 year. The frequency of isolation in intermediate and final products was 2.5%, while frequencies up to 31% were found at specific locations in the processing environment. Nineteen PFGE types could be grouped into six clusters, each containing between 5 and 32 isolates. The majority of isolates were of environmental origin (72.5%) but no cluster was confined to a specific location. These findings suggest that the manufacturing environment serves as a key route for sporadic contamination of powdered infant formula with *Cronobacter*.

A total of 268 RTE foods from retail food shops were microbiologically screened for the presence of *Cronobacter* (Baumgartner *et al.*, 2009). Surveys have revealed the presence of *Cronobacter* in 7 of 25 (26.9%) samples of spices and dried herbs and 3 of 42 (7.1%) samples of confections. To determine if *Cronobacter* persisted in particular products or at specific production sites, follow-up samples of each food found to be positive were analysed. Isolates with identical PFGE profiles were recovered in five samples from two types of confectionery collected over an 11-month period from one bakery. It was concluded that this could be indicative of persistent contamination of the factory or retail premises. It is not known whether persistence in the baked foods was due to survival through the heating process or post-process contamination. These observations provide further evidence of the ubiquitous nature of *Cronobacter*. In contrast to some other members of the *Enterobacteriaceae*, *Cronobacter* has a greater capacity to survive in dry environments for long periods of time (Gurtler and Beuchat, 2007).

Vogel *et al.* (2010) reported a dominant DNA subtype of *L. monocytogenes* that persisted in a fish processing house for years, even during months when no production occurred and where the plant was cleaned and maintained in a dry condition. Examples of the presence and persistence of *Salmonella* and *Cronobacter* in dry food processing and preparation environments are provided in Table 3.

## 4.2 Persistence in biofilm

The survival of resistant and dominant strains of foodborne pathogens in dry processing environments relies on their ability to adapt to high osmotic potentials and dry conditions. Lehner *et al.* (2005) examined 56 strains of *Cronobacter* species for features important to persistence and survival. The ability of the pathogen to form biofilms with the production of cellulose as a component in the extracellular matrix, adherence to hydrophilic and hydrophobic surfaces and production of extracellular polysaccharides along with cell-to-cell signalling molecules are thought to be factors that enable *Cronobacter* species to adapt to physiologically stressful environments and facilitate their persistence.

Persistent strains within a biofilm and cells in the stationary phase of growth may use quorum sensing to modulate the collective activities of the bacterial population, thereby promoting enhanced resistance to adverse environments (e.g., cleaning and sanitising agents, dehydration) (Davis *et al.*, 1998; Huber *et al.*, 2001; Irie and Parsek, 2008; Lazazzera, 2000; Lehner *et al.*, 2005). Other factors which may increase bacterial resistance and cross-resistance to many chemical and physical stresses include activation of stress genes, exposure to non-lethal stresses, senescence and synthesis of stress-related proteins such as chaperones (Boor, 2006; Rodriguez-Romo and Yousef, 2005). Also, genetic islands of pathogenicity which can be present on bacterial chromosomes can control synthesis of chaperone proteins, so that exposure to sub-lethal stress or quorum sensing signals may enhance production of pathogenic traits, e.g., toxin production and invasion proteins (Maurer and Lee, 2005; Mihaljevic *et al.*, 2007).

Table 3. Presence and persistence of foodborne pathogens in dry food processing and preparation environments

Pathogen	Environment	Remarks	Reference
<i>Cronobacter</i> species (formerly <i>Enterobacter sakazakii</i> )	Dairy and dry blending facilities	Detected in 4 of 50 (8%) samples	Restaino <i>et al.</i> , 2006
	Hospital, milk, kitchen	Samples (256) from spoons, jars, bottles, blenders, sieves, and surfaces in infant formula preparation areas were analysed; found in residue from nursing bottle and in cleaning sponge	Palcich <i>et al.</i> , 2009
		Isolate from a blender noted to have a small crack at base; tested positive 5 months after being used to prepare formula	Bar-Oz <i>et al.</i> , 2001; Block <i>et al.</i> , 2002
	Powdered milk facility	Frequency of isolation in product was 2.5%, while frequencies up to 31% were found at specific locations in the processing environment, suggesting that the environment serves as a key route for sporadic contamination	Mullane <i>et al.</i> , 2007
		Genotyping of 200 isolates over 25 months showed 70% had same finger print, which indicates persistence; of the 156 isolates from the processing environment, most were from surfaces surrounding the dryer (floor, steps, walls, or cyclones), blenders, storage silo areas, silo vacuum, platform, and floors in packing areas and canning room	Proudy <i>et al.</i> , 2008
		Detected in 18 of 152 (12%) environmental samples (scrapings from dust, vacuum cleaner bags, spilled product near equipment) taken from three factories	Kandhai <i>et al.</i> , 2004a
	Retail confectionery shop	Isolates with identical PFGE profiles recovered from five samples of two types of confections collected over an 11-month period from one bakery; suggests persistent contamination of the factory or retail premises	Baumgartner <i>et al.</i> , 2009
Various dry food facilities	Detected in four milk powder factories (14 of 68 samples, 21%) and a chocolate factory (2 of 8, 25%), cereal factory (4 of 9, 44%), potato factory (4 of 15, 27%), and pasta factory (6 of 26, 23%)	Kandhai <i>et al.</i> , 2004b	
<i>Salmonella</i>	Egg conveyor belt	Higher numbers remained on hemp-plastic belt than on vinyl belt after washing and disinfection; rdar morphotype, a conserved physiology associated with aggregation and long-term survival, was involved in colonisation but not essential for persistence	Stocki <i>et al.</i> , 2007

While dry foods are generally regarded as low risk, enteric pathogens originating from dry foods, especially in foods containing dry dairy ingredients (Elson, 2006; Reynolds, 2007; Rowe *et al.*, 1987), have been implicated in a number of outbreaks. Usually these outbreaks result from failures that arose in preventive systems. In such a case the origins of contamination (ICMSF, 1998) may include the presence of water in the process, facilitating the multiplication of bacteria, or manufacturing zones that are difficult to maintain in a hygienic state such as in the case of a drying tower or poorly designed equipment, one or more of which may contribute to failure of the system.

### **4.3 Consideration of spores**

Foods of plant origin, particularly seeds that may be contaminated with dust, soil, insects or faeces, can be expected to contain spore-forming bacteria and moulds. Dried foods and dry food processing environments, upon exposure to low levels of moisture, can support the growth of moulds, while higher levels of moisture are needed to support the growth of bacteria and most yeasts. Bacterial spores show high resistance to dry environments by virtue of their very low internal water content. Thus, spores of *Bacillus* spp. are frequently found in rice, flour, spray-dried milk powders, infant formulae, soya flour, dried soups, potato powder, cocoa powder and spices. Clostridial spores are less frequent but can be present in these products.

## 5. OUTBREAKS, ALERTS AND RECALLS ASSOCIATED WITH DRY FOODS

### 5.1 Outbreaks associated with dry foods

A partial list of documented outbreaks of foodborne illnesses that have been traced back to consumption of dry foods and food ingredients is shown in Table 4. An analysis of the table gives some interesting insights into the problem. It is clear that a large majority of these outbreaks have been caused by *Salmonella* contamination. Of the 33 outbreaks listed, 27 were reported to be caused by *Salmonella*, four by *C. sakazakii*, one by *E. coli* O157:H7, and one by *S. aureus*. Outbreaks of salmonellosis have been caused by several different serotypes and while the more common Enteritidis and Typhimurium serotypes do appear, there have been a number of rarer serotypes involved, potentially indicating the wide geographical origin of these foods and food ingredients. Considering all food and ingredient types, powdered milk and dried food/formula were implicated in the highest number (10 of 33, 30.3%) of outbreaks; this could be because the (infant) consumer is more likely to become ill, or due to the nature of the product, which may be rehydrated and then stored before consumption, thus potentially allowing pathogens to grow, increasing the risk of causing illness. Nuts and seeds (and their products – peanut butter, savoury snacks, halva and tahini) caused eleven outbreaks, and chocolate caused four. Although herbs and spices do not feature greatly, it is interesting that some issues linked to other foods may have originated with these items used in their manufacture. For example, the outbreak attributed to potato crisps (chips) was caused by contaminated paprika used as a seasoning (Lehmacher *et al.*, 1995). Some issues may not originate from the indicated raw material. At least one of the peanut butter-associated outbreaks and one chocolate outbreak were probably caused by contamination of equipment in the production area, which then cross-contaminated products that were being manufactured, the dry nature of those products allowing the pathogen to survive for a long time.

Table 4. Partial list of outbreaks of foodborne pathogen infections associated with consumption of contaminated dry foods

Food	Year	Pathogen	Location	Number affected	Remarks	Reference
Almonds	2000	<i>S. Enteritidis</i> PT30	USA, Canada	168	Outbreak strain was detected in raw almonds collected from orchards and distribution, warehouse, retail, and home locations, and from processing equipment 6 - 7 months after being used. Frequent and prolonged recovery suggests diffuse and persistent contamination.	Isaacs <i>et al.</i> , 2005
	2003	<i>S. Enteritidis</i> PT9c	USA, Canada	29	Raw almonds from an opened package, one environmental sample collected at the manufacturer, and tree samples from two huller-shellers that supplied the manufacturer were positive for <i>Salmonella</i> .	CDC, 2004
	2005	<i>S. Enteritidis</i>	Sweden	15	Phage type of isolates from patients was the same as the phage type of isolates from the 2000 almond-associated outbreak.	Ledet Müller <i>et al.</i> , 2007



Cereal (toasted)	1998	S. Agona	USA	209	An opened box of cereal yielded a <i>S. Agona</i> isolate with a PFGE pattern indistinguishable from the predominant PFGE pattern among outbreak-associated clinical isolates. Cereal from unopened boxes was also positive for <i>S. Agona</i> .	CDC, 1998
Cereal (puffed)	2008	S. Agona	USA	28	Puffed rice and puffed wheat cereals implicated in the outbreak were manufactured at the same plant that manufactured toasted oat cereal implicated in a 1998 outbreak of <i>S. Agona</i> infections. <i>S. Agona</i> was isolated from the plant and from bags of puffed rice cereals.	CDC, 2008
Chocolate	1973	S. Eastbourne	Canada, USA	95	Levels of 2 - 9 salmonellae per chocolate bar were estimated. Deficiencies in plant operations coupled with inadequate quality control contributed to spread of <i>Salmonella</i> to different areas of the plant.	D'Aoust <i>et al.</i> , 1975
	1982	S. Napoli	UK	245	Chocolate bars produced on at least 11 days over a 6-week period contained <i>Salmonella</i> . Bars containing a low number of <i>Salmonella</i> caused illness at least 7 months after manufacture. <i>S. Napoli</i> was isolated from bars 12 months after manufacture.	Gill <i>et al.</i> , 1983
	1985	S. Nima	USA, Canada	33	Suspect chocolate contained 4.3 - 24 <i>S. Nima</i> per 100 g, suggesting that small numbers can cause clinical symptoms. Samples were positive for <i>S. Nima</i> at least 19 months after manufacture.	Hockin <i>et al.</i> , 1989
	1987	S. Typhimurium	Norway, Finland	361	Levels of $\leq 10$ <i>S. Typhimurium</i> per 100 g were detected in 91% of positive samples, suggesting that low numbers can cause infections. Other serotypes were isolated from dust collected from rooms in which cocoa beans were stored or rinsed.	Kapperud <i>et al.</i> , 1990
Coconut	1953	S. Typhi, S. Senftenberg, S. Potsdam, S. Orion	Australia	>50	Salmonellae were isolated from packets of desiccated coconut obtained from households and from unopened cartons at retail and wholesale.	Wilson and MacKenzie, 1955
	1999	S. Java PT Dundee	UK	18	Seventy-one percent (128 of 181 samples) of desiccated coconut obtained from retail packets and sacks in warehouses yielded the outbreak strain.	Ward <i>et al.</i> , 1999

Halva (helva)	2001	S. Typhimurium DT104	Australia, Germany, Norway, Sweden, UK	>70	S. Typhimurium DT104 was isolated from jars of halva (plain, pistachio, and chocolate flavours) and other sesame seed-based products.	Aavitsland <i>et al.</i> , 2001; Brockman, 2001; deJong <i>et al.</i> , 2001; Little, 2001; O'Grady <i>et al.</i> , 2001
Infant dried milk product	1985	S. Ealing	UK	76	Source traced to defective factory spray drier. S. Ealing was isolated from scrapings taken from a silo into which waste powder and dust (sweepings) were deposited.	Rowe <i>et al.</i> , 1987
Infant food/cereal	1995	S. Senftenberg	UK	5	Cleaning remains from milling machinery were implicated as sources of contamination. The HACCP system was evaluated and highlighted this hazard.	Rushdy <i>et al.</i> , 1998
Peanuts	2001	S. Stanley, S. Newport	Australia, Canada, UK	109	Asian-style, dry-flavoured or roasted peanuts in their shell were implicated as contaminated sources. Unopened packets were positive for S. Stanley, S. Newport, S. Lexington, S. Kottbus, and an unnamed serotype.	Kirk <i>et al.</i> , 2004
Peanut butter	1996	S. Mbandaka	Australia	54	S. Mbandaka was isolated from opened and unopened jars. Roasted peanuts were implicated as the source of contamination. Positive samples of peanut butter contained <3 - 4 cfu/g. Outbreak highlighted the need for an effective HACCP program throughout the production process.	Scheil <i>et al.</i> , 1998
	2006	S. Tennessee	USA	628	S. Tennessee was isolated from opened and unopened jars. Peanut butter manufactured over at least a 6-month period was positive for the pathogen.	CDC, 2007a
	2008	S. Typhimurium	USA, Canada	684	S. Typhimurium was isolated from opened and unopened jars. S. Tennessee with a PFGE pattern indistinguishable from the peanut butter-associated outbreak strain in 2006 - 2007 outbreak was isolated from an unopened jar.	CDC, 2009
Pepper (black)	1981	S. Oranienburg	Norway	126	Consumption of minced meat and/or minced fish products containing ground black pepper was associated with infection. S. Oranienburg was isolated from an unopened package of pepper. Counts of 10 - > 240/100 g were found in 12 samples positive for S. Oranienburg.	Gustavesen and Breen, 1984

Potato crisps (chips)	1993	S. Saintpaul, S. Rubislaw, S. Javiana	Germany	1,000	Paprika powder and chips seasoned with powder contained salmonellae. Analysis of paprika powder yielded 2.5 salmonellae/g; a second count 8 months later revealed 0.7 salmonellae/g. Snacks with as few as 0.04 salmonellae/g caused infection. The importance of HACCP, including production of paprika powder, was emphasised.	Lehmacher et al., 1995
Powdered infant formula	1986	<i>C. sakazakii</i>	Iceland	3	Four strains of <i>Enterobacter sakazakii</i> ( <i>Cronobacter</i> spp.) isolated from infected neonates were indistinguishable from 22 strains isolated from formula.	Biering et al., 1989
	1988	<i>C. sakazakii</i>	USA	4	Blender used to prepare formula was contaminated with <i>C. sakazakii</i> . Pathogen was found in the powdered milk formula.	Simmons et al., 1989
	1993	S. Tennessee	Canada, USA	3	In addition to powdered infant formula, other spray-dried products (medical food supplement, protein supplement, medical meal replacement, powdered milk, diet beverage, and weaning formula) manufactured at the same plant were recalled.	CDC, 1993
	1998	<i>C. sakazakii</i>	Belgium	12	<i>C. sakazakii</i> was isolated from the implicated prepared milk formula as well as from unopened cans of powdered milk formula. Recommendations for preparing and handling infant milk formula were made, with the goal of enhancing safety.	Van Acker et al., 2001
	2001	<i>C. sakazakii</i>	USA	11	<i>C. sakazakii</i> was isolated from unopened and opened cans of powdered infant formula. PFGE patterns of these isolates were indistinguishable from a clinical isolate.	CDC, 2002
	2008	S. Give	France	8	All cases were associated with consumption of a single brand of powdered infant milk formula.	Jourdan et al., 2008
Powdered milk	1973	S. Derby	Trinidad	3,000	Imported dried milk products were packaged in a single plant. Workers may have been vehicles of S. Derby.	Weissman et al., 1977
	2004	<i>S. aureus</i>	China	150	Old milk powder was suspected to have been repackaged. Powder contained <i>S. aureus</i> toxin.	Anonymous, 2004
Salami	1994	<i>E. coli</i> O157:H7	USA	4	Isolates from intact packages of dry fermented salami collected from the plant warehouse, at retail, and from patients had identical PFGE patterns. Estimated infectious dose was 2 - 45 cells.	Tilden et al., 1994

Snack (rice/corn)	2007	S. Wandsworth, S. Typhimurium	USA	75	S. Wandsworth and S. Typhimurium were isolated from sealed bags of a rice/corn vegetable-coated snack intended for children.	CDC, 2007b
Snack (savoury)	1994	S. Agona PT15	UK, USA, Israel	>2,200	Snacks were manufactured on at least seven separate dates over a 4-month period. Levels were estimated to be 2 - 45 cfu/25 g packet of peanut flavoured snack.	Killalea <i>et al.</i> , 1996; Shohat <i>et al.</i> , 1996
Tahini	2002	S. Montevideo	Australia, New Zealand	68	Three outbreaks occurred. Strains of S. Montevideo with closely related PFGE patterns were isolated from sesame-based products (tahini) imported from two countries. S. Tennessee and S. Orion were also isolated. Salmonellae were isolated from retail samples of tahini in the UK and Canada. Use of HACCP principles in the production of tahini was emphasised.	Unicomb <i>et al.</i> , 2005
Tea (aniseed)	2003	S. Agona	Germany	42	S. Agona was isolated from six brands of tea containing aniseed. Various serotypes were isolated from 61 (11%) of 575 tea and other products containing aniseed. S. Agona survived when exposure to hot water during tea-making.	Koch <i>et al.</i> , 2005

## 5.2 Pathogen Alerts for dry foods in the European Union

The Rapid Alert System for Foods and Feeds (RASFF), used by the EU Members States to alert each other to foods containing pathogens or undesired substances, issued 59 warnings concerning pathogens in low- $a_w$  foods during the period 2008 – August, 2011 (Table 5). Breakdown according to the country of origin yielded no consistent patterns (data not shown). The occurrence of pathogen-food combinations varied considerably with a notable peak of 10 warnings for *Salmonella* in nuts in 2009 but only one in 2010 and one in 2011. Another observation is that *Salmonella* was found twice in infant formula but *C. sakazakii* was found only once. *Salmonella* was clearly the pathogen most often detected, and sesame seeds, nuts and nut products, pepper, herbs and spices (45 out of 62, or 72.6%) were the low- $a_w$  foods and ingredients in which it was most often detected.

Table 5. European food alerts for foodborne pathogens or suspicion of foodborne pathogens in low- $a_w$  foods and ingredients to the Rapid Alert System for Foods and Feeds (RASFF)<sup>a</sup>

Pathogen	Dry food/ingredient	Year			
		2008	2009	2010	2011
<i>Salmonella</i>	Alfalfa seeds		1		
	Baby food		1		
	Buckwheat		1		
	Chocolate (imitation)	1			
	Dried sausage			1	2
	Fruits (exotic)	2	1		
	Grains (organic)		1		
	Herbs and spices	4	4	2	7
	Infant formula	2			
	Nuts (various)		10	1	1
	Pepper	2		2	
	Protein supplement		1	3	
	Sesame seeds	4	6	2	
<i>Cronobacter sakazakii</i>	Infant formula		1		
<i>E. coli</i> (verotoxigenic)	Tea (lime blossom)	2			
	Dried sausage				1
<i>E. coli</i>	Herb		1		
<i>Listeria monocytogenes</i>	Cake			1	
	Dried sausage				2
<i>Staphylococcus aureus</i>	Baked products				1

a) Data were taken from RASFF for the period from January 2008 until August 2011

### 5.3 Recalls and market withdrawal of dry foods containing pathogens in the United States

Table 6 shows a list of recalls and market withdrawals of low-moisture foods due to potential contamination with *Salmonella* that occurred in the United States during the period of 2004 to 2011 (30 June). These data clearly show a predominance of recalls resulting from *Salmonella* contamination of nuts and seeds. Of the 48 types of products noted in Table 6, 19 (39.6%) were nuts, seeds or products produced from these foods. Following these, nine spices and herbs and eight cereal/batter/soup mixes were among the types of products recalled. The latter category is a very heterogeneous mix and the root cause of contamination may be from any one of the ingredients. Another interesting point to note is the large increase in number of individual issues that have occurred in recent years, particularly 2009 and 2010. Whether this is indicative of a worsening safety problem in low-moisture foods or simply that more surveillance is taking place and issues are more likely to be recorded, is not known.

Table 6. Low- $a_w$  foods and food ingredients recalled or withdrawn from the market in the U.S. in 2004 – 2011 (30 June) because of possible contamination with *Salmonella*<sup>a</sup>

Year	Product
2004	Almonds (raw whole and diced) <sup>b</sup> , cheese (hard), mung beans, paprika, pepper (red), sesame seeds
2005	Cake batter dry mix, basil (ground), halva (pistachio), tahini (sesame seeds)
2006	Tea
2007	Chocolate (white), corn snack, pumpkin seed snack mix, tahini (sesame seeds)
2008	Cereal (puffed rice, puffed wheat), pancake mix, peanuts, peanut butter and peanut paste <sup>c</sup> , sesame seeds, waffle mix
2009	Chili (crushed), granola, hazel nuts, meat tenderiser, non-fat dry milk <sup>d</sup> , pecans, pepper (black, white, and red), pistachios <sup>e</sup> , popcorn seasoning, spices (numerous types), tahini (sesame seeds), wasabi powder
2010	Beef soup mix, beef stroganoff sauce mix, chocolate, egg noodles, garlic powder, granola bars, hydrolysed vegetable protein (HVP) <sup>f</sup> , nutmeg, pepper (black and red), pinenuts, pistachios, sesame seeds, soy grits, tortilla soup mix, walnuts, wonton skins
2011 <sup>g</sup>	Herbal tea, peanut butter, powdered protein products, snacks (wheat snack, potato chip, corn curritos), whey protein isolate

a) A detailed list of recalls, market withdrawals, and safety alerts issued by the U.S. Food and Drug Administration in 2004-2011 can be accessed at: <http://www.fda.gov/Safety/Recalls/ArchiveRecalls/default.htm>. Products containing these ingredients included (but were not limited to):

b) Chocolate cake, dietetic snack, fruit and nut mix, fudge, granola bars, mixed nuts, muesli cereal, toffee, and trail mix;

c) 3,980 recalled products, including cakes, cereals, cookies, crackers, ice cream, mixed nuts, snacks, and toppings;

d) Breakfast drink, cappuccino mix, cocoa mix, dietary supplement drink, frosting, gravy mix, nutritional drink, oatmeal, pound cake, and yogurt;

e) 664 recalled products, including cakes, candy, ice cream, mixed nuts, and snacks;

f) 177 recalled products, including bouillon, seasonings, snacks, dips, spreads, soup base, and potato chips.

g) Until 30 June.

## 6. POTENTIAL CONTROL MEASURES

**A** way to increase the safety of low-moisture foods is through proper control of the production conditions. The more sensitive the consumer is to the pathogen(s) that may be in the product, the more strict the end-product specifications should be and the more critical are the control measures during production to ensure that products meet the specifications. Since powdered infant formula has very strict end-product specifications, it is used here as the example to describe the most critical control measures. Control measures for other dry food products with less stringent end-product specifications can then be extrapolated from those discussed here. Section 3 contains additional discussion of sources and routes of entry of pathogens into dry food products and factors to consider for their control.

### 6.1 Initial reduction of contamination

The first microbiological safety control measure for most dry food products occurs before products have been dried. Critical to controlling pathogens in many agricultural commodities and dry foods and ingredients is ensuring that raw and processed materials are quickly and thoroughly dried. Pasteurisation in the wet stage to reduce contamination to acceptable microbial counts is also a critical control point in the process. Perhaps obvious, but not always considered in practice, is that pasteurisation conditions depend on the solids (dry matter) content of liquids. Cream containing 35% fat, for example, requires heating at 80°C for 15 s for pasteurisation, i.e., to reduce *Salmonella*, *Campylobacter* or *L. monocytogenes* by at least 5 log cfu/ml (CAC, 2004). This compares to heating milk with ca. 10% solids at 72°C for 15 s to achieve pasteurisation. The solids content of liquids being pasteurised should always be taken into consideration when defining pasteurisation conditions. Other physical characteristics may also result in the need for higher temperatures or longer treatment times to reach an equivalent reduction.

As long as the product is wet, reducing the number of microorganisms can be achieved by other procedures as well. High pressure is a promising treatment with high potential to inactivate vegetative pathogenic bacteria. When high temperature may adversely affect the sensory quality of a product, high pressure offers a potential alternative to pasteurisation. Irradiation has been considered as a treatment to reduce or eliminate pathogens in dry foods for many years. From a scientific point of view, irradiation is very effective but, from a practical point of view, its commercial application is restricted. In general, the use of irradiation to pasteurise or sterilise foods is not accepted by the consumer and, since the product's label has to show the treatment by law, its use as a commercial process is limited. Alternatively, spices and some other dry foods can be steam treated to achieve decontamination without significant loss of volatile components (Lilie *et al.*, 2007).

### 6.2 Prevention of recontamination

Assuming that low-moisture foods have been decontaminated in a previous processing stage, the main route of entry of pathogens into these foods is by recontamination. All control measures discussed here are aimed at preventing contamination of dry foods in wet as well as in dry stages of production and storage.

#### 6.2.1 Wet process line

To prevent contamination in the wet stage, the line should be designed and constructed as ultra-clean, like lines for UHT products. In that case sieve tubes, located in the line just before the homogeniser for the purpose of protecting it and often constructed in parallel to enable interim cleaning of one of the sieves, should not be cleaned in the wet processing environment but rather in

a special high hygiene room, properly separated from the wet basic hygiene environment. An even better approach would be to clean the sieve tubes in a special clean-in-place cycle, preventing contamination of the sieves during a run. All additions, defined as dry mix ingredients, even if added in the wet phase, should be free from pathogens.

Sometimes, lecithin or mixtures of vegetable and other oils containing long-chain polyunsaturated fatty acids are added to obtain the desired composition and physical properties of the product. Water may need to be added to reduce the content of the solids in the concentrated product, if the evaporation step results in a content that is too high to obtain the desired physical characteristics of the powder. This should be done by means of a hygienically designed delivery system.

## **6.2.2 Dry process line**

### **6.2.2.1 Zoning**

To prevent contamination in the dry stage, proper zoning is crucial. Zoning starts with defining wet and dry zones and subsequently within wet zones, defining basic and medium hygiene zones, and within dry zones, defining basic, medium or high hygiene zones. In the wet part of the processing environment of dry foods, high hygiene zones do not occur, which is contrary to the processing environment of cooked meat products, for example. In principle, all wet activities are carried out in wet zones, regardless whether they are before or after the critical heat treatment. Pasteurisation, evaporation and buffering of concentrated product in a tank are steps that take place in a wet zone. The lines are closed. If not, leakage will rapidly indicate that something is wrong and needs to be corrected. This is especially true for lines designed and constructed as ultra-clean lines. A distinction between a basic hygiene level for the wet part of the line, before the critical heat treatment and a medium hygiene zone for the wet part of the line after the critical heat treatment, might be considered but is not as crucial as separation of the high hygiene zone. Separation of the two hygiene zones in the wet part of the line can be by physical means such as a wall but is not as critical as the separation of high hygiene zones in the dry part.

Wet and dry zones should be physically separated by solid walls. This enables dry zones to be kept dry. Typical dry zones include the spray dryer building, dry mixing department, buildings with silos for storage of intermediate or finished product, packaging department and warehouse. Because the intermediate or finished product is, or can be, exposed to the environment and hence to a risk of contamination, the spray dryer building, dry mixing department, storage silos for dry mix ingredients, intermediate and finished product areas and packaging department for primary packaging are all high hygiene zones. The packaging department for secondary packaging and palletising and the warehouse are basic hygiene zones. The use of packaging materials offers a number of safety risks to food products and must be considered in any risk reduction programme. Primary packaging material is a product contact surface and, as such, should contain no pathogens. Packaging suppliers should be made aware of this and only appropriate food-grade packaging should be used. The mode of entry of primary product packaging materials may have the potential to introduce microbiological hazards into the area or more directly the product. Consideration should be given to areas that store boxes or packing materials that surround the product packaging area and to routes of entry via doors, hatches, etc. into different zones that could result in an increased risk of contamination of end-products.

If personnel or materials will pass from a basic hygiene zone into a high hygiene zone or vice versa, they should always pass through a medium hygiene zone between these zones. Connections between the high hygiene zone and the basic hygiene zone should always function as an air lock, since high hygiene zones should be kept under a positive pressure of at least F7 as defined by filtration performance standards (ECS, 2002).



Dry parts of lines for spray-dried powdered products are seldom tightly closed. It is frequently stated that a line is closed and that it is not therefore necessary to introduce special precautions to prevent contamination, but often powder leaks can be observed, demonstrating that the line is not tightly closed. Only if the spray-dried product, e.g., maltodextrin, is hygroscopic and introduction of any humidity would immediately result in blockage of the line, would the line be tightly closed. If powder can come out of the line, environmental air that may contain dust can easily enter the line, thereby increasing the risk of contamination. For this reason and because some companies may operate with open spray dryers, high hygiene zones are always kept under filtered positive-pressure air of at least F7. High hygiene zones should never open directly to basic hygiene zones or to the outside. Air filters should be constructed such that when they are changed, the filter cloths are removed at the lower hygiene side, preventing contamination of the higher hygiene zone with contaminated dust from the filter cloths.

For worker safety reasons, emergency doors must be present at several locations in various buildings. If emergency doors in the high hygiene zone open directly to the outside, they should be kept tightly closed and sealed to prevent use when not required. Emergency doors should never enable shortcuts; to prevent these shortcuts, emergency doors should be sealed and if possible electronically connected to the control room to alarm operators if used unintentionally. To prevent introduction of potentially contaminated dust and consequently an increased risk of contamination, material should be stripped in the medium hygiene zone before entering the high hygiene zone. For instance, rolls of foil for primary packaging of finished products should pass through a medium hygiene zone and should be stripped before entering the high hygiene primary packaging department. Dry-mix ingredients supplied in bags should be stripped; for this purpose, a special medium-hygiene stripping-zone is created. Dry-mix ingredients in large bags are preferred over small bags, which need more handling, thus introducing greater risk of contamination.

It is becoming common practice that personnel entering a high hygiene zone should change clothes and shoes. It has been shown that operators may introduce *Enterobacteriaceae* and other microorganisms with their clothes and shoes, especially when they operate on the wet evaporator side as well as on the drying side, which is usually the case if the process runs in line and is managed by the same personnel. Since dry processing is done under high hygiene conditions, entrance to the high hygiene zones should be limited as much as possible and controlled by the operators running the drying process. For this reason the control room should be the only entrance into the high hygiene processing area. In general, it is recommended not to have the control room in the high hygiene zone but rather in an area between wet processing and dry processing. It is recommended that operators running various process steps in the high hygiene zone (drying, dry mixing and primary packaging) have their own shoes instead of plastic shoe covers for high hygiene zones. To limit the ingress of microbial contaminants into high hygiene zones by operators working on both sides, a significant distance between basic wet zones and dry high hygiene zones is recommended, thereby enabling reduction of the amount of water on shoes and clothes of operators during transit between zones.

For maintenance purposes, engineers bring tools into the high hygiene zones but these tools are used throughout the production site; one day tools may be used in the high hygiene zone when they had been used the day before in the wastewater treatment plant. To prevent contaminated tools from contributing to the presence of pathogens in critical areas, creation of dedicated tool kits and trolleys for tools that remain within the dedicated high hygiene zone is recommended.

Dry zones should be kept dry. To help achieve this goal, hand-washing facilities should not be present in air locks between basic and high hygiene zones because they create an increase in humidity in the dry zone environment. Hand washing has already been done when entering the production zone, so additional washing is not necessary. Alternatively, dispensers with disinfecting gels (no sprays or liquids) can be placed in the air locks.

### 6.2.2.2 Cleaning and sanitising

Because dry zones should be kept dry, dry cleaning of spray dryers, dry mixers, storage silos, packaging machines and adjacent equipment is essential. Vacuum cleaners are the most common tools for dry cleaning. They can be used for dry cleaning the inside and outside of equipment and the environment. It is essential to dedicate vacuum cleaners and their tools, such as fittings, for different purposes, either for reasons of hygiene (inside of equipment versus outside) or for sampling reasons. Although not yet suitable for all situations, more and more tools have been developed for use in dry cleaning areas. Examples include silo cleaning systems adapted for spray dryers, 'sand' blasters that use lactose or calcium carbonate instead of sand and dry ice (CO<sub>2</sub>) cleaning (condensation is a risk and should be controlled).

If wet cleaning of certain parts of equipment, e.g., nozzles of the spray dryer and heads of the filling units in the filling machine, is necessary in the high hygiene zone, it should be done under controlled conditions in a special room within the dry high hygiene zone, properly separated from the dry zone.

Vacuum cleaners can be used to collect materials from specific areas and thus enable easy distinction between different types of samples, e.g., material from the inside of equipment is a line sample, material from outside of equipment is a critical (Priority 1) environmental sample and material from a floor is a less critical (Priority 2) environmental sample. To enable this distinction, vacuum cleaning should never be done using a central vacuum cleaner system, which results in combining material from all locations and does not allow the origin of a problem to be determined. Moreover, because a system of tubes will collect and maintain dust and cannot easily be cleaned internally, it is prone to harbour insects. Alternatively, a central vacuum system can be installed with distinct small cyclones at locations where vacuum cleaning is necessary. These cyclones enable separation of dust from the air and they are also equipped with filters. Consequently, the tube system will remain free of dust.

If dry cleaning equipment is not possible, but wet cleaning is necessary because of the need for elimination of allergens or because equipment cannot be dismantled for separate wet cleaning, it should be recognised that both the inside and outside of equipment as well as the immediate environment will become wet and that humidity is increased in the environment. For this reason, when wet cleaning is unavoidable, it should include all surfaces of the equipment and its environment. For instance, when wet cleaning a spray dryer, the surrounding floors, walls, ceilings, auxiliary equipment and everything else in the environment where powder and dust may have accumulated should also be cleaned. Otherwise, these remnants may become wet and may allow microorganisms, including pathogens, to grow. For the sake of completeness, it should be stressed that wet cleaning should be followed by sanitising.

After wet cleaning, not only the equipment but also the surrounding environment should be dried to restore the dry conditions before production starts. Usually the equipment is dried by forced hot air. The environment does not become warm, however, if the spray dryer is insulated. To promote drying of the environment in the spray dryer building, elimination of all insulation from the spray dryer is recommended. During spray drying it becomes too hot in the spray dryer building for people to work. However, personnel usually do not need to work in the building if the spray dryer is running. Occasionally they have to enter the building for inspection and sampling for short periods of time. The high temperature in the spray dryer when starting production also promotes rapid drying of the spray dryer environment.

An outbreak of salmonellosis associated with powdered infant formula has been linked with contaminated insulation on a spray dryer (Rowe *et al.*, 1987). Insulation collects dust and powder during production periods. During wet cleaning of the spray dryer, it is inevitable that insulation also becomes wet; consequently microorganisms, including pathogens, may start growing. In the outbreak, *Salmonella* was present in the insulation and was dispersed from time to time into the spray dryer and the product flow through a crack in the spray dryer, resulting in its presence in the finished product.

As dry zones should be kept dry, water does not need to be present, except for extinguishing fires. If spray dryers are dry cleaned, drains are not necessary. If dry cleaning is practiced, eliminate items such as water tubes, hoses and drains that are not necessary. If the spray dryer needs to be wet cleaned, make sure that stagnant water is not present in the environment during production. Drainage in the spray dryer building should simply be done via a tube from the floor to the one below. Drains with water locks should not be present in floors, because they will retain water. Only the very last connection to the sewage system should be properly sealed by means of a water lock. Often, because of poor maintenance, air-handling units equipped with steam give rise to leakage of steam condensate. Proper maintenance of all equipment in high hygiene zones is critical.

Because of the complexity of wet cleaning spray dryers and buildings in which they are housed, the environment should be designed to be as open as possible by putting auxiliary equipment outside the high hygiene zone. Ideally, the high hygiene zone in the spray dryer building should consist of a box containing only the spray dryer connected to all necessary equipment, with the auxiliaries located outside the box.

If, despite all precautions, water has entered the dry zone, it will usually be only in limited quantities. In this situation, water can be removed using paper towels, followed by sanitising. Sanitising in dry environments is usually done with water containing a high concentration of chlorine (ca. 800 µg/ml). The purpose of the high chlorine concentration is to increase the likelihood that sufficient active chlorine is available to kill microbial contaminants. If chlorine is inactivated by organic material, the moist environment may allow growth of microorganisms.

### **6.2.3 Dry mix ingredients**

Ingredients necessary to formulate powdered infant formula are not always combined in the wet phase and subjected to a heat treatment that would result in significant reduction of the pathogens potentially present. The reason for this is often the heat sensitivity of the ingredient (e.g., fat mixes containing polyunsaturated fatty acids). Other reasons may be technological (e.g., mixes of minerals containing copper) or ingredients used to enhance capacity (e.g., lactose). Ingredients such as fat mixes are added during the wet phase just after the critical heat treatment whereas others are added in the dry phase by dry mixing with a base powder.

Dry mix ingredients are added and mixed without any further heat treatment that might result in a significant reduction of pathogens potentially present. Consequently, to ensure that products meet the very strict end-product specifications, the base powder and dry mix ingredients should be produced under strictly controlled conditions.

For statistical, practical and economic reasons, testing of lots of dry mix ingredients for product specifications does not sufficiently guarantee that every unit of the lot will meet the required standards. The only way to meet specifications is to control the production conditions. Certificates of conformity or certificates of analysis do not guarantee that powdered infant formula or any other low-moisture food or ingredient meets these standards. Suppliers will guarantee by a certificate of analysis that products are in accordance with the agreed specifications; otherwise they would not have shipped the lot. The only proper way of ensuring that dry mix ingredients will meet the very strict specifications of the finished products is to control the production conditions.

As a producer of powdered infant formula or other dry food products and having responsibility for safety, it is not sufficient to have the guarantee of the supplier that ingredients meet their specifications. It is necessary to assure by regular audits that production conditions are under control and that consistency of the quality system of the supplier will ensure that this will always be the case. Careful selection, auditing and approval of suppliers of dry mix ingredients are critical.

## 7. VERIFICATION

In the context of food-safety risk-management, there are several definitions of the term verification (CAC, 2008a; ISO, 2005; ILSI Europe, 2004), but they all refer to activities that aim to obtain evidence that control measures have been correctly implemented and that the resulting product meets pre-defined safety criteria.

Statistics show that testing for end-product specifications of dry food products does not give sufficient guarantee that they will be safe for the consumer. Even the strictest specifications for testing, e.g., the one used for powdered infant formulae (absence of *Salmonella* in 60 samples of 25 g), have a certain percentage chance of accepting contaminated lots (e.g., 30% in case of a contamination level of 2% of the units). Less frequent sampling will only increase the chance of accepting contaminated lots. The frequency of sampling that is carried out routinely for practical and economic reasons is relatively limited in this respect. A major way to increase the level of guarantee is by proper control of the production conditions (see Section 6).

Correct implementation of control measures can be verified at the production plant level by regular inspection of control charts, reviewing records to identify potential trends and checking equipment for functionality and calibrations, as well as by internal and external plant audits. Verification of the safety of finished products is accomplished by microbiological analysis, in combination with monitoring consumer complaints and outbreaks of foodborne illnesses. For dry foods and dry-food processing environments, there are some particular constraints that should be taken into account in order to establish an efficient verification approach.

### 7.1 Microbial distribution in dry foods

Although heterogeneous distribution of pathogens is of concern for many types of foods (Habraken *et al.*, 1986; ILSI Europe, 2010; Reij *et al.*, 2009), it is exacerbated in dry foods, rendering testing of finished products an even less reliable tool to verify safety, since it will only serve to identify highly contaminated lots. Thorough mixing of samples before taking an aliquot for analysis or the application of continuous autosamplers will minimise but not eliminate the non-homogeneous distribution of pathogens (CAC, 2008a). To enhance the probability of identifying lots with low contamination levels, testing more samples and increasing the sample size should be considered. However, this requires very sensitive assay methods and the associated increased analytical cost may be disproportionate compared to the benefit. Therefore, rather than focusing only on finished products, it is better also to establish an environmental monitoring programme to test for indicator microorganisms.

### 7.2 Environmental monitoring

Verification of the effectiveness of zoning in dry food processing plants can be achieved by observations obtained by environmental monitoring. In order to make the programme more effective, critical sampling sites and the pathogen(s) of concern must be correctly identified. This requires detailed knowledge of the product and process, as well as detailed zoning in the factory, before establishing a meaningful sampling plan. Depending on the type of production, sampling can be focused on basic hygiene and medium hygiene zones, where pathogens would more likely be detected, and therefore enhance proactive surveillance. Sampling in the high hygiene zone confirms the relevance of the monitoring programme, but is not the area to focus the investigation as long as the environmental pressure is limited to less critical areas. In the production of powdered infant formula, where proper zoning and other preventive measures should already have been implemented, according to CAC (2008b), "monitoring activities should be focused in areas where contamination is likely to occur, i.e., in the dry processing areas located in the high hygiene zones.

Particular attention should be given to the interfaces between these areas and the external areas of a lower hygiene level as well as areas close to the processing line and to equipment where contamination is more likely to occur, e.g., due to the design of equipment, presence of openings such as hatches that may be opened occasionally for inspection. Known or likely harbourage sites should be given priority for monitoring." If a pathogen is found in multiple locations, molecular typing methods are recommended to determine the level of relatedness. This information may be crucial to elucidate the contamination route or routes. Several studies have described how this approach was successfully applied to trace *Cronobacter* in powdered infant formula plants (Craven *et al.*, 2010; Lehner *et al.*, 2010; Mullane *et al.*, 2007; 2008).

### 7.3 Utilisation of indicator microorganisms

Instead of analysing samples for the presence of pathogens, it may be preferable to focus on detecting indicators of the potential presence of pathogens, because this does not require specialised biosafety laboratories and corresponding methods are usually more robust and less expensive. However, it is not always possible to find suitable indicators for pathogens that are relevant for dry foods. For example, it has been reported that testing for the *Enterobacteriaceae* is not reliable to assess whether infant formulae are likely to be free of *Salmonella* (EFSA, 2007), unless a sampling plan with the same stringency is applied and only if the method for detecting *Enterobacteriaceae* is equally or more sensitive than the method for detecting *Salmonella*. However, applying a sampling plan with the same stringency for *Enterobacteriaceae* as for *Salmonella* may not be a realistic option, because this would lead to the unjustified rejection of lots due to the presence of innocuous members of the *Enterobacteriaceae*. In addition, results of monitoring *Enterobacteriaceae* in wet and basic hygiene zones may not be properly interpreted (Cox *et al.*, 1988). Nevertheless, using *Enterobacteriaceae* as an indicator of good hygienic practices can be of value for dry food processing operations, including infant formula manufacturing.

### 7.4 Raw materials

Some dry mixing operations are not followed by a process that is aimed at reducing the presence of pathogens, which implies that the microbiological safety of such products depends directly on the quality of the ingredients. This is often reflected in very strict microbiological specifications for such ingredients. However, microbiological analysis of ingredients to verify their compliance with specifications suffers from the same drawback as testing of finished products: it only serves to detect lots with relatively high levels of contamination. Therefore, it is recommended that resources be dedicated to supplier audits, in particular for ingredients that are considered to represent the highest safety risk.

### 7.5 Analytical methods

Analytical methods used for testing dry foods need to be selected carefully. As mentioned above, methods with very high sensitivities are required to provide meaningful results if the criteria are strict. Since the target microorganisms are likely to be stressed due to processing (e.g., drying and heating), special measures such as the inclusion of a soaking step in the protocol to avoid abrupt rehydration are necessary for recovery of such cells (van Schothorst *et al.*, 1979). It may also be necessary to sample larger quantities of product in order to detect the target pathogen. For example, in an outbreak of salmonellosis associated with powdered infant formula, *Salmonella* Ealing was determined to be present at a level of 2 cfu/450 g packet of product (PHLS, 1995). Recovery can also be enhanced by application of a resuscitation step using pre-enrichment growth media that do not contain selective agents before proceeding with selective enrichment. Due to the large variability of the lag phase of desiccation-stressed cells, an overnight incubation may not be long enough (Stephens *et al.*, 1997). Extending the incubation time, on the other hand, may lead to inactivation of the target microorganism, e.g., due to acidification of the growth medium.

Focusing verification activities on monitoring pathogens in the environment also represents an analytical challenge, because environmental samples often contain large numbers of competitors that can mask the presence of the target microorganism. Nevertheless, standard methods such as ISO 6579 (*Salmonella*) and ISO/TS 22964 (*Cronobacter*) for detection of pathogens specifically cover environmental samples. Likewise, the ISO procedure for validation of alternative methods (ISO 16140) includes evaluation of performance on dry environmental samples if the source using the analytical method wants to claim that the method can be used for this purpose.

## 8. SUMMARY AND CLOSING COMMENTS

Some foodborne pathogens can survive for long periods in low-moisture foods and environments, and in some cases at doses that can cause infections. Many microorganisms can resist drying processes, especially after exposure to non-lethal stress conditions, including sub-lethal heat treatment or acidic environments. If dry foods are reconstituted (rehydrated) before consumption, there is a possibility that bacterial pathogens will grow if products are subsequently stored under inappropriate conditions, potentially resulting in populations sufficient to cause infection or intoxication. Food plants, in which low-moisture foods are produced, are difficult to clean and sanitise effectively, and there are instances of persistence of specific strains of pathogens for extended periods of time.

The 'first law of microbiology' states that everything is everywhere, but the environment selects. So which pathogens are specifically selected to survive in dry food environments? Species encountered in low-moisture foods and dry food processing environments (Tables 2 and 3) are no more than a snapshot taken at the moment of writing. In reality, microorganisms adjust continuously to new environments and new strains of old foes show up in places where they are least expected. Therefore, the microbiological risk management of dry foods, dry food ingredients and dry food processing environments should not limit itself to a number of familiar and well-described species but also must be aimed at countering unknown threats. Microorganisms vary widely in their physiology and thus also in the way they should be controlled. Hence, measures aimed at dealing with pathogens in dry foods and dry food processing environments must be of a general nature and cannot be limited to one or two species.

Drying is an old well-established method for preservation. Microorganisms often do survive the drying process and growth can only resume when water becomes available for biological activities. Hence important factors to be taken into account when establishing microbiological criteria for a dry food include possible ways consumers use the food, which may include rehydration followed by prolonged storage at ambient temperature. The manufacturer cannot expect consumers always to use products in the intended way. Therefore, when setting microbiological criteria, foreseeable use (including abuse) should be considered.

Challenges specific for minimising safety risks associated with dry foods include a much longer survival time of microorganisms (e.g., up to several months or even years), the possibility that spores will germinate during rehydration, enhanced resistance of most microorganisms to dry rather than wet heat, and a general perception that dry foods are sterile, which may lead to careless handling practices. In addition, characteristics unique to dry foods can cause problems when testing for the presence of pathogens. Pathogens cannot be assumed to be homogeneously distributed in dry foods and, during detection or enumeration, they may be outcompeted by non-pathogenic species. As a result, end-product testing is of limited value for verification of the microbiological safety of dry foods and should be complemented by environmental monitoring and audits, including supplier audits.

When end-product testing is not suitable to ensure microbiological quality, how can safety criteria be met? Microorganisms, either pathogenic or spoilage, can enter products via raw materials or by contamination during or after the manufacturing process. These routes of entry must be effectively prevented. To be able to do so, detailed knowledge of the raw materials and their inherent safety risks, and of possible sources and routes of contamination is necessary. This information can be used to identify critical control points and develop an effective HACCP plan. The system should not rely heavily on verification. Control measures should be sufficiently robust to manage unusual and unexpected risks. The end-product must be safe, even if the consumer does not completely follow the instructions on the label or generally accepted kitchen practices.

## 9. REFERENCES

- Aavitsland, P., Alvseike, O., Guérin, P.J. and Stavnes, T.L. (2001). International outbreak of *Salmonella* Typhimurium DT104 – update from Norway. *Eurosurveillance* 5:p1701. Available online: <http://www.eurosurveillance.org/viewArticle.aspx?ArticleId=1701>. Accessed 26 April, 2010.
- Adams, M. (2009). *Staphylococcus aureus* and other pathogenic Gram-positive cocci. In: *Foodborne Pathogens; Hazards, Risk Analysis and Control*. Second edition, Chap. 22, pp. 807-819. Eds. C.deW. Blackburn and P.J. McClure. CRC Press, Boca Raton. Woodhead Publishing Ltd., Oxford, UK.
- Anonymous. (2004). Baby milk poisoning. Available online: <http://www.dairyreporter.com/markets/Baby-milk-poisoning>. Accessed 5 August, 2011
- Arku, B., Mullane, N., Fox, E., Fanning, S. and Jordan, K. (2008). *Enterobacter sakazakii* survives spray drying. *International Journal of Dairy Technology*, 61:102-108.
- Barash, J.R., Tang, T.W.H. and Arnon, S.S. (2005). First case of infant botulism caused by *Clostridium baratii* type F in California. *International Journal of Dairy Technology*, 43:4280-4282.
- Bar-Oz, B., Preminger, A., Peleg, O., Block, C. and Arad, I. (2001). *Enterobacter sakazakii* infection in the newborn. *Acta Paediatrica*, 90:356-358.
- Barrile, J.C. and Cone, J.F. (1970). Effect of added moisture on the heat resistance of *Salmonella anatum* in milk chocolate. *Applied Microbiology*, 19:177-178.
- Baumgartner, A., Grand, M., Liniger, M. and Iversen, C. (2009). Detection and frequency of *Cronobacter* spp. (*Enterobacter sakazakii*) in different categories of ready-to-eat foods other than infant formula. *International Journal of Food Microbiology*, 136:189-192.
- Bell, C. and Kyriakides, A. (2009a). Pathogenic *Escherichia coli*. In: *Foodborne Pathogens: Hazards, Risk Analysis and Control*. Second edition, Chap. 17, pp. 581-626. Eds. C.deW. Blackburn and P.J. McClure. CRC Press, Boca Raton. Woodhead Publishing Ltd., Oxford, UK.
- Bell, C. and Kyriakides, A. (2009b). *Salmonella*. In: *Foodborne Pathogens: Hazards, Risk Analysis and Control*. Second edition, Chap. 24, pp. 844-890. Eds. C.deW. Blackburn and P.J. McClure. CRC Press, Boca Raton. Woodhead Publishing Ltd., Oxford, UK.
- Bell, C. and Kyriakides, A. (2009c). *Listeria monocytogenes*. In: *Foodborne Pathogens: Hazards, Risk Analysis and Control*. Second edition, Chap. 19, pp. 675-717. Eds. C.deW. Blackburn and P.J. McClure. CRC Press, Boca Raton. Woodhead Publishing Ltd., Oxford, UK.
- Beuchat, L.R. and Mann, D.A. (2010). Factors affecting infiltration and survival of *Salmonella* on inshell pecans and pecan nutmeats. *Journal of Food Protection*, 73:1257-1268.
- Biering, G., Karlsson, S., Clark, N.C., Jónsdóttir, E., Lúdvigsson, P. and Steingrímsson, O. (1989). Three cases of neonatal meningitis caused by *Enterobacter sakazakii* in powdered milk. *Journal of Clinical Microbiology*, 27:2054-2056.
- Blackburn, C.deW. and McClure, P.J. (2009). Pathogenic *Bacillus* species. In: *Foodborne Pathogens: Hazards, Risk Analysis and Control*. Second edition, Chap. 24, pp. 844-890. Eds. C.deW. Blackburn and P.J. McClure. CRC Press, Boca Raton. Woodhead Publishing Ltd., Oxford, UK.
- Block, C., Peleg, O., Minster, N., Bar-Oz, B., Simhon, A., Arad, I. and Shapiro, M. (2002). Cluster of neonatal infections in Jerusalem due to unusual biochemical variant of *Enterobacter sakazakii*. *European Journal of Clinical Microbiology and Infectious Disease*, 21:613-616.
- Boor, K.J. (2006). Bacterial stress responses: what doesn't kill them can make them stronger, *PLoS Biology*, 4:e23.
- Breeuwer, P., Lardeau, A., Peterz, M. and Joosten, H.M. (2003). Desiccation and tolerance of *Enterobacter sakazakii*. *Journal of Applied Microbiology*, 95:967-973.



- Brett, M.M., McLaughlin, J., Harris, A., O'Brien, S., Black, N., Forsyth, R.J., Roberts D. and Bolton, F.J. (2005). A case of infant botulism with a possible link to infant formula milk powder: evidence for the presence of more than one strain of *Clostridium botulinum* in clinical specimens and food. *Journal of Medical Microbiology*, 54:769-776.
- Brockmann, S. (2001). International outbreak of *Salmonella* Typhimurium DT104 due to contaminated sesame seed products – update from Germany (Baden-Württemberg). *Eurosurveillance*, 33:p11999. Available online: <http://www.Eurosurveillance.org/ViewArticle.aspx?ArticleId=1699>
- Burnett, S.L., Gehm, E.R., Weissinger, W.R. and Beuchat, L.R. (2000). Survival of *Salmonella* in peanut butter and peanut butter spread. *Journal of Applied Microbiology*, 89:472-477.
- CAC (Codex Alimentarius Commission) (2004). CAC/RCP 57-2004. Code of hygienic practice for milk products, Annex II – Appendix B.
- CAC (Codex Alimentarius Commission) (2008a). Guidelines for the validation of food safety control measures. CAC/GL 69-2008. Available online: <http://www.codexAlimentarius.net/download/./cxg-069e.pdf>
- CAC (Codex Alimentarius Commission) (2008b). CAC/RCP 66-2008. Code of hygienic practice for powdered formulae for infants and young children, Annex III.
- Calicioglu, M., Sofos, J.N., Samelis, J., Kendall, P.A. and Smith, G.C. (2002). Inactivation of acid-adapted and non-adapted *Escherichia coli* O157:H7 during drying and storage of beef jerky treated with different marinades. *Journal of Food Protection*, 65:1394-1405.
- Calicioglu, M., Sofos, J.N., Samelis, J., Kendall, P.A. and Smith, G.C. (2003). Effect of acid adaptation on inactivation of *Salmonella* during drying and storage of beef jerky treated with marinades. *International Journal of Food Microbiology*, 89:51-65.
- Caubilla-Barron, J. and Forsythe, S.J. (2007). Dry stress and survival time of *Enterobacter sakazakii* and other Enterobacteriaceae in dehydrated powdered infant formula. *Journal of Food Protection*, 70:2111-2117.
- CDC (Centers for Disease Control and Prevention) (1993). *Salmonella* serotype Tennessee in powdered milk products and infant formula – Canada and United States, 1993. *Morbidity and Mortality Weekly Report*, 42:516-517.
- CDC (Centers for Disease Control and Prevention) (1998). Multistate outbreaks of *Salmonella* serotype Agona infections linked to toasted oats cereal – United States, April – May, 1998. *Morbidity and Mortality Weekly Report*, 47:462-464.
- CDC (Centers for Disease Control and Prevention) (2002). *Enterobacter sakazakii* infections associated with the use of powdered infant formula – Tennessee, 2001. *Morbidity and Mortality Weekly Report*, 51:297-300.
- CDC (Centers for Disease Control and Prevention) (2004). Outbreak of *Salmonella* serotype Enteritidis infections associated with raw almonds – United States and Canada, 2003-2004. *Morbidity and Mortality Weekly Report*, 53:484-487.
- CDC (Centers for Disease Control and Prevention) (2007a). Multistate outbreak of *Salmonella* Tennessee infections associated with peanut butter – United States, 2006-2007. *Morbidity and Mortality Weekly Report*, 56:521-524.
- CDC (Centers for Disease Control and Prevention) (2007b). *Salmonella* Wandsworth outbreak investigation, June-July, 2007. Available online: <http://www.cdc.gov/print.do?url=http%3A/www.cdc.gov/Salmonella/wandsworth.htm>. Accessed 5 May, 2010.
- CDC (Centers for Disease Control and Prevention) (2008). Investigation of outbreak of infections caused by *Salmonella* Agona. Available online: <http://www.cdc.gov/print.do?url=http%3A/www.cdc.gov/Salmonella/agona>. Accessed 5 May, 2010.

- CDC (Centers for Disease Control and Prevention) (2009). Multistate outbreak of *Salmonella* infections associated with peanut butter and peanut butter-containing products – United States, 2008-2009. *Morbidity and Mortality Weekly Report*, 58:85-90.
- Chen, Y., Scott, V.N., Freier, T.A., Kuehm, J., Moorman, M., Meyer, J., Morille-Hinds, T., Post, L., Smoot, L., Hood, S., Shebuski, J. and Banks, J. (2009a). Control of *Salmonella* in low-moisture foods. III: Process validation and environmental monitoring. *Food Protection Trends*, 29:493-508.
- Chen, Y., Scott, V.N., Freier, T.A., Kuehm, J., Moorman, M., Meyer, J., Morille-Hinds, T., Post, L., Smoot, L., Hood, S., Shebuski, J. and Banks, J. (2009b). Control of *Salmonella* contamination and growth. *Food Protection Trends*, 29:435-445.
- Christian, J.H.B. and Stewart, B.J. (1973). Survival of *Staphylococcus aureus* and *Salmonella* newport in dried foods as influenced by water activity and oxygen. In: *Microbiological Safety of Food, Proceedings*, 8th International Symposium on *Food Microbiology*. pp. 107-119. Academic Press, London.
- Cousin, M.A., Riley, R.T. and Pestka, J.J. (2005). Foodborne mycotoxins: Chemistry, biology, and toxicology. In: *Foodborne Pathogens, Microbiology and Molecular Biology*. Chap. 10, pp. 163-226. Eds. P.M. Fratamico, A.K. Bhunia and J.L. Smith. Caister Academic Press, Norfolk, UK.
- Cox, L.J., Keller, N. and van Schothorst, M. (1988). The use and misuse of quantitative determinations of Enterobacteriaceae in food microbiology. *Journal of Applied Microbiology*, Symp. Suppl., pp. 237S-249S.
- Craven, H.M., McAuley, C.M., Duffy, L.L. and Fegan, N. (2010). Distribution, prevalence and persistence of *Cronobacter* (*Enterobacter sakazakii*) in the non-processing and processing environments of five milk powder factories. *Journal of Applied Microbiology*, 109:1044-1052.
- Dancer, G.K., Mah, J.H., Rhee, M.S. and Kang, D.H. (2009). Resistance of *Enterobacter sakazakii* (*Cronobacter* spp.) to environmental stresses. *Journal of Applied Microbiology*, 107:1606-1614.
- D'Aoust, J.Y., Aris, B.J., Thisdele, P., Durante, P., Brisson N., Dragon, D., Lachapelle, G., Johnston, M. and Laidley, R. (1975). *Salmonella* eastbourne outbreak associated with chocolate. *Canadian Institute of Food Science and Technology Journal*, 8:181-184.
- D'Aoust, J.Y. and Maurer, J. (2007). *Salmonella* species. In: *Food Microbiology: Fundamentals and Frontiers*. Third edition, Chap. 10, pp. 187-236. Eds. M.P. Doyle and L.R. Beuchat. ASM Press. Washington, DC.
- Davies, A.R., Blood, R.M. and Gibbs, P.A. (1990). Effect of moisture level on the heat resistance of *Salmonellae* in cocoa liquor. *British Food Manufacturing Industries Research Association Research Report Number 660*.
- Davis, D.G., Parsek, M.R., Pearson, J.P., Iglewski, B.H., Costerton, J.W. and Greenberg, E.P. (1998). The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science*, 280:295-298.
- de Jong, B., Andersson, Y., Giesecke, J., Hellström, L., Stamer, U. and Wollin, R. (2001). *Salmonella* Typhimurium outbreak in Sweden from contaminated jars of helva (or halva) *Eurosurveillance*, 29:p=1715. Available online: <http://www.Eurosurveillance.org/ViewArticle.aspx?ArticleId=1715> Accessed 26 April, 2010.
- Deng, Y., Ryu, J.H. and Beuchat, L.R. (1998). Influence of temperature and pH on survival of *Escherichia coli* O157:H7 and growth in reconstituted infant rice cereal. *International Journal of Food Microbiology*, 45:173-184.
- DiPersio, P.A., Kendall, P.A., Yoon, Y. and Sofos, J.N. (2005a). Influence of blanching treatments on *Salmonella* during home-type dehydration and storage of potato slices. *Journal of Food Protection*, 68:2587-2593.
- DiPersio, P.A., Yoon, Y., Sofos, J.N. and Kendall, P.A. (2005b). Inactivation of *Salmonella* during drying and storage of carrot slices prepared using commonly recommended methods. *Journal of Food Science*, 70:230-235.

- D'Souza, D.H., Moe, C.L. and Jaykus, L.A. (2007). Foodborne viral pathogens. In: *Food Microbiology: Fundamentals and Frontiers*. Third edition, Chap. 27, pp. 581-607. Eds. M.P. Doyle and L.R. Beuchat. ASM Press, Washington, DC.
- Duizer, E. and Koopmans, M. (2009). Gastroenteritis viruses. In: *Foodborne Pathogens: Hazards, Risk Analysis and Control*. Second edition, Chap. 32, pp. 1161-1179. Eds. Blackburn, C.deW., and P.J. McClure. CRC Press, Boca Raton. Woodhead Publishing Ltd., Oxford, UK.
- ECS (European Committee for Standardization) (2002). EN779-2002. Particulate air filters for general ventilation – Determination of the filtration performance. European Committee for Standardization, Brussels.
- Edelson-Mammel, S.G., Porteous, M.K. and Buchanan, R.L. (2005). Survival of *Enterobacter sakazakii* in a dehydrated powdered infant formula. *Journal of Food Protection*, 68:1900-1902.
- EFSA (European Food Safety Authority) (2007). Opinion of the Scientific Panel on Biological Hazards (BIOHAZ) on the request for review of the opinion on microbiological risks in infant formulae and follow-on formulae with regard to *Enterobacteriaceae* as indicators. *The EFSA Journal*, 444:1-14. <http://www.efsa.europa.eu/en/scdocs/doc/444.pdf>.
- Elson, R. (2006). UK Health Protection Agency view on food alert announcing the recall of a number of confectionery products. *Eurosurveillance*, 11:pii=2986. Available at: <http://www.Eurosurveillance.org/ViewArticle.aspx?ArticleId=2986> Accessed 16 June, 2010.
- Forsythe, S., Sutherland, J. and Varnam, A. (2009). *Yersinia, Shigella, Aeromonas, Plesiomonas, Cronobacter, Enterobacter, Klebsiella* and *Citrobacter*. In: *Foodborne Pathogens; Hazards, Risk Analysis and Control*. Second edition, Chap. 21, pp.763-801. Eds. C.deW. Blackburn and P.J. McClure. CRC Press, Boca Raton. Woodhead Publishing Ltd., Oxford, UK.
- Gibbs, P.A. (2009). Pathogenic *Clostridium* species. In: *Foodborne Pathogens; Hazards, Risk Analysis and Control*. Second edition, Chap. 23, pp. 820-843. Eds. C.deW. Blackburn and P.J. McClure. CRC Press, Boca Raton. Woodhead Publishing Ltd., Oxford, UK.
- Gill, O.N., Sockett, P.N., Bartlett, C.L.R., Vaile, M.S.B., Rowe, B., Gilbert, R.J., Dulake, C., Murrell, H.C. and Salmaso, S. (1983). Outbreak of *Salmonella* napoli infection caused by contaminated chocolate bars. *The Lancet*, 12:574-577.
- Glass, K.A., Loeffelholz, J.M., Ford, J.P. and Doyle, M.P. (1992). Fate of *Escherichia coli* O157:H7 as affected by pH or sodium chloride in fermented, dry sausage. *Applied and Environmental Microbiology*, 58:2513-2516.
- Goepfert, J.M. and Biggie, R.A. (1968). Heat resistance of *Salmonella* senftenberg 775W in milk chocolate. *Applied Microbiology*, 16:1939-1940.
- Granum, P.E. (2007). *Bacillus cereus*. In: *Food Microbiology: Fundamentals and Frontiers*. Third edition, Chap. 20, pp. 445-455. Eds. M.P. Doyle and L.R. Beuchat. ASM Press, Washington, DC.
- Gurtler, J.B. and Beuchat, L.R. (2007). Survival of *Enterobacter sakazakii* in powdered infant formula as affected by composition, water activity, and temperature. *Journal of Food Protection*, 70:1579-1586.
- Gustavsen, S. and Breen, O. (1984). Investigation of an outbreak of *Salmonella* Oranienburg infections in Norway, caused by black pepper. *American Journal of Epidemiology*, 119:806-812
- Habraken, C.J.M., Mossel, D.A.A. and van den Reek, S. (1986). Management of *Salmonella* risk in the production of powdered milk products. *Netherlands Milk and Dairy Journal*, 40:99-116.
- Hockin, J.C., D'Aoust, J.Y., Bowering, D., Jessop, J.H., Khannna, B., Lior, H. and Milling, M.E. (1989). An international outbreak of *Salmonella* nima from imported chocolate. *Journal of Food Protection*, 52:51-54.
- Huber, B., Riedel, K., Hentzer, M., Heydorn, A., Gotschlich, A., Givskov, M., Molin, S. and Eberl, L. (2001). The *cep* quorum sensing system of *Burkholderia cepacia* H111 controls biofilm formation and swarming motility. *Microbiology*, 147:2517–2528.

- ICMSF (International Commission on Microbiological Specifications for Foods) (1996). *Microorganisms in Foods*, Volume 5: Microbiological Specifications of Food Pathogens. Eds. ICMSF. Blackie Academic & Professional, London.
- ICMSF (International Commission on Microbiological Specifications for Foods) (1998). *Microorganisms in Food*. 6: Microbial Ecology of Food Commodities. Eds. ICMSF. Blackie Academic & Professional, London.
- ILSI Europe (International Life Sciences Institute – Europe) (2002). Foodborne viruses: An emerging pathogen. *ILSI Europe report series*. Available online: <http://www.ilsi.org/Europe/Publications/>
- ILSI Europe (International Life Sciences Institute – Europe) (2004). A simple guide to understanding and applying the HACCP concept. *ILSI Europe concise monograph series*. Third edition. Available online: [http://www.ilsi.org/Europe/Publications/C2004Simp\\_GuiEng.pdf](http://www.ilsi.org/Europe/Publications/C2004Simp_GuiEng.pdf)
- ILSI Europe (International Life Sciences Institute – Europe) (2009). Animal-borne viruses of relevance to the food industry. *ILSI Europe report series*. Available online: <http://www.ilsi.org/Europe/Publications/>
- ILSI Europe (International Life Sciences Institute – Europe) (2010). Impact of Microbial distributions on food safety. *ILSI Europe report series*. Available online: <http://www.ilsi.org/Europe/Publications/Microbial%20Distribution%202010.pdf>
- Irie, Y. and Parsek, M.R. (2008). Quorum sensing and microbial biofilms. In: *Bacterial Biofilms. Current Topics in Microbiology and Immunology*. Ed. T. Romeo. Springer, Berlin and Heidelberg.
- Isaacs, S., Aramini, J., Ciebin, B., Farrar, J.A., Ahmed, R., Middleton, D., Chandran, A.U., Harris, L.J., Howes, M., Chan, E., Pichette, A.S., Campbell, K., Gupta, A., Lior, L.Y., Pearce, M., Clark, C., Rodgers, F., Jamieson, F., Brophy, I. and Ellis, A. (2005). An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella Enteritidis*. *Journal of Food Protection*, 68:191-198.
- ISO (International Organisation for Standardisation) (2005). ISO 22000:2005 Food Safety Management Systems – Requirements for any organization in the food chain.
- Jaquette, C.B. and Beuchat, L.R. (1998). Survival and growth of psychrotrophic *Bacillus cereus* in dry and reconstituted infant rice cereal. *Journal of Food Protection*, 61:1629-1635.
- Johnson, E.A. (2007). *Clostridium botulinum*. In: *Food Microbiology: Fundamentals and Frontiers*. Third edition, Chap. 18, pp. 401-421. Eds. M.P. Doyle and L.R. Beuchat. ASM press, Washington, DC.
- Johnson, E.A., Tepp, W.H., Bradshaw, M., Gilbert, R.J., Cook, P.E. and McIntosh, E.D.G. (2005). Characterization of *Clostridium botulinum* strains associated with an infant botulism case in the United Kingdom. *Journal of Clinical Microbiology*, 43:2602-2607.
- Jourdan, N., LeHello, S., Delmas, G., Clouzeau, J., Manteau, C., Désaublianus, B., Chagnon, V., Thierry-Bled, F., Demare, N., Weill, F.X. and deValk, H. (2008). Nationwide outbreak of *Salmonella enterica* serotype Give infections in infants in France, linked to infant milk formula, September 2008. *Eurosurveillance*, 13:pii=18994. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18994>.
- Jung, Y.S. and Beuchat, L.R. (1999). Survival of multidrug-resistant *Salmonella typhimurium* DT104 in egg powders as affected by water activity and temperature. *International Journal of Food Microbiology*, 49:1-8.
- Juven, B.J., Cox, N.A., Bailey, J.S., Thomson, J.E., Charles, O.W. and Shutze, J.V. (1984). Survival of *Salmonella* in dry food and feed. *Journal of Food Protection*, 47:445-448.
- Kandhai, M.C., Reij, M.W., van Puyvelde, K., Guillaume-Gentil, O., Beumer, R.R. and van Schothorst, M. (2004a). A new protocol for the detection of *Enterobacter sakazakii* applied to environmental samples. *Journal of Food Protection*, 67:1267-1270.
- Kandhai, M.C., Reij, M.W., Gorris, L.G.M., Guillaume-Gentil, O. and van Schothorst, M. (2004b). Occurrence of *Enterobacter sakazakii* in food production environments and households. *The Lancet*, 363:39-40.

- Kapperud, G., Gustavsen, S., Hellesnes, I., Hansen, A.H., Lassen, J., Hirn, J., Jahkola, M., Montenegro, M.A. and Helmuth, R. (1990). Outbreak of *Salmonella* typhimurium infection traced to contaminated chocolate and caused by a strain lacking the 60-megadalton virulence plasmid. *Journal of Clinical Microbiology*, 28:2597-2601.
- Kenney, S.J. and Beuchat, L.R. (2004). Survival, growth, and thermal resistance of *Listeria monocytogenes* in products containing peanut and chocolate. *Journal of Food Protection*, 67:2205-2211.
- Keto-Timonen, R., Tolvanen, R., Lunden, J. and Korkeala, H. (2007). An eight-year surveillance of the diversity and persistence of *Listeria monocytogenes* in chilled food processing plant analyzed by amplified fragment length polymorphism. *Journal of Food Protection*, 70:1866-1873.
- Killalea, D., Ward, L.R., Roberts, D., de Louvois, J., Sufi, F., Stuart, J.M., Wall, P.G., Susman, M., Schweiger, M., Sanderson, P.J., Fisher, I.S.T., Mead, P.S., Gill, O.N., Bartlett, C.L.R. and Rowe, B. (1996). International epidemiological and microbiological study of outbreak of *Salmonella agona* infection from a ready to eat savoury snack – I: England and Wales and the United States. *British Medical Journal*, 313:1105-1107.
- Kirk, M.D., Little, C.L., Lem, M., Fyfe, M., Genobile, D., Tan, A., Threlfall, J., Paccagnella, A., Lightfoot, D., Lyi, H., McIntyre, L., Ward, L., Brown, D.J., Surnam, S. and Fisher, I.S.T. (2004). An outbreak due to peanuts in their shell caused by *Salmonella enterica* serotypes Stanley and Newport – sharing molecular information to solve international outbreaks. *Epidemiology and Infection*, 132:571-577.
- Koch, J., Schrauder, A., Alpers, K., Werber, D., Frank, C., Prager, R., Rabsch, W., Broll, S., Feil, F., Roggentin, P., Bochemuhl, J., Tschape, H., Ammon, A. and Stark, K. (2005). *Salmonella Agona* outbreak from contaminated aniseed, Germany. *Emerging Infectious Diseases*, 11:1124-1127.
- Kornacki, J.L. (2006). Microbiological sampling in the dry foods environment. *Food Safety Magazine*, 12(1):66-72.
- Kotzekidou, P. (1998). Microbial stability and fate of *Salmonella* Enteritidis in halva, a low-moisture confection. *Journal of Food Protection*, 61:181-185.
- Kusumaningrum, H.D., Riboldi, G., Hazeleger, W.C. and Beumer, R.R. (2003). Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. *International Journal of Food Microbiology*, 85:227-236.
- Labbe, R.G. and Juneja, V.K. (2006). *Clostridium perfringens* gastroenteritis. In: *Foodborne Infections and Intoxications*. Third edition, Chap. 4, pp. 137-184. Eds. H.P. Riemann and D.O. Cliver. Academic Press, London.
- Lazizzera, B.A. (2000). Quorum sensing and starvation: signals for entry into stationary phase. *Current Opinion in Microbiology*, 3:177-182.
- Ledet Müller, L., Hjertqvist, M., Payne, L., Pettersson, H., Olsson, A., Plym Forshell, L. and Andersson, Y. (2007). Cluster of *Salmonella* Enteritidis in Sweden, 2005-2006 – suspected source: almonds. *Eurosurveillance*, 12:153-155. Available online: <http://www.Eurosurveillance.org/ViewArticle.aspx?ArticleId=718>
- Lehmacher, A., Bockemühl, J. and Aleksic, S. (1995). Nationwide outbreak of human salmonellosis in Germany due to contaminated paprika and paprika-powdered potato chips. *Epidemiology and Infection*, 115:501-511.
- Lehner, A., Riedel, K., Eberl, L., Breeuwer, P., Diep, B. and Stephan, R. (2005). Biofilm formation, extracellular polysaccharide production, and cell-to-cell signaling in various *Enterobacter sakazakii* strains: Aspects promoting environmental persistence. *Journal of Food Protection*, 68:2287-2294.
- Lehner, A., Fricker-Feer, C., Gschwend, K. and Stephan, R. (2010). Identification of Enterobacteriaceae and *Cronobacter* spp. in raw milk, milk concentrate and milk powder: prevalence and genotyping. *Archives Lebensmittelhygiene*, 61:22-26.

- Lilie, M., Hein, S., Wilhelm, P. and Mueller, U. (2007). Decontamination of spices by combining mechanical and thermal effects – An alternative approach for quality retention. *International Journal of Food Science and Technology*, 42:190-193.
- Lin, L.C. and Beuchat, L.R. (2007). Survival of *Enterobacter sakazakii* in infant cereal as affected by composition, water activity, and temperature. *Food Microbiology*, 24:767-777.
- Little, C. (2001). International outbreak of *Salmonella* Typhimurium DT104 – update from United Kingdom. *Eurosurveillance*, 5:1700. Available online: <http://www.Eurosurveillance.org/ViewArticle.aspx?ArticleId=1700>.
- Mattison, K., Biawid, S. and Farber, J. (2009). Hepatitis and emerging viruses. In: *Foodborne Pathogens; Hazards, risk analysis and control*. Chap. 25, pp. 891-929. Eds. C.deW. Blackburn and P.J. McClure. CRC Press, Woodhead Publishing Ltd., Cambridge, UK.
- Maurer, J. and Lee, M. (2005). *Salmonella*: virulence, stress response and resistance. In: *Understanding Pathogen Behaviour: Virulence, Stress Response, and Resistance*. Chap. 9, pp. 215-239. Ed M. Griffiths. Woodhead Publishing Ltd., Oxford, UK.
- McClane, B.A. (2007). *Clostridium perfringens*. In: *Food Microbiology: Fundamentals and Frontiers*. Third edition, Chap. 19, pp. 423-444. Eds. M.P. Doyle and L.R. Beuchat. ASM Press, Washington, DC.
- McClure, P. and Blackburn, C. (2002). *Campylobacter* and *Arcobacter*. In: *Foodborne Pathogens*. Third edition, Chap. 20, pp. 363-384. Eds. Blackburn, C.W., and P.J. McClure. Woodhead Publishing, Oxford, UK.
- McLauchlin, J., Grant, K.A. and Little, C.J. (2006). Food-borne botulism in the United Kingdom. *Journal of Public Health*, 28:337-342.
- Meng, J., Doyle, M.P., Zhao, T. and Zhao, S. (1994). Detection and control of *Escherichia coli* O157:H7 in foods. *Trends in Food Science and Technology*, 5:179-85.
- Meng, J., Doyle, M.P., Zhao, T. and Zhao, S. (2007). Enterohemorrhagic *Escherichia coli*. In: *Food Microbiology: Fundamentals and Frontiers*. Third edition, Chap. 12, pp. 249-269. Eds. M.P. Doyle and L.R. Beuchat. ASM Press, Washington, DC.
- Mihaljevic, R.R., Sikic, M., Klancnik, A., Brumini, G., Mozina, S.S. and Abram, M. (2007). Environmental stress factors affecting survival and virulence of *Campylobacter jejuni*. *Microbial Pathogenesis*, 43:120-125.
- Montville, T.J. and Matthews, K.R. (2008). *Listeria monocytogenes*. In: *Food Microbiology – An Introduction*. pp. 173-88. Eds. T.J. Montville and K.R. Matthews. ASM Press, Washington, DC.
- Morita, T., Kitazawa, H., Idia, T. and Kamata, S. (2006). Prevention of *Salmonella* cross-contamination in an oilmeal manufacturing plant. *Journal of Applied Microbiology*, 101:464-473.
- Mullane, N.R., Whyte, P., Wall, P.G., Quinn, T. and Fanning, S. (2007). Application of pulsed-field gel electrophoresis to characterise and trace the prevalence of *Enterobacter sakazakii* in an infant formula processing facility. *International Journal of Food Microbiology*, 116:73-81.
- Mullane, N.R., Healy, B., Meade, J., Whyte, P., Wall, G. and Fanning, S. (2008). Dissemination of *Cronobacter* spp. (*Enterobacter sakazakii*) in a powdered milk protein manufacturing facility. *Applied and Environmental Microbiology*, 74:5913-5917.
- O'Grady, K.A., Powling, J., Tan, A., Valcanis, M., Lightfoot, D., Gregory, J., Lalor, K., Guy, R., Ingle, B., Andrews, R., Crerar, S. and Stafford, R. (2001). *Salmonella* Typhimurium DT104 – Australia, Europe. *Archive No. 20010822.1980*. Available online: <http://www.promedmail.org>. Accessed 20 April, 2010.
- Ortega, Y.R. (2007). Protozoan parasites. In: *Food Microbiology: Fundamentals and Frontiers*. Third edition, Chap. 31, pp. 663-681. Eds. M.P. Doyle and L.R. Beuchat. ASM Press, Washington, DC.
- Pagotto, F.J., Lenati, R.F. and Farber, J.M. (2007). *Enterobacter sakazakii*. In: *Food Microbiology: Fundamentals and Frontiers*. Third edition, Chap. 13, pp. 271-291. Eds. M.P. Doyle and L.R. Beuchat. ASM Press, Washington, DC.

- Palcich, G., Gillio, C.deM., Oragon-Alegro, L.C., Pagotto, F.J., Farber, J.M. and Destro, M.T. (2009). *Enterobacter sakazakii* in dried infant formulas and milk kitchens of maternity wards in Sao Paulo, Brazil. *Journal of Food Protection*, 72:37-42.
- Petrignani, M., Verhoef, L., van Hunen, R., Swaan, C., van Steenberghe, J., Boxman, I., Ober, H.J., Vennema, H. and Koopmans, M. (2010). A possible foodborne outbreak of hepatitis A in the Netherlands, January – February 2010. *Eurosurveillance*, 15:pii=19512. Available online: <http://www.Eurosurveillance.org/ViewArticle.aspx?ArticleId=19512>.
- PHLS (Public Health Laboratory Service) (1995). Schedules for the examination of food. In: *Practical Food Microbiology*. Eds. D. Roberts, W. Harper and M. Greenwood. Public Health Laboratory Service, London.
- Podolak, R., Enache, E., Stone, W., Black, D.G. and Elliott, P.H. (2010). Sources and risk factors of contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. *Journal of Food Protection*, 73:1919-1936.
- ProMed Mail. (2009a). *E. coli* O157:H7 – USA (07): refrigerated cookie dough. Archive no. 20090710.2473. Available online: <http://www.promedmail.org>.
- ProMed Mail. (2009b). Hepatitis A virus, semi-dried tomatoes – Australia: recall. International Society for Infectious Diseases, 22 May, 2009. Archive no. 20090522.1917. Available online: <http://www.promedmail.org>.
- Proudy, L., Bouglé, D., Leclercq, R. and Vergnaud, M. (2008). Tracing of *Enterobacter sakazakii* isolates in infant milk formula processing by BOX-PCR genotyping. *Journal of Applied Microbiology*, 105:550-558.
- Ray, B., Jezeski, J.J. and Busta, F.F. (1971). Isolation of *Salmonellae* from naturally contaminated dried milk products. II. Influence of storage time on isolation of *Salmonellae*. *Journal of Milk and Food Technology*, 34:425-427.
- Rayman, M.K., D'Aoust, J.Y., Aris, B., Maishment, C. and Wasik, R. (1979). Survival of microorganisms in stored pasta. *Journal of Food Protection*, 42:330-334.
- Reij, M., Jongenburger, I., Gkogka, E., Gorris, L.G.M. and Zwietering, M.H. (2009). Perspective on the risk to infants in the Netherlands associated with *Cronobacter* spp. occurring in powdered infant formula. *International Journal of Food Microbiology*, 136:232-237.
- Restaino, L., Frampton, E.W., Lionberg, W.C. and Becker, R.J. (2006). A chromogenic plating medium for the isolation and identification of *Enterobacter sakazakii* from foods, food ingredients, and environmental sources. *Journal of Food Protection*, 69:315-322.
- Reynolds, G. (2007). Food Production Daily, 2007. Available online: <http://www.foodproductiondaily.com/Quality-Safety/ConAgra-estimates-peanut-butter-recall-will-cost-60m>. Accessed 16 June, 2010.
- Rieschel, H. and Schenkel, J. (1971). Das Verhalten von Mikroorganismen, speziell Salmonellen in Schokoladenwaren. *Alimenta*, 10:57-66.
- Rodriguez-Romo, L. and Yousef, A. (2005). Cross-protective effects of bacterial stress. In: *Understanding Pathogen Behaviour Virulence, Stress Response and Resistance*. Ed. M. Griffiths. CRC Press, Boca Raton, FL.
- Rowe, B., Hutchinson, D.N., Gilbert, R.J., Hales, B.H., Begg, N.T., Dawkins, H.C., Jacob, M., Rae, F.A. and Jepson, M. (1987). *Salmonella* Ealing infections associated with consumption of infant dried milk. *The Lancet*, 330:900-903.
- Rushdy, A.A., Stuart, J.M., Ward, L.R., Bruce, J., Threlfall, E.J., Punia, P. and Bailey, J.R. (1998). National outbreak of *Salmonella* Senftenberg associated with infant food. *Epidemiology and Infection*, 120:125-128.
- Ryu, J.H., Deng, Y. and Beuchat, L.R. (1999). Survival of *Escherichia coli* O157:H7 in dried beef powder as affected by water activity, sodium chloride content, and temperature. *Food Microbiology*, 16:309-316.

- Scheil, W., Cameron, S., Dalton, C. and Wilson, D. (1998). A South Australian *Salmonella* Mbandaka outbreak investigation using a database to select controls. *Australian and New Zealand Journal of Public Health*, 22:536-539.
- Schraft, H. and Griffiths, M.W. (2005). *Bacillus cereus* gastroenteritis. In: Foodborne Infections and Intoxications. Third edition, Chap. 15, pp. 563-582. Eds. H.P. Riemann and D.O. Cliver. Academic Press, London.
- Scott, V.N., Chen, Y., Freier, T.A., Kuehm, J., Moorman, M., Meyer, J., Morille-Hinds, T., Post, L., Smoot, L., Hood, S., Shebuski, J. and Banks, J. (2009). Control of *Salmonella* in low-moisture foods. I: Minimizing entry of *Salmonella* into a processing facility. *Food Protection Trends*, 29:342-353.
- Seo, K.S. and Bohach, G.A. (2007). *Staphylococcus aureus*. In: *Food Microbiology: Fundamentals and Frontiers*. Third edition, Chap. 22, pp. 493-518. Eds. M.P. Doyle and L.R. Beuchat. ASM Press, Washington, DC.
- Shohat, T., Green, M.S., Merom, D., Gill, O.N., Reisfeld, A., Matas, A., Blau, D., Gal, N. and Slater, P.E. (1996). International epidemiological and microbiological study of outbreak of *Salmonella agona* infection from a ready to eat savoury snack – II: Israel. *British Medical Journal*, 313:1107-1109.
- Silva, F.V.M. and Gibbs, P.A. (2010). Non-proteolytic *Clostridium botulinum* spores in low-acid cold-distributed foods and design of pasteurisation process. *Trends in Food Science and Technology*, 22:95-105.
- Simmons, B.P., Gelfand, M.S., Hass, M., Metts, L. and Ferguson, J. (1989). *Enterobacter sakazakii* infections in neonates associated with intrinsic contamination of a powdered infant formula. *Infection Control and Hospital Epidemiology*, 10:390-401.
- Sinha, K.K. and Bhatnagar, D. (1998). *Mycotoxins in Agriculture and Food Safety*. Marcel Dekker, Inc., New York.
- Smith, H. and Evans, R. (2009). Parasites: *Cryptosporidium*, *Giardia*, *Cyclospora*, *Entamoeba histolytica*, *Toxoplasma gondii* and free-living amoebae (*Acanthamoeba* spp. and *Naegleria fowleri*) as foodborne pathogens. In: *Foodborne Pathogens: Hazard, Risk Analysis and Control*. Second edition, Chap. 26, pp. 930-1008. Eds. C.deW. Blackburn and P.J. McClure. CRC Press, Boca Raton. Woodhead Publishing Ltd., Oxford, UK.
- Stephens, P.J., Joynson, J.A., Davies, K.W., Holbrook, R., Lappin-Scott, H.M. and Humphrey, T.J. (1997). The use of automated growth analyser to measure recovery times of single heat-injured *Salmonella* cells. *Journal of Applied Microbiology*, 83:445-455.
- Stocki, S.L., Annett, C.B., Sibley, C.D., McLaws, M., Checkley, S.L., Singh, N., Surette, M.G. and White, A.P. (2007). Persistence of *Salmonella* on egg conveyor belts is dependent on the belt type but not on the rdar morphotype. *Poultry Science*, 86:2375-2383.
- Swaminathan, B., Cabanes, D., Zhang, W. and Cossart, P. (2007). *Listeria monocytogenes*. In: *Food Microbiology: Fundamentals and Frontiers*. Third edition, Chap. 21, pp. 457-491. Eds. M.P. Doyle and L.R. Beuchat. ASM Press, Washington, DC.
- Tamminga, S.K., Beumer, R.R., Kampelmacher, E.H. and van Leusden, F.M. (1976). Survival of *Salmonella eastbourne* and *Salmonella typhimurium* in chocolate. *Journal of Hygiene (London)*, 74:41-47.
- Tamminga, S.K., Beumer, R.R., van Leusden, F.M. and Kampelmacher, E.H. (1975). Overleving van *Salmonella eastbourne* en *Salmonella typhimurium* in chocolate. *Voedingsmiddelentechnologie*, 8:22-24.
- Taormina, P.J. and Beuchat, L.R. (1999). Comparison of chemical treatments to eliminate enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa seeds. *Journal of Food Protection*, 62:318-324.



- Teunis, P.F.M., Kasuga, F., Fazil, A., Ogden, I.D., Rotariu, O. and Strachan, J.J.C. (2010). Dose-response modelling of *Salmonella* using outbreak data. *International Journal of Food Microbiology*, 144:243-249.
- Tilden, J., Young, W., McNamara, A.-M., Custer, C., Boesel, B., Lambert-Fair, M.A. Majkowski, J., Vugia, D., Werner, S.B., Hollingsworth, J. and Morris, J.G. (1996). A new route of transmission for *Escherichia coli*: infections from dry fermented salami. *American Journal of Public Health*, 86:1142-1145.
- Uesugi, A.R., Danyluk, M.D. and Harris, L.J. (2006). Survival of *Salmonella* Enteritidis phage type 30 on inoculated almonds stored at -20, 4, 23, and 35°C. *Journal of Food Protection*, 69:1851-1857.
- Unicomb, L.E., Simmons, G., Merritt, T., Gregory, J., Nicol, C., Jelfs, P., Kirk, M., Tan, A., Thomson, R., Adanopoulos, J., Little, C.L., Currie, A. and Dalton, C.B. (2005). Sesame seed products contaminated with *Salmonella*: three outbreaks associated with tahini. *Epidemiology and Infection*, 133:1065-1072.
- van Acker, J., de Smet, F., Muyldermans, G., Bougateg, A., Naessens, A. and Leuwers, S. (2001). Outbreaks of necrotizing enterocolitis associated with *Enterobacter sakazakii* in powdered milk formula. *Journal of Clinical Microbiology*, 39:293-297.
- van Schothorst, M., van Leusden, F.M., de Gier, E., Rijnierse, F.M. and Veen, A.J.D. (1979). Influence of reconstitution on isolation of *Salmonella* from dried milk. *Journal of Food Protection*, 42:936-937.
- Vogel, B.F., Hansen, L.T., Mordhorst, H. and Gram, L. (2010). The survival of *Listeria monocytogenes* during long term desiccation is facilitated by sodium chloride and organic material. *International Journal of Food Microbiology*, 140:192-200.
- Ward, L., Duckworth, G. and O'Brien, S. (1999). *Salmonella* Java phage type Dundee – rise in cases in England: update. *Eurosurveillance*, 3:p=1435 Available online: <http://www.Eurosurveillance.org/ViewArticle.aspx?ArticleId=1435>.
- Weissman, J.B., Deen, R.M.A.D., Williams, M., Swanston, N. and Ali, S. (1977). An island wide epidemic of salmonellosis in Trinidad traced to contaminated powdered milk. *West Indies Medical Journal*, 26:135-143.
- WHO/FAO (World Health Organization/Food and Agriculture Organization) (2007). Guidelines for the safe preparation, storage and handling of powdered infant formula. Available online: [http://www.who.int/foodsafety/publications/micro/pif\\_guidelines.pdf](http://www.who.int/foodsafety/publications/micro/pif_guidelines.pdf).
- Wilson, M.M. and MacKenzie, E.F. (1955). Typhoid fever and salmonellosis due to consumption of infected desiccated coconut. *Journal of Applied Bacteriology*, 18:510-521.

## 10. ACRONYMS

$a_w$	Water activity
cfu	Colony-forming Unit
CODEX	FAO/WHO Codex Alimentarius Commission
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GHP	Good Hygiene Practice
GI	Gastrointestinal
GMP	Good Manufacturing Practice
HEPA	High-Efficiency Particulate Air
HACCP	Hazard Analysis and Critical Control Points
HAV	Hepatitis A
HVP	Hydrolyzed Vegetable Protein
MPN	Most Probable Number
PCR	Polymerase Chain Reaction
PFGE	Pulsed-field Gel Electrophoresis
RTE	Ready-to-Eat
RAPD PCR	Rapid Amplified Polymorphic Deoxyribonucleic Acid Polymerase Chain Reaction
RASFF	Rapid Alert System for Foods and Feeds
UHT	Ultra-high Temperature
VTEC	Verotoxin-producing Escherichia coli
WHO	World Health Organization of the United Nations



## Other ILSI Europe Publications

### Concise Monographs

- Alcohol – Health Issues Related to Alcohol Consumption
- A Simple Guide to Understanding and Applying the Hazard Analysis Critical Control Point Concept
- Calcium in Nutrition
- Carbohydrates: Nutritional and Health Aspects
- Caries Preventive Strategies
- Concepts of Functional Foods
- Dietary Fibre
- Food Allergy
- Food Biotechnology – An Introduction
- Food, Glycaemic Response and Health
- Functional Foods – From Science to Health and Claims
- Genetic Modification Technology and Food – Consumer Health and Safety
- Healthy Lifestyles – Nutrition and Physical Activity
- Microwave Ovens
- Nutrition and Genetics – Mapping Individual Health
- Nutrition and Immunity in Man
- Nutritional and Health Aspects of Sugars – Evaluation of New Findings
- Nutritional Epidemiology, Possibilities and Limitations
- Oral and Dental Health - Prevention of Dental Caries, Erosion, Gingivitis and Periodontitis
- Oxidants, Antioxidants, and Disease Prevention
- Principles of Risk Assessment of Food and Drinking Water Related to Human Health
- The Acceptable Daily Intake – A Tool for Ensuring Food Safety
- Threshold of Toxicological Concern (TTC)
- Type 2 Diabetes – Prevention and Management

### Reports

- Addition of Nutrients to Food: Nutritional and Safety Considerations
- An Evaluation of the Budget Method for Screening Food Additive Intake
- Animal-Borne Viruses of Relevance to the Food Industry
- Antioxidants: Scientific Basis, Regulatory Aspects and Industry Perspectives
- Applicability of the ADI to Infants and Children
- Application of the Margin of Exposure Approach to Compounds in Food which are both Genotoxic and Carcinogenic
- Approach to the Control of Enterohaemorrhagic *Escherichia coli* (EHEC)
- Assessing and Controlling Industrial Impacts on the Aquatic Environment with Reference to Food processing
- Assessing Health Risks from Environmental Exposure to Chemicals: The Example of Drinking Water
- Beyond PASSCLAIM – Guidance to Substantiate Health Claims on Foods
- Campylobacters as Zoonotic Pathogens: A Food Production Perspective
- Considering Water Quality for Use in the Food Industry
- Consumer Understanding of Health Claims
- Detection Methods for Novel Foods Derived from Genetically Modified Organisms
- Emerging Technologies for Efficacy Demonstration
- Evaluation of Agronomic Practices for Mitigation of Natural Toxins
- Evaluation of the Risks Posed in Europe by Unintended Mixing of Food Crops and Food Crops Developed for Non-Food Uses
- Exposure from Food Contact Materials
- Foodborne Protozoan Parasites
- Foodborne Viruses: An Emerging Problem
- Food Consumption and Packaging Usage Factors
- Food Safety Management Tools

- Food Safety Objectives – Role in Microbiological Food Safety Management
- Frontiers in Food Allergen Risk Assessment
- Functional Foods in Europe – International Developments in Science and Health Claims
- Functional Foods – Scientific and Global Perspectives
- Guidance for the Safety Assessment of Botanicals and Botanical Preparations for Use in Food and Food Supplements
- Impact of Microbial Distributions on Food Safety
- Markers of Oxidative Damage and Antioxidant Protection: Current status and relevance to disease
- 3-MCPD Esters in Food Products
- Method Development in Relation to Regulatory Requirements for the Detection of GMOs in the Food Chain
- Micronutrient Landscape of Europe: Comparison of Intakes and Methodologies with Particular Regard to Higher Consumption
- *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and the Food Chain
- Nutrition in Children and Adolescents in Europe: What is the Scientific Basis?
- Overview of the Health Issues Related to Alcohol Consumption
- Overweight and Obesity in European Children and Adolescents: Causes and consequences – prevention and treatment
- Packaging Materials: 1. Polyethylene Terephthalate (PET) for Food Packaging Applications
- Packaging Materials: 2. Polystyrene for Food Packaging Applications
- Packaging Materials: 3. Polypropylene as a Packaging Material for Foods and Beverages
- Packaging Materials: 4. Polyethylene for Food Packaging Applications
- Packaging Materials: 5. Polyvinyl Chloride (PVC) for Food Packaging Applications
- Packaging Materials: 6. Paper and Board for Food Packaging Applications
- Packaging Materials: 7. Metal Packaging for Foodstuffs
- Packaging Materials: 9. Multilayer Packaging for Food and Beverages
- Recontamination as a Source of Pathogens in Processed Foods – A Literature Review
- Recycling of Plastics for Food Contact Use
- Safety Assessment of Viable Genetically Modified Microorganisms Used in Food
- Safety Considerations of DNA in Foods
- *Salmonella* Typhimurium definitive type (DT) 104: A multi-resistant *Salmonella*
- Significance of Excursions of Intake above the Acceptable Daily Intake (ADI)
- The Safety Assessment of Novel Foods
- The Safety Assessment of Novel Foods and Concepts to Determine their Safety in use
- Threshold of Toxicological Concern for Chemical Substances Present in the Diet
- Transmissible Spongiform Encephalopathy as a Zoonotic Disease
- Trichothecenes with a Special Focus on DON
- Using Microbiological Risk Assessment (MRA) in Food Safety Management
- Validation and Verification of HACCP
- Water Use of Oil Crops: Current Water Use and Future Outlooks

### To order

ILSI Europe a.i.s.b.l.

Avenue E. Mounier, 83, Box 6

B-1200 Brussels, Belgium

Phone: (+32) 2 771 00 14 • Fax: (+32) 2 762 00 44

E-mail: [publications@ilsieurope.be](mailto:publications@ilsieurope.be)

ILSI Europe's Concise Monographs and Report Series can be downloaded from: [www.ilsieurope.eu](http://www.ilsieurope.eu)

ISBN 9789078637325



9 789078 637325