

**LACORS/HPA Co-ordinated Food Liaison Group Studies:  
An Assessment of the Microbiological Safety of Ready-To-Eat Dried Seeds  
From Retail Premises in the United Kingdom with a focus on *Salmonella* spp.**

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**On behalf of the Local Authorities Co-ordinators of Regulatory Services  
(LACORS) and the Health Protection Agency**

## Abstract

Sesame seed products have been associated with a number of *Salmonella* outbreaks from 2001 to 2003, in the UK and elsewhere. Aside from sesame seeds, there is little published information on the prevalence of *Salmonella* spp. in edible seeds. A study of retail edible dried seeds in the UK was therefore carried out to assess their microbiological safety in relation to *Salmonella* contamination and levels of *Escherichia coli*, an indicator of faecal contamination.

Between October 2007 and March 2008, 3735 samples of ready-to-eat (RTE) dried seeds were collected from 3390 retail premises in the UK. Overall, *Salmonella* was detected in 23 samples (0.6%). Of the samples contaminated with *Salmonella*, over half were sesame seeds (57%). Other seeds contaminated with *Salmonella* were linseed (one sample, 0.4%), sunflower (one sample, 0.1%), alfalfa (one sample, 1.7%), melon (four samples, 8.5%) and mixed seeds (three samples, 0.9%). *Escherichia coli* was detected in 9% of samples, with 1.5% containing unsatisfactory levels ( $\geq 10^2$  /g). These included melon, pumpkin, sesame, hemp, poppy, linseed, sunflower and mixed seeds.

The UK retailers affected by the detection of *Salmonella* in their products publicly recalled the contaminated batches, and Food Standard Agency food alerts were also issued, advising consumers not to eat the affected products. This study highlights the potential public health risk associated with the consumption of ready-to-eat seeds, and the need for good hygiene practices and effective decontamination procedures during the production of these products.

## Introduction

Dried seeds are popular as a ready-to-eat food, particularly due to reports of their health benefits (Cooney et al., 2001; Ollis et al., 1999; Yu et al., 2005). However, in recent years there have been a number of reports of *Salmonella* incidents related to edible seeds and their products. For example, in 2001, an international *Salmonella* Typhimurium outbreak in Sweden, Norway, Germany and Australia was linked with the consumption of helva, a sesame seed-based confectionery (de Jong et al., 2001). In 2002 and 2003, three outbreaks of *Salmonella* Montevideo infection were identified in Australia and New Zealand (Unicomb et al., 2005), with tahini (sesame seed paste) imported from Egypt and Lebanon being identified as the source of infection. In Germany, *Salmonella* Agona from an untreated batch of aniseed imported from Turkey was found to be the cause of 36 cases of infantile gastroenteritis (Koch et al., 2005; Rabsch et al., 2005). Sprouted seeds, and alfalfa sprouts in particular, have also been associated with outbreaks of *Salmonella* (Proctor et al., 2001; Van Beneden et al., 1999; Winthrop et al., 2003), including two outbreaks in the EU in 2007 (Emberland et al., 2007; Werner et al., 2007).

Different seed types require varying climates and environments for growth and are therefore grown in many different areas of the world. Linseed, poppy and hemp crops are grown in large fields, and harvested, cleaned and sorted mechanically in a similar way to cereal crops. Similarly, sunflower seeds are harvested from large fields and hulled mechanically. However, sunflowers are harvested at such a height from the ground that they tend to be cleaner than other seed crops (Pierce, 1970). The moisture content of sunflower seeds after harvest may be up to 20%. These are dried to less than 10% moisture, usually by the use of forced air through the batch of seeds, before the hulls are removed mechanically (Salunkhe et al., 1992). In contrast, pumpkin and melon seeds are removed from the fruit in a relatively moist condition (approx. 38%) (Pierce, 1970). The drying process

for these crops may involve spreading the seeds on the ground to dry in the sun, or alternatively drying in an oven or with the use of smoke (Bankole et al., 2005) before they are hulled with the aid of water and then re-dried. Sesame seeds are harvested and then subjected to a cleaning process to remove debris before being hulled by means of water (aqua-hulling), mechanical friction or a chemical process (using a caustic soda solution) (Brockmann et al., 2004, Wittenberg, 2007). Finally, they are dried, often mechanically in a stream of hot air.

During growth, the crops are likely to be exposed to a wide range of microbial contamination from many sources including soil, manure, irrigation water, wild birds and animals (Doyle and Erickson, 2008). Further potential for microbial contamination may occur during post-harvest processing. Whilst the drying process for some seed products involves a heat treatment, this is not always the case, and where heat is used it is not necessarily at a sufficient temperature to ensure that all pathogenic bacteria are killed.

There is little published data on the microbiological quality of edible dried seeds. Some surveys of sesame seed products have been carried out in the context of international outbreaks of *Salmonella* associated with the consumption of these products in 2001 and 2003. For example, Brockmann et al. (Brockmann et al., 2004) examined 117 sesame seed products on retail sale in Germany in 2001. Of these, 11 (9.4%) were found to be contaminated with *Salmonella* species. Two investigations of sesame seed products from retail premises in England and Wales were carried out in 2001 and 2003. In 2001, *Salmonella* Typhimurium DT 104 was detected in 6 of 151 halva samples. These consisted of two brands, both produced by a single manufacturer in Turkey (C Little, HPA, unpublished observations). In 2003, 160 sesame products were examined, of which five serotypes of *Salmonella* (Cubana, Lille, Mbandaka, Senftenberg, Tennessee) were detected in 25 tahini samples from Lebanon and Cyprus (S Surman-Lee, HPA personal communication). In Australia, the National Enteric Pathogen Surveillance Scheme reported the isolation of 17

different *Salmonella* species from sesame seeds and their products on 30 occasions between 1985 and 2001 (O'Grady et al., 2001). In 2006, a pan-London study of edible seeds showed that 2.0% (7/367 of samples) were contaminated with *Salmonella* (Ahmadi, Give, Senftenberg, Shangani, Muenster) (S Surman-Lee, HPA personal communication). These findings demonstrate that edible seeds are a potential source of microbial contamination in the UK.

In response to these investigations, the Local Authorities Co-ordinators of Regulatory Services (LACORS) and the Health Protection Agency (HPA) Co-ordinated Food Liaison Group programme undertook a microbiological study with the aim of assessing the microbiological safety of edible dried seeds on retail sale in the UK, with a particular focus on the detection of *Salmonella* species. A range of ready-to-eat dried seeds were sampled and examined over a six month period to provide further data on the microbiology of these products and to highlight potential problems with their production and use.

## **Materials and Methods**

### ***Sample Collection***

A total of 3735 samples of edible seeds were randomly collected from 3390 retail premises by sampling officers from 317 Environmental Health Departments (EHD) in 55 Local Authority Food Liaison Groups (as shown in Annex 1) between 1 October 2007 and 31 March 2008. Sampling officers were requested to include at least one sample of sesame seeds on every occasion that they collected samples. This level of scrutiny was deemed necessary because of the previous reports of *Salmonella* contamination of sesame seed products. Seeds coated with chocolate, yoghurt or other coatings, flavoured with seasonings (spices, salt, etc.), or those containing dried fruits were specifically excluded from the study.

Registered retail premises lists held by EHDs were used to derive an approach to sampling. Retail premises were selected at random from EHDs'

database of food businesses via a random number generator or every 10<sup>th</sup> entry, and if suitable, samples were collected. Samples (of at least 50g) were collected and transported in accordance with the Food Standards Agency Food Law Code of Practice (Food Standards Agency, 2006) and the Local Authorities Co-ordinators of Regulatory Services (LACORS) guidance on microbiological food sampling (Local Authorities Co-ordinators of Regulatory Services, 2006). These were examined by 32 Official Food Control Laboratories in the UK.

Information on samples and premises was obtained by observation and enquiry and recorded on a standard questionnaire (Annex 2). This included information on the type of premises, type of seeds, packaging, country of origin and whether or not the product was labelled as organic.

### **Sample Examination**

*Escherichia coli* was enumerated and the presence of *Salmonella* sought in accordance with HPA Standard Methods F22 and F13 respectively (Health Protection Agency, 2005; Health Protection Agency, 2008). Two representative (one 25g sample (*Salmonella* test) and one 10g sample (*Escherichia coli* test)) sub-samples from the edible seeds sample were required for microbiological examination. Where *Salmonella* was detected, and sufficient sample was available, *Salmonella* was also enumerated by a Most Probable Number (MPN) 10-tube method. This involved preparing a 1 in 10 dilution of the sample by adding 900 ml of Buffered Peptone Water to 100 g of sample. Ten aliquots, each of 100 g, of this homogenate were then dispensed into separate sterile containers. These were incubated and sub-cultured as described in HPA Standard Method F13 (Health Protection Agency, 2008). The number of aliquots from which *Salmonella* was detected was compared to an MPN 10-tube table (adapted from ISO/FDIS 7218:2007 (International Organisation for Standardisation, 2007)).

All isolates of *Salmonella* were sent to the Laboratory of Enteric Pathogens (LEP), HPA Centre for Infections, for further characterisation. This included serotyping (Bale et al., 2007; Popoff and Le Minor, 2001), phage typing (Chambers et al., 1987) and antimicrobial sensitivity testing (Frost, 1994). Typing data for isolates of *Salmonella* were compared with those from isolates from human cases of salmonellosis which occurred in England and Wales over the same time period of this study.

Microbiological results were compared to the HPA (PHLS) Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale (Table 1) (PHLS, 2000).

**Table 1: Criteria for the interpretation of microbiology results, according to the HPA (PHLS) Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale (PHLS, 2000)**

	Satisfactory	Acceptable	Unsatisfactory	Unacceptable/ Potentially Hazardous
<i>E. coli</i> /g	<20	20 - <100	≥100	N/A <sup>a</sup>
<i>Salmonella</i> in 25g	Not Detected	N/A	N/A	Detected <sup>b</sup>

<sup>a</sup> N/A, Not applicable

<sup>b</sup>, Potentially injurious to health and/or unfit for human consumption (contravenes Article 14 Food Safety Requirements of Regulation (EC) No.178/2002 (the General Food Law Regulation))

#### ***Exclusion of inappropriate seed samples***

A further 349 samples that were collected and examined as part of this survey were not included in the final analysis of results as they did not fit the study criteria (i.e. ready-to-eat dried seeds that have not undergone a cooking process). These

excluded samples were tree nuts, dried spices, beans and sprouted seeds. Of these, *Salmonella* Bareilly was detected in a sample of dried spice (fennel seeds).

### **Statistical Analysis**

Descriptive and statistical analysis of the data was undertaken using Microsoft Excel. Relative proportions were compared using the Chi squared test ( $\chi^2$ ) or Fisher's Exact Test. A probability value of less than 5% was defined as significant.

### **Results**

#### ***Salmonella contamination of edible seeds***

The types of seeds sampled are shown in Table 2. Overall, *Salmonella* spp. were detected in 23 of 3735 (0.6%) samples. This included 20 samples of a single seed type (alfalfa, linseed, melon/"egusi", sesame and sunflower) and three samples of mixed seeds - two containing five seed types (pumpkin, sunflower, sesame, linseed and hemp) and one containing three types (pumpkin, sunflower and sesame).

Over half (57%; 13/23) of samples containing *Salmonella* were sesame seeds. Moreover, mixed seeds that contained sesame seeds were contaminated with *Salmonella* (1.3%; 3/228), whereas those without sesame seeds were not (0%; 0/122). However, the frequency of contamination of sesame seeds with *Salmonella* (1.7%; 13/771) was equal to the contamination rate of alfalfa seeds (1.7%; 1/58) and significantly lower than that of melon seeds (8.5%;  $p=0.013$ ; Fisher's Exact test).

*Salmonella* was enumerated in six samples. In four of these, the Most Probable Number was  $<0.1$  per gram, whilst counts in the other two samples were 0.1 and 0.2 per gram respectively.

**Table 2. Edible dried seed types sampled in relation to presence of *Salmonella* and unsatisfactory levels of *E. coli* (i.e.  $\geq 10^2$  per gram)**

Seed type	No. of samples		No. with <i>Salmonella</i>		No. with <i>E. coli</i>	
	N=3735	(%)	detected in 25g	(%)	$\geq 10^2$ /g	(%)
<b>Single seed types</b>	<b>3385</b>	<b>(90.6)</b>	<b>20</b>	<b>(0.6)</b>	<b>47</b>	<b>(1.4)</b>
Alfalfa	58	(1.6)	1	(1.7)	-	-
Hemp	121	(3.2)	-	-	2	(1.7)
Linseed (flax)	284	(7.6)	1	(0.4)	1	(0.4)
Melon (egusi)	47	(1.3)	4	(8.5)	6	(12.8)
Poppy	202	(5.4)	-	-	3	(1.5)
Pumpkin	886	(23.7)	-	-	23	(2.6)
Sesame	771	(20.6)	13	(1.7)	8	(1.0)
Sunflower	976	(26.1)	1	(0.1)	4	(0.4)
Other (water melon, celery )	40	(1.1)	-	-	-	-
<b>Mixed seed types</b>	<b>350</b>	<b>(9.4)</b>	<b>3</b>	<b>(0.9)</b>	<b>8</b>	<b>(2.3)</b>
2 seed types	57	(1.5)	-	-	1	(1.8)
3 seed types	80	(2.1)	1	(1.3)	4	(5.0)
4 seed types	139	(3.7)	-	-	3	(2.2)
5 seed types	71	(1.9)	2	(2.8)	-	-
>5 seed types	3	(0.1)	-	-	-	-
<b>Total</b>	<b>3735</b>		<b>23</b>	<b>(0.6)</b>	<b>55</b>	<b>(1.5)</b>

### ***Salmonella* subtypes present in edible seeds**

Seventeen different *Salmonella* subtypes were isolated from 23 samples in this study (Table 3). *Salmonella* Drypool was isolated from five separate samples, of

which four were sesame seeds from India and one was a mixture of pumpkin, sunflower and sesame seeds (country of origin unknown). *Salmonella* Unnamed (I 47: z4,z23 :-) was detected in 3 samples, of which two were sesame seeds from India and one was egusi seed (country of origin unknown). Although *S. Agona* and *S. Virchow* were also isolated from multiple samples, no single type was isolated from more than one sample.

Twenty two of the 23 *Salmonella* isolates were sensitive to all antimicrobials tested (Table 3). However, one (*S. Sculcoates*) demonstrated resistance to ampicillin.

Although there is no direct evidence that these contaminated edible seeds were responsible for any cases of human illness, during the period of the study six of the identified subtypes were reported in cases of human infection in England and Wales: *S. Montevideo* (27 cases), *S. Newport* (62), *S. Schwarzengrund* (24), *S. Senftenberg* (14), *S. Tennessee* (8) and *S. Virchow* PT31 (2).

#### ***Escherichia coli levels in edible seeds***

*E. coli* was present at a level of  $\geq 3$  /g in 339 samples (9%). Counts of  $\geq 10^2$  /g (range  $1.0 \times 10^2$  -  $>1.0 \times 10^3$  /g) were detected in 55 samples (1.5%, Table 2). Of these, 23 samples (42%) were pumpkin seed products. Only two samples in which *Salmonella* species were detected also had an *E. coli* count of  $\geq 10^2$  /g (240/g, 1100 /g), whilst in 17 samples contaminated with *Salmonella*, *E. coli* was not detected ( $<3$  /g) ( $p>0.05$ ).

**Table 3: *Salmonella* subtypes isolated from edible dried seed samples**

<i>Salmonella</i> sero / phage type (PT)	Number of samples	Antibiotic Resistance Profile
S. Agona	3	
- PT 3	- 1	Sensitive
- PT UT <sup>a</sup>	- 1	Sensitive
- PT RDNC <sup>b</sup>	- 1	Sensitive
S. Bergen	1	Sensitive
S. Binza	1	Sensitive
S. Chittagong	1	Sensitive
S. Drypool	5	Sensitive
S. Montevideo	1	Sensitive
S. Newport	1	Sensitive
S. Schwarzengrund	1	Sensitive
S. Sculcoates	1	A <sup>c</sup>
S. Senftenberg	1	Sensitive
S. Tennessee	1	Sensitive
S. Unnamed (I 47: z4,z23 :-)	3	Sensitive
S. Unnamed (I 3,10: y :-)	1	Sensitive
S. Virchow	2	
- PT 31	- 1	Sensitive
- PT 51	- 1	Sensitive

<sup>a</sup> UT, Untypeable

<sup>b</sup> RDNC : Reacts with typing phages, but does not conform to a known type

<sup>c</sup> A: Ampicillin resistant

#### ***Relationship between country of origin and microbiological quality of seeds***

The country of origin of seeds sampled is presented in Table 4. Samples were produced in at least 42 countries, with 281 samples containing seeds of more than one country. One fifth (20%; 750/3735) of all samples were produced in China, and 7% (249/3735) in India.

**Table 4: Country of origin of edible dried seeds in relation to the presence of *Salmonella* and elevated levels of *E. coli* (i.e.  $\geq 10^2$  per gram)**

Country	No. of samples N = 3735 (%)		No. with <i>Salmonella</i> detected in 25g (%)	No. with <i>E. coli</i> $\geq 10^2$ /g (%)
<b>EC Countries</b>	<b>490</b>	<b>(13.3)</b>	<b>0</b>	<b>5 (1.0)</b>
Austria	17	(0.5)	-	1 (5.9)
Czech Republic	3	(0.1)	-	-
France	136	(3.6)	-	1 (0.7)
Germany	38	(1.0)	-	-
Greece	6	(0.2)	-	-
Hungary	3	(0.1)	-	-
Italy	16	(0.4)	-	-
Netherlands	79	(2.1)	-	1 (1.3)
Spain	5	(0.1)	-	-
UK	180	(4.8)	-	2 (1.1)
Other EC countries	7	(0.2)	-	-
<b>Non EC Countries</b>	<b>1472</b>	<b>(39.4)</b>	<b>15 (1.0)</b>	<b>22 (1.5)</b>
Africa	25	(0.7)	-	-
Argentina	13	(0.3)	1 (7.7)	1 (7.7)
Australia	16	(0.4)	-	-
Burkina Faso	18	(0.5)	1 (5.6)	-
Canada	8	(0.2)	-	-
China	750	(20.0)	-	8 (1.1)
Egypt	12	(0.3)	-	-
Guatemala	22	(0.6)	1 (4.5)	-
India	249	(6.7)	8 (3.2)	7 (2.8)
Nicaragua	25	(0.7)	-	-
Nigeria	34	(0.9)	1 (2.9)	-
Paraguay	28	(0.7)	-	-
South America	15	(0.4)	-	-
Thailand	8	(0.2)	-	-
Turkey	26	(0.7)	-	-
Uruguay	8	(0.2)	-	-
USA	182	(4.9)	1 (0.5)	2 (1.1)
Venezuela	10	(0.3)	-	-
West Africa	7	(0.2)	2 (28.6)	4 (57.1)
Other non-EC countries	16	(0.4)	-	-
More than one country	281	(7.5)	3 (1.1)	9 (3.2)
Not known	1492	(39.9)	5 (0.3)	19 (1.3)
<b>Total</b>	<b>3735</b>		<b>23 (0.6)</b>	<b>55 (1.5)</b>

*Salmonella* spp. were detected in seeds produced in Argentina, Burkina Faso, Guatemala, India, Nigeria, USA and West Africa (Table 4). *Salmonella* spp. were also detected in three samples which were labelled as produce of more than one country and five for which the country of origin was unknown, but were not detected in any samples originating from individual countries within the European Union (p=0.03).

*E. coli* levels of  $\geq 10^2$  /g were detected in seeds produced in Argentina, Austria, China, France, India, Netherlands, UK, USA and West Africa. Of the seed samples labelled as produce of more than one country, 3.2% contained *E. coli* at  $\geq 10^2$  /g, as did 1.3% of samples of unknown country of origin.

#### ***Relationship between packaging and microbiological quality of seeds***

The majority (97.2%; 3632/3735) of samples collected were pre-packed, with 2.8% (103) of samples being taken from open display containers. All samples contaminated with *Salmonella* and *E. coli* at  $\geq 10^2$  /g were pre-packed.

*Salmonella* spp. were detected in 12 different brands of seed products, with the maximum number of contaminated samples of a single brand being five. However, eight samples of sesame seeds contaminated with *Salmonella* comprised two different brands, both produced in India. No duplication of batch numbers was observed between contaminated samples.

#### ***Association between organic status and microbiological quality of seeds***

Almost one quarter of seed samples (24%; 893/3735) were labelled as organic. There was no significant difference in the proportion of seeds labelled as organic (0.2%; 2/893) or not (0.7%; 21/2842) that were found to be contaminated with *Salmonella*. However, a significantly higher proportion of organically produced seed samples had unsatisfactory levels of *E. coli* ( $\geq 10^2$  /g) (2.4%; 21/893) compared to those that were not (1.2%; 34/2842) (p = 0.017).

### ***Association between premises type and microbiological quality of seeds***

Almost half (46.8%; 1747/3735) of the seeds sampled were collected from health food shops, whilst a further 37.6% (1405/3735) were from supermarkets. The remainder were from convenience shops (5.5%; 206/3735), delicatessens (2.0%; 73/3735), greengrocers (2.3%; 86/3735), market stalls (1.7%; 64/3735) and other premises (4.1%; 154/3735; including farm shops, department stores, cash and carry shops and garden centre food halls).

*Salmonella* spp. were detected in samples from convenience shops (2.4%; 5/206), greengrocers (2.3%; 2/86), health food shops (0.6%; 10/1747) and supermarkets (0.4%; 6/1405). Unsatisfactory *E. coli* levels were found in samples from convenience shops (1.0%; 2/206), greengrocers (2.3%; 2/86), health food shops (1.4%; 24/1747), supermarkets (1.8%; 25/1405) and other premises (1.3%; 2/154).

### **Discussion**

Products of plant origin are increasingly being associated with outbreaks of infection of both *Salmonella* and other pathogens (Little and Gillespie, 2008; Sivapalasingam et al., 2004). There are many opportunities for microbial contamination of these products pre-, during and post-harvest, and in many cases, the products are consumed raw or following minimal processing. In the case of edible dried seeds, these are frequently eaten as snack foods or incorporated into meals without further cooking. Results from this study have demonstrated that, whilst the vast majority of edible dried seeds sampled at retail were of satisfactory or acceptable microbiological quality (98.0%; 3659/3735), a small proportion of samples (0.6%) were contaminated with *Salmonella* spp.; this is unacceptable. Any ready-to-eat foods contaminated with *Salmonella* spp. are of unacceptable risk and unsafe. They are considered to be injurious to health and/or unfit for human consumption and they therefore contravene the food safety requirements (Article 14) of Regulation (EC) No.178/2002 (European Commission, 2002). Where *Salmonella* was detected

from samples examined as part of this study, the UK retailers affected publicly recalled the contaminated batches and full investigations were undertaken. A number of Food Standard Agency food alerts were also issued, advising consumers not to eat the affected batches (Food Standards Agency, 2007a; 2007b; 2007c; 2007d; 2007e; 2007f; 2008a; 2008b; 2008c; 2008d; 2008e).

The recent international outbreaks of salmonellosis have demonstrated that major health problems can arise from consumption of contaminated ready-to-eat sesame seed products if hygiene practices break down (de Jong et al. 2001, Unicomb et al. 2005). The prevalence of *Salmonella* spp. in sesame seeds in the UK in 2007/8 (1.7%) was significantly lower than that previously found in Germany in 2001 (9.4%) (Brockmann et al. 2004) ( $p < 0.0001$ ) and in England in 2006 (2.5%) (S Surman-Lee, HPA, personal communication) ( $p = 0.0144$ ). Where *Salmonella* was enumerated from seeds in this study, counts were low (all  $< 1$  per gram). However, small numbers (ranging from  $< 0.03$  to 0.46 organisms per gram) of salmonellae in a sesame-seed product (tahini) were reported to cause a large outbreak of salmonellosis in Australia and New Zealand (Unicomb et al., 2005). This indicates that even low doses of *Salmonella* in these types of product may lead to infection.

The most frequently contaminated seed type was melon seeds, also known as egusi. Eight and a half percent of the melon seeds tested contained *Salmonella* and 12.8% contained unsatisfactorily high levels of *E. coli* (i.e.  $\geq 10^2$  /g). In contrast, 1.7% of both sesame and alfalfa seeds were contaminated with *Salmonella*, whilst 1% of sesame seeds and none of the alfalfa seeds examined contained unsatisfactory *E. coli* levels. Although labelling on some of the packs of melon seeds included instructions to cook thoroughly, this seed type was included in the study as the cooking instructions did not include specific time and temperature directions, and it was considered that the seeds might be eaten with no or inadequate heat treatment. It is interesting to note that in 2007 the EC was notified of 17 batches of melon seeds (all from Nigeria) contaminated with aflatoxins (European Commission,

2008). Aflatoxins are a further indicator of poor control of microbial growth during production and storage of these seeds.

*Escherichia coli* was included in this study as it is traditionally considered to be a useful marker of faecal contamination of food products, and therefore indicates the potential risk of contamination with faecal pathogens (Roberts and Greenwood, 2003). However, results obtained in this study indicate that there was no significant association between an elevated *E. coli* count and the detection of *Salmonella* in seeds. This finding is supported by a study carried out in the United States in 1977 (Andrews et al., 1979), which involved the microbiological analysis of 1,960 samples of “health foods” including seeds. *Salmonella* was detected in four packs of sunflower seeds of a single brand and a single sample of alfalfa seeds. In the sunflower seed samples, faecal coliforms were not detected, and in the alfalfa seeds, the faecal coliform count (Most Probable Number) was 0.4 per gram. Moreover, a recent LACORS/HPA study of fresh herbs also found that *E. coli* was not a reliable indicator for *Salmonella* presence (Elviss et al., 2008).

The contaminated seed samples in this study were from several different producers, indicating that the results were not simply due to hygiene lapses by a single producer, but rather that contamination problems may be relatively widespread throughout the industry and in many different countries. These results highlight the importance of good hygiene practices at all stages of production in order to avoid contamination of the final product with pathogenic bacteria. However, there is little published guidance on good hygiene practices for use by the seed-producing industry.

Critical points for controlling the microbiological quality of edible seeds appear to be the cleaning and drying processes. The method and speed of drying seeds are variable, with seeds such as pumpkin and melon frequently being laid out on the ground to dry in the sun, and sesame and sunflower seeds being dried by means of a hot air stream whilst being agitated mechanically (Bankole et al., 2005; Salunkhe et

al., 1992). Where appropriate, a heat treatment as part of the drying process is desirable and, in some instances, is insisted on by purchasers. In one study, optimum drying of pumpkin seeds was found to be achieved by an airflow speed of 0.8 m/s and an air temperature of 60°C (Sito et al. , 1999). Temperatures of 80 or 100°C impaired the quality of the seeds. However, if a temperature of 60°C were to be used, a holding time of several minutes would be required to ensure a significant reduction in numbers of pathogenic organisms. For example, at 65°C, it has been demonstrated that numbers of *Salmonella* can be reduced by 1 log per minute, and therefore a 10 minute heat treatment would ensure a 10 log reduction in counts (Adams and Moss, 1995).

This study has highlighted the potential public health risk associated with the consumption of edible dried seeds, due to contamination with *Salmonella* spp., and has drawn attention to the requirement for clearer guidance on good hygiene practices throughout all stages of seed production, pre-, during and post-harvest. Such guidance would aid purchasers and suppliers of seed products in ensuring that appropriate practices are put in place by producers worldwide.

### **Acknowledgements**

The authors would like to thank the staff in Environmental Health Departments throughout the UK and staff in the HPA laboratories and other Official Food Control Laboratories for their contributions to this study. Thanks are extended to LEP, HPA Centre for Infections for typing *Salmonella* isolates, to Gemma Cantelo at LACORS for co-ordinating the participation of Environmental Health Practitioners and advice from the LACORS Food Examination Focus Group and Food Hygiene Focus Group, to the HPA Regional Food, Water and Environmental Co-coordinators Forum for their contribution and advice on the sampling protocols of this study, and to Lillian Hucklesby for co-ordinating data entry and validation.

## References

- Adams, M. and Moss, M. (1995). Food Microbiology. Royal Society of Chemistry, Cambridge.
- Andrews, W., Wilson, C., Poelma, P., Romero, A. and Mislivec, P. (1979). Bacteriological survey of sixty health foods. *Applied and Environmental Microbiology* **37**, 559-566.
- Bale, J., de Pinna, E., Threlfall, E. J. and Ward, L. (2007). Kauffmann-White Scheme - 2007: Salmonella identification - Serotypes and antigenic formulae. Health Protection Agency, London.
- Bankole, S., Osho, A., Joda, A. and Enikuomehin, O. (2005). Effect of drying method on the quality and storability of 'egusi' melon seeds (*Colocynthis citrullus* L.). *African Journal of Biotechnology* **4**, 799-803.
- Brockmann, S., Piechotowski, I. and Kimmig, P. (2004). Salmonella in sesame seed products. *Journal of Food Protection* **67**, 178-180.
- Chambers, R., McAdam, P., de Sa, J., Ward, L. and Rowe, B. (1987). A phage-typing scheme for *Salmonella virchow*. *FEMS Microbiology Letters* **40**, 155-157.
- Cooney, R., Custer, L., Okinaka, L. and Franke, A. (2001). Effects of dietary sesame seeds on plasma tocopherol levels. *Nutrition and Cancer* **39**, 66-71.
- de Jong, B., Andersson, Y., Giesecke, J., Hellstrom, L., Stamer, U. and Wollin, R. (2001). Salmonella Typhimurium outbreak in Sweden from contaminated jars of helva (or halva). *Eurosurveillance Weekly* **5**, 1715.
- Doyle, M. and Erickson, M. (2008). Summer meeting 2007 - the problems with fresh produce: an overview. *Journal of Applied Microbiology* **105**, 317-330.
- Elviss, N., Little, C., Hucklesby, L., Sagoo, S., Surman-Lee, S., de Pinna, E. and Threlfall, E. J. (2008). LACORS/HPA Co-ordinated Food Liaison Group Studies: Microbiological Assessment of Fresh Herbs from Retail Premises in the United Kingdom Uncovers an International Outbreak of Salmonellosis. *International Journal of Food Microbiology*, Submitted August 2008.
- Emberland, K., Etherlberg, S., Kuusi, M., Vold, L., Jensvoll, L., Lindstedt, B., Nygard, K., Torpdahl, M., Sorensen, G., Jensen, T., Lukinmaa, S., Niskanen, T. and Kapperud, G. (2007). Outbreak of Salmonella Weltevreden infections in Norway, Denmark and Finland associated with alfalfa sprouts, July-October 2007. *Eurosurveillance* **12**, 3321.
- European Commission. (2002). Regulation (EC) No 178/2002 of the European Parliament and of the council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. *Official Journal of the European Communities* **L 31 1.2.2002**, 1-24.
- European Commission. (2008). The Rapid Alert System for Food and Feed (RASFF) Annual Report 2007. European Communities, Luxembourg.
- Food Standards Agency. (2006). Food Law Code of Practice. FSA, London., Available at: [www.food.gov.uk/enforcement/foodlaw/foodlawcop/copengland](http://www.food.gov.uk/enforcement/foodlaw/foodlawcop/copengland); Accessed 20 July 2008.
- Food Standards Agency. (2007a). The Health Store recalls certain batches of their own brand sesame seeds due to possible salmonella contamination, 9 November 2007. Ref 38/2007. Available at: <http://www.food.gov.uk/enforcement/alerts/2007/nov/healthstoresalmonella>.
- Food Standards Agency. (2007b). Holland and Barrett withdraws a batch of their Nature's Harvest Sesame Seeds product due to *Salmonella* contamination, 12 October 2007. Ref 31/2007. Available at: <http://www.food.gov.uk/enforcement/alerts/2007/oct/hollandbarrett>.
- Food Standards Agency. (2007c). Tesco recalls a batch of its Natural Seed Mix product due to salmonella contamination, 31 October 2007. Ref 35/2007.

- Available at:  
<http://www.food.gov.uk/enforcement/alerts/2007/oct/tescoseedmix>.
- Food Standards Agency. (2007d). Tesco recalls certain batch codes of its wholefood seed mix and wholefood savoury seed mix products due to salmonella contamination, 22 November 2007. Ref 42/2007. Available at:  
<http://www.food.gov.uk/enforcement/alerts/2007/nov/savouryseedrecall>.
- Food Standards Agency. (2007e). TRS Wholesale Co. Limited recalls two batches of its sesame seed product due to salmonella contamination, 21 December 2007. Ref 49/2007. Available at:  
<http://www.food.gov.uk/enforcement/alerts/2007/dec/trsseeds>.
- Food Standards Agency. (2007f). Waitrose recalls certain date codes of its Wholesome Mixed Seeds due to salmonella contamination, 21 November 2007. Ref 41/2007. Available at:  
<http://www.food.gov.uk/enforcement/alerts/2007/nov/waitroseseeds>.
- Food Standards Agency. (2008a). Imperial Snack Foods Ltd recalls a batch of its premier quality seed mix due to salmonella contamination, 22 February 2008. Ref 06/2008. Available at:  
<http://www.food.gov.uk/enforcement/alerts/2008/feb/imperial>.
- Food Standards Agency. (2008b). Julian Graves Ltd recalls a batch of sesame seeds due to possible salmonella contamination, 29 February 2008. Ref 08/2008. Available at: <http://www.food.gov.uk/enforcement/alerts/2008/feb/graves>.
- Food Standards Agency. (2008c). Suma Wholefoods recalls certain batches of its organic sesame omega mixed seeds due to salmonella contamination, 4 March 2008. Ref 09/2008. Available at:  
<http://www.food.gov.uk/enforcement/alerts/2008/mar/suma>.
- Food Standards Agency. (2008d). TRS Wholesale Co. Limited recalls a batch of its sesame seed product due to salmonella contamination, 11 March 2008. Ref 12/2008. Available at:  
<http://www.food.gov.uk/enforcement/alerts/2008/mar/trssesame>.
- Food Standards Agency. (2008e). Waitrose Ltd recalls its own brand alfalfa seeds due to presence of salmonella, 4 April 2008. Ref 16/2008. Available at:  
<http://www.food.gov.uk/enforcement/alerts/2008/apr/alfalfa>.
- Frost, J. (1994). In *Methods in Practical Laboratory Bacteriology* (H. Chart, ed.), pp. 73-82. CRC Press, New York.
- Health Protection Agency. (2005). Enumeration of beta-glucuronidase positive *Escherichia coli* – Most Probable Number method. National Standard Method F 22; [http://www.hpastandardmethods.org.uk/pdf\\_sops.asp](http://www.hpastandardmethods.org.uk/pdf_sops.asp).
- Health Protection Agency. (2008). Detection of *Salmonella* species. National Standard Method F13; [http://www.hpa-standardmethods.org.uk/pdf\\_sops.asp](http://www.hpa-standardmethods.org.uk/pdf_sops.asp).
- International Organisation for Standardisation. (2007). ISO/FDIS 7218: 2007. Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations. ISO, Brussels.
- Koch, J., Schrauder, A., Alpers, K., Werber, D., Frank, C., Prager, R., Rabsch, W., Broll, S., Feil, F., Roggentin, P., Bockemuhl, J., Tschape, H., Ammon, A. and Stark, K. (2005). *Salmonella* Agona outbreak from contaminated aniseed, Germany. *Emerging Infectious Diseases* **11**, 1124-1127.
- Little, C. and Gillespie, I. (2008). Prepared salads and public health. *Journal of Applied Microbiology*, Published article online: 4 Apr 2008 doi: 10.1111/j.1365-2672.2008.03801.x.
- Local Authorities Co-ordinators of Regulatory Services. (2006). LACORS Guidance on Food Sampling for Microbiological Examination; Issue 2. Available at: [www.lacors.com](http://www.lacors.com); Accessed 20 July 2008.
- O'Grady, K., 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; No. 20010822.1980. Available at: [www.promedmail.org](http://www.promedmail.org) Accessed 20 July 2008.
- Ollis, T., Meyer, B. and Howe, P. (1999). Australian food sources and intakes of omega-6 and omega-3 polyunsaturated fatty acids. *Annals of Nutrition and Metabolism* **43**, 346-355.
- PHLS. (2000). Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. *Communicable Disease and Public Health* **3**, 163-167.
- Pierce, R. (1970). Sunflower processing techniques. *Journal of American Oil Chemists Society* **47**, 248A-269A.
- Popoff, M. and Le Minor, L. (2001). Antigenic formulas of the Salmonella serovars, 8th edition. WHO collaborating centre for reference and research on Salmonella, Institut Pasteur, Paris.
- Proctor, M., Hamacher, M., Tortorello, M., Archer, J. and Davis, J. (2001). Multistate outbreak of Salmonella serovar Muenchen infections associated with alfalfa sprouts grown from seeds pretreated with calcium hypochlorite. *Journal of Clinical Microbiology* **39**, 3461-3465.
- Rabsch, W., Prager, R., Koch, J., Stark, K., Roggentin, P., Bockemuhl, J., Beckmann, G., Stark, R., Siegl, W., Ammon, A. and Tschape, H. (2005). Molecular epidemiology of *Salmonella* enterica serovar Agona: characterization of a diffuse outbreak caused by aniseed-fennel-caraway infusion. *Epidemiology and Infection* **133**, 837-844.
- Roberts, D. and Greenwood, M. (2003). Practical Food Microbiology. Blackwell Publishing Ltd.
- Salunkhe, D., Chavan, J., Adsule, R. and Kadam, S. (1992). World Oilseeds: Chemistry, Technology and Utilization. Springer.
- Sito, S., Barcic, J. and Ivancan, S. (1999). Effect of different airflow speeds upon the duration of the drying process of high moisture pumpkin seeds. *Agriculturae Conspectus Scientificus* **64**, 133-136.
- Sivapalasingam, S., Friedman, C., Cohen, L. and Tauxe, R. (2004). Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *Journal of Food Protection* **67**, 2342-2353.
- Unicomb, L., Simmons, G., Merritt, T., Gregory, J., Nicol, C., Jelfs, P., Kirk, M., Tan, A., Thomson, R., Adamopoulos, J., Little, C., Currie, A. and Dalton, C. (2005). Sesame seed products contaminated with *Salmonella*: three outbreaks associated with tahini. *Epidemiology and Infection* **133**, 1065-1072.
- Van Beneden, C., Keene, W., Strang, R., Werker, D., King, A., Mahon, B., Hedberg, K., Bell, A., Kelly, M., Balan, V., MacKenzie, W. and Fleming, D. (1999). Multinational outbreak of *Salmonella* enterica serotype Newport infections due to contaminated alfalfa sprouts. *JAMA* **281**, 158-162.
- Werner, S., Boman, K., Einemo, I., Erntell, M., Helisola, R., de Jong, B., Lindqvist, A., Lofdahl, S., Meeuwisse, A., Ohlen, G., Olsson, M., Persson, I., Runehagen, A., Rydevik, G., Stamer, U., Sellstrom, E. and Andersson, Y. (2007). Outbreak of *Salmonella* Stanley in Sweden associated with alfalfa sprouts, July-August 2007. *Eurosurveillance* **12**, 3291.
- Winthrop, K. L., Palumbo, M. S., Farrar, J. A., Mohle-Boetani, J. C., Abbott, S., Beatty, M. E., Inami, G. and Werner, S. B. (2003). Alfalfa sprouts and *Salmonella* Kottbus infection: a multistate outbreak following inadequate seed disinfection with heat and chlorine. *Journal of Food Protection* **66**, 13-17.
- Wittenberg, M.M. (2007). Nuts and Seeds. In *New Good Foods: Essential Ingredients for Cooking and Eating Well*. pp. 141-166. Ed. Wittenberg, M.M. Ten Speed Press, Canada.
- Yu, L., Zhou, K. and Parry, J. (2005). Antioxidant properties of cold-pressed black caraway, cranberry and hemp seed oils. *Food Chemistry* **91**, 723-729.

**Annex 1: Participating Laboratories and Local Authority Food Liaison Groups and number of samples**

**Table 1a. Participating HPA and HPA Collaborating Laboratories and number of samples**

<b>HPA Region</b>	<b>HPA/HPA Collaborating Laboratory</b>	<b>Number of samples</b>
East	Chelmsford	126
	Norwich	117
East Midlands	Leicester	90
	Lincoln	104
London	London FWEM <sup>1</sup>	224
South East	Ashford	169
	Haywards Heath	276
	WEMS <sup>2</sup>	407
North East and Yorkshire and the Humber	Leeds	171
	Newcastle	127
North West	Sheffield	164
	Carlisle	35
South West	Chester	287
	Preston	441
	Bristol	47
	Exeter	91
	Gloucester	35
West Midlands	Plymouth	84
	Truro	34
	Birmingham	49
	Coventry	117
	Shrewsbury	152
	Stoke on Trent	40
	Hereford	22
<b>Total</b>		<b>3409</b>

<sup>1</sup> London Food, Water & Environmental Microbiology Services Laboratory

<sup>2</sup> Wessex Environmental Microbiology Services

**Table 1b. Other participating Official Food Control Laboratories in Wales, Scotland, Northern Ireland & England and number of samples examined**

<b>Country</b>	<b>Laboratory</b>	<b>Number of samples</b>
Wales	Bangor	47
	Cardiff	89
	NPHS-W Microbiology Rhyl	63
Ireland	Belfast City Hospital	71
Scotland	Aberdeen City Council Public Analysts	13
	Edinburgh Analytical and Scientific Services	12
	Glasgow Scientific Services	22
England	Kings Lynn & West Norfolk	9
<b>Total</b>		<b>326</b>

**Table II: Participating Food Safety Liaison Groups and number of samples**

<b>Local Authority Food Liaison Group</b>	<b>Number of Samples</b>
Berkshire	72
Buckinghamshire	53
Cambridgeshire	74
Cheshire	101
Cornwall	34
Cumbria	40
Derbyshire	72
Devon	131
Dorset	42
Durham	36
East Sussex	99
Essex	70
Gloucestershire	35
LFCG <sup>1</sup> Greater London NE Sector	52
LFCG Greater London NW Sector	68
LFCG Greater London SE Sector	20
LFCG Greater London SW Sector	15
Greater Manchester	157
Hampshire & Isle Of Wight	122
Hereford & Worcester	28
Hertfordshire & Bedfordshire	58
Humberside/North Lincoln	75
Kent	169
Lancashire	271
Leicestershire	90
Lincolnshire	47
Merseyside	161
North Yorkshire	51
Northamptonshire	57
Northern Ireland Food Group <sup>2</sup>	71
Norfolk	74
Nottinghamshire	57
Northumberland	16
Oxfordshire	115
Scottish Food Enforcement Liaison Committee <sup>3</sup>	48
Shropshire	98
Somerset	48
South East Wales	89
South West Wales	11
South West Yorkshire	140
Staffordshire	45
Suffolk	26
Surrey	111
Tees Valley	43
Tyne & Wear	29
Warwickshire	68
West Midlands	81
West of England	38
West Sussex	88
Wiltshire	5
<b>Total</b>	<b>3735</b>

<sup>1</sup> London Food Co-ordinating Group

<sup>2</sup> Northern Ireland Food Group consists of Eastern, Northern, Southern & Western Groups

<sup>3</sup> SFELG consists of Central Scotland, Lothian & Scottish Borders, North Scotland, East of Scotland and West of Scotland

Annex 2

LACORS/HPA RTE Dried Seeds Study with a Focus on *Salmonella* spp. : October 2007 – March 2008

LABORATORY NAME.....

Laboratory Sample Number.....

Annex 3



**Study 32: LACORS/HPA CO-ORDINATED FOOD LIASION GROUP STUDY: QUESTIONNAIRE**



**LACORS/HPA Microbiological Examination of Ready-to-Eat Dried Seeds from Retail Premises with a Focus on *Salmonella* spp**

All information must be correctly and clearly entered on the form using black ink to facilitate clear photocopying

1. Local Authority..... 2. Food Liaison group.....  
 3. Samplers Name..... & contact number:..... 4. Sample collected at..... (time) on (date) .../.../...  
 5. LA Premises Reference Number..... 6. LA Sample Reference Number(s).....

**Premises details:**

7. Name of premises.....  
 8. Address..... Postcode.....  
 9. Type of Premises: Supermarket  Greengrocers  Convenience shop  Health food shop   
 Delicatessen  Market stall  Other  (Specify).....

**Sample Details:**

10. Are the dried seeds made up of a: Single seed type  Go to Q11, then Q14  
 Mixed seed types  Go to Q12  
 11. Please indicate the type of single seed (tick one): Pumpkin  Sunflower  Sesame  Linseed (flax)   
 Melon (Egusi)  Hemp  Poppy  Other  (specify).....  
 12. Please specify the number of seed types in the seed mix: 2  3  4  5  >5   
 13. Please indicate the type of seeds within the mix (tick all applicable): Pumpkin  Sunflower  Sesame  Linseed (flax)   
 Melon (Egusi)  Hemp  Poppy  Other  (specify).....  
 14. Were the dried seeds: Pre- Packed  Open (e.g. from display container) Go to Q16  Other  (Specify).....  
 15. What is the pack size: <50g  (specify weight).....  
 (Note: In the case of packaged product more than 1 pack from the same batch may be needed to make up the necessary 50 g) 50-<100g  (specify weight).....  
 ≥100g  (specify weight).....

**Product Details:**

16. Specify (where available) Product Name exactly as it appears on the label/container/ display card:.....  
 17. Brand/ Producer (specify):..... Not known   
 18. Product Date (specify): Best before date .../.../... Not Known   
 19. Is the product labelled as organic? YES  NO   
 20. What is the country of origin?..... Not known   
 21. Is there a batch code? YES  NO ; If YES please specify code.....

COPY TO: Laboratories to retain a copy of the questionnaire for the purposes of validating data within the Excel Workbook Page 1 of 2

LACORS/HPA RTE Dried Seeds Study with a Focus on *Salmonella* spp. : October 2007 – March 2008

LABORATORY NAME.....

Laboratory Sample Number.....

Annex 3

**RESULTS**

**Recording results**

Please record the results of the *E. coli* count/g test as **ACTUAL NUMBERS** in the appropriate box within the table.  
Only place ticks columns headed ND (Not Detected) and Detected for the *Salmonella* test results.

Laboratory Sample No.....

	ND	Detected	>3 /<20	20-<10 <sup>2</sup>	10 <sup>2</sup> -<10 <sup>3</sup>	10 <sup>3</sup> -<10 <sup>4</sup>
<i>Escherichia coli</i> / g						
<i>Salmonella</i> spp. 25/g*						

Microbiological Quality: Satisfactory  Acceptable  Unsatisfactory  Unacceptable/Potentially hazardous

*\*Salmonella spp. detected in RTE dried seeds are of unacceptable quality using published HPA (PHLS) public health Guidelines and are also covered by Regulation (EC) No. 178/2002 (General Food Law Regulation)*

Date *Salmonella* isolates sent to the Laboratory of Enteric Pathogens, HPA Centre for Infections or in Scotland to the Scottish Reference Laboratory (Stobhill Hospital Glasgow) .....

MICROBIOLOGISTS COMMENTS.....

Signature ..... Date reported .....

**WHERE POSSIBLE THE LABORATORY SHOULD TAKE A DIGITAL PHOTO OR MAKE AND RETAIN A PHOTOCOPY OF THE PRODUCT LABEL FOR FUTURE REFERENCE OR RETAIN THE REMAINING PRODUCT UNTIL THE END OF THE STUDY**

Methods as defined in LACORS/HPA RTE Dried Seeds (Study 32); Annex 4

COPY TO: Laboratories to retain a copy of the questionnaire for the purposes of validating data within the Excel Workbook  
Page 2 of 2