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Hazard identification and characterisation, and dose response assessment of spore forming pathogens in cooked chilled food containing vegetables

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Abstract

A hazard identification and characterisation, including a preliminary dose response assessment, of sporeforming pathogens in cooked chilled food containing vegetables was performed according to the structure and principles for a quantitative microbiological risk assessment as described by the Codex Alimentarius Commission.

In cooked chilled food containing vegetables *Clostridium (C.) botulinum* group II and *Bacillus (B.) cereus* were identified and characterised as a hazard with a high risk. *C. botulinum* group I was identified as a hazard with a median risk, only at refrigerated storage with mild temperature abuse. *B. subtilis* and related species, and *C. perfringens* were identified and characterised as hazards with a low risk in these type of products.

A relation between exposure to these organisms and the occurrence of adverse health effects could be determined, but not be quantified mainly due to incomplete and no standardised registration of these adverse health effects, and the inability to assess the involved dose of organisms and/or toxic substances.

At a concentration of botulinal toxin of 0.06 $ng/kg_{bodyweight}$ adverse health effects were found. A “safe” dose of botulinal toxin of 0.004-0.008 $ng/kg_{bodyweight}$ was estimated.

Preface

This report describes the hazard identification and hazard characterisation, including a dose response assessment, of spore-forming pathogenic bacteria in cooked chilled foods containing vegetables. Both, the hazard identification as well as the hazard characterisation, are steps in the risk assessments and were part of a project “Research on factors allowing a risk assessment of spore-forming bacteria in cooked chilled foods containing vegetables (RASP)”, which at RIVM is classified under project 149106 “Quantitative aspects of pathogens in food”. The RASP project has been carried out with financial support from the Commission of the European Communities, Agriculture and Fisheries (FAIR) specific RTD programme, CT97-3159. It does not necessarily reflect its view and in no way anticipates the Commission’s future policy in this area.
The author wishes to thank the participants in the RASP project for providing additional literature for and fruitful comments on the above mentioned steps in the risk assessment.

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Samenvatting

In dit rapport wordt de gevaren identificatie en gevaren karakterisering, inclusief een summiere dosis respons schatting, beschreven voor sporenvormende pathogene bacteriën (SPB) in gekookte voeding, welke als hoofdbestanddeel groente bevat en gekoeld moet worden bewaard.
Clostridium (C.) botulinum (inclusief *C. butyricum), C. perfringens, Bacillus (B.) cereus* en *B. subtilis* en verwante species moeten worden beschouwd als een potentieel gevaar in bovengenoemde producten. Deze gevolgtrekking is gebaseerd op het voorkomen van SPB op of in groente, ten gevolge van besmetting vanuit het milieu gedurende groei, en bewijs van het vermogen van de SPB om voedsel gerelateerde ziekte te veroorzaken in de humane bevolking.

Indien ook aanvullende beperkingen en omstandigheden, zoals het vermogen om te kunnen groeien gedurende opslag bij een normale koelkasttemperatuur (4 ^{o}C) of bij een iets te hoge koelkasttemperatuur (10 ^{o}C), aantallen explosies per jaar, aantal ziektegevallen per jaar, mate van fataal zijn van de ziekte, en een relatie met explosies door voedsel dat groente bevat, mee worden genomen dan zijn *C. botulinum* van groep II en *B. cereus* gevaren met een hoog risico in gekookte voeding, welke als hoofdbestanddeel groente bevat en gekoeld moet worden bewaard. *C. botulinum* van groep I vormt dan een gemiddeld risico bij bewaring bij een te hoge koelkasttemperatuur. *B. subtilis* en verwante species en *C. perfringens* geven een laag risico in dit type producten.

Er is een duidelijke relatie tussen blootstelling aan deze organismen en gezondheidseffecten. Deze effecten kunnen varieren van milde gastro-intestinale klachten tot ernstige neurologische afwijkingen. Een kwantitatieve relatie tussen blootstelling aan een bepaalde concentratie van de SPB of haar toxine en bepaalde effecten die de gezondheid schaden kon niet worden vastgesteld. Dit is voornamelijk toe te schrijven aan incomplete en niet gestandaardiseerde beschrijving van de klinische verschijnselen en het onvermogen om de dosis van organismen en/of toxinen waaraan de patient is blootgesteld te kunnen vaststellen.

Op grond van gegevens bij het therapeutisch gebruik van *C. botulinum* toxine als spierverslapper bij de mens, is een poging gedaan een "veilige" dosis te bereken die in voedsel zou mogen voorkomen. Deze "veilige" dosis van *C. botulinum* toxine is geschat op 0,004-0,008 ng/kg $_{\text{lichaamsgewicht}}$.

Summary

This report describes the hazard identification and characterisation, including a preliminary dose response assessment, of sporeforming pathogens (SFP) in cooked chilled food containing vegetables as the main ingredient.

Clostridium (C.) botulinum (including *C. butyricum), C. perfringens, Bacillus (B.) cereus* and *B. subtilis* and related species, have to be considered as a potential hazard in the above mentioned products. This conclusion is based on the prevalence of SFP on/in vegetables, due to a contamination from the environment during growth, and evidence of a potential of the SFP to cause foodborne illness in the human population.

Looking at additional limitations or conditions, such as growth potency during storage at refrigeration temperature (4 °C) or at mildly improper cooled storage (10 °C), number of outbreaks per year, cases of illness per year, case fatality rate and the relation with outbreaks from vegetable products, *C. botulinum* group II and *B. cereus* are hazards with a high risk in cooked chilled food containing vegetables stored under proper refrigeration temperature. C. *botulinum* group I forms a medium risk, only during storage with mild temperature abuse. *B. subtilis* and related species, and *C. perfringens* are of low risk in these type of products. There is an evident relation between exposure to these organisms and adverse health effects, ranging from mild gastro-intestinal complaints to severe neurological symptoms. A quantitative relation between a certain concentration of the SFP or its toxin, and adverse health effects could not be determined. This is mainly due to incomplete and not standardised registration of the adverse health effects and the inability to assess the involved dose of organisms and/or toxic substances.

Based on data from therapeutic use of *C. botulinum* toxin in relaxation of muscles in man, an attempt was made to calculate a “safe” dose of toxin in food. This “safe” dose of *C. botulinum* toxin was estimated at 0.004-0.008 ng/kg $_{\text{bodyweight}}$.

1 Introduction

Today's consumer tends more and more to the use of ready to use or eat food products, like cooked chilled foods. This is confirmed by a dramatic increase in the production and sales of ready-to-use food stored at low temperature. To preserve taste and freshness cooked chilled foods are mildly heat-treated and rely on refrigeration for preservation. Spore-forming pathogens (SFP), like *Clostridium (C.) botulinum*, *C. perfringens* and *Bacillus (B.) cereus* can survive this mild heat treatment. The psychrotrophic strains have opportunities for growth despite chilled storage and the mesophilic strains have also opportunities for growth when there is some temperature abuse during the period of chilled storage.

Vegetables can form a substantial ingredient in cooked chilled foods and can be contaminated with SFP from natural sources like soil, fertiliser, etc. So, cooked chilled foods containing vegetables can harbour a risk due to the SFP. The objective of the FAIR-CT97-3159 project is to evaluate this risk in doing a formal quantitative microbial risk assessment.

1.1 Outline

Quantitative microbiological risk assessment (QMRA) is the scientific evaluation of known or potential adverse health effects resulting from human exposure to, within the RASP project, foodborne microbial hazards. It was decided to perform the QMRA according to the structure and principles as described by the Codex Alimentarius Commission [32]. The QMRA includes four components: hazard identification, hazard characterisation, exposure assessment and risk characterisation. The component hazard characterisation can include a dose-response assessment when data are available and obtainable.

This report covers the hazard identification and characterisation of SFP in cooked chilled food containing vegetables, and the dose response assessment for the identified hazards. Data for these components were extracted from literature review. The extracted information was sent to the other participants in the RASP project, mainly involved in the exposure assessment, to complete it.

2 Hazard identification and hazard characterisation

2.1 Introduction

In this project the hazard identification and hazard characterisation fit in the framework of the assessment of the risk from exposure to a defined product. In this case the question of interest is which pathogen the product may transmit. In such circumstances microbiological and epidemiological data have to be available to determine which pathogens have been, or potentially could be, associated with the product. This is also the case for information on the occurrence and levels of the pathogenic organisms in the product of concern. Expert systems to support the hazard identification with respect to food products are very helpful.
For example Van Gerwen *et al.* (1998) [48] proposed a stepwise procedure that starts simple before going into detail of a quantitative risk assessment (QRA). Product and process specifications, for example, limit the width of the hazard identification and characterisation. The products studied in this QRA are cooked chilled foods containing vegetables as the main ingredient. Cooking of the product exclude all non spore-forming organisms from the QRA. So, storage of the product after production at refrigeration temperature could narrow the spore-forming pathogens (SFP) to those able to multiply by these temperatures. But, because of conceivable temperature abuse during storage those SFP with growth potency at 10-15 ^{o}C, were included in the QRA too. Likewise, the raw vegetables have to be contaminated with SFP.

2.2 Outline literature review

The above mentioned limitations were the basic principle for the literature review. The electronic databases Medline and Toxline (U.S. department of Health and Human Services: Public Health Service: National Library of Medicine, 8600 Rockville Pike, Bethesda, MD 20894, USA), Current Contents (Institute for Scientific Information, 3501 Marked Street, Philadelphia, PA 19104, USA) and FSTA (International Food Information Service, Lane End House, Shinfield, Reading RG2 9BB, UK) were searched for SFP with the potency to cause foodborne diseases. The most important searchstrings were: “Bacillus or Clostridium” in combination with “food or fruit or vegetable” or “outbreak or poisoning or intoxication or botulism” or “contamination or incidence or occurrence or presence” or “toxicity or pathogenicity”. Information extracted from the collected literature was included in the QRA when it concerned 1) original data (no citations); 2) a relation or a possible translation to vegetables or fruit and 3) traceable information (description of methods, calculations etc.).

The definition of hazard identification and hazard characterisation according to the principles and guidelines for the application of microbiological risk assessment of the Codex Alimentarius Commission [32] are as follows:

Hazard identification: The identification of biological agents capable of causing adverse health effects and which may be present in a particular food or group of foods;

Hazard characterisation: The qualitative and/or quantitative evaluation of the nature of adverse health effects associated with biological agents which may be present in food. For biological agents, a dose-response assessment should be performed if data are obtainable.

Following these definitions and the limitations described in 2.1. the selected literature is reviewed for the following domains of interest:

1. prevalence of SFP in the environment;
2. incidence of SFP on vegetables;
3. prevalence of food-borne outbreaks of toxico-infections and intoxications caused by SFP and;
4. food-borne outbreaks of toxico-infections and intoxications caused by SFP and adverse health effects.

2.3 Results

2.3.1 Prevalence of SFP in the environment

Drowbniewski [40] showed that *Bacillus (B.) anthracis, B. cereus, B. licheniformis, B. pumilis, B. subtilis and B. thuringiensis* could be isolated from the environment as well as from food (see table 1).

Table 1: Non-clinical sources of isolation of Bacilli

	Isolated from		
Species	**Environment**	**Food**	**Reference**
B. anthracis	+	+	40
B. cereus	+	+	40
B. licheniformis	+	+	40
B. pumilus	+	+	40
B. subtilis	+	+	40
B. thuringiensis	+	+	40

B. anthracis is considered as zoonotic. Foodborne infections by *B. anthracis* are mainly due to the consumption of meat from infected animals.

Hobbs *et al.* [57], reviewed literature for the prevalence of Clostridia in faeces, soil and water, marine sediments and food. Only, *Clostridium (C.) botulinum, C. butyricum, C. difficile, C. perfringens* and *C. sporogenes* were found in faeces, soil and water, and food (see table 2).

Table 2: Non-clinical sources of isolation of Clostridia

Species	Isolated from: Faeces	Soil, water	Marine sediment	Food	Reference
C. bifermentans	+	+	+		57
C. botulinum	+	+	+	+	57
C. butyricum	+	+		+	57
C. carnis		+			57
C. chauvoei	+				57
C. colinum	+				57
C. difficile	+	+		+	57 3
C. fallax		+			57
C. histolyticum		+			57
C. novyi	+	+	+		57
C. perfringens	+	+	+	+	57
C. septicum	+	+			57
C. sordellii		+			57
C. spiroforme	+				57
C. sporogenes	+	+		+	57
C. tetani	+	+			57

Data of reference [57] were extracted from:

Borriello, S.P. and Carman, R.J. (1985) Clostridial diseases of the gastro-intestinal tract in animals. In: Clostridia in gastro-intestinal diseases (S.P. Borriello ed.). CRC Press Inc., Boca Raton, Florida, 195-221.

Cato, E.P., George, W.L. and Finegold, S.M. (1986) Genus *Clostridium*Prazmowski 1880. In: Bergey's Manual of systemic bacteriology. Williams and Wilkis, Baltimore, 1141-1200.

Haagsma, J. (1979) Clostridial disease in Europe. In: CRC Handbook Series in Zoonoses (J.H. Steel, ed.). CRC Press Inc., Boca Raton, Florida, 225-236.

Hill, E.O. (1981). The genus *Clostridium* (medical aspects). In: The prokaryotes. A handbook on habitats, isolation and identification of bacteria (M.P. Starr et al. eds.). Springer-Verlag, Berlin, 1756-1766.

Data concerning the quantitative prevalence in soil and sediments were extracted from the literature only for *C. botulinum* (see table 3). The number of organisms in soil varied from 1 to 25000 kg^{-1} soil. In Asia and North America all *C. botulinum* types (A to F) were found. In Europe *C. botulinum* type A was hardly isolated from soil and sedimens.

Table 3: Quantitative prevalence of C. botulinum in soil and sediments

Region	% of samples positive	MPN/kg	Remarks
Asia	0 – 78	<2 - 25000	type A to F isolated
Europe	0 – 100	1 - >1750	hardly type A isolated
North America	1 – 74	9 - 1280	type A to F isolated
Reference:[39], citation			

2.3.2 Incidence of SFP on vegetables

Data concerning the incidence of pathogenic SFP on fruit and vegetables, spices and vegetable containing products are shown in table 4. For detailed information see appendix 2: table 6 “Incidence of bacilli in food”, and table 7 “Incidence of clostridia in food”.
The condition of the products varied from raw to completely processed. In 60 to 70% of the examined products the number of B. *subtilis* and *B. cereus* was less than 100 colony forming

Table 4: Incidence of SFP in fruit, vegetables, spices and vegetable containing food.

Organism	Number of samples	Number of samples with colony forming units ($\log_{10}$) g^{-1}		
		<2	**2 – 5**	**>5**
B. cereus [5, 20, 28, 49, 52, 62, 63, 70, 88, 89, 95, 96, 101, 106]	1007	697	304	27
B. subtilis [20, 49, 61]	281	160	81	40
C. perfringens [43, 62, 88, 89, 97, 118]	4040	3998	42	0
		Number of samples with absence or presence		
		absence	**presence**	**MPN (>)**
C. botulinum [23, 38, 39, 51, 58]	>1112	1043	>69	41/100 g (mushroom) 2100/kg mushroom 0.8-1.6/kg mushroom 0.63/kg potato

units (CFU) g^{-1}. CFUs of more than 10^5 were seen in respectively ca. 2 and 3% of the samples. *C. perfringens* was found in numbers of $<10^2$ CFUs g^{-1} and 10^2 to 10^5 CFUs g^{-1} in 99% respectively 1% of the examined samples. Because it is very difficult and laborious to count *C. botulinum* in products, most data on the incidence are qualitative in stead of quantitative. Only in a few experiments the number of organisms was counted. Of the 1112 samples tested for presence / absence of *C. botulinum* only 69 (ca. 6%) were positive. In those products, tested quantitatively for *C. botulinum* , the number of organisms was 41 g^{-100}, 2100 kg^{-1}, 0.8 – 1.6 kg^{-1}, and 0,63 kg^{-1} respectively.

2.3.3 Prevalence of food-borne outbreaks of toxico-infections and intoxications caused by SFP

In the years 1967 to 1998 *Bacillus (B.) cereus* (n~38) [22,69,79,80,90,103,121], *Bacillus subtilis* (n=11*)* [7,19,102]*, Bacillus licheniformis* (n=24) [60], *Bacillus pumilus* (n=?) [60] and *Clostridium (C.) botulinum* (n>1000) [6,8,9,10,11,14,15,16,17,26,29,30,35,46,47,51,53,58,68,76,78,82,83,86,87,92,93,99,107,120] were related to outbreaks of food poisoning after consumption of products with a high probability of containing vegetables (see appendix 3: table 8, 9 and 10). In the same period only one outbreak of food poisoning caused by *Clostridium perfringens* related to a vegetable product was reported [91] (see appendix 3: table 11).
The majority of the published outbreaks occurred in Canada, the USA, Croatia, France, Spain, the UK, and East Asia (see appendix 3, table 12). Over the period 1967 to 1998 the average number of outbreaks per year for these countries or regions was for *C. botulinum* 2.0, 13.0, 14.9, 4, no information, and 1.2 respectively. For *C. perfringens* the figures were 11.1, 14.7, no information, no information, 36, and no information. In case of *B. cereus* the number of average outbreaks per year was 4.9, 4.0, no information, no information, 10.5, and 11.1. Average number of outbreaks of *B. subtilis* could only be calculated for Canada and the UK and was 1.8 for both countries.

2.3.4 Food-borne outbreaks of toxico-infections and intoxications caused by SFP and adverse health effects

Studying the outbreaks of intoxication or toxico-infections caused by *B. cereus, B. subtilis* and related species, *C. botulinum* and *C. perfringens* (appendix 3, tables 8, 9, 10 and 11) it is clear that the information concerning the exposed population in relation to illness, death, the duration of illness, and adverse health effects is incomplete.

In three out of 37 outbreaks of *B. cereus* intoxication death occurred (in total 5 patients). The most important clinical signs were vomiting (30 out of 37), followed by diarrhoea, nausea and abdominal cramps. In one outbreak, probably a toxico-infection, the main adverse health effect was diarrhoea (96% of the cases), followed by abdominal cramps

(90%), nausea (50.6 %) and vomiting (13.8%). Also in this outbreak neurological signs (muscle weakness 24.7%) were recorded.

In the outbreaks caused by *B. subtilis* and related species (n = about 35) no fatal cases were recorded. In most outbreaks diarrhoea and vomiting were noticed, followed by abdominal cramps and fever. In one of these outbreaks also neurological signs (headache) were noticed.

In the outbreak of *C. perfringens* toxico-infection related to food containing vegetables the most important clinical sign was diarrhoea (94%), followed by abdominal cramp (91%) and the neurological signs headache (63%) and weakness (60%). In 3 out of the other 38 outbreaks related to meat and fish products death occurred in 4 outbreaks. Two times it concerned elderly or inhabitants of institutions or hospitals.

In the outbreaks of botulism the neurological signs were detected more frequently than gastro-intestinal complaints. Within the neurological signs no particular symptom was seen with a higher frequency in the patients.

From the yearly reports of food-borne outbreaks of intoxications and toxico-infections (appendix 3, table 12) it was calculated per outbreak per year that for *C. botulinum* an avarage of 4.5 to 29.3 cases and 1 to 3.3 times death was involved. This gives a fatality rate of 3.4 to 11.1. The figures for *C. perfringens* are: cases 476 – 1627, death 0.07, and fatality rate < 0.1. For *B. cereus* and *B. subtilis* and related species these figures are: cases 37 – 411, death not recorded, fatality rate 0, respectively cases> 10 – 17, death not recorded, fatality rate 0.

2.4 Discussion and conclusions

In this chapter of the report two steps in the formal process of risk assessment, namely hazard identification and hazard characterisation, have been worked out for SFP in cooked chilled food containing vegetables, with the main focus on vegetables.
It was assumed that vegetables were the most important source for SFP in cooked chilled food and were contaminated from the environment during growth. *B. anthracis, B. subtilis* and related species, *C. botulinum, C. butyricum, C. difficile, C. perfringens* and *C. sporogenes* were detected in the environment as well as in food. *B. anthracis* was excluded as a hazard in cooked chilled food containing vegetables due to its zoonotic character. *C. difficile* and *C. sporogenes* were not recognised as a hazard because of a lack of evidence being a causative agent for gastro-intestinal infection, intoxication or toxico-infection. *C.*

butyricum is not discussed separately. This organism causes a type E botulinal intoxication and is considered as a *C. botulinum* group I organism.

Fruit, vegetables and vegetable containing food were contaminated with *B. cereus* and *B. subtilis* in numbers of > 10^2 CFU g^{-1} in 30% respectively 40% of the examined samples. *C. perfringens* was present in numbers of > 10^2 CFU g^{-1} in 1% of the products. Because counting of *C. botulinum* is very difficult and time consuming data of quantitative contamination of products are very rare. In 6% of the examined products *C. botulinum* was present. The quantitative contamination levels varied from 0.6 to 2100 CFU kg^{-1} product (mushroom and potato).

The information concerning hazards in relation to exposed population, illness, death, duration of illness, and other adverse health effects, is incomplete. In outbreaks of *B. cereus* intoxication the most important clinical sign was vomiting, for the toxico-infection this was diarrhoea. In outbreaks caused by *B. subtilis* and related species the most recognised clinical signs were diarrhoea and vomiting. In outbreaks of botulism neurological adverse health effects were seen more frequently than gastro-intestinal complaints. In outbreaks of *C. perfringens* toxico-infection the main adverse health effects were diarrhoea and abdominal cramps. The data available from the literature do not allow to quantify the adverse health effects in a proper way.

From the yearly reports of food-borne outbreaks of intoxications and toxico-infections of the above mentioned organisms, it was possible to calculate per year the average number of outbreaks, and per outbreak the average number of persons with illness, of death, and the fatality rate.

Data concerning the fatality rate, outbreaks per year, cases per year, relation to vegetables and vegetable products, and growth temperatures can be integrated to categorise the risk of the SFP. As can been seen from table 5 the non-proteolytic *C. botulinum* (group II) and psychrotrophic *B. cereus* can be classified as high risk. This classification is based on their relation to the product, the high number of outbreaks per year and especially the property of these organisms to grow at temperatures of respectively 3 ^{o}C and 4^{o}C.

As medium risk can be considered the proteolytic *C. botulinum* (group I). This classification is based on the difference in minimal growth temperature with group II, namely 10 ^{o}C. This means that this type of organism will be a risk only by temperature abuse.

Table 5. Categories of the risk of the SFP

	Attributive value of the different characteristics to risk of:				
	C. botulinum		***C. perfringens***	***B. cereus***	***B. subtilis* and related species**
Characteristic	group I	group II			
fatality rate	high (3-11)		low (<1)	low (0)	low (0)
outbreaks/year	high (2-15)		high (11-36)	high (4-11)	low (1.8)
cases/year	low (4-39)		high (476-1627)	median (37-411)	low (>10-17)
growth at low temperature	median (10^{o}C)	high (3^{o}C)	median	high (4^{o}C)	low/high
relation to vegetables	high		low	high	high
Risk	**median**	**high**	**low**	**high**	**low**

The other SFP, mentioned in table 5, are merely of low risk. This classification is based for *C. perfringens* on the low relation with vegetables: most *C. perfringens* intoxications are related to meat and meat containing products

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3 Dose-response assessment

3.1 Introduction

When pathogenic organisms or their toxic products enter the human body via ingestion with food, they meet a system of barriers of the host. The organism or its toxic product has to reach the parts of the gastro-intestinal tract that are suitable to attachment, growth, sporulation, toxin production and/or absorption, before it is capable to cause adverse health effects. At present, the basis for dose response infection models is that at least one of the ingested pathogenic organisms must survive the system of barriers mounted by the host, to start colonisation [109]. A similar system cannot be applied to microbial intoxications, like botulism and the vomiting type of intoxication of *B. cereus* . Although in case of microbial intoxications a certain concentration of toxins must survive a system of barriers and reach the suitable part of gastro-intestinal tract to start the intoxication, the single hit (molecule) model cannot be used. In traditional toxicological procedures a safe level of exposure is defined as some arbitrary fraction of that dose level at which no effects are observed in any of the animals tested [34]. On the grounds that the observed no effect level will depend on the sample size, with response rates of 0/10, 0/100 and 0/1000 obviously having different interpretations, this procedure has been criticised [33]. Implicit in this approach is the assumption of the existance of a threshold dose below which no adverse health effects will occur. Such thresholds are likely to vary among individuals.

For toxico-infections, caused by *C. perfringens* and *B. cereus*, diarrhoeal type, the basis for dose response models is also more complex than the single hit model. In these cases a large number of vegetative cells has to survive the system of barriers followed by sporulation, respectively a certain number of spores has to survive the barriers, followed by germination, growth and toxin production.

3.2 Outline literature review

See chapter 2.2

The definition of dose response assessment according to the principles and guidelines for the application of microbiological risk assessment of the Codex Alimentarius Commission [32] is as follows:

Dose response assessment: The determination of the relationship between the magnitude of exposure (dose) to a biological agent and the severity and/or frequency of associated adverse health effects (response).
Following this definition the selected literature is reviewed for the following domains of interest:

1. the safe use of botulinal toxin as a therapeutic in man;
2. dose response experiments in human volunteers;
3. dose response experiments in animals which possibly could be translated to man;
4. relations between dose and adverse health effects in food-borne outbreaks of toxico-infections and intoxications caused by SFP.

3.3 Results, discussion and conclusions

Two animal dose response experiments for B. cereus [55 104] and one human volunteers experiment [65] were extracted from the literature. In both animal experiments the vomiting type of toxin was studied. Only in one group of animals the toxin was administered orally with emesis as adverse health effect. A dose dependent relation was noticed. In experiment with human volunteers symptoms occurred, but were not related to B. cereus toxico-infection and showed no dose response relation.
In most animal dose response experiments for botulism only the number of dead animals as result of exposure to a certain concentration of toxin was studied. No dose response experiments in human volunteers were extracted from the literature.
From the dose response data recorded in outbreaks (appendix 2, table 8 and 10) caused *by C. botulinum* or *B. cereus* no quantitative relation was seen between dose and any of the health effects, or incubation time.
Both high-risk SFP cause food poisoning. *C. botulinum* produces a heat-labile toxin in the food. After consumption of insufficiently reheated food the neurotoxin creates botulism. The LD_{50} is for mice, guinea pigs, rabbits, monkeys, and man [98 45] ca 1 ng/kg $_{bodyweight}$. Neurotoxin of *C. botulinum* is used in therapeutics as a medicine to paralyse muscles. The intramuscular dose for local paralysis [98] is 10 – 20 Units. These units equal ca 0.33-0.66 ng of toxin. When an intramusculair dose of about 5 ng is administered, the paralysis is not restricted to the injected muscle but spread also to muscles at a distance. Also, subclinical effects like obstruction of the upper airways can occur by this dose [45 98]. This may mean that a safe dose is 0.004-0.008 ng/kg $_{bodyweight}$ and at concentrations of ca 0.06 ng/kg $_{bodyweight}$ effects can appear.

B. cereus can produce two different types of toxins, namely emetic and diarrhoeal. The emetic toxin is mainly related to rice and rice-products, and is therefore of no importance in this QRA. The diarrhoea causing toxins are produced in the intestine during growth of *B. cereus*. This supposes growth and sporulation of *B. cereus* in the food, because vegetative cells will be killed during passage through the stomach. Generally it is accepted, based on epidemiological data, that at least 10^5 spores have to be consumed with the food to cause a *B. cereus* food poisoning of the diarrhoeal type. This is confirmed by the number of *B. cereus* in food involved in outbreaks (appendix 2, table 8). The lack of methods to detect the different complexes of this type of toxins and the lack of knowledge of concentrations of toxin produced in the intestine made it impossible to assess a dose response relation.
Both the lack of data concerning a dose-response relation of the toxins of both organisms and the lack of a clear relation between numbers of organisms in the food and the amount of toxin produced in the product or in the intestine will limit the quality of the QRA.

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Appendix 1 Mailing list

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4. Keuringsdienst van Waren, VWS, drs. J.T. Jansen
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20. Directie, RIVM
21. Prof. dr. ir. D. Kromhout
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Appendix 2 Incidence of SFP on vegetables

Table 6: Incidence of bacilli in food

Ref.	Product	Condition	Year	Country	Nr. of samples	Organism	Pre-sence	Range of colony forming units (log 10) <1	1-2	2-3	3-4	4-5	5-6	6-7	Remarks
63	convenience meals		<89	UK	72	Bacillus cereus		<	71	1					
2	dates		(96)	Schotland	13	Bacillus cereus		<	11	2					
106	mung been sprouts (4 days)		(82)	USA	16	Bacillus cereus		<	<	14		2			
63	mushroom	blanced	<89	UK	45	Bacillus cereus		<	45						
5	soup	dries	(88)	Spain	10	Bacillus cereus		<	5	5					asparagus, leek, pie with ham, mushroom
81	spices	dried	(87)	Nigeria	75	Bacillus cereus					<	75	>		peppers, curry, thyme
89 88	spices	processed	<76	USA	110	Bacillus cereus		<	65	30	15				bay leaves, pepper, chili/garlic/mustard powder, cinnamon, oregano
101	spices		(94)	India	75	Bacillus cereus		60			15				
49	spices		(96)	Italy	200	Bacillus cereus		192				<	8	>	pepper, chilli, blends, nutmeg, miscellaneous
61	spices, herbs		(96)	Netherlands	6	Bacillus cereus				<	<	6	>		garlic/chilli powder, pepper, cinnamon, oregano, thyme
70	vegetable and fruit juice	sterilised	(90)	Austria	40	Bacillus cereus		<	40						
95	vegetable products		(94)	Malaysia	3	Bacillus cereus					3				
63	vegetable salads		<89	UK	54	Bacillus cereus		<	53	1					
52	vegetable seeds	unsprouted	(86)	USA	99	Bacillus cereus		30	56	13					alfalfa, mung bean, wheat
52	vegetable sprouts (2-3 days)		(86)	USA	65	Bacillus cereus		<	<	24	10	12	12	7	
101	vegetables	cooked	(94)	India	25	Bacillus cereus		19			6				
28	vegetables	raw and cooked	(87)	Italy	50	Bacillus cereus		<	50						
62	vegetables	raw and cutted	(89)	Japan	38	Bacillus cereus					38				Average log 10
95	vegetables		(94)	Malaysia	11	Bacillus cereus				<	11	>	>		
				Total:	*1007*			*301*	*396*	*90*	*98*	*95*	*20*	*7*	
94	fruit		(76)	Germany	6	Bacillus spp.			4	2					appel
94	mushroom	fresh	(76)	Germany	2	Bacillus spp.			1	1					
21	pasta, filled with cheese and vegetables	fresh	(86)	Italy	79	Bacillus spp.				20	59				B. circulans, B. laterosporus, B. licheniformis, B. subtilis, B. stearothermophilus
5	soup	dried	(88)	Spain	12	Bacillus spp.				2	2	8			asparagus, leek, pie with ham, mushroom
49	spices		(96)	Italy	200	Bacillus spp.		25		<	<	175	>	>	pepper, chilli, blends, nutmeg, miscellaneous
56	vegetables	fresh and cutted	(89)	Germany	113	Bacillus spp.		<	<	<	113	>	>	>	
94	vegetables, flower, fresh	fresh and washed	(76)	Germany	4	Bacillus spp.			3	1					cauliflower
94	vegetables, immature fruit	fresh and washed	(76)	Germany	3	Bacillus spp.			3						pepper
94	vegetables, leafy	fresh and washed	(76)	Germany	27	Bacillus spp.		1	13	4	2	7			brussels sprout, parsley, cabbage, spinach, lettuce
94	vegetables, mature fruit	fresh and washed	(76)	Germany	4	Bacillus spp.			3	1					tomato
94	vegetables, root	fresh and washed	(76)	Germany	20	Bacillus spp.		4	10	2		4			radish, sellery root, onions, carrot, potato
				Total:	*470*			*30*	*37*	*33*	*176*	*194*	*0*	*0*	
20	spices	dried	(87)	Nigeria	75	Bacillus subtilis				<	75	>			peppers, curry, thyme
49	spices		(96)	Italy	200	Bacillus subtilis		160				<	40	>	pepper, chilli, blends, nutmeg, miscellaneous
61	spices, herbs		(96)	Netherlands	6	Bacillus subtilis					<	6			
				Total:	*281*			*160*	*0*	*0*	*75*	*6*	*40*	*0*	

Table 7: Incidence of clostridia in food

Ref.	Product	Condition	Year	Country	Nr. of sam-ples	Organism	Presence	Range of colony forming units (log 10) <1	1-2	2-3	3-4	4-5	5-6	6-7	Remarks
39	fruit and vegetables		(22)	USA	189	Clostridium botulinum A,B	29								(citation table 4: ref. 1)
39	fruit and vegetables		(22)	USA	431	Clostridium botulinum A,B	54								(citation table 4: ref. 2)
58	mushroom	fresh, lightly rinsed	(75)	Canada	?	Clostridium botulinum	41/100g								citation Hauschild et al. 1975 Can. Inst. Fd. Sci. Tech. J. (8) p84
38	mushrooms		(75)	Canada	?	Clostridium botulinum B	2100/kg								(citation table 4: ref. 9)
38	mushrooms		(89)	Netherlands	?	Clostridium botulinum	0.8-1.6/kg								(citation table 4: ref. 11)
38	potatoes	cooked, vacuum packed	(85)	Netherlands	?	Clostridium botulinum	0.63/kg								(citation table 4: ref. 7)
23	potatoes	pasteurised, vacuum packed	(87)	Germany	48	Clostridium botulinum	0								
38	potatoes	raw and cooked, vacuum packed	(87)	Germany	122	Clostridium botulinum	0								(citation table 4: ref. 8)
23	potatoes	raw, vacuum packed	(87)	Germany	72	Clostridium botulinum	0								
38	potatoes (n=27), carrots (n=18)		(79)	USSR	45	Clostridium botulinum	0								(citation table 4: ref. 6)
51	soil		89-90	Spain	10	Clostridium botulinum B	1								
51	vegetables		89-90	Spain	88	Clostridium botulinum B	1								
38	vegetables		(83)	Italy	296	Clostridium botulinum B	13								(citation table 4: ref. 1)
				Total:	*1112*		*69*								
97	food	cooked chilled	85-86	UK	2517	Clostridium perfringens		<	2517						
118	pasta dumpling		86	Italy	60	Clostridium perfringens		60							stuffing among other things: fresh vegetables, herbs
43	salad, rice, cooked	cooked	90	Spain	144	Clostridium perfringens		<	142	2	?				
89 88	spices	processed	<76	USA	114	Clostridium perfringens		<	101	11	2				bay leaves, pepper, chili/garlic/mustard powder, cinnamon, oregano
41	spices		<87	Germany	45	Clostridium perfringens		32	13	>					black pepper, coriander, majoram, paprika
64	spices		(71)	India	58	Clostridium perfringens		28	7	23					
43	vegetables	cooked chilled	90	Spain	226	Clostridium perfringens		<	222	4	?				
97	vegetables	cooked chilled	85-86	UK	876	Clostridium perfringens		<	876						
62	vegetables	raw and cutted	(89)	Japan	38	Clostridium prefringens	3								
				Total:	*4078*			*120*	*3878*	*40*	*2*	*0*	*0*	*0*	
94	fruit		(76)	Germany	6	Clostridium spp.		6							appel
94	mushroom	fresh	(76)	Germany	2	Clostridium spp.		2							
1	pasta, stuffed (ravioli, tortellini)	fresh and hand made	(96)	Italy	585	Clostridium spp.		497	65	18	2	3			
23	potato	pasteurised, vacuum packed	(87)	Germany	48	Clostridium spp.		<	48						Cl. sporogenes>felsineum=scatalogenes
23	potato	raw, vacuum packed	(87)	Germany	72	Clostridium spp.		40	32						Cl. sporogenes>perfringens>bifermentand>scatologenes
23	spices		(87)	Germany	45	Clostridium spp.				45	>				Cl. perfringens>sporogenes>bifermentans=histolyticum>sordellii
56	vegetables	fresh and cutted	(89)	Germany	113	Clostridium spp.		<	113						
94	vegetables, flower, fresh	fresh and washed	(76)	Germany	4	Clostridium spp.		4							cauliflower
94	vegetables, immature fruit	fresh and washed	(76)	Germany	3	Clostridium spp.		3							pepper
94	vegetables, leafy	fresh and washed	(76)	Germany	27	Clostridium spp.		17	6	3	1				brussels sprout, parsley, cabbage, spinach, lettuce
94	vegetables, mature fruit	fresh and washed	(76)	Germany	4	Clostridium spp.		3	1						tomato
94	vegetables, root	fresh and washed	(76)	Germany	24	Clostridium spp.		15	7		2				radish, sellery root, onions, carrot, potato
				Total:	*933*			*587*	*272*	*66*	*5*	*3*	*0*	*0*	

Appendix 3: (Prevalence of) food-borne toxico-infections and intoxications and adverse health effects

Table 8: Bacillus cereus

Ref. nr,	Incriminated food (ingredient)	Year	Country	Place	Out-break	Expo-sed	Ilness	Death	Mortality %	Dose log10 Faeces	Dose log10 Vomitus	Dose log10 Food	Incu-bation time hrs	Clinical signs: duration time hrs	Gastrointestinal: abdo-minal cramp	Gastrointestinal: diar-rhoea	Gastrointestinal: nausea	Gastrointestinal: vomit-ing	Neurol. (muscle) weakness
69	spaghetti, pesto	96	Switzerland	home	1	2	2	1	50			2.6 (p)	0.5		f+	f+		s+	
103	chicken, roasted	78	Japan/Osaka			1	1					6.1	0.5-1					+	
103	rice, fried	77	Japan/Osaka		1	2	2	1	50			6.1	0.5-1					+	
103	rice and omelet	81	Japan/Osaka		1	4	4					+	0.5-1					+	
103	lunch box, catered	77	Japan/Osaka		1	13	9					7.1	0.5-2					+	
103	rice and curry	77	Japan/Fukuoka		1	2	2					7.8	0.5-2					+	
103	rice, fried	79	Japan/Osaka		1	4	4			5.3			0.5-2					+	
103	rice ball	77	Japan/Osaka		1	6	6					7.2	0.5-3					+	
90	rice, boiled	75	Finland	canteen	1	36	18			neg		8.2 (r) / 6.0 (m)	0.5-4	<24	+	+		+	
79	rice fried with curried shrimps	71	UK	chinese restaurant			1						1.5			+		+	
103	rice, fried	78	Japan/Aichi		1	2	2					+	1.5-2					+	
103	rice, fried	78	Japan/Osaka		1	2	2			4.3			1.5-2					+	
77	meatloaf	69	USA/California	fraternity house	1	31	15					7.8	10 m	<24	+	+	+	(+)	
103	rice, fried	82	Japan/Miyagi		1	2	2					+	1-1.5					+	
103	rice, fried	81	Japan/Kyoto		1	53	35					6.1	1-2					+	
103	rice, fried	79	Japan/Osaka		1	6	6					7.9	1-2					+	
103	yakisoba (dumpling with cabbage and beef)	74	Japan/Aichi		1	52	51					8.2	1-2					+	
103	rice and chicken	81	Japan/Aichi		1	61	46				6		1-2.5					+	
22	chicken enchilada with gravy	85	USA/Tennessee	cafetaria, hospital	1	249	160						12.5 m	m 24.3	90%	96.3%	50.6%	13.8%	24.7%
103	lunch box, catered	75	Japan/Osaka		1	48	24				6.1		1-3					+	
103	rice, fried	78	Japan/Osaka		1	206	94			4.4			1-4					+	
103	soy bean curd	81	Japan/Chiba		1	338	172					9.7	1-5					+	
79	rice fried with beef. bean shoots	71	UK	chinese restaurant			5						1-6			+ (3x)		+	
79	rice fried with beef, beanshoots or curried shrimps	71	UK	chinese restaurant			3						2	24		+		+	
79	rice fried with chickenegg foo yung	71	UK	chinese restaurant			1						2			+		+	
103	lunch box, catered	74	Japan/Osaka		1	33	12					7.5	2-12			+			
80	milk, pasteurized	86-89	Netherlands		1	4200	280					5.6	2-14	<36			+	+	
103	lunch box, catered	75	Japan/Yamagata		1	422	130	3	2.3			+	2-17			+			
103	rice ball and shushi	77	Japan/Osaka		1	1809	211					8.9	2-2.5					+	
103	tempura (fried vegetables with shrimps and/or fish)	74	Japan/Osaka		1	3	3					5.7	2-3					+	
103	rice, fried	73	Japan/Osaka		1	5	5					6.8	2-4					+	
103	rice and omelet	82	Japan/Saitama		1	6	5					7.0	2-4					+	
103	pudding	71	Japan/Osaka		1		89					6.1	3-7			+			
80	vegetable pie, home made	86-89	Netherland	home	1	3	3					5.3	4	24	+	+			
79	rice fried with curried shrimps, beanshoots	71	UK	chinese restaurant			2						4			+ (1x)		+	
103	lunch box, catered	74	Japan/Mie		1	1407	194					6.8	4-10			+			
121	roastbeef	67	Netherlands	eldery home	1	150	18					3.5	8-20	<24	+	++			

Table 9: Bacillus subtilis, Bacillus licheniformis and Bacillus pumilus

Ref. nr.	Incriminated food (ingredient)	Year	Country	Place	Outbreaks	Exposed	Illness	Death	Mortality %	Dose log10 Faeces	Dose log10 Vomitus	Dose log10 Food	Incubation time hrs	Clinical signs: duration time	Gastrointestinal: abdominal cramp	Gastrointestinal: diarrhoea	Gastrointestinal: fever	Gastrointestinal: nausea	Gastrointestinal: vomit-ing	Neurol.: headache
	B. subtilis																			
7	chicken and stuffing	79	Australia/New Zealand		1		5					5.6	1.5			+			+	
102	vegetable with mustard	80	Japan	school luncheon	1	923	580			7		8	2-25		73%	33%	39%		50%	84%
7	chinese meal	79	Australia/New Zealand		1		1					5.4	2-3		+				+	
7	pizza	76	Australia/New Zealand		1		12					large numbers	4			11			12	
7	milk shake	77	Australia/New Zealand		1		6			+			6			+				
7	bread	79	Australia/New Zealand		1		1					6	7-8		+	+			+	
7	turkey and stuffing	76	Australia/New Zealand		1		4					9	10			3			1	
7	chinese meal	75	Australia/New Zealand		1		2					5.4	12		+				+	
7	reconst. dehydr. milk / meat and sauce	79	Australia/New Zealand		1		several						18			+			+	
7	unknown	78	Australia/New Zealand		1		20			+			?			+				
19	ham sandwich	98	UK	restaurant	1		2													
	B. licheniformis																			
60(cit)	cooked meat and vegetables	75-86	UK		24		>218					>6	8 m			+			+	
	B. pumilus																			
60 (cit)	meat, scotch eggs, cheese sandwich, canned tomato juice	75-86	UK				5					large numbers				+			+	

Tabel 10: Clostridium botulinum

Ref. nr.	Type	Incriminated food (ingredient)	pH	Year	Country	Place	Outbreaks	Exposed	Illness	Death	Mortality %	Dose log10: Faeces	Dose log10: Food	Toxin detection: Faeces	Toxin detection: Food	Toxin detection: Serum	Incubation time hrs	Clinical signs – Gastrointestinal: abdominal cramps	constipation	diarrhea	nausea	vomiting	Neurological symptoms: blurred vision	double vision	dizziness	fatique	headache	nasal/slurred speaking	muscle weakness	respiratory problems	swallowing problems
31	A	peanuts		86	Taiwan		1		7	1					+ (10E4 dilution)		1-4														
47	E	mushroom (macaroni)		41	USA	home	1	6	3	1					+ E		1-20	?						+	+			+		+	+
68	A	onions, sauteed (served on patty melt sandwich)	5.6	83	USA/Illinois	restaurant	1		28			+		+	+	+	7-115				37%		90%	78%		60%		60%	36%	23%	78%
82	B prot.	hazelnut (hazelnut yochurt)	5.0-5.5	89	UK		1		27	1		+	+		14-30 MLD		2-120							+				+	+		+
83	A	fried lotus-rhizome solid mustard		84	Japan		2		36	11					0.0023-0.549 mug		10					+	+	+	+					+	
86	Unknown	asparagus			Australia	home			1								12						+	+				+	+		
107	B prot.	garlic chopped, in soybean oil, commercial (garlic bread)	>4.6	85	Canada/Br.C.	restaurant	2		36			+		-		-	19-240	48%	48%	21%	59%	18%	88%	91%	82%		38%	82%	100%		91%
58	B	mushroom, home canned		74	USA	home	1		3	2		+	+		+		<24	1			1	1						1	1	2	2
92	E	nv: fish		94	USA, Alaska				1			+ A and E	+ E		+ E		24				+	+		+						+	
17 16	A	stew (potatoes and roast beef)		94	USA/Oklah.	home			1						+ (p)		24				+		+		+			+			+
35	B prot.	egg plant, sliced and roasted, commercial	3.9-5.1	93	Italy	rest./home		15	7(9)			+				-	24-112		+	(+)		(+) +		+					+	+	+
99	A	potato salad		78	USA/Color.	restaurant	1	13	7								24-144							+				+	+		
15	B	eggplant in oil, roasted	> 4.6	93	Italy	restaurant	2	14	7			+			+		>24			+		+		+				+			
78	A	garlic in oil, commercial (garlic bread)	5.7	89	USA/N.York	home	1	4	3				>1.0		20 MLD		35-40			+	+							+		+	
8 9	A	turkey loave (cereal, onions, green pepper)		84	USA/Calif.	home	1	4	2					+			36							+					+		
8 9	A	stew (meat, unpeeled potatoes and carrots)		84	USA/Calif.	home	1	2	1							+	40					+	+					+		+	
30	B	pesto	5.3-5.8	97	Italy	home	1		2				5.0				148			+		+		+				+			
51	A+B, B, D	asparagus, preserved		69-85	Spain		7		37	7																					
120	A,B,E,?	beancurd, preserved (74%), broad-bean sauce (12.5%)		58-83	China		986		4377	548					+ tf	+								+	+			+			+
120	A	beancurd, preserved (74%), broad-bean sauce (12.5%)		58-83	China				3062/227	239/24	7.5/10.6																				
120	B	beancurd, preserved (74%), broad-bean sauce (12.5%)		58-83	China				367/18	34/2	9.2/11.1																				
120	E	beancurd, preserved (74%), broad-bean sauce (12.5%)		58-83	China				5/41	1/16	20.0/39.0																				
120	Unknown	beancurd, preserved (74%), broad-bean sauce (12.5%)		58-83	China				433/224	28/213	6.5/95.1																				
29	A	coconut cream, canned		85	USA				1			+	+	+		+															
87	Unknown	green beans		96	Spain		1		2						+																
93		ham, home cured (83%)		65-90	France			108							+ (49) - (33)					62%	62%	?	93%	17%					97%	5.6%	
46	AB, B	macrobiotic food, canned		95	Italy				1	1					1300 MLD A, 50 MLD B			+				+								+	
10 11 74	A	mushrooms (chantarelle) (home prepared)		87	Canada/Br.C.	restaurant	1	31	11						+																
53	B	mushrooms, commercial canned		71-84	Canada		1																								
53	A, B, E	non vegetable and vegetable		71-84	Canada		61		122	21																					
30	B	pesto		97	Italy	home									500 MLD																
26	A	potato salad		92	USA/Color.	home	1	2				+	+	+	+ (10E2 dilution)						+		+	+				+	+	+	+
6	A	potato salad, bean salad		78	USA/New M.	restaurant	1		32						+ (p)																
14	A	potato, dip of (skordalia)		94	US/El Paso	restaurant	1	240	23						+																
76	A,B, ?	vegetables		70-80	USA		13																								
53	A	vegetables, home canned (a.o.t. corn)		71-84	Canada		2																								

Table 11: C. perfringens outbreaks

Ref. nr.	Type	Incriminated food (ingredient)	Year	Country	Place	Outbreaks	Exposed	Illness	Death	Mortality %	Dose log10 Faeces	Dose log10 Food	Incubation time hrs	Clinical signs: Gastrointestinal: duration time hrs	abdominal cramp	diarrhoea	fever	nausea	vomiting	Neuroligical symptoms: blurred vision	fatique	headache	nasal/slurred speaking	(muscle) weakness	respiratory problems	swallowing problems
27		mutton	82	UK	Old people's home	1		9			+	+	1			+			+							
59		turkey, pies, gravy	70	USA/N.Jersey	home	1	18	17	2	11.8		5.7 (turkey) / 3.7 (stuffing)	1-18		94%	100%	16%	71%	100%	24%	53%	29%	5.9%		11.8%	11.8%
91		minnestrone soup	90	USA	conference center	1	42	32					1.5-15	11-60	91%	94%		9%				63%		60%		
12		roast beef	89	Canada	corporate lunch	1	420	300			+	2.3	2-18	12	+	+		+								
119	A	crab salad or egg soup with spinach (suspected)	80	Japan	hospital	1		61			+ (89%)		3-12(24)	24-28	41%	100%	0%		5%							
85		turkey, gravy, mashed potatoes	95	USA/Cal.	cafetaria	1		100				6	5-19	8-72	90%	100%		63%	30%							
27		chicken	78	UK	hospital	1		106			+	+	6-24			+			(+)							
54		salmon, boiled (served as hors d'ouvre)	84	UK	hotel dinner	1	455	>284			+		6-30		+	+		+								
54		salmon, boiled (served as hors d'ouvre)	84	UK	hotel dinner	1	702	>209			+ (tox. +)		6-30	24-72	+	+		+								
108		turkey	66	UK	restaurant	1	120	27	1		+		8-12		+	+										
36		minced meat	84	Belgium	eldery home kitchen	1		119	7	5.9		+	8-20		100%	100%	10%		90%							
105	A	meat, sauce, potatoes and vegetables	75	Norway	cafetaria	2		15+1			7.7 (100% spores)	7.7 (1% spores)	9		+	+										
108		tonques, cooked	67	UK	school canteen	1	225	88			5.3-7.5	7	9-10		+	+										
54		salmon, boiled (served cold with salad)	75	UK	masonic dinner	1	219	56			5.0-8.0	4.8-5.6	9-12	24-72		+										
4		tongue	77	New Zealand	student residential hall	1		20			5.0-6.0	4.6	9-15		+	+										
27		roast lamb	85	UK	Training centre	1		12			+	+	9-16		+	+										
12		ground beef, cooked	89	Canada	restaurant	1	5	4			6.2-7.3	3.1	12	12	+	+										
27		mince?	92	UK	Nursing home	1		4			+		12			+										
108		roast beef, rolled	66	UK	school canteen	1	200	30			6.8-8.0	+ (moderate level)	10-11		+	+										
108		ox liver, grilled	66	UK	restaurant	1	4	4			5.2-5.7		10-14		+	+										
27		minced beef	77	UK	geriatric hospital	1		>6			+		12			+										
27		steak and kidney	75	UK	luncheon club	1		18			+	+	12			+										
108	A	meat	66	UK	school	1	400	50			5.9-7.4	+	12-15	12-24	+	+										
27		roast beef	76	UK	geriatric hospital	1		68			+		12-15			+										
54		salmon, boiled (served with mayonaise)	84	UK	hotel lunch	1	850	>60			+	2.0-3.0	12-16		+	+										
27		chicken	76	UK	party	1		14			+	+	12-18			+			+							
54		salmon, boiled (served as hors d'ouvre)	74	UK	festival supper	1		50			4	8	16-20			+										
105	A	meat, sauce, potatoes and vegetables	75	Norway		1		1			5.3 (100% spores)		25			pasty faeces										
19		beef	98	UK	residential institution	1		3				+														
13		meat, poultry, soup, unknown	93	UK	institutions, hospitals	8	455	124	1																	
27		roast lamb and gravy	74	UK	hospital	1		>105			+															

Table 12: Foodborne outbreaks of intoxications and toxico-infections caused by SFP

Ref. nr.	Country	Year	Incriminated food	Etiologic agent: Clostridium botulinum					Clostridium perfringens					Bacillus cereus					Bacillus subtilis or spp.				
				Outbreak	Cases	Death	Fatality rate	Remarks	Outbreak	Cases	Death	Fatality rate	Remarks	Outbreak	Cases	Death	Fatality rate	Remarks	Outbreak	Cases	Death	Fatality rate	Remarks
110 73	Canada	73		2	5				7	535				1	2				3	19			spp.
110 73	Canada	74		4	10				3	200				4	86				4	86			spp.
111 73	Canada	75		2	9				12	556				0	0				1	2			spp.
112 73	Canada	76		2	13				19	603				3	17				5	13			spp.
113	Canada	77		2	5				13	498				5	24				2	10			spp.
114	Canada	78		4	9				6	262				5	22				0				spp.
115	Canada	79		0					11	480				13	61				1	21			
116	Canada	80		1	3				17	752				8	48				0				
116	Canada	81		1	2				12	396				5	77				0				
			mean per year:	*2.0*	*6.2*				*11.1*	*475.8*				*4.9*	*37.4*				*1.8*	*16.8*			
42	USA	50-79		161																			
			fruit&vegetables (67%)	112																			
67 44	USA	69		10	17				65	18527				1	5			NYcity, mushrooms					
76 67	USA	70		6	13	5	38.5		54	6942			ca. 90% meat involved										
76	USA	71		10	23	8	34.8																
75	USA	71		6	15				3	106													
76	USA	72		4	24	4	16.7																
75	USA	72		2	24				9	973													
76	USA	73		10	31	4	12.9																
76	USA	70-73	vegetables (56.1 %)	13				6x A, 4x B, 0x E, 3x ?															
45	USA	74		21	32				15	863													
45	USA	75		14	19				16	419													
45	USA	76		23	40				6	50													
25	USA	73-87		231	494	47	9.5		190	12234	12	0.1		58	1123	0	0.0						
25	USA	73-87	fruit&vegetables (46.3%)	107	231				1	6				3	58								
117	USA	75-84																	16	59			spp.
24	USA	88		20	49	1	2.0		0					5	51	0	0.0						
24	USA	89		13	24	2	8.3		7	436	0	0.0		3	61	0	0.0						
24	USA	90		12	22	5	22.7		11	1240	0	0.0		5	43	0	0.0						
24	USA	91		1	25	2	8.0		10	1231	1	0.1		5	253	0	0.0						
24	USA	92		4	13	1	7.7		12	912	0	0.0		3	25	0	0.0						
			mean per year:	*13.0*	*29.3*	*3.3*	*11.1*	*(mean 70-92)*	*14.7*	*1627.1*	*0.7*	*0.0*		*4.0*	*77.8*	*0.0*	*0.0*						
27	Croatia	86-92	bean or potato salad, coleslaw							1025	cases involved in outbreaks												
100	France	56-70		134	337	17	5.0	>/17 death															
100	France	71-78		256	567	15	2.6	>/ 15 death															
100	France	79		21	30	0	0.0																
100	France	56-79	fruit&vegetables (56-79) (4.4%)	18																			
100	France	56-79	unknown (15.6%)	64																			
18	France	87-89		21																			
18	France	90-92		31																			
18	France	93-95		30																			
18	France	93-95	meat products	25				all type B															
			vegetables, preserved	4				all type B															
			mean per year:	*14.9*	*38.9*	*1.3*	*3.4*																
71 72	Spain	69-73		5																			
71 72	Spain	74-78		6																			
71 72	Spain	79-83		19																			
71 72	Spain	84-88		33																			
71 72	Spain	89-91		15																			
71 72	Spain	92-94		21																			
71 72	Spain	92-94		26	34	2	5.9	episodes															
71 72	Spain	92-94	vegetables (75%)	18	25	2	8.0																
			mean per year:	*4*	*11*	*1*	*5.9*	(including single cases)															
7	UK	75-79																	9	>51			
50	UK	80		nd					55	1056				13	64								
37	UK	92-94		nd					89					29									
37	UK	92-94	salad, vegetables and fruit						5					7									
			mean per year:						*36*					*10.5*					*1.8*	*>10*			
66	Japan	81-90	grain, vegetables																				
102	Japan	82-86		8	49	12	24.5							63	1323			11.2 % not rice or noodles					
102	Japan	70	tofu																1	?			
102	Japan	80	vegetable with mustard																1	580			
66	Korea	81-90	grain, vegetables																				
7	New Zealand/Australia	64-78	3x vegetable																15				incidents
84	Taiwan	86-95		10	19			3x A, 6x B, 1 E	nd					104	4844								
			mean per year:	*1.2*	*4.5*									*11.1*	*411.1*								