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Abstract

Survey analysis of the microbial contamination of fresh produce and ready to-eat mixed salads and the associated relative risk to consumers in the Netherlands

The risk of ready-to-eat mixed salads in Dutch supermarkets being contaminated with the bacteria *Campylobacter, Salmonella, Escherichia (E.) coli* O 157 and *Listeria monocytogenes* is slight, less than 0.26 percent. It has been estimated that approximately 22 persons fall ill each year from *Campylobacter* infection following consumption of these products. This is a fraction of the number of people who fall ill from *Campylobacter* following the consumption of poultry (about 12,000 per year).

This information has been revealed in a survey conducted by the National Institute for Public Health and the Environment (RIVM) and the Food and Consumer Product Safety Authority (VWA). In this survey, the risk for contamination in the whole production chain of these mixed salads was studied. The ingredients for these mixed salad products were produced mainly in the Netherlands.

The survey included 4,180 samples. The unprocessed produce and ready-to-eat mixed salads were examined for the presence (qualitative and quantitative) of the above-mentioned pathogens at fixed places in the production chain. In addition, the risk of illness from eating these products was calculated. On this point, the main uncertainty relates to a lack of dose response data – in other words, what is the number of bacteria needed before people actually become ill?

Key words:
microbial contamination, fresh produce, consumer risk

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Rapport in het kort

Analyse van onderzoek naar de microbiële besmetting van onbewerkte groente en kant en klare gemengde salades, en de daarmee samenhangende risico's voor de Nederlandse consument

De kans dat voorverpakte gemengde salades uit Nederlandse supermarkten de bacterie *Campylobacter, Salmonella, E. coli* O157 of *Listeria monocytogenes* bevatten is gering (minder dan 0,26 procent). Geschat is dat per jaar circa 22 mensen ziek worden door *Campylobacter* na het eten van deze producten. Dit is een fractie van het geschatte aantal mensen die ziek worden van *Campylobacter* nadat zij kip hebben gegeten (circa 12000 per jaar).

Dit blijkt uit onderzoek van het RIVM en de Voedsel en Waren Autoriteit (VWA). Hierin is de kans onderzocht dat deze salades in de gehele productieketen van deze groenten tot het moment dat ze worden geconsumeerd met deze bacteriën besmet raken. De ingrediënten van deze salades zijn hoofdzakelijk in Nederland geteeld en verwerkt.

Het onderzoek omvat 4180 monsters. Op bepaalde plaatsen in de productieketen is gekeken naar de mate waarin ziekteverwekkende micro-organismen in de producten en grondstoffen voorkomen. Daarnaast is gekeken naar de kans om ziek te worden door deze producten te eten. De belangrijkste bron van onzekerheid hierbij blijft de vraag bij hoeveel bacteriën een mens ziek wordt.

Trefwoorden:
microbiële besmetting, rauw geconsumeerde groente, consumentengevaar

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Summary

A survey was performed from October 2006 till October 2007 to get insight in the microbial contamination of fresh produce for raw consumption in the Netherlands. From a tracing and tracking perspective it was decided to sample both the produce (separate raw vegetables) and products (ready-to-eat mixed salads) from two large vegetable processing companies in the Netherlands. Produce (n = 1900) was sampled at the distribution hall of these companies from where any (positive) sample could be traced back to a specific Dutch, or EU, primary production site. Products were sampled at two sites further down the production chain for tracking purposes (*i.e.* in following the dynamics of microbes throughout the chain), namely at the packing stage in the processing companies (n = 780) on the same day as the produce was sampled to assess the effect of "food handling", and, in the retail (n = 1500) to assess any further distribution effects. The pathogens of concern were *Campylobacter* spp., *Salmonella* spp., *E. coli* O157 and *Listeria monocytogenes*.

In raw produce *Campylobacter* spp. were detected in three samples, *Salmonella* spp. in six samples, *E. coli* O157 in one sample and *Listeria monocytogenes* also in one sample. The pathogens of concern were not detected in any of the samples taken at the packing stage in the processing companies. At retail level one sample was found positive for *Salmonella* spp.
Positive samples were further quantified following Most Probable Number (MPN) in order to obtain an initial public health estimate. That is: the MPN for *Campylobacter* spp. was in the range of 0.025 – 0.096 colony forming units (cfu) g^{-1}, *Salmonella* spp. was found in the range of 0.019 - >0.281 cfu g^{-1}, *E. coli* O157 was estimated at 0.052 cfu g^{-1} and *Listeria monocytogenes* at 250 cfu g^{-1}.

A sQMRA model was used to calculated the point estimate for the number of ill people associated with the pathogens of concern in mixed salads.
Assuming that washing and diluting through mixing of produce has an effect on the contamination levels (reduction-factor 86 %) the point estimates are as follows: for *Campylobacter* spp. 22 per $year^{-1}$ (y^{-1}), for *Salmonella* spp. ≥ 0 y^{-1}, for *E. coli* O157 100 y^{-1} and for *Listeria monocytogenes* 330 y^{-1}.

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1 Introduction

In the last decades outbreaks with pathogenic micro organisms on raw produce (e.g. *E. coli* O157:H7 in spinach and *Salmonella* spp. in tomatoes) (Anonymous 2005, 2006, 2008; Bowen et al. 2006; Burke 2008; Cooley et al. 2007; Greene et al. 2008; Johnston et al. 2006; Johnston et al. 2005; Manuel et al. 2000; Naimi et al. 2003; Nuorti et al. 2004; Scavia et al. 2008; Sewell and Farber 2001; Sivapalasingam et al. 2004) have increased the interest to characterize the microbiological hazards associated with fresh fruits and vegetables. A literature review in the EU provides, however, no clear data regarding food borne infections related to fresh produce. Moreover, reports are not standardized between countries for commodity-specific outbreak investigations (Anonymous 2007a). Both the Rapid Alert System for Food and Feed (RASFF) of the EU (Anonymous 2007b) and the CDC in the USA (Bean et al. 1996) report approximately 4 % of reported food infections to be attributable to vegetables. General figures show that the consumption of raw vegetables does not pose an increased microbiological health risk based on epidemiological data from the EU and the USA. Furthermore, the probability of an outbreak due to the consumption of raw vegetables is comparable with other product groups such as eggs, milk products and shell en shellfish. However, when relating the incidence of outbreaks to the number of food infections involved, vegetables become the second most important commodity, after "Meat and Meat Products", for severity of microbiological infections. The hazard is enclosed in those cases where an outbreak does occur as then many patients are involved. The current increase in the production of ready-to-eat foods can further increase the potential risk even though reported prevalences are low. A literature review on surveys related with these food commodities revealed prevalences of pathogens in the range of 0-4.5 % (Jansen and In 't Veld 2002; Jansen et al. 2002; Erickson and Doyle 2007; Park and Sanders 1992).

The following simple calculation reveals the potential microbial risk involved in relation to the large production of ready-to-eat-foods (specifically, mixed salads containing at least two lettuce varieties) for the Dutch situation.

# packages in retail	Prevalence in %		
Per month $30 \cdot 10^6$	1	0.1	0.01
# Contaminated samples	$30 \cdot 10^4$	$30 \cdot 10^3$	$30 \cdot 10^2$
# possible infections*	$30 \cdot 10^1$	30	3

* assumption: 1 colony forming unit (cfu) per package results in 1 patient : 1000 packages

Recently reported outbreaks, a lack of instant effective intervention strategies together with increasing production figures resulted in a framework for the study presented in this paper. That is, a survey for ready-to-eat salads in order to get insight in:

1. microbial contamination sources;
2. the dynamics of pathogenic micro-organisms throughout the production chain;
3. the relative public health risk; and
4. intervention strategies to reduce the risk of contamination.

The pathogens of concern were *Campylobacter* spp., *Listeria monocytogenes*, *Salmonella* spp., and *E. coli* O157. This selection was based on literature review on the occurrence of pathogenic micro-organisms on fresh produce (Long et al. 2002) and their biological characteristics (e.g. contamination via soil, survival and growth at low temperatures). As for the traceability aspect of the study, vegetables from known primary production sites being packed into labeled ready-to-eat mixed salads were the primary produce/product combination of concern.

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2 Sample design

To meet the specific goals of this project, namely, traceability of contamination sources and the possibility of tracking a contamination event up to human exposure, this study required a process directed sampling approach. Hereto, a profound sampling plan was developed considering the relevant steps in the production chain of ready-to-eat salads, space and time effects, specific produce and product selection, and number of samples to be analysed. The sample plan is explained in the following subsections.

2.1 Sampling sites in the production chain

The supply chain of fresh produce in the Netherlands is highly diverse and ranges from individual farmers producing for specific green groceries through large contract producers for the retail up until intercommunity trade and import. From a tracing and tracking perspective it was decided to sample both the produce (separate raw vegetables) and products (ready-to-eat mixed salads) from two large vegetable processing companies in the Netherlands. Produce was sampled at the distribution hall of these companies from where any (positive) sample could be traced back to a specific Dutch, or EU, primary production site. Products were sampled at two sites further down the production chain for tracking purposes (i.e. in following the dynamics of microbes throughout the chain). That is, at the packing stage in the processing companies on the same day as the produce was sampled (to assess the effect of "food handling"), and, in the retail (to assess any further distribution effects). Retail samples were obtained from supermarkets directly related to the processing companies. These supermarkets were sampled over five regions, in the area surrounding the five departments of the Dutch Food and Consumer Product Safety Authority (VWA), in the Netherlands.

2.2 Selected produce / product combinations

The decision to focus sampling on leafy raw vegetables and pre-packed, ready-to-eat, mixed salads in the cutting plant is based on the following reasons:

1. Leafy greens form the major part of pre-packed, ready-to-eat, mixed salads.
2. The contamination level of the raw produce and the effects of processing can both be measured in the cutting plants.
3. Packed products are completely traceable in the Dutch produce processing chain;
4. The production of ready-to-eat mixed salads is increasing.

As the variety in supply of these salads is substantial it would be an impossible task to sample the whole range of salads and still come to accurate prevalence estimates (see also section 2.3). As a consequence, a subset of products was selected for sampling based on their potential to contain hazardous microbes. These were the mixed salads, i.e. salads containing at least two leafy produce. Again, the number of

mixed salads was too large to optimize the trade-off between laboratory (analysis) capacities and accurate prevalence estimates. A further selection was then based on the sales volumes of the companies. This narrowed the sampling strategy down to twelve carefully selected products from two Dutch processing companies, like oak leaf lettuce mélange, mixed iceberg lettuce, Italian salad, et cetera. Products were sampled proportional to production numbers. Also produce was sampled proportional to production numbers and the distribution of produce in the selected products. Specific vegetables were selected for sampling accounting for a) contaminated produce samples found in earlier studies and b) the amount of specific produce types being distribution over the twelve selected products. This resulted in thirteen vegetable types being sampled at the entrance hall of the companies (Table 1).

Table 1 Numbers (50 per week) and variety (13) of raw produce selected for sampling at the entrance hall over two Dutch processing companies

Raw produce	# samples investigated per week
Iceberg lettuce	15
Endive	12
Lollo rosso	5
Curly endive	4
Lollo bionda	3
Red pepper	2
Green oak leaf lettuce	2
Red oak leaf lettuce	2
Baby leaf	1
Cucumber	1
Red lettuce	1
Radicchio rosso	1
Rucola	1

This approach accounted for a direct tracing link between product contamination at the retail level, the processing companies, possible effect of food handling and primary production. Actual sample sizes will be further specified in the next section.

2.3 Number of samples

The number of samples to be taken in studies where the prevalence is considered to be low is often a trade-off between time/money aspects and the accuracy of the resulting prevalence estimates. Preliminary literature search revealed the prevalence range from 0 to 4.5 % (Jansen and In ‘t Veld 2002;

Jansen et al. 2002; Erickson and Doyle 2007; Park and Sanders 1992). This presumed prevalence range, together with the capacities for sample analysis at the participating laboratories formed the basis to decide on the amount of samples to be taken during this one year trial. Ultimate decisions were based on this trade-off between "work load" and "accuracy of estimates" which could be quantified using the methodology as described in Evers (2001). The basic principle here is to consider a Binomial process describing the number of positive samples ("successes") using a known number of samples (n) and prevalence (d). The properties of this process can be used to calculate the number of samples to be tested *negative* to assess an upper prevalence level with some confidence (p). This is useful information to answer the question "What will new insights in a prevalence estimate contribute to existing information". Assuming a 'worst case scenario' in which all samples are tested negative, the question is then: How many samples should be taken (and subsequently tested negative) in order to make sure (with a reasonable confidence level) that the upper level of the prevalence estimate will be below the 4.5 % already known from literature (Jansen and In 't Veld 2002; Park and Sanders 1992). The equation is:

$$n = \frac{{}^{10}\log(1-p)}{{}^{10}\log(1-d)},$$

(Eq. 1)

where both *p* and *d* have values between 0 and 1.

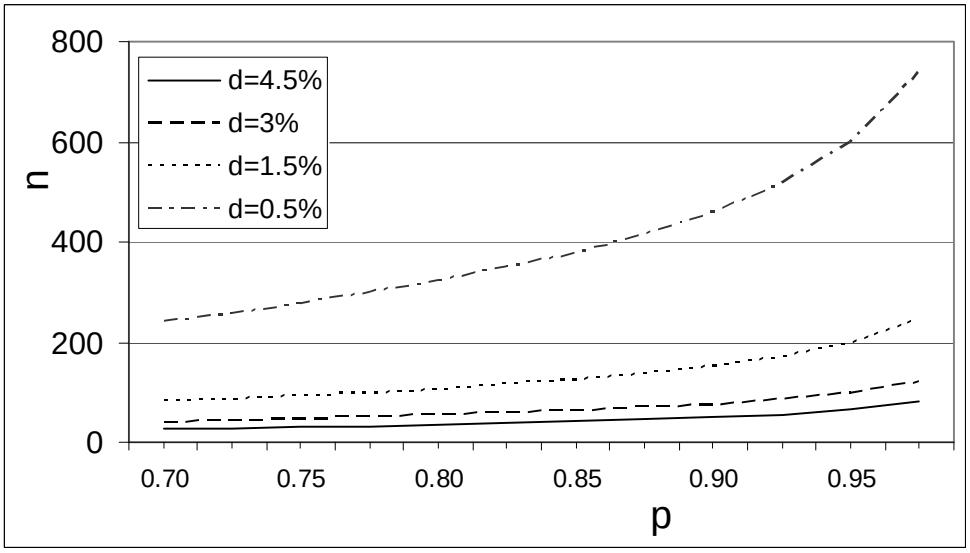

Figure 1 Number of consecutive samples to be tested negative, n, in order to assess the upper prevalence level, d, with confidence p.

Figure 1 illustrates the number of samples to be tested negative for a pathogen according to Equation 1 in order to assess an upper level for the prevalence with some confidence. This figure illustrates, for example, that 600 samples need to be tested negative in order to assess a prevalence level between 0 - 0.5 % with 95 % confidence.

A point estimate for the prevalence can be assessed if a positive sample is found during the predefined sampling period. Again, the Binomial distribution forms the basis for this estimate, whereas the uncertainty about *d*, due to sampling variability, can be assessed with a Beta distribution (Vose 2000). Table 2 illustrates how both the prevalence *(d)* and the number of positive samples (*k*) affect the choice for a sample size (*n*). The total number of samples is determined by the presumed prevalence. That is, in order to be able to estimate a prevalence of 0.5 % one should, at least, collect 200 samples, because one positive sample would then result in this 0.5 % point estimate for the prevalence. However, the accuracy of this estimate is determined by the number of positive samples from the total number collected. Table 2 also shows that the relative confidence interval (representing the accuracy) decreases from 265 % to 117 % as the number of positive samples increases from 1 to 10 for a constant *d*.

Table 2 Sampling size (*n*) affected by the presumed prevalence (*d*) and the accuracy of the point estimate for *d* when positive samples (*k*) are found.

K	*n*	*d*	*95 % Confidence Interval* *Lower*	*Upper*	*Relative Confidence*[1]
1	200	0.5 %	0.12 %	2.74 %	265 %
10	200	5.0 %	2.76 %	8.96 %	114 %
10	2000	0.5 %	0.27 %	0.92 %	117 %

[1] Relative Confidence is $\frac{|\,d - Lower\,|}{d} + \frac{|\,d - Upper\,|}{d}$

Considering:

1. a presumed prevalence in the range of 0-4.5 %,
2. the accuracy of a resulting prevalence estimate,
3. laboratory capacity and
4. budget capacity,

resulted in the following initial sampling plan: at least 1900 raw produce samples at the entrance hall of two processing companies, 780 product samples at the end of the processing chain in the companies and 1500 product samples in the retail. This would, at least, result in the following prevalence estimates:

Sample	Number	Prevalence estimate with 95 % confidence (interval)	
		$k = 0$	$k = 1$
Raw produce	1900	≤ 0.16 %	0.10 % (0.013, 0.29)
Product at company	780	≤ 0.38 %	0.26 % (0.031, 0.71)
Product in retail	1500	≤ 0.20 %	0.13 % (0.016, 0.37)

This survey will result in useful information in the light of obtaining new insights in the microbial contamination of fresh produce in addition to current knowledge.

2.4 The sampling process

Samples at the processing companies were taken evenly spread over one year from October 2006 through to October 2007, in order to reveal possible seasonality influences on contamination levels. The sampling consisted of a three-weekly cycle with sampling on Mondays and Wednesdays and the analysis starting on Tuesdays and Thursdays. In total, 50 raw produce samples were collected at the two processing companies in each sampling week (see Table 1 for numbers per produce).

Raw produce samples were randomly selected from incoming trays at the processing companies, packed in separate bags by the responsible quality manager and stored at 4 ºC. Subsequently, a certified courier delivered the samples at RIVM on Monday and Wednesday evening where the samples were stored overnight in the fridge (4 ºC) to be prepared for analysis early the following day.
The same procedure was followed for the products being sampled at the end of the processing line (22 samples over 12 different products per week) and investigated at one of the 5 VWA departments.
In addition, product sampling in the supermarkets from two retail branches was done on a monthly basis as this fitted the regular sampling protocols of the VWA. Each of the 5 departments was to collect 25 samples (over 13 different products) at supermarkets in their region with a direct link to the processing companies.
This finally resulted in a total of 1950 produce, 858 product samples from the processing companies and 1500 retail samples to be investigated over a one year period.

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3 Sample analysis

A preliminary public health risk estimate was to be assessed through the quantification of contamination levels with a Most Probable Number (MPN) approach in addition to prevalence estimates of *Campylobacter* spp., *Salmonella* spp., and *E. coli* O157. This study required a modified MPN method to keep a manageable amount of sample analysis. The chosen method was related to 1) portion size at consumption, 2) the lab capacity (both in storage space and labour time) and 3) the ability to come to an improved risk estimate over current knowledge from earlier studies. This resulted in a MPN method using the following matrix:

Source material (g)	Replicate 1	Replicate 2
25	+ / -	+ / -
2.5	+ / -	+ / -

A different approach was used to assess the occurrence of *Listeria monocytogenes* in raw produce. This decision stemmed from the height of the infectious dose and food safety standard for *Listeria monocytogenes* (count < 100 colony forming units (CFU)) in the Netherlands. Hereto, 10 g of sample was mixed with 90 ml of BPW. Subsequently, 1 ml and 0.1 ml of the mixture was plated in duplicate, grown and cfu's counted. A detailed description of the sample analysis is presented below.

All materials used to process the samples, e.g. mixing bowls of the food processor, knives and chopping boards were decontaminated before each use to prevent (cross)contamination. The vegetables were preprocessed in accordance with the methods used by the production companies. In brief, before mincing and homogenization in a Braun K-650 Combimax food processor the following actions were taken with the various produces. From the lettuce heads and endive heads the stems were cut off and discarded, and the outer leaves were removed and discarded. Red peppers were sliced in half, and the seeds and membranes were removed. From the cucumbers the ends were cut, iceberg lettuce heads and radicchio rosso heads were cut in half and the stalk and the outer leaves were removed. Rucola and baby greens were minced and homogenized without preparation. After mincing and homogenization the produce samples were examined for the presence of *Salmonella* species, *Campylobacter* species and *Escherichia coli* O157. An enumeration method was used to assess the presence of *Listeria monocytogenes*. Methods, as described below, were based on the following international standards: ISO 6579 (*Salmonella* spp.), ISO 10272 (*Campylobacter* spp.), ISO 16654 (*Escherichia coli* O157) and ISO 11290-2 (*Listeria monocytogens*).

For *Salmonella*, 225 ml and 22.5 ml Buffered Peptone Water (BPW) were inoculated in duplicate with 25 g and 2,5 g of the homogenized sample, respectively, and incubated at 37 °C for 18-20 hours. Subsequently, Modified Semi solid Rappaport Vassiliadis (MSRV) plates were inoculated with 100 µl BPW culture divided over three drops and incubated at 41.5 °C for 2 x 24 hours. Plates were evaluated after 24 and 48 hours, and if suspected for *Salmonella* spp. Brilliant Green Agar (BGA) was inoculated and incubated at 37 °C for 24 hours. MSRV was regarded negative if after 2 x 24 hours of incubation no

suspected colonies had developed. Biochemical confirmation was done with Triple Sugar Iron (TSI) agar, Urea (UA) agar and L-Lysine Decarboxylase medium (LDC). Confirmed isolates were serotyped. For *Campylobacter*, 225 ml and 22.5 ml Bolton Broth with laked horse blood were inoculated in duplicate with 25 g and 2.5 g of the homogenized samples, respectively, and incubated at 41.5 °C for 48 hours in a microaerophilic atmosphere (10 % O_2). Subsequently, a sample from the Bolton Broth culture was plated out on Charcoal Cefoperazone Deoxycholate Agar (CCDA) plates and incubated for another 48 hours in a microaerophilic atmosphere. Suspected colonies were tested for their microscopic appearance (motile cork-screw like microorganisms) and oxidase reaction. Further determination was done with a *Campylobacter* Test kit (Oxoid, Basingstoke, UK) according to the manufacturer's instructions.
Escherichia coli O157 was examined by means of an immuno magnetic separation test. For this purpose 225 ml and 22.5 ml of modified Tryptone Soya Broth containing Novobiocin (mTSB+Nov) was inoculated in duplicate with 25 g and 2.5 g homogenized sample, respectively, and incubated at 41.5 °C for 22 hours. Subsequently, 1 ml of mTSB+Nov culture was used for separation and concentration with Dynabeads anti-*E. coli* O157 test kit (Dynal Biotech ASA, Oslo Norway) according to the manufacturer's instructions. Cefixime Tellurite Sorbitol MacConkey (CT-SMAC) agar plates were used for detection. Presumptive colonies were confirmed with eosine methylene blue agar plates and Wellcolex *E.coli* O157 latex test (Remel Europe Ltd, Kent UK).
An enumeration method was used for *Listeria monocytogenes*. Hereto, 90 ml of BPW was inoculated with 10 g of homogenized sample and left at room temperature for resuscitation for one hour. Subsequently, 1.0 and 0.1 ml were plated out in duplicate on respectively 14 cm and 9 cm agar plates of Agar Listeria according to Ottaviani & Agosti (ALOA) and incubated for 48 hours at 37 °C. Confirmation of the suspected colonies was done by means of a heamolysis test, a katalase reaction, motility test at 25 °C and the fermentation of L-Rhamnose and D-Xylose. From the number of counted and confirmed colonies the cfu/ml was calculated.
Unless stated otherwise all materials were from Biotrading, Mijdrecht, the Netherlands.

Ready to eat products, collected at the end of the lines at the production companies and at retail level were minced and homogenized without further pre-treatment, and investigated as described.

Salmonella spp. were serotyped by the Laboratory for Infectious Diseases and Perinatal Screening of the RIVM.

4 Results

4.1 Prevalence estimation

Tables 3 and 4 show the net realization of numbers of investigated samples with accompanying prevalence estimates for the separate microbes on total produce and product level. Net realizations differ from the indicated numbers at the end of section 2.4 due to occasionally missing produce/product samples, failed analysis, et cetera. The results for the samples from the processing companies are shown in Table 3, the results for the retail samples in Table 4. As no micro organisms were detected in the products from the processing companies (see Table 3) only an upper prevalence level could be determined (in this case, the 95 % confidence upper prevalence level was below 0.40 % for all pathogens). The prevalence point estimate for the micro organisms in raw produce varied from 0.11 % for *L. monocytogenes* and *E. coli* O157 to 0.38 % for *Salmonella* spp. Only one retail sample was found positive (Table 4), which resulted in a *Salmonella* spp. prevalence point estimate of 0.17 % and an upper prevalence level estimate of 0.26 % for the other pathogens (with 95 % confidence).

Table 3 Results of the sample analysis from the processing companies for a) total raw produce and b) total products. Where, *k* is the number of positive samples, *n* is the total number of samples analysed per pathogen/produce or product combination, *d* is the prevalence point estimate. In those cases where *k* > 0, the last two columns represent the *Lower* (2.5 %) and *Upper* (97.5 %) bound of the 95 % Confidence Interval around *d*. In those cases where *k*=0, *d* gives the upper bound of the prevalence estimate with 95 % confidence.

				95 % Confidence Interval	
a) Raw produce	*k*	*n*	*d*	*Lower*	*Upper*
Salmonella spp.	6	1860	0.38 %	0.15 %	0.70 %
Campylobacter spp.	3	1810	0.22 %	0.06 %	0.48 %
E. coli O157	1	1833	0.11 %	0.01 %	0.30 %
L. monocytogenes	1	1860	0.11 %	0.01 %	0.30 %
b) Products					
Salmonella spp.	0	751	< 0.40 %	95 % Confidence level	
Campylobacter spp.	0	764	< 0.39 %	,,	
E. coli O157	0	760	< 0.39 %	,,	
L. monocytogenes	0	781	< 0.38 %	,,	

Table 4 Results of the product sample analysis from retail. Where, *k* is the number of positive samples, *n* is the total number of samples analysed per pathogen/product combination, *d* is the prevalence point estimate. In those cases where *k* > 0, the last two columns represent the *Lower* (2.5 %) and *Upper* (97.5 %) bound of the 95 % Confidence Interval about *d*. In those cases where *k*=0, *d* gives the upper bound of the prevalence estimate with 95 % confidence.

				95 % Confidence Interval	
Retail	*k*	*n*	*d*	*Lower*	*Upper*
Salmonella spp.	1	1151	0.17 %	0.02 %	0.48 %
Campylobacter spp.	0	1151	< 0.26 %	95 % Confidence level	
E. coli O157	0	1151	< 0.26 %	,,	
L. monocytogenes	0	1151	< 0.26 %	,,	

Table 5 shows the prevalence estimates of the separate microbes according to the produce they were associated with. Endive appeared to be the most susceptible raw produce, as *Salmonella* spp. was detected in three heads, *Campylobacter* spp. in two heads and *E. coli* O157 in one head.

Table 5 Prevalence estimates for the separate microbes on produce level. Where, *k* is the number of positive samples, *n* is the total number of samples analysed per pathogen/produce combination, *d* is the prevalence point estimate. The last two columns represent the *Lower* (2.5 %) and *Upper* (97.5 %) bound of the 95 % Confidence Interval about *d*.

				95 % Confidence Interval	
Microbe / Produce	*k*	*n*	*d*	*Lower*	*Upper*
Salmonella spp.					
Endive	3	370	1.10 %	0.29 %	2.30 %
Cucumber	1	37	5.10 %	0.64 %	13.8 %
Iceberg lettuce	2	565	0.53 %	0.11 %	1.27 %
Campylobacter spp.					
Endive	2	360	0.83 %	0.17 %	2.00 %
Oak tree lettuce green	1	72	2.70 %	0.33 %	7.40 %
E. coli O157					
Endive	1	370	0.54 %	0.06 %	1.50 %
L. monocytogenes					
Frisee fine	1	111	1.77 %	0.22 %	4.87 %

Table 6 shows further results, i.e. serotyping and concentration estimates of the positive samples as found throughout the sampling period. All *Salmonella* types found in raw produce appeared to be *S. Typhimurium* DT104 strains. Further discrimination is still under investigation.

Table 6 Time during survey, number of positive (*k*) products/produce with a further serotype specification and concentration estimates (MPN) together with *Lower* (2.5 %) and *Upper* (97.5 %) values of the 95 % Confidence Interval about the MPN for the positive samples as found throughout the sampling period.

					95 % Confidence Interval	
2006	*k*	*Product*	*Pathogen*	*MPN (cfu g $^{-1}$)*	*Lower*	*Upper*
November 10	1	Oak tree lettuce	*Salmonella Montevideo*	0.02	0.001	0.11
2007		*Produce*				
May 2	2	Endive	*Campylobacter* spp.	0.024	0.0014	0.112
		Endive	*Campylobacter* spp.	0.024	0.0014	0.112
May 21	1	Oak tree lettuce green	*Campylobacter* spp.	0.096	0.0133	0.518
June 20	1	Curly endive	*L. monocytogenes*	*see Table 7*		
July 18	1	Endive	*E. coli* O157	0.052	0.0084	0.171
September 3	2	Cucumber	*Salmonella Typhimurium* DT104	0.019	0.0011	0.082
		Endive	*Salmonella Typhimurium* DT104	0.019	0.0011	0.082
September 10	1	Iceberg lettuce	*Salmonella Typhimurium* DT104	0.024	0.0014	0.112
October 17	3	Endive	*Salmonella Typhimurium* DT104	> 0.281[1]		
		Endive	*Salmonella Typhimurium* DT104	0.024	0.0014	0.112
		Iceberg lettuce	*Salmonella Typhimurium* DT104	0.281	0.041	1.31

[1] In this case only a lower limit could be estimated since all dilutions and replicates of the MPN were tested positive.

The result of the quantitative assessment for *L. monocytogenes* (i.e for a curly endive sample in which the microorganism was detected) is presented in Table 7. The concentration of *L. monocytogenes* could be calculated based on the number of colonies on the 14 and 9 cm ALOA plates.

Table 7 Result of the analysis (cfu's observed) on 10 g frisee fine for L. monocytogenes (left hand side). The contamination level point estimate together with the Lower (2.5 %) and Upper (97.5 %) values of the 95 % Confidence Interval about the point estimate are given in the right hand side of the table.

Dilution	cfu observed	Estimated cfu g^{-1} produce[1]	95 % Confidence Interval	
			Lower	Upper
10^0	26	250	194	320
10^0	28			
10^{-1}	2			
10^{-1}	5			

[1] This estimate is calculated following a Bayesian approach using a Uniform Prior and Poisson Likelihood distribution (Vose 2000)

4.2 Microbiological Risk Assessment

This section will reveal insight in the production chain processes of "ready to eat" mixed salads and its microbial dynamics up until human consumption inclusive. An initial point estimate of the potential microbial risk involved in the consumption of "ready to eat" mixed salads is calculated using a "swift Quantitative Microbiological Risk Assessment" (sQMRA) Tool developed by Evers and Chardon (2008b, 2008a). The sQMRA Tool involves several calculation procedures which result in a relative risk point estimate. This risk refers to the number of people in a predefined population becoming ill on a yearly basis relative to the number stemming from *Campylobacter* in poultry as result of a quantitative risk assessment (including variability) performed during the CARMA project (Nauta et al. 2005). We refer to the paper of Evers and Chardon (submitted) for a full Tool description. Here, only the output sheet of the tool will be explained where appropriate for our purposes. That is, applying this Tool will give insight in the number of variables and uncertain parameters in the chain under study. Scenario analysis will give insight in the need for a full quantitative chain approach risk assessment concerning these pathogen/product combinations.

The tool shows the pathogen matrix combination and the population at risk in the left hand corner at the top (Figure 2). Further relevant model input parameters are to be filled out in the left hand column at the bottom. General parameters apply to the behavior of the population at risk, such as consumption and preparation properties. Specific pathogen characteristics are to be filled out, in this case using the survey results. Further parameters concern cross-contamination and pathogen survival events during preparation that refer to both the population hygiene standards and pathogen/matrix characteristics. Sub-procedures give point estimates for contamination figures throughout the food chain, such as contamination at retail and human exposure levels, to ultimately come to a relative risk point estimate.

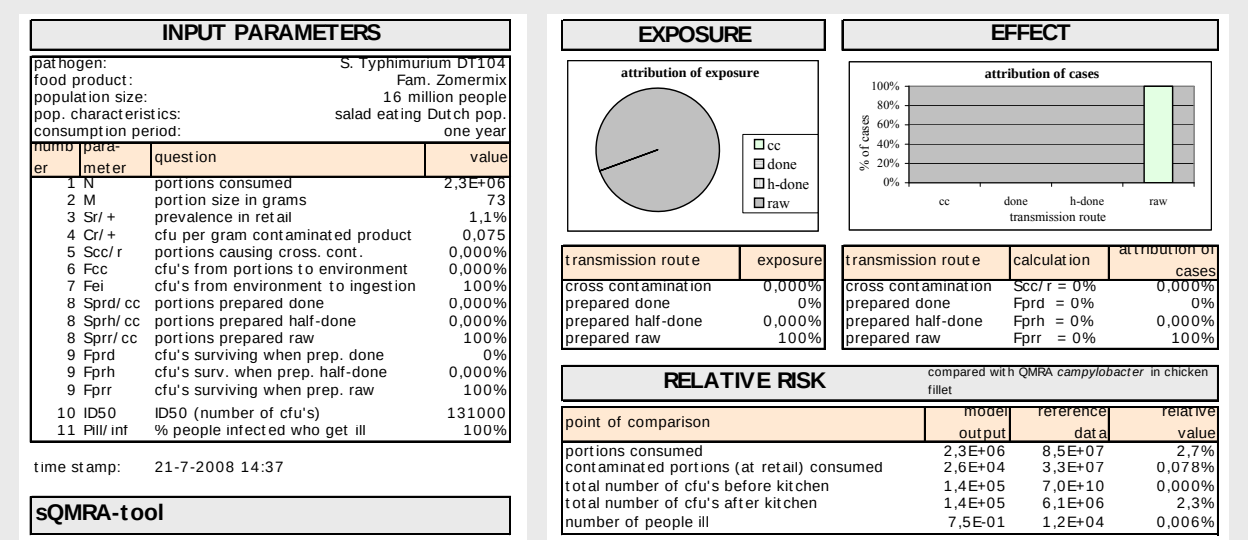

Figure 2 Overview of the sQMRA Tool giving a relative risk estimate (0.006 %) and a point estimate of < 1 cases per year for the Dutch population being exposed (relative value of 2.3 % and an exposure level of 1.4E+05 cfu y^{-1}) to *Salmonella Typhimurium* DT104 in "Family Summermix". The relative risk is calculated using the reference data: estimates on campylobacteriosis from chicken filet (Nauta et al., 2005, 2007)). The effect of produce washing on the contamination level of the product has not been incorporated in this figure.

The data filled out here relate to the risk assessment concerning ready-to-eat raw mixed salads. Scenario's represent the relative microbial risk from products associated with contaminated produce as found during the survey. An important factor in the conversion of contaminated produce to cfu per gram contaminated product (parameter no. 4 in the Tool, Figure 2) is the effect of washing before different produce are merged into a mixed salad. The low number of products found positive for microbial contamination (only one sample positive for *Salmonella* in the retail (Table 4) could indeed indicate a food handling effect, i.e. reducing contamination levels, mainly due to washing. In addition, dilution of the concentration occurs as only mixed salads were tested, i.e salads containing ≥ 2 produce. Still, one should account for the fact that only a limited number of samples was tested and this one positive sample might be a consequence of the product sample numbers. This reasoning can be tested with a probability calculation (see footnote in Table 8), i.e. what would be the probability of testing all samples negative if the prevalence through the chain would stay constant (so, if a washing and dilution effect from produce up to a mixed salad package could be ignored). Table 8 shows the probability that all products (i.e. ~760 samples at the end of the production chain and 1151 samples in the retail) are found negative, given the prevalence estimates as found in the produce (when no food handling effect was to be assumed).

Table 8 Probability of finding all product samples negative (both at the end of the processing chain, n~760, and at retail, n=1151), given the boundary of the prevalence estimates as a result of the produce survey (Table 3a).

Prevalence (%), d	Number of samples, n	Probability *all* negative (%)*
Salmonella spp.		
0.38[1]	760	5
0.38	1151	1
0.15[2]	760	32
0.15	1151	18
0.70[3]	760	0.5
0.70	1151	0.03
Campylobacter spp.		
0.22[1]	760	19
0.22	1151	8
0.06[2]	760	63
0.06	1151	50
0.48[3]	760	2.6
0.48	1151	0.4

[1,2,3] Refer respectively to the point estimate for the mean and lower and upper bound of the 95 % confidence interval about the mean (Table 3a)

* Calculated following $(1-d)^n$

The small probabilities of finding only negative product samples given a constant prevalence as shown in Table 8 indicate a likely reduction of pathogenic contamination during processing. The reduction factor can be estimated from the results on microbial contamination levels in the survey, comparing the MPN estimates for the produce with those of the products.

As the input for the sQMRA Tool is solely based on point estimates, the washing factor was initially calculated using the MPN point estimates from the survey results. Hereto, all presence/absence results of the produce were combined and an MPN was estimated to come to an average MPN for the produce. The same was done for the lab results of all products under study. Subsequently, the overall MPN point estimate for produce compared to the products was used to calculate this factor. In this we assume that the reduction factor is microbe independent. The results are shown in Table 9.

Table 9 Input values to calculate the effect of produce washing and dilution through mixing on product contamination.

Average Sample size	Source material (g)	Positives	MPN	Reduction
Produce			$1.69 \cdot 10^{-4}$	86 %
1835	$2 \cdot 25$	11		
1835	$2 \cdot 2.5$	6		
Products			$2.40 \cdot 10^{-5}$	
759	$2 \cdot 25$	1		
759	$2 \cdot 2.5$	0		

4.2.1 Application of the sQMRA Tool

Figure 2 shows an overview of the input and output variables of the sQMRA tool. The first scenario analysis will be explained in detail below and represents the case in which the relative risk (y^{-1}) for the Dutch population exposed to *Salmonella Typhimurium* DT104 from the consumption of a mixed salad "Family Summermix" is assessed. All subsequent calculations will be based on the following procedure.

Initial calculations to obtain the input parameters for the sQMRA tool consist of:

1. Specify the pathogen under study (here, *Salmonella Typhimurium* DT104)
2. Define the mixed salad product under study (here, "Family Summermix")
3. Describe the population under study (here, the potential "Family Summermix" consuming Dutch population of 16 million people and its exposure over a one year period)
4. Define the ingredients and their division over the product (here, Iceberg lettuce (70 %), carrots (3 %), white cabbage (7 %), red lettuce (2 %))
5. Define the weight of the product (here, 400 g)
6. Calculate the number of portions produced per year from the production figures (here, 2.3E+06)
    a. That is, the average weight of all products under study is 220 g. Assuming, on average, 3 persons eat from one mixed salad then the average portion size is 73 g.
    b. Production figures are assumed to be equal to consumption figures.
7. Estimate the prevalence for the separate microbes in the product under study (The "Family Summermix" contains Iceberg lettuce which was found positive for *Salmonella Typhimurium* DT104 as shown in Table 6. More specifically, 1 of the 185 Iceberg lettuce heads in the cutting company that produces this mixed salad was found positive (Table 6, October 17). This results in a prevalence point estimate of 1.1% of the "Family Summermix" to be contaminated with *Salmonella*.
8. Estimate the concentration of the pathogen in the product under study.
    a. In this case, the MPN estimate resulted in 0.281 cfu g^{-1} Iceberg lettuce (Table 6). Yet, the produce Iceberg lettuce is the ingredient for 4 products under study from this processing company. Once the cut Iceberg lettuce is well mixed and subsequently equally divided over the 4 products one can assume that the number of microbes will be spread accordingly. In reality, however, some products contain more Iceberg lettuce than others and, therefore, the number of microbes was divided proportionally to the amount of this produce in the separate products. This resulted in a point estimate for the concentration of *Salmonella Typhimurium* DT104 in the "Family Summermix" salad to be 0.075 cfu g^{-1}.
    b. The possible effect of a further dilution of the concentration due to washing of the produce on the resulting risk estimate has been incorporated in running 2 scenario's: I. without a reduction factor, i.e. a constant contamination level throughout the chain as calculated under 8a. II. with a reduction facter, i.e. the contamination level reduces to $0.14 \cdot$ [conc. on produce]. (here, parameter no. 4 in Figure 2 then becomes 0.0105 cfu g^{-1}).
9. As mixed salads are assumed to be consumed raw, no further handling effects have to be considered as input for the tool and the final estimates concern the dose-response parameters.
10. The ID50 value (i.e. the number of *Salmonella* to be ingested upon which 50 % of the exposed people will become infected) for *Salmonella Typhimurium* DT104 is assumed to be 1.31E+5 (this is an average value calculated from Berk (2008)).
11. The percentage of people becoming ill upon infection (Pill/inf) is assumed to be 100 %.

reduction factor) and its relative risk would become >0.32 or >0.04 % (with reduction factor), values not shown.
Although absolute illness point estimates for *Campylobacter* are somewhat higher compared to *Salmonella*, still the values relative to *Campylobacter* in chicken filet are low. The same reasoning is true for *E. coli* O157 and *L. monocytogenes*. Yet, recall that a difference in the ID50 value would change the risk linearly in this part of the dose response curve. So, for example, when an ID50 value of 1.0E+04 would be applied for *L. monocytogenes*, values would change to 2.1E+04 (relative risk 168 %) or 3.2E+03 (relative risk 26 %) (without and with application of the reduction factor respectively, values not shown).

Table 10 Summary of the sQMRA tool exposure scenario's applied to all products that contain at least one of the produce found positive for the microbes under study. Effect of produce washing has not been included here. The "Total" numbers under the horizontal line indicate the point estimates for human exposure (cfu's) to the separate tested microbes over all products. The "Total" numbers to the right of the vertical line present the same figures for the separate products over all the microbes tested.

Pathogen	*Salmonella* spp.		*Campylobacter* spp.		*E. coli* O157		*L. monocytogenes*		Total
Product	Exposure (cfu y^{-1})	Rel. risk (%)	Exposure (cfu y^{-1})	Rel. risk (%)	Exposure (cfu y^{-1})	Rel. risk (%)	Exposure (cfu y^{-1})	Rel. risk (%)	Exposure (cfu y^{-1})
Variegated lettuce mix 1* (Bonte slamix 1*)	3.9E+04	0.634	7.6E+04	1.2	1.1E+05	1.8			≥8.5E+05
Variegated lettuce mix 2* (Bonte slamix 2*)	4.9E+04	0.803							
Variegated lettuce mix 3* (Bonte slamix 3*)	≥5.8E+05	9.5							
Family Summermix (Familie Zomermix)	1.4E+05	2.3							1.4E+05
Dutch raw vegetables (Hollandse rauwkost)	5.1E+03	0.083							5.1E+03
Iceberg lettuce frisee mix (IJsbergsla friseemix)	1.4E+05	2.3							1.4E+05
Salad dish (Saladeschotel)	9.6E+04	1.6							9.6E+04
Lamb's lettuce mix (Veldslamix)	2.9E+04	0.479							2.9E+04
Oakleaf lettuce blend (Eikenbladslamelange)			1.2E+05	2.0					1.2E+05
Oakleaf lettuce blend (retail) (Eikenbladslamelange (retail))	6.9E+02	0.011							6.9E+02
Mixed Iceberg lettuce (Gemengde ijsbergsla)	2.4E+04	0.386							2.4E+04
Iceberg lettuce frisee mix (IJsbergsla friseemix)	1.4E+04	0.231					3.4E+08	5539	3.4E+08
Italian salad (Italiaanse salade)			4.3E+05	7.1					4.3E+05
Economy size mixed lettuce (Voordeelverpakking gemengde salade)	1.9E+04	0.304							1.9E+04
Total	≥1.1E+06		6.3E+05		1.1E+05		3.4E+08		≥3.4E+08

* Variegated lettuce mix (Bonte slamix) 1, 2 and 3 refer to sQMRA output for the 3 different concentrations initially found on the produce (i.e. 1. Endive with 0.019, 0.024 and >0.281 cfu g^{-1} respectively)

Table 10a Summary of the sQMRA tool exposure scenario's applied to all products that contain at least one of the produce found positive for the microbes under study. Effect of produce washing has been included here (86 %, Table 9). The "Total" numbers under the horizontal line indicate the point estimates for human exposure (cfu's) to the separate tested microbes over all products. The "Total" numbers to the right of the vertical line present the same figures for the separate products over all the microbes tested.

Pathogen Product	*Salmonella* spp.		*Campylobacter* spp.		*E. coli* O157		*L. monocytogenes*		Total
	Exposure (cfu y^{-1})	Rel. risk (%)	Exposure (cfu y^{-1})	Rel. risk (%)	Exposure (cfu y^{-1})	Rel. risk (%)	Exposure (cfu y^{-1})	Rel. risk (%)	Exposure (cfu y^{-1})
Variegated lettuce mix 1* (Bonte slamix 1*)	5.4E+03	0.089	1.1E+04	0.175	1.5E+04	0.246			
Variegated lettuce mix 2* (Bonte slamix 2*)	6.9E+03	0.112							≥1.2E+05
Variegated lettuce mix 3* (Bonte slamix 3*)	≥8.1E+04	1.3							
Family Summermix (Familie Zomermix)	2.0E+04	0.322							2.0E+04
Dutch raw vegetables (Hollandse rauwkost)	7.1E+02	0.012							7.1E+02
Iceberg lettuce frisee mix (IJsbergsla friseemix)	1.9E+04	0.316							1.9E+04
Salad dish (Saladeschotel)	1.3E+04	0.22							1.3E+04
Lamb's lettuce mix (Veldslamix)	4.1E+03	0.067							4.1E+03
Oakleaf lettuce blend (Eikenbladslamelange)			1.7E+04	0.275					1.7E+04
Oakleaf lettuce blend (retail) (Eikenbladslamelange (retail))	9.6E+01	0.002							9.6E+01
Mixed Iceberg lettuce (Gemengde ijsbergsla)	3.3E+03	0.054							3.3E+03
Iceberg lettuce frisee mix (IJsbergsla friseemix)	2.0E+03	0.032					4.7E+07	775	4.7E+07
Italian salad (Italiaanse salade)			6.1E+04	0.994					6.1E+04
Economy size mixed lettuce (Voordeelverp. gemengde salade)	2.6E+03	0.043							2.6E+03
Total	≥1.6E+05		8.9E+04		1.5E+04		4.7E+07		

* Variegated lettuce mix (Bonte slamix) 1, 2 and 3 refer to sQMRA output for the 3 different concentrations initially found on the produce (i.e. 1. Endive with 0.019, 0.024 and >0.281 cfu g^{-1} respectively)

Table 11 Summary of the sQMRA tool response scenario's applied to all products that contain at least one of the produce found positive for the microbes under study. Effect of produce washing has not been included here. The "Total" numbers under the horizontal line indicate the point estimates for the number of people ill from the separate tested microbes over all products. The "Total" numbers to the right of the vertical line present the same figures for the separate products over all the microbes tested. Furthermore, the assumed ID50 values and Fill values have been indicated. Note that a change in the ID50 or Pill/inf will result in a linear change of the risk in this linear part of the dose-response curve.

Pathogen Product	*Salmonella* spp. Ill (y^{-1})	 Rel. risk (%)	*Campylobacter* spp. Ill (y^{-1})	 Rel. risk (%)	*E. coli* O157 Ill (y^{-1})	 Rel. risk (%)	*L. monocytogenes* Ill (y^{-1})	 Rel. risk (%)	Total Ill (y^{-1})
Variegated lettuce mix 1* (Bonte slamix 1*)	0	0.002	1.9E+01	0.157	7.4E+02	6.0			≥7.6E+01
Variegated lettuce mix 2* (Bonte slamix 2*)	0	0.002							
Variegated lettuce mix 3* (Bonte slamix 3*)	≥3	>0.025							
Family Summermix (Familie Zomermix)	1	0.006							1
Dutch raw vegetables (Hollandse rauwkost)	0	0							0
Iceberg lettuce frisee mix (IJsbergsla friseemix)	1	0.006							1
Salad dish (Saladeschotel)	1	0.004							1
Lamb's lettuce mix (Veldslamix)	0	0.001							0
Oakleaf lettuce blend (Eikenbladslamelange)			3.1E+01	0.248					3.1E+01
Oakleaf lettuce blend (retail) (Eikenbladslamelange (retail))	0	0							0
Mixed Iceberg lettuce (Gemengde ijsbergsla)	0	0.001							0
Iceberg lettuce frisee mix (IJsbergsla friseemix)	0	0.001					2.3E+03	19	2.3E+03
Italian salad (Italiaanse salade)			1.1E+02	0.896					1.1E+02
Economy size mixed lettuce (Voordeelverpakking gemengde salade)	0	0.001							0
Total	≥6	>0.004	1.6E+02	0.100	7.4E+02	0.461	2.3E+03	1.461	≥3.2E+03
ID50	1.31E+05		897		100		1.00E+05		
Pill/inf	100 %		33 %		100 %		100 %		

* Variegated lettuce mix (Bonte slamix) 1, 2 and 3 refer to sQMRA output for the 3 different concentrations initially found on the produce (i.e. 1. Endive with 0.019, 0.024 and >0.281 cfu g^{-1} respectively)

Table 11a Summary of the sQMRA tool response scenario's applied to all products that contain at least one of the produce found positive for the microbes under study. Effect of produce washing has been included here (86 %, Table 9). The "Total" numbers under the horizontal line indicate the point estimates for the number of people ill from the separate tested microbes over all products. The "Total" numbers to the right of the vertical line present the same figures for the separate products over all the microbes tested. Furthermore, the assumed ID50 values and Fill values have been indicated. Note that a change in the ID50 or Pill/inf will result in a linear change of the risk in this linear part of the dose-response curve.

Pathogen Product	*Salmonella* spp. Ill (y^{-1})	 Rel. risk (%)	*Campylobacter* spp. Ill (y^{-1})	 Rel. risk (%)	*E. coli* O157 Ill (y^{-1})	 Rel. risk (%)	*L. monocytogenes* Ill (y^{-1})	 Rel. risk (%)	Total Ill (y^{-1})
Variegated lettuce mix 1* (Bonte slamix 1*)	0	0	3	0.022	1.0E+02	0.843			≥1.0E+02
Variegated lettuce mix 2* (Bonte slamix 2*)	0	0							
Variegated lettuce mix 3* (Bonte slamix 3*)	≥0	≥0.003							
Family Summermix (Familie Zomermix)	0	0.001							0
Dutch raw vegetables (Hollandse rauwkost)	0	0							0
Iceberg lettuce frisee mix (IJsbergsla friseemix)	0	0.001							0
Salad dish (Saladeschotel)	0	0.001							0
Lamb's lettuce mix (Veldslamix)	0	0							0
Oakleaf lettuce blend (Eikenbladslamelange)			4	0.035					4
Oakleaf lettuce blend (retail) (Eikenbladslamelange (retail))	0	0							0
Mixed Iceberg lettuce (Gemengde ijsbergsla)	0	0							0
Iceberg lettuce frisee mix (IJsbergsla friseemix)	0	0					3.3E+02	2.7	3.3E+02
Italian salad (Italiaanse salade)			1.5E+01	0.125					1.5E+01
Economy size mixed lettuce (Voordeelverpakking gemengde salade)	0	0							0
Total	≥0	≥0	2.2E+01	0.014	1.0E+02	0.065	3.3E+02	0.208	
ID50	1.31E+05		897		100		1.00E+05		
Pill/inf	100 %		33 %		100 %		100 %		

* Variegated lettuce mix (Bonte slamix) 1, 2 and 3 refer to sQMRA output for the 3 different concentrations initially found on the produce (i.e. 1. Endive with 0.019, 0.024 and >0.281 cfu g^{-1} respectively)

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5 Conclusions and discussion

Output of the sQMRA tool for general microbial exposure levels from mixed salads in the Netherlands is in the range of 1E+05 to (≥) 1E+06 y^{-1} (and 1E+04 to 1E+05 y^{-1} when including the produce washing and dilution through mixing reduction-factor of 86 %) for *Salmonella* spp., *Campylobacter* spp. and *E. coli* O157. The exposure risk point estimate for *L. monocytogenes* turned out to be considerably higher, i.e. 1E+08 (and 1E+07) y^{-1} without (and with) using the reduction factor. The individual (per pathogen/mixed salad combination) exposure risk point estimates are, except for *L. monocytogenes* in "iceberg lettuce frisee mix" and *Salmonella* spp. in "variegated lettuce mix", all below 10 % compared to the risk estimate for *Campylobacter* spp. in chicken filet. The relative exposure level risk point estimate for *L. monocytogenes* in "iceberg lettuce frisee mix" is > 5000 % (and > 700 % including the reduction factor) compared to the reference data.

The point estimate for the number of ill people associated with microbes in mixed salads, assuming washing of produce has no effect on the contamination levels, is for *Salmonella* spp. ≥ 6 y^{-1}, for *Campylobacter* spp. 160 y^{-1}, for *E. coli* O157 740 y^{-1} and for *L. monocytogenes* 2300 y^{-1}. If the point estimate for the washing and dilution through mixing step reduction-factor (86 %) is applied then the number of cases become ≥ 0 y^{-1} for *Salmonella* spp., 22 y^{-1} for *Campylobacter* spp., 100 y^{-1} for *E. coli* O157 and 330 y^{-1} for *L. monocytogenes*. Still, the individual (per pathogen/mixed salad combination) risk point estimates are all below 20 % compared to the risk estimate for *Campylobacter* spp. in chicken filet.

In calculating a relative risk estimate using the sQMRA tool one should be aware that the input parameters only reflect point estimates of the variables and uncertain parameters. The tool does not account for variability of the process parameters and microbial dynamics nor for uncertainty in the prevalence and concentration estimates.
Important sources of variability and uncertainty for a quantitative microbial risk estimate from the consumption of raw salads turned out to be:

Variability:

1. the division of produce over products;
2. product production figures;
3. human consumption patters (*i.e.* portion size, fraction consumed in relation to what is produced);
4. dilution of the pathogen concentration on the produce due to the and mixing step in the cutting companies;
5. pathogen prevalence;
6. pathogen concentration;
7. dose response relation in the Dutch population for the separate pathogen/matrix combinations.

Uncertainty:

1. contribution of the large cutting companies to the total production/consumption figures of mixed salads;
2. dilution of the pathogen concentration on the produce due to the washing step in the cutting companies;
3. reduction of the pathogen concentration per mixed salad bag with respect to the separate produce concentration due to mixing;
4. pathogen prevalence estimates from a survey;
5. pathogen concentration estimates from a survey;
6. dose response relation in the Dutch population for the separate pathogen/matrix combinations.

A further model assumption is that pathogens are homogeneously spread over the produce.

A full quantitative microbial risk assessment will quantify the variability and uncertainty that underlie the risk estimate for exposure to pathogens from the consumption of mixed salads. Such a full risk assessment would reveal the risk as a probability density distribution reflecting natural population variability. With that, such a distribution would show the possibility of extreme exposure values and human cases of illness (*i.e.* the tails of the distributions having a low probability of occurrence). Including uncertainty distributions for the parameter estimates in a full QMRA will increase insight in the tails of the distribution even more. And, it is often the tail of these distributions that determine the risk (Nauta and Havelaar 2008; Rosenquist et al. 2003; Rieu et al. 2007). Including variability and uncertainty distributions in a full QMRA would enable us to get insight in the current reasoning concerning the likeliness of relating recent outbreak data, e.g. Friesema et al. (in preparation), to current survey results. In other words, could the results of this survey, with generally low prevalence and concentration estimates, result in an outbreak or are outbreaks associated with microbial contamination of raw produce more likely to stem from incidentally high point contaminations?

The drawback of the potential of increasing insight in the human risk when composing a full QMRA is still in the lack of data, particularly in the dose response relationship for these pathogen/mixed salad combinations.
Data gaps for the dose response relationship will also in a full QMRA still form a large source of uncertainty.
Dose-response models are part of a RA and used in translating exposure assessment data into a risk on infection. Information on dose-response processes, however, is scarce and the little data available is mostly derived from non-representative human volunteer studies or from studies using an animal system. In these studies, usually laboratory adapted strains are used, cultured under conditions different from those found in food products. More and more information becomes available showing that the behaviour of micro-organisms grown under laboratory conditions strongly differs from that of bacteria present in food products (De Jong et al. 2008; Wijnands et al. accepted). This was found both for the level of heat resistance and for the ability of food borne pathogens to survive the human stomach. Recently, the behaviour of Campylobacter on the surface of chicken meat was found to differ significantly from the behaviour in broth upon heating (De Jong et al. 2008). In a recent *Salmonella* outbreak, cheeses produced from raw milk were found to be the vehicle of *Salmonella*. The level of contamination of these cheeses appeared to be as low as 4 cfu's of *Salmonella* per kilo. Moreover, the

microorganism was very unevenly distributed in the cheese, indicating that the matrix and the distribution of a microorganism in a matrix do play important roles. What role do food products play in the dose response relationship? Do storage conditions affect the virulence? These are questions that need to be answered in order to build more process based dose response models which can then be used in QMRA.

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