



Risk-Benefit Assessment of Cold-Smoked Salmon: Microbial Risk versus Nutritional Benefit

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Authors' contributions

This work was carried out in collaboration between all authors. Author FLB designed the study, did literature research, developed the model, performed the statistical analysis, and wrote the first draft of the manuscript. The other authors contributed by discussing the model, adding expertise from various research disciplines and helping in finalizing the paper. In addition, authors MN and JH assisted in the model development and statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

The objective of the study is to perform an integrated analysis of microbiological risks and nutritional benefits in a fish product, Cold Smoked Salmon (CSS). Literature study identified the major health risks and benefits in connection with CSS consumption. The reduction of the risk of Coronary Heart Disease (CHD) mortality and stroke, as well as enhanced cognitive (IQ) development of unborns following maternal intake, are identified as the main health benefits of omega-3 fatty acid from CSS. Contrary, risk of meningitis, septicemia and abortion/stillborn are identified as a major health risk endpoints due to exposure to the pathogen *L. monocytogenes*.

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Two consumption scenarios were considered: a reference scenario (23g/day and 20g/day for man and woman respectively) and an alternative scenario (40g/day for both sexes). In order to evaluate and compare the risks and benefits, the Disability Adjusted Life Years (DALY) method has been used as a common metric.

Results show that the overall health benefits outweigh the risk, foremost contributed by the effect of decreased CHD mortality and IQ increase. A sensitivity analysis indicated that this result was robust for the analyzed parameters, except the storage time: the adverse effect of consumption of CSS prevails over the beneficial effect if the storage time of CSS is increased from two weeks to five weeks or more, due to an increased risk of listeriosis.

This study demonstrates how microbial risks can be integrated in risk-benefit assessment, and shows that a sensitivity analysis has an added value, even if the benefits largely outweigh the risk in the initial analysis.

Keywords: Cold-smoked salmon; Listeria monocytogenes; omega-3 fatty acids; DALY.

1. INTRODUCTION

Risk-benefit assessment is the weighing of the probability of an adverse health effect against the probability of a beneficial effect as a result of exposure/intake of food (EFSA, 2010). Examples and a guidance of how to perform risk-benefit assessment of foods have recently been provided (Hoekstra et al., 2010; EFSA, 2010). Nonetheless, risk-benefit methods need further development. There is currently no internationally agreed method to perform human health risk-benefit assessment of food and so far only a few risk-benefit assessments studies included microbiological hazards (Havelaar et al., 2000; Magnússon et al., 2012). Typical aspects of microbiological risk assessment, like the inclusion of the impact of storage and processing on the weighing of the risk and benefit, are therefore rarely included in published risk-benefit assessments.

In this paper we present a risk-benefit assessment on a fish product. Several studies have assessed the risk of toxic contaminants and benefits of nutrients following the consumption of fish (Gladyshev et al., 2008; Cohen et al., 2005b; Guevel et al., 2008; FAO/WHO, 2011; Hoekstra et al., 2012) and found that in general the public health benefits are larger than the risks. However, microbial risks have not been integrated into these risk-benefit assessments. The present study aims to illustrate how a microbiological hazard can be included in a typical risk-benefit assessment and how this may add to the existing risk-benefit assessment tools and methodologies. Furthermore, we included a sensitivity analysis to evaluate the impact of some of the model parameters on the assessment.

2. RISK-BENEFIT ASSESSMENT OF COLD-SMOKED SALMON: MODEL

2.1 Scope

The risk of the bacterial pathogen (*L. monocytogenes*) is evaluated against the benefits of the intake of omega-3 fatty acids in a risk-benefit assessment of CSS consumed in Denmark. Salmon is an oily fish containing considerable amount of omega-3 fatty acids, it is a popular ready-to-eat food in most part of the world and it is consumed in many European countries (WHO/FAO, 2004).

The assessment compares a reference scenario with an alternative scenario, as in (Hoekstra et al., 2010). In this comparison it is assumed that CSS is added to the normal diet in an isocaloric way and substitution by other food items with potential health effects is neglected. Best estimates are applied for the various model parameters. Disability Adjusted Life Years (DALYs) are used as an integrated health measure to compare risks and benefits (Hoekstra et al., 2010).

All statistical and mathematical modelling is implemented in Microsoft Office, version 2007 except for the dose-response modelling of CHD mortality and stroke (Appendix) which was performed on statistical software R, version 2.10.1.

2.2 Hazard Identification, Selected Health Effects and Affected Subpopulation

Cold-smoked fish products may be contaminated with *L. monocytogenes*, the agent that causes foodborne listeriosis. Vacuum-packed cold-smoked fish has a long shelf-life and can support the growth of *L. monocytogenes* (WHO/FAO, 2004). The contribution of salmon for the cases of listeriosis has been reported (Pouillot et al., 2007; Lindqvist and Westoo, 2000; WHO/FAO, 2004). Recently an increasing incidence of invasive listeriosis, primarily septicemia and meningitis have been reported in several European countries (Allerberger and Wagner, 2010; Jensen et al., 2010). Listeriosis during pregnancy is also a serious threat to the unborn child, which can lead to abortion/ stillborn (Smith, et al., 2009). Hence, meningitis, septicemia and abortion/stillborn are selected as the endpoints following exposure to *L. monocytogenes*.

Elderly, immunocompromized and pregnant women and/or their unborn fetuses are the most susceptible groups for listeriosis (WHO/FAO, 2004; Allerberger and Wagner, 2010). Therefore, both sexes aged 60 are selected for septicemia and meningitis. The population of interest for abortion/stillborn, are potentially pregnant women aged 20-45.

2.3 Benefit Identification, Selected Health Effects and Affected Subpopulation

The nutrients in fish that have plausible and significant health benefits for human are omega-3 fatty acids, principally eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) (Mozaffarian, 2006). Intake of fish may protect against CHD mortality and stroke (Mozaffarian, 2006). In addition, an association is found between the maternal intake of DHA and a beneficial effect in cognitive development of their unborn child, measured as an increase in IQ (Cohen et al., 2005a). Zeilmaker et al. (2012) investigated both the adverse (MeHg) and beneficial (DHA) effect of fish intake on IQ and found a very small IQ gain for salmon intake. Therefore, reduction of CHD mortality and total stroke, and improved cognitive development are selected endpoints in this paper.

Most of the studies mentioned in the Appendix (Table 8 and 9), which are incorporated in the dose-response modeling of CHD mortality and total stroke, included adults of both sexes older than 35 years. Hence, both sexes aged 35 are selected for both endpoints as a target population. For the benefit of maternal intake of DHA on the child's IQ, it is assumed that women aged 20-45 give birth with different probabilities depending on age (Table 3).

2.4 Intake and Exposure Assessment

The current mean fish intake is 20 and 23 g of fish/day for women and men respectively (Pedersen et al., 2010). In the reference scenario it is assumed that every individual consumes the current mean fish consumption as CSS i.e. 20 and 23 g for women and men. For the alternative scenario it is assumed that every person consumes 40 g CSS/day. The intake of CSS is assumed the same for all age groups in each scenario.

Omega-3 fatty acid intake is computed from the official Danish Food Composition Database in combination with the consumption scenarios (Denmark Technical University, 2011).

For *L.monocytogenes*, an exponential growth model is applied to assess the distribution of concentrations at consumption as a function of initial concentration, storage time, growth rate and lag-time (Table 1, eq. 7). The 10-based logarithm of initial concentrations (N_0) of *L.monocytogenes* {0.5: 1.5: 2.5: 3.5} and their prevalences {0.28: 0.05: 0.01: 0} are taken from Jørgensen and Huss (1998). For the exposure assessment, these results are combined with the consumption scenarios.

2.5 Integration of the Health Effects

To combine the health outcomes of the risk and the benefit we have chosen the DALY model of (Hoekstra et al., 2010). For an individual of age CA, the amount of DALY per person per year is:

$$DALY_{a,s} = P_{eff,a,s} [(P_{rec} * YLD_{rec} * w + P_{die} (YLD_{die} * w + LE_{a,s} - CA - YLD_{die})) + (1 - P_{die} - P_{rec}) * (LE_{a,s} - CA) * w]$$

Where:

$DALY_{a,s}$	disability adjusted life years at age, a and sex, s
$P_{eff,a,s}$	probability of onset of the disease at age, a and sex, s, per year
P_{rec}	probability of recovery from the disease
P_{die}	probability the disease causes death
YLD_{rec}	duration of disease for those who recover
YLD_{die}	duration of disease for those who die
CA	current age of individual in year of disease onset (years)
$LE_{a,s}$	normal life expectancy for an individual of age CA ¹
w	disability weight for disease.

2.6 Dose-Response Relationship

After a literature survey, eleven and eight studies are incorporated for the dose-response modeling of CHD mortality and total stroke respectively (Appendix, Tables 8 and 9). The results from studies that are included in the dose-response relation of CHD mortality and total stroke have been implemented by a relation where the relative risk (RR) of the health outcomes is a function of fish intake. Different functions are analyzed in order to select the best model based on the best fit statistics (Appendix 1).

¹ LE is interpreted here as expected age at death not as the also commonly used expected remaining years of life

Table 1. Model equations applied and point estimates of the parameters

Model equation	Parameter values	Description (unit)
1. $I_{DHA} = F_{intake} * DHA$	F_{intake} , scenarios DHA = 1.16g/100g	I_{DHA} , intake of DHA (g/d); F_{intake} , fish intake (g/d); DHA content of CSS (g DHA per g CSS) (DTU, 2011).
2. $IQ = d * I_{DHA}$ (Cohen et al. 2005a)	$d = 1.3$, uncertainty interval (0.8-1.8)	IQ, change in intelligent quotient; d , coefficient.
3. $P_{eff(IQ)}$ = Probability of a woman giving a birth,	$P_{eff(IQ)}$, vary depending on age of a women giving a birth	$P_{eff(IQ)}$, probability of onset of IQ effect which is equivalent to probability of a woman giving a birth (Table 3).
4. $\ln(RR) = a + b * \ln(F_{intake})$	RR, $a=0.17$, $b=0.137$ for CHD mortality and $a=0.113$, $b=0.094$ for stroke	RR, relative risk of CHD mortality and total stroke; a and b are estimated in the meta-analysis in appendix1.
5. $P_{eff,ep,r(a,s)} = Inc_{(a,s)} / N_{(a,s)}$	Variable with age a , sex s and endpoint ep	$P_{eff,ep,r(a,s)}$ probability of onset of endpoints ep , (CHD mortality and total stroke) at reference intake r , in 5 year age class a , for sex s ; $Inc_{(a,s)}$, current incidence of endpoint ep in (a,s) ; $N_{(a,s)}$, number of population in (a,s) . Note: It is assumed that the probability of effect is the current incidence rate for reference intake for both endpoints.
6. $P_{eff,ep,a(a,s)} = RR_{(s)a} * P_{eff,ep,r(a,s)} / RR_{(s)r}$	Varies with scenarios, ages and sexes	$P_{eff,ep,a(a,s)}$, probability of onset of endpoint at alternative intake at age and sex; $RR_{(s)a}$, relative risk of alternative scenario at sex, s ; $RR_{(s)r}$, relative risk of reference scenario at sex, s .
7. $\log N_t = \log N_0 + \mu(t - \tau)$	N_0 , (see section 2.4), $t=14$; $\mu=0.113$; $\tau=0.167$ (WHO/FAO, 2004)	N_t , concentration of <i>Listeria</i> after storage (CFU/g); N_0 , initial concentration (CFU/g); μ , growth rate (log CFU/d); t , storage time (day); τ , lag-time (day). At storage temperature of 5°C
8. $D_{listeria} = F_{intake} * N_t$		$D_{listeria}$, dose of listeria (CFU/d).
9. $P_{inf} = 1 - e^{-rD_{listeria}}$	$r = 5.85 * 10^{-12}$	P_{inf} , probability of infection and illness of <i>Listeria</i> ; r , dose-response parameter specific to <i>Listeria</i> for susceptible population (WHO/FAO, 2004).
10. $P_{eff(mengi)} = K_{mengi} * P_{inf}$	$K = 0.24$	$P_{eff(mengi)}$, probability of onset of meningitis; K_{mengi} , proportion of meningitis cases among those infected with <i>Listeria</i> .
11. $P_{eff(septi)} = K_{septi} * P_{inf}$	$K_{septi} = 0.74$	$P_{eff(septi)}$, probability of onset of septicemia; K_{septi} , proportion of septicemia cases among those infected with <i>Listeria</i> .
12. $P_{eff(abo/stl)} = K_{(abo/stl)} * P_c * P_{inf}$	$K_{(abo/stl)} = 0.266$; P_c , variable with age	$P_{eff(abo/stl)}$, probability of onset of abortion/stillborn; $K_{abo/stl}$, proportion of abortion/stillborn among pregnant women infected with <i>Listeria</i> . P_c , probability of giving birth

In addition, the models are validated using residual analysis and QQ-plot (Ekstrøm and Sørensen, 2011). Based on this, the log-linear model has been selected to estimate the RR based on the intake scenarios. Then, the estimated RR are converted into absolute risk by combining the RR's and the current incidence rates of CHD mortality and total stroke, which are obtained from Denmark Statistics (2011). To characterize the benefit of maternal DHA intake to the cognitive development (IQ) of their offspring, the relation described in Table 1, eq. 1 is applied.

The exponential dose-response model for *L. monocytogenes* is used to characterize and estimate the probability of infection (Table 1, eq. 9).

The DALY calculation has been performed for each sex, age and scenario. From these, the total DALYs are calculated for the Danish population by summation; population's data are shown in Table 2 and obtained from Denmark Statistics (2011). Parameter estimates for DALY computation are also presented in Table 5 and explained in sections 2.7 and 2.8.

Table 2. Number of population by age and gender (Denmark Statistics, 2011)

		Sex		
		Man	Woman	Total
Age	60	582589	642706	1225295
	18-49		1155573	1155573
	35	1556888	1631905	3188793
				5569661

2.7 Estimation of DALY Parameters for Listeriosis

The probability of developing septicemia ($P_{eff(septi)}$) and meningitis ($P_{eff(mengi)}$) depends on the infection probability, P_{inf} , which depends on fish intake. In the reference scenario P_{inf} is $8.9 \cdot 10^{-6}$ for women (20g CSS) and $1 \cdot 10^{-05}$ for men (23 g CSS). In the alternative scenario (40 g CSS) it is $1.78 \cdot 10^{-05}$. In this study it is assumed that the percentage of septicemia and meningitis (K_{septi} , K_{mengi}) is 74% and 24% respectively (Gerner-Smidt et al., 2005). Studies reported that the percentage of abortion/stillborn ($K_{abo/still}$) is about 15-25% (Mylonakis et al., 2002) and 33.3% (Smith et al., 2009). Consequently, we take the mean (26.6%) of the two reported percentages to estimate the abortion/stillborn percentage.

P_{die} is assumed to equal published case fatality rates, 20.8% and 25.4% of the patients died of septicemia and meningitis respectively within a month of diagnosis in a 10-years follow up study period (Gerner-Smidt et al., 2005). For septicemia it is assumed that people who do not die will recover, so $P_{rec} = 1 - P_{die}$. For meningitis the sequela is taken into account, so $P_{rec} = 1 - P_{die} - 0.14$ (Aouaj et al., 2002).

YLD_{die} is computed for meningitis and septicemia from (Gerner-Smidt, et al., 2005). YLD_{rec} and w for both meningitis and septicemia are obtained from (Kemmeren et al., 2006).

Abortion/stillborn implies that the life of a newborn is lost. Therefore, YLD_{die} , YLD_{rec} , CA and P_{rec} are 0 and P_{die} is 1. Obviously abortion/stillborn can only happen with pregnant women therefore the probability of a pregnancy, P_c is included and $P_{eff(abo/stl)}$ is estimated using eq. 12 on Table 1. It is assumed that women give birth with different probabilities depending on age. The probabilities of pregnancy at age below 20 and above 45 are assumed zero.

Table 3. The annual probability that a woman gives birth depending on age (Denmark Statistics, 2011)

Age mother	P_c , Probability of giving birth
20 - 25 year	0.039
25 - 30 year	0.1139
30 - 35 year	0.127
35 - 40 year	0.057
40 - 45 year	0.01

2.8 Estimation of DALY Parameters for CHD Mortality, Stroke and IQ

The case fatality rate of total stroke is assumed to be the same as for ischemic stroke, so $P_{die}=0.26$ (Andersen et al., 2009). P_{rec} and YLD_{rec} are set to zero, assuming no one can recover from stroke. We also assumed that the YLD_{die} is associated with highest mortality period which is within 30 days, this leads to an estimate of approximately 0.082 (Ingall, 2004; Andersen et al., 2009). The disability weight of stroke varies depending on the stages of stroke. WHO estimated that for the first-ever stroke cases and long-term stroke survivors, w is 0.92 and 0.266 respectively (WHO, 2008). In our case, we take the rounded mean of the two values ($w=0.6$). P_{eff} depends on RR , age and sex (eq. 4 in Table 1). RR for stroke for 20, 23 and 40 g fish/day is 0.84, 0.83 and 0.79.

For fatal CHD, because no one recovers from fatal CHD, P_{rec} and YLD_{rec} are set to 0. By definition P_{die} of fatal CHD is 1. P_{eff} depends on RR and age and sex (eq. 4 in Table 1). RR for fatal CHD for 20, 23 and 40 g fish/day is 0.79, 0.77 and 0.71 respectively.

For IQ, $P_{eff(IQ)}$ is assumed to be the probability that a woman delivers a baby (P_c , Table 3). The probability of having a particular IQ (from the definition of IQ, normal distributed, mean 100, standard deviation 15) resulting from the change in IQ obtained from Table 1 eq. 2 linked with the disability weight of a particular IQ (Table 4) results in a weighted average w depending on IQ change as in (Hoekstra et al., 2012). For the IQ effect, the parameters YLD_{die} , YLD_{rec} , P_{die} , P_{rec} and CA are 0.

Table 4. Disability weights of IQ levels (Stouthard et al., 1997)

IQ	W
>85	0
70-84	0.09
50-69	0.29
35-49	0.43
20-34	0.82
0 <20	0.76

Table 5. Parameter values for the DALY calculations as estimated from epidemiological data

Health effects	Estimated parameters				
	P_{rec}	YLD_{rec}	P_{die}	YLD_{die}	W
Meningitis	0.625	0.5	0.254	0.08	0.32
Septicemia	0.792	0.02	0.208	0.08	0.93
Abortion/stillborn	0.0	-	1	-	-
CHD mortality	0.0	-	1	-	-
Total stroke	0.0	-	0.26	0.082	0.6
IQ	0.0	-	0.0	-	X^*

X^* is dependent on IQ (Table 4) which depends on the maternal intake of DHA (Table 1 eq 2).

The net DALY is calculated using:

$$DALY = DALY_{alt} - DALY_{ref}$$

Where, $DALY$ is change in DALY; $DALY_{alt}$, summation over all persons in the population of DALY's for the alternative scenario; $DALY_{ref}$, summation over all persons in the population of DALY's for the reference scenario.

DALY represents health loss; therefore, if the estimation of $DALY$ results in a positive value then the change in consumption has an adverse health effect. If the $DALY$ is negative, then the change in consumption has a beneficial effect (Hoekstra et al., 2010).

2.9 Sensitivity Analysis

A sensitivity analysis is performed to explore the impact of modifying some of the model parameter estimates on the risk-benefit assessment. Targeted parameters are the d -value for the effect of DHA intake on IQ change (Table 1, eq. 2), the parameters a and b defining RR of CHD and total stroke (Table 1, eq. 4), the storage time t (Table 1, eq. 7) and the lag-time of *L. monocytogenes*, (Table 1, eq. 7). These parameters relate to different endpoint and are known to be variable and/or uncertain. Moreover, for the estimation of RR of CHD mortality and total stroke one more function (exponential function, $\ln(RR) = b * F_{intake}$) is analysed to see the difference in DALY estimate of the two endpoints compared to the DALY estimate obtained from function, $\ln(RR) = a + b * \ln(F_{intake})$.

For example, the storage time of CSS was wide in range in various studies (Hansen et al., 1998; Leroi et al., 2001; WHO/FAO, 2004). *L. monocytogenes* relative lag time in foods is in the range of 0–40h, with a peak value near 2.5. Lag-times in laboratory broths had a similar range, but the peak value was nearer to 4.5h (Ross, 1999). In this study 4h is selected as a baseline lag-time value and converted to day unit (Table 1, eq. 7) and for the sensitivity analysis 2.5h and 4.5h is used from the peak value of foods and laboratory broths.

Furthermore, the uncertainty interval of the d -value in Table 1, eq. 2 and the 95% CI of the selected (bold) function in the appendix on Table 10 for the parameter a and b of RR of both CHD mortality and stroke are analyzed for sensitivity. The sensitivity analysis is done by varying one variable at the time (OAT) while keeping the others constant at their baseline value. More sophisticated sensitivity methods are possible (Saltelli et al., 2000), but in this relatively simple model the OAT approach is sufficient to identify the greatest sources of uncertainty and their approximate influence on the end result.

3. RISK-BENEFIT ASSESSMENT OF COLD-SMOKED SALMON: RESULTS

3.1 Baseline

The assessment shows that increasing the consumption of CSS has an overall health gain with respect to the selected endpoints, as the beneficial effects of fatty acids clearly outweigh the adverse health effect of *Listeria* (Table 7).

The extra cases of the hazardous endpoint and the prevented cases of the beneficial endpoint due to the change in consumption are presented in Table 6 below.

Table 6. The number of extra/prevented cases when change in consumption per year

Endpoints	Reference	Alternative	Extra/prevented cases
Septicemia	8.66	16.2	7.54
Meningitis	2.83	5.25	2.42
Abortion/stillborn	1.5	3	1.5
CHD mortality	5435	4953	-482
Stroke	3787	3580	-207

The number shows the number of cases per year at the different scenario. The last column shows that the additional cases (positive value) due to listeriosis and the prevented number of cases (negative value) due to omega-3 fatty acid when change in consumption.

When comparing the hazardous endpoints, for listeriosis there are more life years lost due to septicemia and meningitis in women compared to men. This is due to a larger increase in intake of CSS for women compared to men. The amount of healthy life years lost is largest for septicemia, followed by abortion/stillborn and meningitis.

Table 7. The baseline DALY's for each sex and scenario

	Men		Women			Sum of DALY	
	Ref	Alt	DALY	Ref	Alt		DALY
Septicemia	13	23	9.7	14.6	29	14.5	24.
Meningitis	6	10.6	4.5	6.74	13.5	6.8	11
Abortion/Stillborn*				11	23	12	12
CHD mortality	32093	29592	-2501	23402	21032	-2370	-4871
Stroke	11874.7	11302	-572	15192.5	14284	-908	-1480.5
IQ*				-3139	-6181	-3042	-3042
Net DALY							-9343

Ref, reference scenario; Alt, alternative scenario

**Because abortion/stillborn and IQ endpoints are result of maternal consumption on their fetus, the DALY is only reported for women.*

On the other hand, there is a large gain in healthy life years for both sexes due to reduction of CHD mortality and stroke. Likewise, a large benefit is obtained due to the IQ effect.

Women achieve more benefit than men by the prevention of stroke and men attain more benefit from the prevention of CHD mortality than women.

As a result, the net public health effect of the change of consumption of CSS leads to a gain of 9343 healthy life years in the population of approximately 5570000.

3.2 Sensitivity Analysis

The sensitivity analysis shows quantitative and qualitative changes in DALY depending on the parameters. As illustrated in Fig. 1, increasing the storage period leads to higher risks of listeriosis and has no effect on fatal CHD, stroke and IQ change in newborns. The net DALY shows that the adverse effect of consumption of CSS prevails over the beneficial effect from five weeks of storage time on. It leads to a loss of 1677 and 58391 healthy life years in the population at five and six weeks storage period respectively.

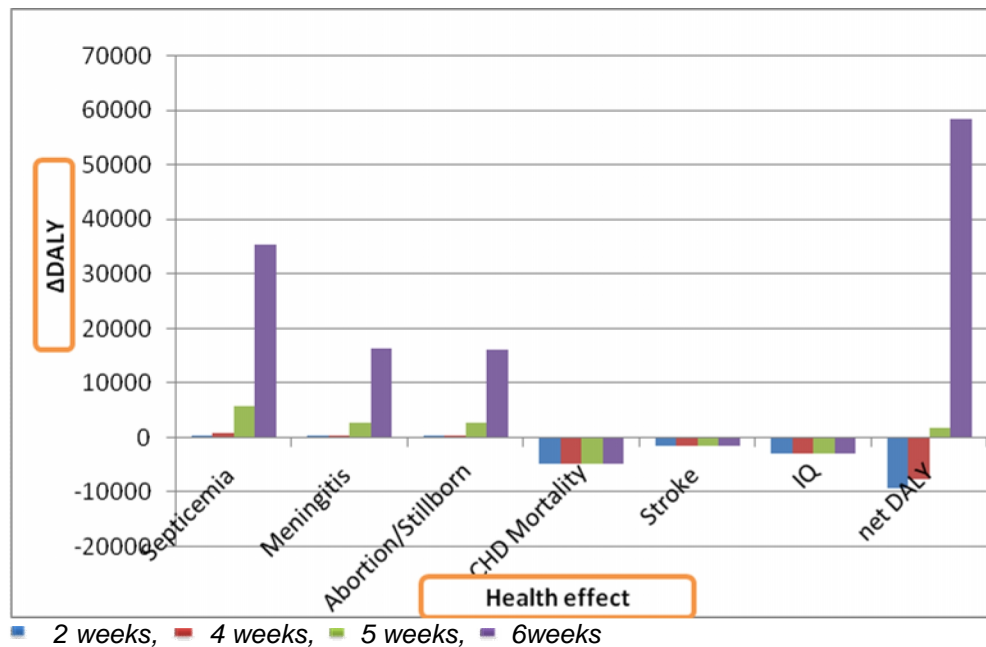


Fig. 1. Sensitivity analysis for the effect of storage time.

The sensitivity analysis for the RR for CHD mortality and total stroke, lag-time of listeria and the d-value uncertainty interval shows no change in the overall balance of the risk-benefit. The result of all the parameters analysed for sensitivity is presented in Fig. 2 below, including the net DALY when using the exponential RR model of CHD mortality and total stroke as an alternative model. The figure includes the net DALY for the baseline scenario given in Table 7, which is represented by “baseline”. The various parameters and their values that are used to estimate the baseline net DALY are presented in Table 1.

In Fig. 2 it appears that there is no difference between the result of the lag-time sensitivity analysis and the baseline result. The other parameters (RR and d) show a beneficial effect of net DALY which is similar to baseline result qualitatively. However, there can be seen a

shift of beneficial to hazardous effect at which the bar extends to the positive direction at storage time of 5 weeks and further.

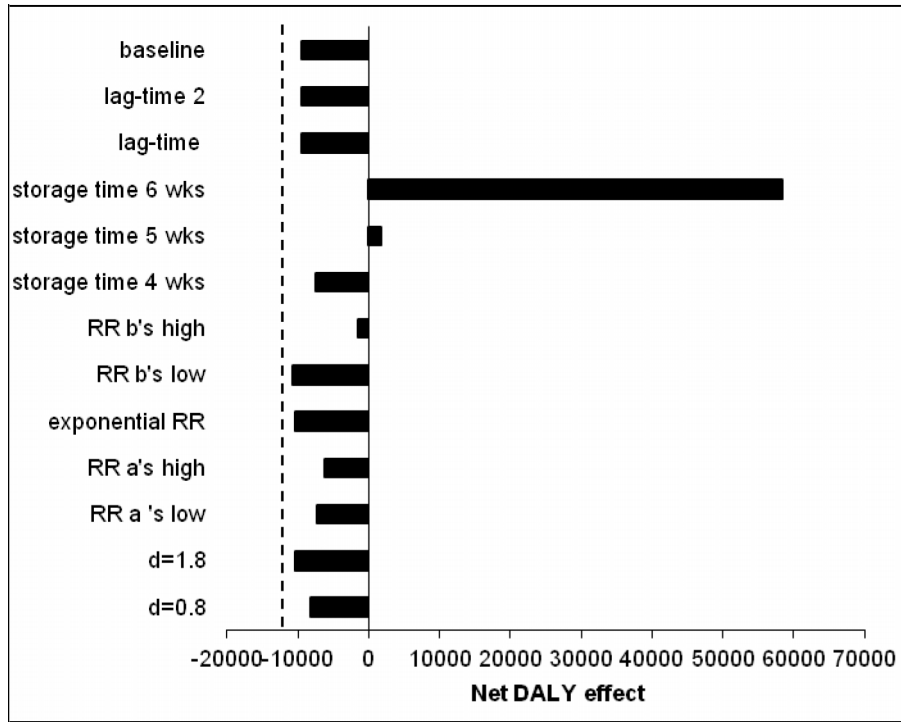


Fig. 2. Result of sensitivity analysis for all selected parameters

When using the exponential function ($\ln(RR) = b * F_{intake}$) the predicted DALY decrease to -5580 and -1964 for CHD mortality and total stroke respectively compared to the DALY estimate obtained by $\ln(RR) = a + b * \ln(F_{intake})$ (Table 7).

4. DISCUSSION

4.1 The CSS Study

In this study with CSS, major health benefit is obtained from the prevention of CHD mortality and the IQ increment of newborns. This is in accordance with other studies on fish consumption and/or omega-3 fatty acid intake (Cohen et al., 2005b; Guevel et al., 2008; Hoekstra et al. 2012). In Hoekstra et al. (2012) the health benefit was higher for stroke than IQ effect, in this study the health benefit is higher for IQ than stroke. This may be related to the difference in dose-response model used for stroke and because salmon is an oily fish that have a significant effect on IQ. As a part of sensitivity analysis the dose-response model used by Hoekstra et al., (2012) for stroke is applied to see the difference and the outcome indicate that the benefit would step up but still the health benefit is higher for IQ gain than for stroke.

Our paper is the first that shows that these health benefits also outweigh the risk of listeriosis, unless the storage time is too long (>4 weeks) and leads to the exposure to high

concentrations of the pathogen. Note though that if abortion was valued less severe than in our analysis storage time can be longer before risks outweigh benefits.

As common in microbiological risk assessment, different parameters (storage time, lag-time, growth rate, etc) that affect the concentration of the pathogen have been considered in the exposure assessment of *L. monocytogenes*. However, in the intake assessment of omega-3 fatty acid, the different parameters that affect its concentration have not been considered in the same way. For instance, processing and storage of CSS, and the bioavailability of the compound could affect its concentration before it reaches to target organ. In a comparative approach of the microbial risk and the nutritional benefit, the exposure assessment for nutrients and chemical contaminants should consider all the factors that affect the concentration until the compound in question reaches the target organ to exert an action.

The dose-response models that are applied for CHD mortality and stroke used aggregate data and different models have been used and validated to optimize the output. Nevertheless, the conversion of the estimated RRs to absolute risk is associated with uncertainty (Table 1, eq. 5 and 6). On the other hand, a few aggregate data have been used to model the dose-response relationship of the hazardous endpoints. In addition, in this study the intake assessment uses a point estimate of CSS and DHA intake for all age groups but take into account the differences between sexes. These all together might have an impact on the final outcome.

The result is expressed in DALY (morbidity, mortality and recovery) and it appears that, the septicemia to meningitis DALY ratio is approximately 2:1 (Table 7). Compared to men, the DALY changes are higher for women for both septicemia and meningitis cases this could be linked to the increase in intake of CSS on women than men. Looking back the history of invasive listeriosis in Denmark, in most cases men have higher incidence of invasive listeriosis than women (Gerner-Smidt et al. 2005). Septicemia has been the highest morbidity compared to meningitis. On the other hand, the mortality rate is higher for meningitis than septicemia (Gerner-Smidt et al. 2005). However, mostly in comparative studies on overall invasive listeriosis (septicemia vs meningitis), the ratio of septicemia to meningitis is 5:1 (Jensen et al., 2010); 3:1 (Gerner-Smidt et al. 2005).

In this study four parameters have been tested for sensitivity. The most sensitive parameter was the storage time. The shift of a net public health benefit to a net public health risk is observed when CSS is consumed at five weeks of storage and further (Fig. 2). The shift is mainly because of the increased concentration of *L. monocytogenes* that entails increased incidence of listeriosis. According to our model prediction, maximum benefit with minimum risk can be attained from the consumption of CSS within two weeks of production. The risks increase with storage time whereas benefits remain unchanged. Further study may encompass stochastic analysis of all the uncertain parameters presented in the study.

The study was not meant to thoroughly address all beneficial and hazardous components; neither includes the all related endpoints in connection to CSS consumption. Instead, focus was on the integration of microbial hazards and nutritional benefits. Although there could be more endpoints due to the intake/exposure of the selected risk and benefit, this paper assess health outcomes with strong evidences that enable quantitative evaluation. In addition, we evaluated only endpoints that we expected to have relatively high public health impact. For example, febrile gastroenteritis is usually caused by *L. monocytogenes* (WHO/FAO, 2004); but reported quantitative data with respect to this endpoint are insufficient to do a risk-benefit assessment and moreover, febrile gastroenteritis has less

public health impact than septicemia and meningitis. Thus, febrile gastroenteritis is not considered in this assessment. Other health effects related to fish/omega-3 fatty acid intake, for instance neuropsychiatric disorders (Young and Conquer, 2005) are not considered for the same reason. If our study would have included chemical hazards as well (dioxin-like compounds and mercury) and would have included all the endpoints, the net DALY would change quantitatively, and the balance between risk and benefit might change as well.

4.2 Implications for Future Risk-Benefit Assessment Methodology

If a pathogen is selected as a hazard, it is essential that the specific associated endpoints are considered instead of considering the generic clinical syndrome. Nevertheless, most Quantitative Microbial Risk Assessments (QMRA) report the generic clinical syndrome cases only. For example, if the assessment includes listeria then the endpoint is cases of listeriosis (Pouillot et al., 2007; Lindqvist and Westoo, 2000; WHO/FAO, 2004). However, these types of data are insufficient if one intends to integrate pathogens in risk-benefit assessment because of the exclusion of the mortality, morbidity, recovery and/or sequela of the specific diseases. In this risk-benefit assessment, the specific major clinical syndromes of foodborne listeriosis (meningitis, septicemia and abortion/stillborne) are included, instead of the generic endpoint "cases of listeriosis". On the other hand, this could also be a problem in dose-response modeling as most studies on the pathogenesis of pathogens have dose-response parameters only for general cases like listeriosis, salmonellosis, campylobacteriosis. In this case, different epidemiological data could be used to extrapolate the percentage of the specific endpoints as in section 2.7.

In addition, the integration of the health effects of microbial hazard into risk-benefit assessment may need further refining in the distinction of pathogenesis from the pathogens itself and the microbial toxins especially with regard to exposure assessment and dose-response modeling. Depending on the pathogens/toxins, the exposure assessment and the dose-response relationship require additional investigation like for example the stomach and small intestine dynamics as explained by (Pielaat et al., 2005) for *Bacillus cereus*.

Moreover, in this study the DALY is used as a common health metric to integrate microbial hazard in risk-benefit assessment. Here a thorough quantitative assessment has been done to the end, as opposed to the EFSA, 2010; Hoekstra, et al., 2010) where the assessment stops when the benefit outweighs the risk or vice versa. Had we followed the tiers of BRAFO tiered approach (Hoekstra et al., 2010) in this study, we might have stopped at the earlier stage. For example, in our assessment the baseline result showed that, the benefit clearly outreaches the risk (Table 7); as of Hoekstra, et al., (2010), we could have stopped at this point. However, further quantitative analysis gives a different result in connection with the change in storage time of CSS (Fig. 1), which, in our view, is an important result. This shows that the integration of microbial risk and/or benefit into risk-benefit assessment may require a more elaborate quantitative assessment to reach to best estimation of public health impact.

Furthermore, in general some disease may have some major secondary disease (sequela) that result from the primary clinical syndrome. For instance, if someone gets listeriosis meningitis then there is some probability that person would get neurological sequela (Aouaj et al., 2002). The DALY model applied in this study does not consider this kind of endpoints. However it can be extended to do so easily.

Consequently, the current risk-benefit assessment framework/models need more refinement in regard to the aforementioned points.

5. CONCLUSION

This paper evaluated and integrated the major risk and benefits in connection with the change in CSS consumption. Our risk-benefit assessment predicted that the overall health impact of change in consumption of CSS from reference to alternative intake provide more health benefits for the Danish population.

The model predictions depend on the assumptions taken during the analysis and the sensitivity analysis reveals that the most sensitive parameter was the storage time. If CSS is consumed after two weeks of storage, the benefit remains the same but the risk increases significantly with storage time.

This study provides an insight for future improvement of the methodologies with regard to exposure assessment of the different component, dose-response relationship and common health metric and general framework for risk-benefit assessment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

1. The Relative Risks of CHD Mortality and Total Stroke

Tables 8 and 9 present the results of studies on the relation between fish intake and the relative risks of CHD mortality and stroke. These results are used for dose response modeling. The most appropriate model is selected after analysis from the five functions mentioned below, based on best fit statistics using statistical computing R-programme.

- $RR = a + b * F_{intake}$
- $RR = a + b * \ln(F_{intake})$
- $\ln(RR) = a + b * F_{intake}$
- $\ln(RR) = b * F_{intake}$
- $\ln(RR) = a + b * \ln(F_{intake})$

Where, RR is relative risk; *a* and *b* are parameters and F_{intake} , is fish intake per day (Dose).

Table 8. The studies incorporated in the dose-response modeling of CHD mortality: fish intake (g/d) and relative risk (RR)

Reference	Dose (g/d)	RR (95% CI)	Reference	Dose (g/d)	RR (95%CI)
Kromhout et al., 1985	0	1	Mozaffarian et al., 2003	0	1
	7.5	0.63		7	0.78 (0.47, 1.28)
	22.5	0.56		14	0.77 (0.45, 1.32)
	37.5	0.36		29	0.53 (0.30, 0.96)
	75	0.39		71	0.47 (0.27, 0.82)
Ascherio et al., 1995	0	1	Mann et al., 1997	0	1
	7	0.74 (0.38, 1.45)		7	1.21(0.62, 2.38)
	18	0.86 (0.5, 1.47)		29	1.23 (0.7, 2.17)
	37	0.71 (0.41, 1.21)	Oomen et al., 2000, The Netherlands	0	1
	69	0.54 (0.29, 1.00)		10	1 (0.59, 1.68)
	119	0.77 (0.41, 1.44)	35	1.1 (0.68, 1.79)	
Daviglus et al., 1997	0	1	Oomen et al., 2000, Finland	10	1
	9	0.88 (0.63, 1.22)		30	0.97 (0.68, 1.38)
	26	0.84 (0.61, 1.17)		70	1.25 (0.89, 1.76)
	67.5	0.62 (0.4, 0.94)	Jarvinen et al. 2006	4	1
Albert et al., 1998	0	1		12.0	0.91 (0.55, 1.35)
	7.5	1.18 (0.59, 2.26)		19.8	0.77 (0.48, 1.23)
	21	0.82 (0.45, 1.51)		32	0.68 (0.42, 1.12)
	50	0.91(0.5, 1.66)		70.0	0.59 (0.36, 0.99)
	86	0.81(0.41, 1.61)	Hu et al., 2002	0	1
Oomen et al., 2000, Italy	0	1		7	0.8 (0.56, 1.15)
	10	0.94 (0.55,1.59)		14	0.65 (0.46, 0.91)
	30	0.93 (0.53, 1.63)		43	0.72 (0.48, 1.09)
	70	0.67 (0.33, 1.39)		86	0.55 (0.33, 0.91)

Table 9. The studies incorporated in the dose-response modeling of total stroke: Fish intake (g/d) and relative risk (RR)

Reference	Dose (g/d)	RR (95% CI)	Reference	Dose (g/d)	RR (95% CI)
He et al., 2002	0	1	Mozaffarian et al., 2005)	0	1
	7	0.73 (0.48-1.10)		7	0.88 (0.66–1.17)
	14	0.74 (0.52-1.04)		36	0.74 (0.56–0.98)
	43	0.67 (0.46-0.96)		86	0.77 (0.56–1.07)
	86	0.83 (0.53-1.29)		Orencia et al., 1996	0
Iso et al., 2001	0	1		9	0.98 (0.61, 1.59)
	7	0.93 (0.65-1.34)		26	0.94 (0.59, 1.52)
	14	0.78 (0.55-1.12)		50	1.26 (0.74, 2.16)
	43	0.73 (0.47-1.14)	Larsson et al., 2011	0	1
	86	0.48 (0.21-1.06)		17	0.87 (0.75, 1.01)
Gillum et al., 1996	0	1		25	0.92 (0.82, 1.09)
	7	0.78 (0.54, 1.12)		36	0.88 (0.76, 1.02)
	14	0.77 (0.53, 1.13)		64	0.84 (0.71, 0.98)
	60	0.55 (0.32, 0.93)	Wang et al., 2011	7	1
Gillum, et al., 1996	0	1		29	0.7 (0.5, 0.98)
	7	1.27 (0.83, 1.96)		80	0.82 (0.61, 1.11)
	14	1.23 (0.79, 1.91)			
	60	0.85 (0.49, 1.46)			

Table 10. The model outputs for the relative risk of CHD mortality and stroke

Models	CHD	Stroke
$RR = a + b * F_{intake}$	RR= 0.9-0.0028* F_{intake} CI 95%: (0.79, 1.01) and (-0.005, -0.0004) p-value: (<0.001) and (0.023)	RR= 0.935-0.0024* F_{intake} CI 95%: (0.82, 1.05) and (-0.005, 0.0001) p-value: (<0.001) and (0.0593)
$RR = a + b * \ln(F_{intake})$	RR= 1.11-0.0964* $\ln(F_{intake})$ CI 95%: (0.86, 1.36) and (-0.172, -0.021) p-value: (<0.001) and (0.014)	RR= 1.09-0.075* $\ln(F_{intake})$ CI 95%: (0.83, 1.34) and (-0.15, 0.003) p-value: (<0.001) and (0.059)
$\ln(RR) = a + b * F_{intake}$	$\ln(RR) = -0.13-0.004 * F_{intake}$ CI 95%: (-0.271, 0.02) and (-0.007, -0.0008) p-value: (0.081) and (0.016)	$\ln(RR) = -0.08-0.003 (F_{intake})$ CI 95%: (-0.21, 0.05) and (-0.006, 0.0002) p-value: (0.235, 0.038)
$\ln(RR) = b * F_{intake}$	$\ln(RR) = -0.006 * F_{intake}$ CI 95%: (-0.008, -0.0041) p-value: <0.001	$\ln(RR) = -0.0045 * F_{intake}$ CI 95%: (-0.006, -0.003) p-value: <0.001
$\ln(RR) = a + b * \ln(F_{intake})$	$\ln(RR) = 0.17-0.137 * \ln(F_{intake})$ CI 95%: (-0.16, 0.5) and (-0.24, -0.04) p-value: (0.3) and (0.008)	$\ln(RR) = 0.113-0.094 * \ln(F_{intake})$ CI 95%: (-0.184, 0.41) and (-0.184, -0.004) p-value: (0.44) and (0.04)

The last model is used to estimate the relative risk for each scenario and endpoints.

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