Method development in risk-benefit assessment and burden of disease estimation of food

Firew Lemma Berjia
PhD Thesis
2013

DTU Food
National Food Institute

Method development in risk-benefit assessment and burden of disease estimation of food

Firew Lemma Berjia

PhD Thesis

December 2013

Technical University of Denmark

National Food Institute

Division of Epidemiology and Microbial Genomics

Group of Epidemiology and Risk Modelling

Mørkhøj Bygade 28, DK-2860 Søborg

Denmark

Preface

This thesis was conducted between October 2010 to December 2013 at the Group of Epidemiology and Risk Modeling, the National Food Institute, Technical University of Denmark. A part of the work in this thesis was performed during the three months stay at the National Institute for Public Health and the Environment (RIVM), The Netherlands. The project was funded by Technical University of Denmark through the FoodDTU programme.

The thesis focuses on method development in risk-benefit assessment and burden of disease estimation of food.

Supervisors:

Senior researcher, Dr. Maarten Nauta (principal supervisor), Epidemiology and Risk Modelling Group, Division of Epidemiology and Microbial Genomics, National Food Institute, Technical University of Denmark, Denmark.

Senior researcher, Dr. Morten Poulsen (co-supervisor), Division of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark, Denmark.

Scientist, Dr. Rikke Andersen (co-supervisor), Division of Nutrition, National Food Institute, Technical University of Denmark, Denmark.

Assessment Committee:

Dr. Andy Hart, The Food and Environment Research Agency (FERA), Risk Analysis Team, UK

Prof. Paw Dalgaard, Division of Industrial Food Research, National Food Institute, Technical University of Denmark, Denmark.

Dr. Bjarke Christensen, Head Department of Food Science, University of Copenhagen, Denmark.

Acknowledgments

Quantitative integrated risk-benefit assessment of food and burden of foodborne disease study is a first of its kind at the Technical University of Denmark. It is a complex, demanding and multidisciplinary study that needs an intensive interaction between the PhD student, supervisors and other expertise from several disciplines. Despite the complexity and challenges during the study period, the PhD project comes to the end.

For the accomplishment of this PhD project, in addition to the PhD student several scientists from different disciplines contributed significantly. I primarily thank **Dr. Maarten Nauta** (principal supervisor) for providing a considerable scientific support and technical efforts for the completion of this PhD project. In addition, I would like to extend my gratitude for the co-supervisors for their unreserved scientific inputs, interaction and language translation during the study period. Moreover, I pay a special appreciation for all the supervisors for establishing interdepartmental and institutional collaboration from the birth to the end of the PhD project. I thank DTU, the National Food Institute secretariats and colleagues for their excellent collegiality spirit, social activities, for supporting me in scientific as well as administrative issues which altogether makes me feel Denmark my second home.

Moreover, I thank the National Institute for Public Health and the Environment (RIVM), The Netherlands collaborative partners, particularly **Dr. Jeljer Hoekstra** and **Prof. Hans Verhagen** for facilitating the three months external research stay at RIVM and for extensive scientific support. I also thank RIVM secretariat (Maya Kartasasmita), staffs and interns for making my research stay at RIVM charming and cosy.

Finally, I am very grateful to Alemayehu Fekadu, Surafel Kifle (Mulu), Dr. Aberham Hailu, Daxious Mengistu (Dax), Samson Asfaw, Takele Admasu (Meku), Dagne Mamo, Ephrem Mamo, Mahder Feru and Wendsen Taye (emerging Menfes) for their precious moral support and energizing me during the stressful period.

List of abbreviations

25(OH)D - 25-hydroxyvitamin D

BBN - Bayesian belief network

DALY - Disability adjusted life year

DHA - Docosahexanoic acid

EFSA - European Food Safety Authority

EPA - Eicosapentaenoic acid

FAO - Food and Agriculture Organization

HCA - Heterocyclic amine

HR - Hazard ratio

IQ - Intelligence quotient

IU - International unit

LE - Life expectancy

OR - Odd ratio

PAH - Polycyclic aromatic hydrocarbon

PCBs - Polychlorinated biphenyls

QALY - Quality adjusted life year

QMRA - Quantitative microbial risk assessment

RCT - Randomized controlled trial

RIVM - National Institute for Public Health and the Environment

RR - Relative risk

UV - Ultraviolet

VKM - Norwegian scientific committee for food safety

WHO - World Health Organization

Table of Contents

Summary

Due to the increasing interest of food authorities, producers and consumers in knowing the health outcomes of food consumption, risk-benefit assessment of food and burden of foodborne disease studies have become major topics for scientists in recent years. Health risk-benefit assessment of food and burden of foodborne disease studies are used to estimate the health outcomes related to food consumption. Both studies combine food consumption, epidemiological and population statistics data to predict the health outcomes of food consumption. While risk-benefit assessment of food integrates the health outcomes of the beneficial and hazardous components of food, the burden of foodborne disease study typically focuses on the health outcomes of the hazardous components of food.

Progressively, the risk and benefit assessment of food is shifting from a separate qualitative assessment to an integrated quantitative risk-benefit assessment. During the last decade, methods for health risk-benefit assessment of food have been developed. Most of these methods focus on comparison of two or more scenarios and predict the relative health outcome of the scenarios.

This thesis aims to further develop the existing methods for health risk-benefit assessment of food. The present thesis comprises of three studies. Given that microbial hazards were not well integrated in previous health risk-benefit assessment of food, the first study of this thesis illustrates 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 (CHAPTER 6). Then, a method for health risk-benefit assessment of food is developed, using vitamin D as an example (MANUSCRIPT I). In addition, burden of disease estimates related to the various red meat cooking practices are performed (MANUSCRIPT II).

In PAPER I, an integrated quantitative health risk-benefit assessment was conducted, using DALY (disability adjusted life years) as a common health metric. The health outcomes of *Listeria monocytogenes* and omega-3 fatty acids were estimated due to the consumption of CSS in Denmark using two consumption scenarios. 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 are identified as health risk endpoints due to exposure to *L. monocytogenes.* Results show that the overall health benefits outweigh the risk, foremost contributed by the effect of decreased CHD mortality and improved IQ. The study demonstrated 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. This suggests that modulating the food processing parameter can have a significant impact in the reduction of the net health loss.

In CHAPTER 7, a method for health risk-benefit assessment of food was developed, using vitamin D as an example. The method focuses on finding an optimum scenario that provides maximum net public health gain. The method is based on multiple scenario simulation. In addition to the reference scenario, several alternative scenarios are simulated to detect the scenario that provides maximum net health gains. As a common health metric, Disability Adjusted Life Years (DALY) has been used to project the net health effect by using the QALIBRA (Quality of Life for Benefit Risk Assessment) software. The example on vitamin D illustrates the applicability of the developed method. The method is illustrated only on nutrient, vitamin D. The method may include food processing parameter optimization (temperature – time of cooking, storage, handling condition and addition of preservatives) and scenario simulation as an integral part of the assessment to maximize the net health gain.

In MANUSCRIPT II, the burden of disease estimates for the different red meat cooking practices was performed. The health impacts of barbecued, fried and roasted red meat were compared using DALY as a common health metric. The selected health effect linked to cooked red meat consumption includes colorectal, prostate, breast and pancreatic cancer. The result reveals that the healthy life year's loss is considerable due to the consumption of barbecued red meat compared to the other cooking practices. This study indicates that the choice of cooking practice has an impact on the reduction of health loss. The method used to quantify the difference in disease burden consequential to different cooking practices can help to inform the consumer to

make a choice whether the benefit of a preferred cooking style is worth the associated health loss. This study is the first to show burden of disease estimate related to red meat cooking practices and the method applied in this study can be used as a basis for similar studies in the future.

To conclude, this thesis contributes for further development of the existing risk-benefit assessment of food methodologies. Parts of the thesis also suggest that food processing parameter optimization and the choice of the cooking practice contribute for the improvement of public health linked to food consumption.

In the future, additional case studies need to be performed to further apply the proposed method. For efficient integrated risk-benefit assessment, a database that encompasses data of nutrients, chemical contaminants, pathogens and their disease epidemiology needs to be constructed. Future risk-benefit assessment of food may incorporate emerging nutrigenomics, toxicogenomics and pathogenomics data, to take into account population genetic variation in response to intake and exposure to nutrients, chemical contaminants and pathogens. Additional studies are needed to investigate the impact of food cooking practices (in different food products) on the total burden of foodborne diseases.

Sammendrag

Da fødevaremyndigheder, producenter og forbrugere har stigende interesse i at kende det sundhedsmæssige udfald af indtaget af fødevarer, er risk-benefit vurderinger og studier i fødevarerelateret sygdomsbyrde blevet et væsentligt emne for forskere i de senere år. Sundhedsmæssige risk-benefit vurderinger af fødevarer og studier i fødevarerelateret sygdomsbyrde bruges til at estimere det sundhedsmæssige udfald relateret til indtaget af fødevarer. Begge slags vurderinger/studier kombinerer fødevareindtag, epidemiologi og statistiske populationsdata til at forudsige det sundhedsmæssige udfald af indtaget af fødevarer. Risk-benefit vurderinger af fødevarer integrerer sundhedsmæssige udfald af både de gavnlige og de skadelige komponenter i fødevarer, hvorimod studier i fødevarerelaterede sygdomme typisk fokuserer på de sundhedsmæssige effekter af de skadelige komponenter i fødevarer.

Vurdering af de gavnlige og skadelige effekter af fødevarer er gradvist skiftet fra at være separate kvalitative vurderinger til at være en integreret kvantitativ risk-benefit vurdering. Metoder til sundshedsmæssige risk-benefit vurderinger af fødevarer er blevet udviklet i løbet af det sidste årti. De fleste af disse metoder har fokuseret på at sammenligne to eller flere scenarier og forudsige det relative udfald af disse scenarier.

Denne afhandlings formål er at videreudvikle de eksisterende metoder til sundhedsmæssig risk-benefit vurdering af fødevarer. Afhandlingen består af tre studier. Da mikrobielle risici ikke er godt integreret i tidligere sundhedsmæssige risk-benefit vurderinger af fødevarer, illustrerer denne afhandlings første studie, hvordan mikrobielle risici kan inkluderes i en risk-benefit vurdering og hvordan dette kan forbedre de eksisterende risk-benefit værktøjer og metoder (KAPITEL 6). Dernæst er der udviklet en metode til sundhedsmæssig risk-benefit vurdering af fødevarer. Metoden er anvendt i et eksempel med D-vitamin (MANUSKRIPT I). Derudover er der gennemført sygdomsbyrde estimater relateret til forskellige tilberedningsmetoder af rødt kød (MANUSKRIPT II).

En integreret kvantitativ sundhedsmæssig risk-benefit vurdering, hvor DALY (disability adjusted life years) blev brugt som en fælles sundhedsenhed, blev udført i ARTIKEL 1. De sundhedsmæssige effekter af Listeria monocytogenes og omega-3-fedtsyrer blev estimeret på baggrund af indtaget af koldrøget laks i Danmark ved at bruge to indtags scenarier. Nedsat risiko for dødelighed af hjertekarsygdom (CHD) og slagtilfælde samt øget kognitiv (IQ) udvikling af ufødte afhængende af moderens indtag blev identificeret som de væsentligste gavnlige effekter af omega-3-fedtsyrer fra koldrøget laks. Risiko for meningitis, sepsis og abort/dødfødte blev identificeret som sundhedsmæssige risici, når man bliver udsat for L. monocytogenes. Resultaterne viser at overordnet er der samlet er en sundhedsmæssig fordel, især pga. effekten af nedsat CHD dødelighed og forbedret IQ. Dette studie viser, hvordan mikrobielle risici kan integreres i risk-benefit vurderinger, og viser at sensitivitetsanalyser har en øget værdi, selvom de gavnlige effekter i høj grad overgår risikoen i de indledende analyser. Dette viser, at ændringerne i fødevareproduktionen (modulering af en fødevareproces parameter) kan have signifikant indflydelse på reduktionen af netto sundhedsmæssige tab.

I Kapitel 7 er der udviklet en metode til at udføreen risk-benefit vurdering, hvor D-vitamin er brugt som eksempel. Metoden fokuserer på at finde det scenario, der giver flest sundhedsmæssige fordele for befolkningen, og er baseret på en simulation af mange scenarier. Udover referencescenariet er der simuleret flere alternative scenarier for at finde det scenarie, der giver den største sundhedsmæssige gevinst. Som en sundhedsmæssig fællesnævner benyttes ”Disability adjusted life years” (DALY), der udregnes vha. QALIBRA software (Quality of Life for Benefit Risk Assessment). Eksemplet med D-vitamin viser at den udviklede metode er anvendelig. Metoden er kun vist for et enkelt næringsstof, D-vitamin. I vurderingen kan der inddrages fødevareproduktionsparametre (temperatur, tilberedningstid, håndtering, opbevaring, og brug af tilsætningsstoffer) og simulation af scenarier som en integreret del af vurderingen for at optimere den sundhedsmæssige gevinst. Som eksempel og for at validere den foreslåede måde at vurdere risk-benefit på, har vi vurderet D-vitamin (Manuskript I). Eksemplet med D-vitamin viser at metoden er anvendelig.

I Manuskript II er sygdomsbyrden for forskellige måder at tilberede rødt kød på blevet vurderet. Den sundhedsmæssige betydning af grillet, pandestegt og ovnstegt rødt kød udtrykt som DALY er blevet sammenlignet. Kolorektal-, prostata-, og brystkræft samt kræft i bugspytkirtlen er de sundhedsmæssige effekter der er blevet kædet sammen med indtag af tilberedt rødt kød. Resultatet af sammenligningen viser at tabet af livsår er størst ved indtag af grillet rødt kød. Vurderingen viser at valget af tilberedelsesmetode har betydning for sundheden. Den anvendte måde at vurdere kvantificere sygdomsbyrde for forskellige tilberedelsesmetoder kan hjælpe forbrugerne til at træffe beslutningen, om fordelen ved en bestemt tilberedelsesmetode er risikoen værd. Dette forsøg er det første til at vise sygdomsbyrden relateret til indtag af tilberedt rødt kød, og demonstrerer at den anvendte metode kan bruges fremover.

Det kan konkluderes at denne afhandling bidrager til den fortsatte udvikling af eksisterende værktøjer til risk-benefit vurderinger indenfor fødevareområdet. Afhandlingen viser også at optimering af fødevareproduktionsparametre og valget af tilberedelsesmetode bidrager til at øge folkesundheden.

Der vil fremadrettet være behov for at udføre yderligere forsøg for at validere den foreslåede metode. I forhold til en integreret risk-benefit vurdering er det nødvendigt at lave en database der indeholder data om næringsstoffer, kemiske forureninger, patogener og deres sygdomsepidemiologi. Fremtidige helhedsvurderinger af fødevarer bør inddrage kommende nutrigenomics, toxicogenomics og pathogenomics data og medtage variationer i befolkningens genetik i forhold til indtag af, og eksponering til næringsstoffer, kemiske forureninger og patogener. Yderligere forsøg er nødvendige for at undersøge betydningen af tilberedelsesmetoder for forskellige fødevarer på den totale sygdomsbyrde.

List of Publications

Papers in peer-reviewed journals included in the thesis:

1. Berjia, FL., Andersen, R., Hoekstra, J., Poulsen, M., Nauta, M. (2012). Risk-benefit assessment of cold-smoked salmon: microbial risk versus nutritional benefit. EJFRR, 2, 49-68.
2. Berjia, FL., Hoekstra, J., Verhagen, H., Poulsen, M., Andersen, R., Nauta, M. (2013). Finding the optimum scenario in risk-benefit assessment: an example on vitamin D. (MANUSCRIPT I, on preparation). European Journal of Nutrition & Food Safety
3. Berjia, F., Poulsen, M., Nauta, M. (2013). Burden of diseases estimates associated to different red meat cooking practices. (Submitted). Food and Chemical Toxicology.

Conference presentations (poster and oral)

1. Berjia, FL., Andersen, R., Hoekstra, J., Poulsen, M., Nauta, M. (2013). Integration of microbial health risks and nutritional health benefits. Advances in predictive modeling and quantitative microbiological risk assessment of foods. Sao Paulo, Brazil. (Oral Presentation).
2. Berjia, FL., Andersen, R., Hoekstra, J., Poulsen, M., Nauta, M. (2012). Integration of nutritional health benefits and microbial health risks: Quantitative risk-benefit assessment of cold-smoked salmon. European Federation of Food Science and Technology (EFFoST), Montpellier, France. (Poster Presentation).
3. Berjia, FL., Andersen, R., Hoekstra, J., Poulsen, M., Nauta, M. (2011). Risk-Benefit Assessment of Food: How Healthy is Cold-Smoked Salmon?. FOOD Denmark PhD Congress, University of Copenhagen, Denmark. (Oral Presentation).
4. Berjia, FL., Andersen, R., Hoekstra, J., Poulsen, M., Nauta, M. (2011). Risk-benefit assessment of cold-smoked salmon. Workshop within BEPRARIBEAN (Best Practices in Benefit-Risk Analysis of Foods). Maastricht University, The Netherlands. (Poster Presentation).

Chapter 1

Introduction

1. Introduction

A number of food products contain both beneficial and hazardous components. The weighing of risks and benefits of food or food components has thereby become a main public health issue (Hoekstra et al., 2008). Risk-benefit assessments of food focus on interventions and policies in connection with food consumption and health outcomes (Hoekstra et al. 2012). Food policy makers, scientists, nutritionists, dieticians, food industries as well as consumers are interested to know the health impact of food.

The degree of the detrimental effect of a hazard on the health of the consumer is often established by risk assessment. Likewise, the extent of the beneficial effect of a food on the health of the consumer may be established by benefit assessment. In 1995, a risk assessment paradigm had been established (FAO/WHO, 1995). Until some time ago, the risk and benefit assessments of food have been separate processes with different methods. Due to the increasing interest of estimation of the overall health impact of food consumption, development of methods that integrate both the health benefits and risks of food in one go have gained interest. Gradually, it has been recognized that a similar paradigm as used for the risk assessment can be used for the benefit assessment and in addition for the integrated risk–benefit assessment approach (EFSA, 2006a, EFSA, 2010).

Usually, the health risk-benefit assessments of food have been performed qualitatively using a gauge such as tolerable upper intake level or acceptable daily intake or recommended daily intake level without integrating them in common health metrics (Sand et al., 2008). Thus, the result of the assessment about the risks and benefits of food are presented separately. Qualitative risk-benefit assessment will not provide information about the healthy life years that could be gained or lost. Therefore, preferably the risk and benefit have to be combined to provide a signal about the overall health effect of a certain food or food components (Hart et al., 2010).

During the last decade, scientists had worked to develop an approach that enables to perform a holistic quantitative risk-benefit assessment of foods (Hoekstra et al., 2008; Fransen et al., 2010;

Hoekstra et al., 2012; EFSA, 2010; Hart et al., 2013). Besides methodology development studies, several case studies have been conducted on risk-benefit assessment of different foods or food components (Gladyshev et al., 2008; Cohen et al., 2005; Guevel et al., 2008; Hoekstra et al., 2013b; Watzl et al., 2011; Schutte et al., 2012; Verhagen et al., 2012, Berjia et al., 2012).

Recently, a progressively quantitative risk-benefit assessment approach that integrates the positive and negative health effects has been developed in an EU-funded project (Hoekstra et al., 2012; Hart et al., 2013). However, these approaches and other similar quantitative case studies focus on comparison of two or more scenarios and state whether one scenario prevails over the other/s in terms of health effect (Cohen et al., 2005; Hoekstra et al., 2013b). Ideally, it is imperative to make a statement about the best scenario that provides maximum health benefit to the public.

To integrate the health effects of beneficial and hazardous components, DALY and QALY (disability/quality adjusted life years) is the best known health outcome measurement in the population. DALY is a measure of potential healthy life years lost due to premature death and poor health or disability (Murray and Lopez, 1996). QALY measure the total number of years of perfect heath in a population (Murray and Lopez, 1996). The detail of DALY and QALY is presented in chapter 5.1.

DALY has mostly been used to estimate the burden of disease attributed to the environment, nutrition, foodborne pathogens and infectious diseases (Murray and Lopez, 1996; Murray et al., 2013; Gkogka et al., 2011; Havelaar et al., 2012). It has also been applied in risk-benefit assessment of food (Hoekstra et al., 2008; Berjia et al., 2012; Hoekstra et al., 2013b). Nevertheless, the burden of disease study as well as risk-benefit assessment study related to the consequences of the different food preparation was not studied. The common features of burden of disease study and health risk-benefit assessment of food study is that both studies deal with health outcomes interpreted using common health metric and are used to support decision makers in relation to public health issues.

1.1 Objectives of the thesis

The overall objective of the PhD project was to further develop a risk-benefit assessment of foods or food components approach.

The specific aims are:

- To perform a case study in risk-benefit assessment of food. This case study deals with the benefit of omega-3 fatty acids and the risk of listeriosis due to the consumption of cold-smoked salmon (PAPER I).
- To develop a method for risk-benefit assessment of food which focus on finding optimum scenario that provides maximum net health gain (MANUSCRIPT I).
- To perform a case study to develop a method that enable to estimate burden of disease related to different food cooking practices of the same food (MANUSCRIPT II).

1.2 Outline of the thesis

This PhD thesis is divided into 10 chapters. **Chapter 1** (this chapter) provides general introduction about risk-benefit assessment and burden of disease. In addition, it contains the objectives of the PhD project and outline of the thesis. **Chapter 2** presents definitions in risk-benefit assessment of food and literature review related to food safety, food health hazards, food health benefits and risk-benefit assessment. **Chapter 3** portrays the existing food health risk-benefit assessment methodologies and other risk-benefit assessment studies. In addition, it describes the similarities and differences of the existing food risk-benefit assessment methodology studies. **Chapter 4** depicts the application of health risk-benefit assessment of food and selected qualitative and quantitative risk-benefit assessment of fish studies. In addition, it presents an opinion about fish risk-benefit assessment studies. **Chapter 5** discusses other issues related in risk-benefit assessment of food including common scale, data, uncertainty and sensitivity analysis, expertise needed and consumer perception in risk-benefit assessment food. **Chapter 6** presents a case study

that deals risk-benefit assessment of cold-smoked salmon: microbial risk versus nutritional benefit (PAPER I). **Chapter 7** presents a method to find the optimum scenario in risk-benefit assessment: an example on vitamin D (MANUSCRIPT I). **Chapter 8** details a study on burden of disease estimate of the different red meat cooking practices in Denmark (MANUSCRIPT II). **Chapter 9** discusses the results obtained from PAPER I and the MANUSCRIPTS produced during the PhD study. In addition, it discusses in broad context what need to be considered to find the optimum scenario that provides maximum net health gain in holistic risk-benefit assessment of food. Furthermore, it discusses the challenges in performing holistic integrated quantitative risk-benefit assessment of food. **Chapter 10** summarizes the overall conclusion of the PhD study and future perspectives.

Chapter 2

Definitions in risk-benefit assessment of food, burden of foodborne disease and concept of food safety hazard and benefit

2. Definitions in risk-benefit assessment of food, burden of foodborne disease and concept of food safety hazard and benefit

This PhD thesis considers the health risk and benefit associated with the consumption of a particular food or food component. The health effects related to food consumption are a result of exposure to pathogens, chemical contaminants and intake of nutrients or combinations. Therefore, it is essential to have a general understanding of the different health hazards and beneficial components linked to food consumption. The definitions given hereunder include a general definition used in risk-benefit assessment of food, burden of disease and definitions specific to this PhD thesis.

Hazard: The inherent property of a food that potentially causes detrimental health effects when an individual is exposed to it (IPCS, 2004).

Risk: The probability of an adverse health effect in an individual in response to exposure to a hazard that may be present in a food or nutrient (IPCS, 2004).

Benefit: The probability of a positive health effect and/or the probability of a reduction of an adverse health effect in a population when exposed to the food (EFSA, 2010; Hoekstra et al., 2012).

Risk-benefit assessment: The weighing of probability of an adverse health effect against the probability of benefit as a consequence of exposure, if both are known to be present (EFSA, 2010).

Optimum health effect: The maximum health gain obtained by several scenario simulations, fortification, food processing parameter optimization or combinations, when relevant. The achievement of best scenario that provides optimum health effect gain may involve the reduction of the risk of the potential hazardous component of the food and maximizing the positive health effect of the beneficial component, when both hazardous and beneficial components are known to be present in a food.

Food processing parameter optimization: The process of identification of the parameters in food processing pathway (and in some cases after intake or exposure) that affect the concentration of the hazardous and beneficial components. Followed by incorporating those parameters in the model and quantify them in order to optimize the net health impact of food consumption. Examples of food processing parameters: cooking time, temperature and storage conditions.

Burden of foodborne disease: The health impact of food consumption in an individual or a population measured by common health metrics. It is often quantified in terms of QALY or DALY by combining food consumption, mortality, morbidity, recovery and population statistics data (Murray and Lopez, 1996; Hoekstra et al., 2012; Hart et al., 2013).

DALY: An acronym for disability adjusted life year, is a measure of disease burden, expressed as the number of years lost due mortality, morbidity or recovery (Murray and Lopez, 1996; Hoekstra et al., 2012; Hart et al., 2013)

Duration of the disease: is the period at which from the onset of the disease until recovery or death, it may also take into account recurrence period of the disease.

Disability or severity weight: is a weight factor that reflects the severity of the disease on a scale from 0 (perfect health) to 1 (equivalent to death).

Life expectancy: is the expected age at death (Hoekstra et al., 2012).

The following section elucidates the various hazardous and beneficial components linked to food consumption.

2.1 Food safety health hazards

Foodborne illness is a serious problem all over the world, causing a considerable morbidity, mortality and substantial economic losses. There are three known food safety hazards: biological,

chemical and physical hazards (Alli, 2004). With regard to health risk-benefit assessment of food and burden of foodborne disease, biological and chemical hazards are a major concern.

2.1.1 Biological hazards

The majority of the biological hazards linked to food are pathogenic bacteria and their toxins. Foodborne pathogenic bacteria are accountable for a great proportion of foodborne illness outbreak (IFT, 2004). Often, large shares of the reported cases are caused by *Salmonella* spp., *Eschericha coli 0157:H7, Lysteria monocytogenes, Clostridium perfringens, Clostridium botulinum, Staphylococcus aureus,* and *Campylobacter jejeuni* (IFT, 2004). Many severe and fatal incidents happen in infants, elderly and immunocompromised.

In addition, certain viruses such as hepatitis A and E viruses, the Norwalk viruses and rotavirus can cause foodborne illness (Koopmans and Duizer, 2004). Moreover, several human parasites such as *Cryptosporidium parvum, Giardia lambia* and *Ascaris lumbricoides* (Dawson, 2005) and to a lesser extent fungi (such as moulds) can cause foodborne illness (Udagawa, 2005).

2.1.2 Chemical Hazards

The sources of the chemical contaminants in food are diverse. For example, food additives are a hazard when taken beyond the tolerable level. Food may contain naturally occurring chemical contaminants (oxalate in rhubarb, alkaloids in potatoes, toxins in mushrooms) (Watson, 2001). Agricultural residues such as pesticides, herbicides, fungicides, antibiotics and other veterinary drugs can also be food safety hazard (Schrenk, 2012). Industrial activity contaminants such as heavy metals (lead, mercury and arsenic), organochlorinated compounds such as polychlorinated biphenyls (PCBs) are a known chemical food safety hazards (Watson, 2001).

Furthermore, some chemical contaminants can be formed during food processing especially during high temperature cooking of certain food products. Polyaromatic hydrocarbons, hetrocyclic amines, hetrocyclic amines and acrylamide are good examples of chemical contaminants formed

during high temperature processing of food (NTP, 1999; NTP, 2002; SCF, 2002; EFSA, 2008; WHO/IARC, 2010; EFSA, 2011).

Other chemical food safety hazards related to the consumption of food includes food allergens that cause allergic reaction in some subpopulation (Schrenk, 2012) and mycotoxin formed due to fungal activities in food (Watson, 2001).

2.1.3 Other food safety issue

In addition to the biological and chemical hazards, some nutrients can be deleterious when the intake is too low (deficiency) or too high (excessive). This can be the case for folic acid, vitamin D, iodine, niacin, selenium, vitamin A and zinc (Vanderveen, 2006). Therefore, for nutrients that can induce adverse health effect at a lower or higher intake, determining the intake level that provides optimum health effect could be crucial.

2.2 Concept of food safety risk analysis

Food safety risk analysis is used to estimate the risks to human health due to the exposure to hazards during food consumption. Its primary aim is to estimate the probability of the occurrence of foodborne illness (FAO/WHO, 2005). In addition, it supports to find and apply appropriate measures to regulate the risks and to communicate with stakeholders about the possible risk mitigation (FAO/WHO, 2005). A risk analysis includes risk assessment, risk management and risk communication. In this PhD thesis more focus is given to risk-benefit assessment than to risk-benefit management and communication.

2.3 Food component health benefits

Food contains various nutritional constituents that are indispensable for human health. The nutrients that contribute to the growth, development and well-being of human health are carbohydrates, fats, proteins, vitamins, minerals and water (Kohlstadt, 2009). The health benefits

of these components are diverse, for example supplying energy, built and repair body tissues, improve immune system, prevent or reduce several chronic and acute diseases (Kohlstadt, 2009).

Several nutritional epidemiological studies reported the role of various nutrients in the prevention of several diseases, for instance omega-3 fatty acids prevent or reduce risk of cardiovascular diseases (Kris-Etherton et al., 2003), cobalamin in the prevention of megaloblastic anemia (Bolaman et al., 2003) or the role of vitamin D in the prevention of bone diseases (Bischoff-Ferrari et al., 2010).

In addition to the nutrients, certain microbes could provide health benefits. For instance, probiotics may prevent necrotizing enterocolitis and mortality in preterm very low-birth-weight infants (Wang et al., 2012).

On the other hand, some nutrients may be the source of both health benefits and risks. For instance, folic acid reduces the incidence of neural tube diseases (IOM 1998; Ray et al., 2002), and colorectal cancer (Kim et al., 2001; Sanjoaquin et al., 2004). On the contrary, folic acid may cause health problem such as masking vitamin B12-deficiency leading to progression of irreversible neurological symptoms (IOM, 1998; SCF, 2000).

2.4 Risk-benefit assessment of food

Food could contain both health benefits and risks. So, the weighing of the benefits against the risks becomes an interesting issue (Hoekstra et al., 2008). If the intake of a particular food or food component provides both health risk and benefit, it is essential to delineate an intake level within which the balance of risk and benefit is acceptable for risk management purposes (Hoekstra et al., 2008).

Due to the increasing interest of the integration of the health risk and benefit of food consumption among food chain stakeholders, significant progresses have been achieved in method development of health risk-benefit assessment of food during the last decade.

With regard to method development for human health risk-benefit assessment of food, EFSA has taken the initiative to organize a scientific colloquium on risk-benefit analysis of foods in July 2006 to have an open scientific debate on the methods and approaches for risk-benefit analysis of foods. The outcome of the colloquium was that risk-benefit analysis and assessment should mirror the old paradigm of risk analysis and risk assessment. In addition, to facilitate the communication of the result of health risk-benefit assessment of food, a common scale particularly DALY and QALY, to combine the positive and negative health effect have been suggested (EFSA, 2006a).

Later, it was proposed that guidance on risk-benefit assessment of food with respect to methodology, approaches, tools and potential limitations in the risk-benefit assessment should be documented. Since then, various EU-funded projects such as the Beneris, Qalibra, Brafo, Bepraribean and PlantLIBRA projects, have been launched to address health risk-benefit assessment of food and food ingredients.

Among these projects, QALIBRA and BRAFO provide a quantitative integrated health risk-benefit assessment approach using common health metrics.

The following chapter describes the existing methods in health risk-benefit assessment of food and other similar studies. In addition, the similarities and differences of the existing methods are elaborated in section 3.7.

Chapter 3

The existing methods and other similar studies in risk-benefit assessment of food

3. The existing methods and other studies in risk-benefit assessment of food

During the last decade, different approaches have been proposed to integrate the health risk and benefit of food or food component intake. Most of the risk-benefit approaches are established in EU-funded projects. These methods and other risk-benefit studies are presented hereunder.

3.1 EFSA: Guidance on human health risk-benefit assessment of foods

Probably, EFSA is the pioneer in conceptualizing the health risk-benefit assessment of food. EFSA suggests a progressively integrated health risk-benefit assessment of food by using the same risk assessment paradigm for benefit assessment (EFSA, 2010). A stepwise approach is suggested for the risk-benefit assessment following a problem formulation. The aim of problem formulation is to elucidate what is included in the assessment (EFSA, 2010).

The EFSA guidance comprises of three steps: i) initial assessment, addressing whether the health risks clearly outweigh the health benefits or vice versa, ii) refined assessment, aiming at providing semi-quantitative or quantitative estimates of risks and benefits at relevant exposure, by considering different populations and refining the dose-response; iii) comparison of risks and benefits using a composite metric such as DALYs or QALYs to express the outcome of the risk-benefit assessment as a single net health impact value. At the initial assessment, the classical risk assessment paradigm is mirrored for benefit assessment. It includes: 1) hazard-positive health effect identification; 2) hazard-positive health effect characterization; 3) exposure assessment; 4) risk-benefit characterization. At each step of the process, the assessment could stop when it is clear that the risk outweighs the benefit or vice versa (EFSA, 2010).

In EFSA guidance, various specific aspects of risk-benefit assessment issues including exposure scenarios, data, common health metrics and uncertainty analysis have been discussed. In addition, simple examples on fish and selenium have been performed to illustrate the method.

3.2 BRAFO tiered approach for benefit–risk assessment of foods

BRAFO (Benefit–risk analysis for foods) is an EU project conducted from 2007 to 2010. The main aim of BRAFO was to develop a framework that allows for the comparison of human health risks and benefits in relation to foods and food compounds by using a common scale (when needed) (Hoekstra el al., 2012).

The approach starts with pre-assessment and problem formulation, followed by a separate risk and benefit assessment as proposed by EFSA (2010). Like EFSA, it mirrors the classical risk assessment paradigm for benefit assessment. The approach consists of four tiers. In Tier 1, each risk and benefit is assessed independently. In Tier 2, risks and benefits are compared qualitatively without using common metric. In Tier 3, risks and benefits are combined quantitatively using a common metric, by a deterministic approach. In Tier 4, risks and benefits are integrated quantitatively in a common metric by a probabilistic approach. It should be noted that, at each tier the assessment could stop when it is clear that the benefit prevails over the risk or vice versa.

In addition, a DALY and QALY model is developed in the BRAFO project, to integrate the health risk and benefit. The model considers three possible health outcomes after the onset of a disease: recovery from the disease, death as a result of the disease, or survive with the disease until the normal life expectancy (Hoekstra et al., 2012). This DALY model has been used throughout this PhD thesis to combine the health risk and benefit.

Moreover, various issues related to risk-benefit assessment including DALY calculation for continuous and quantal health effects, dose-response for different health effects, data, presentation and interpretation of the outcome, and uncertainty have been described (Hoekstra et al., 2012).

Furthermore, several case studies have been conducted to validate the BRAFO tiered approach, and in most of the case studies it has been observed that the assessment stopped at early stage before the health effects are integrated using common health metrics (Schütte et al., 2012;

Verhagen et al., 2012; Watzl et al., 2012). The case studies demonstrate the applicability of the BRAFO tiered approach for health risk-benefit assessment of food.

3.3 QALIBRA

Qalibra (Quality of Life – Integrated Benefit and Risk Analysis) is also an EU project conducted from 2006 to 2009. Qalibra aimed to provide a user-friendly tool for a deterministic and/or probabilistic (when needed) risk-benefit assessment of food (Hart et al., 2013). The software is developed for quantitative assessment of risks and benefits corresponding to tier 3 and 4 of the BRAFO tiered approach (Hoekstra el al., 2012). It quantifies uncertainties and variabilities at each stage of the process, if necessary.

The case studies performed by using QALIBRA tool includes fish risk-benefit assessment (Hoekstra et al., 2013); a simple example on fish risk-benefit assessment to illustrate the output (Hart, et al., 2013); benefit–risk assessment of plant sterols in margarine (Hoekstra et al., 2013a) and the vitamin D optimum scenario (chapter 7 of this thesis). The case studies illustrate how the QALIBRA tool can be used in a quantitative risk-benefit assessment.

3.4 BENERIS: Benefit-risk assessment for food

Beneris (Benefit-risk assessment for food) is an EU project conducted from 2006 to 2009. The general objective of BENERIS was to create a framework for handling complicated benefit-risk situations and apply it for analysis of the benefits and risks of certain foods using Bayesian belief networks (BBN) (Tuomisto, 2010).

The main outcome of Beneris is the enhanced methodology (open assessment) for benefit-risk assessments in the web workspace Opasnet. The benefit-risk assessment method is described on the web workspace Opasnet (http://en.opasnet.org). A risk-benefit case study on fish was performed in order to illustrate the developed method and BBN in practice (Tuomisto, 2010).

3.5 BEPRARIBEAN: Best practices for risk-benefit analysis

BEPRARIBEAN (best practices for risk-benefit analysis) is a state-of-the-art of benefit-risk analysis project conducted from 2009 to 2011. The BEPRARIBEAN project aims to advance benefit–risk analysis in the area of food and nutrition by learning from other fields. It describes the state of the art in benefit-risk analysis in medicine (Luteijn et al., 2012), food microbiology (Magnússon et al., 2012), food and nutrition (Tijhuis et al., 2012), environmental health (Pohjola et al., 2012), economics and marketing–finance (Kalogeras et al., 2012) and consumer perception (Ueland et al., 2012).

3.6 PlantLIBRA: plant food supplements: levels of intake, benefit and risk assessment

PlantLIBRA (plant food supplements: levels of intake, benefit and risk assessment is an ongoing EU project, aims to foster the safe use of food supplements containing plants or botanical products, by increasing science-based decision-making by regulators and food chain operators. PlantLIBRA is structured to develop, validate and disseminate data and methodologies for risk and benefit assessment and implement sustainable international cooperation (http://www.plantlibra.eu/web/node/44, 2013).

3.7 Similarities and differences of the existing risk-benefit assessment methodologies

The food risk-benefit assessment methodologies considered for comparison in this section includes EFSA guidance (EFSA, 2010), a tiered approach for risk-benefit assessment of foods, example on folic acid (Fransen et al., 2010; Hoekstra et al., 2008), the BRAFO tiered approach (Hoekstra et al., 2012), BENERIS (Tuomisto, 2010) and QALIBRA (Hart et al., 2013).

The methodologies mentioned above are splitted into two categories depending on the resemblance of the study. Category 1) includes EFSA guidance, a tiered integrated approach for risk-benefit assessment of foods, example on folic acid and the BRAFO tiered approach. They provide risk-benefit assessment of food methodologies. Category 2) includes BENERIS and

QALIBRA, which are web-based tools that assist to perform quantitative risk-benefit assessment of food.

Category 1): The commonality of these risk-benefit assessment methodologies is the application of the classical risk assessment paradigm for risk-benefit assessment of food. In category 1 the methodologies initially assess the risk and the benefit separately without integrating them in a common health metrics. Then, they follow a progressively integrated quantitative risk-benefit assessment, using a common scale. When there is sufficient information to answer the risk-benefit assessment question (whether the risk outweighs the benefit or vice versa), the assessment could stop at early steps. This implies that, the health impact of the beneficial and hazardous component of food may not be integrated using a common health metric, if the assessment stops at earliest steps. Furthermore, category 1 methodologies in most cases consider comparison of two or more scenarios.

Category 2): Includes QALIBRA and BENERIS. QALIBRA helps to run quantitative risk-benefit assessment described in BRAFO tier 3 and 4 by either a deterministic or a probabilistic approach. QALIBRA considers both variability and uncertainty present in the assessment. In QALIBRA one can integrate the risk and benefit by using either QALY or DALY. QALIBRA quantifies and reports the health impact (DALY or QALY) explicitly for those who die from a disease, survive with a disease until the normal life expectancy and for those who recover from a disease. Also, QALIBRA makes it possible to quantify quantal (effects that are modelled as either absent or present, e.g. cancer) or continuous (effects expressed as a change in a continuous variable, such as a change in body weight) health effects and produce either graphical or tabulated results (Hart et al., 2013). In addition to quantifying the DALY or QALY of each health effect, scenarios and the net DALY or QALY, QALIBRA automatically computes the incidences of the endpoints for each scenario. Even though in QALIBRA one can run a comparison of only two scenarios at a time, it is shown in MANUSCRIPT I that it is possible to run as many scenarios as possible by repeating the run for the different scenarios. This is especially useful if one aims to identify the scenario that provides maximum net health gain by several scenario simulations. Once the input data are ready, QALIBRA quantifies the health impact within short time as opposed to manual computation that usually

takes longer time. Moreover, QALIBRA makes it possible to handle large amount of data and share it with interested users. In QALIBRA tool, the user is guided through the assessment and it is made clear in advance which data is needed.

Experiences show that QALIBRA may not be limited to only quantification of risk-benefit assessment of food, it would also probably be used to compute burden of disease including the sequelae's from different attribution as long as the input data are readily sorted. QALIBRA does not calculate the intake, dose-response and the parameters needed for DALY or QALY on its own. The values for each parameter should be calculated and saved on MS-Excel as a CSV (comma delimited) file and need to be uploaded in the software. Also, a single parameter value can also be used to run in QALIBRA without uploading matrix.

BENERIS provides a tool to collect, organize and distribute information on issues relevant for benefit risk analyses of food and environmental issue. It allows for the integration of data from food consumption and nutrient intake studies with chemical contaminant to evaluate exposure to both contaminants and nutrients in food. BENERIS has developed and applied Bayesian belief networks (BBNs). BENERIS does not apply QALY or DALY for the integration of health effect and provide no information about the net health impact of hazardous and beneficial components of food. In addition, it is limited to the assessment of nutrient and toxicants.

In general, the method developed by Fransen et al. (2010); Hoekstra et al. (2008); Hoekstra et al. (2012); Hart et al. (2013) explicitly guide how to perform a progressive quantitative integrated risk-benefit assessment by using a common scale. These approaches do not contradict each other, but rather they are complementary (Hart et al., 2013). In this PhD thesis a part of BRAFO approach QALIBRA were used.

Chapter 4

Application of risk-benefit assessment in food and selected case studies

4. Application of risk-benefit assessment in food and selected case studies

In addition to the above risk-benefit methodologies and case studies, several risk-benefit assessment studies have been conducted especially on fish. This section presents the application of risk-benefit assessment in food and food components. Besides, selected qualitative and quantitative risk-benefit assessment case studies are discussed.

Risk-benefit assessment can be applied to food or food components in different circumstances. EFSA, (2010) and Fransen et al. (2010) suggest different situations where risk-benefit assessment can be applied in food or food components. Risk-benefit assessment is needed when a single food constituent induces both positive and negative health effects, for example folic acid (Hoekstra et al., 2008). If a certain level of dietary exposure is associated with both risk and benefit in different subpopulations, risk-benefit assessment is needed to define the preferred intake level for the different subpopulations (Fransen et al., 2010). Also, if the positive and negative health effects are a result of the different components of a food product, risk-benefit needs to be performed, for example in the case of fish omega-3 fatty acid versus chemical contaminants or a pathogen (Cohen et al., 2005; Hoekstra et al., 2013b; Berjia et al., 2012). In addition, risk-benefit assessment can be applied to determine the optimum scenario that provides maximum health gain (MANUSCRIPT I).

Moreover, risk-benefit assessment can be applied when a substantial modification of dietary consumption patterns occur; for instance in case of food substitution (Verhagen et al., 2012). Risk-benefit assessment is essential when chemicals are used to reduce microbial contamination (Havelaar et al., 2000). Risk-benefit assessment can be applied when enhanced bioavailability of nutrient by improving processing is associated with an increased survival of foodborne pathogens (EFSA, 2010). When a new food product is developed (that contains both beneficial and hazardous components), risk-benefit assessment can be applied to define the intake level to maximize the benefit and minimize the potential associated risk (EFSA, 2010).

4.1 Selected qualitative risk-benefit assessment of fish consumption studies

Risk-benefit assessment of food, especially of fish, has gained interest in the recent years in Europe and USA. The risk-benefit assessment of fish studies in the Nordic countries are mostly evaluated in qualitative terms, which do not consider integration using common health metrics. The summary of risk-benefit assessment studies related to fish consumption in Denmark, Sweden, Norway, Finland, UK and Belgium is presented below.

4.1.1 Fish risk-benefit assessment in Denmark

In the Danish fish risk-benefit assessment, several nutritional benefits (such as vitamin A, D, selenium and iodine) and hazardous chemical contaminants (such as methyl mercury, cadmium, lead, arsenic and PCB) were reviewed. Nevertheless, the benefit of omega-3 fatty acids and risk of methyl mercury were highlighted (Fødevaredirektoratet, 2003).

The Danish assessment recommends the intake of fish should be 200-300 g/week, with special consideration for certain fish species and subpopulations. As certain fish species contain high level of methylmercury, it was suggested that pregnant and breast-feeding women and should avoid the consumption of fish that contain high level methylmercury (Fødevaredirektoratet, 2003). For the elderly, the beneficial cardiovascular effects of omega-3 fatty acids outweigh risks associated with exposure to contaminants (Fødevaredirektoratet, 2003).

4.1.2 Fish risk-benefit assessment in Finland

In the Finnish fish risk-benefit assessment, the benefit of omega-3 fatty acids and the risk of chemical contaminants such as dioxins and dioxin-like PCBs and mercury have been studied. Fish should be consumed at least 200g/week and consumption should be varied between different fish species in order to minimize the intake of any individual contaminant. (Livsmedelssäkerhetsverket, 2006). In addition, due to the contaminant levels, children, young people and persons at fertile age should only eat large Baltic herring, salmon, or pike (Livsmedelssäkerhetsverket, 2006).

4.1.3 Fish risk-benefit assessment in Norway

The Norwegian assessment stressed the positive health effect of omega-3 fatty acids and the negative health effect of dioxins and dioxin-like PCBs and mercury. Norwegians are generally recommended to eat more fish, varied between lean and fatty fish. A higher consumption is mainly advised due to beneficial cardiovascular effects in the older population and also beneficial effects on pregnancy and foetus development. From a toxicological point of view, it is reported that there is no danger associated with a high consumption (4 meals/week or more) if consumption is varied and fatty fish does not exceed 2 meals/week. (VKM, 2006).

4.1.4 Fish risk-benefit assessment in Sweden

The Swedish risk-benefit assessment of fish consumption focuses the risk of dioxin and dioxin-like PCBs, and methyl mercury and the benefit of omega-3 fatty acids and vitamin D. The Swedish assessment recommends an increased fish consumption. The Swedish assessment concludes that an increased fish consumption in line with the general dietary advice of 2–3 meals a week (250–375 g/week), with consumption of different fish, is supported (Livsmedelsverket, 2007). Nevertheless, intake of certain fish (e.g. fish from Baltic sea) that contain high contaminant levels could lead to intake that surpasses the tolerable intake limits; this primarily concern children and women of child bearing age (dioxins/PCBs), and pregnant women (methyl mercury) (Livsmedelsverket, 2007).

In general, in all the Nordic assessments the chemical contaminant risks are assessed in terms of the likelihood to exceed tolerable intake limits. All assessments take in account the positive health effect of omega-3 fatty acid on the cardiovascular system to be the most important and probable beneficial effects on foetus development are also pointed out (Sand et al., 2008).

4.1.5 Fish risk-benefit assessment in United Kingdom

In the UK assessment, it was concluded that British consumers should eat more fish and recommends at least two portions per week, of which one should consist of fatty fish. This is because the positive effects on reducing the risk of cardiovascular disease and foetal development due to intake of omega-3 fatty acids (SACN/COT, 2004).

4.1.6 Fish risk-benefit assessment in Belgium

In the Belgian fish risk-benefit assessment, the benefits of omega-3 fatty acids, vitamin D, and iodine were stressed. A broad range of chemical contaminants such as dioxin, mercury were considered. It was concluded that a regular consumption of oily fish together with intake of EPA and DHA enriched margarine is advised to increase omega-3 fatty acids intake without exceeding the tolerable intake of contaminants (Sioen, 2007).

In general, the consumption of fish seems the benefit outweighs the risk. However, pregnant women and children need to minimize the consumption of fish species that contain high concentration of chemical contaminants.

4.2 Selected quantitative risk-benefit assessment of fish consumption studies

In this section the quantitative risk-benefit assessment study performed using common health metrics is presented. It includes the fish risk-benefit assessment study in The Netherlands, France and USA.

4.2.1 Fish risk-benefit assessment in The Netherlands

The Netherlands fish risk-benefit assessment study is a quantitative study that integrates the health effects using a common health metric, DALY. The nutritional benefit and the risk of chemical contaminants were compared. The model was implemented in the QALIBRA tool. It was

concluded that the overall benefits of eating 200 g of fish per week, instead of the current consumption amounts, outweigh the risks. Eating 500 g of fish per week is even more beneficial, but the associate risks would also increase (Hoekstra et al., 2013b).

4.2.2 Fish risk-benefit assessment in USA

In USA, a risk-benefit analysis of fish consumption is performed that focusses on omega-3 fatty acids and mercury, using QALY to integrate the health effects. The result of the assessment shows that, replacement of fish with high mercury level with fish containing less mercury in childbearing age women provide considerable developmental benefits with few negative impacts. But, if women reduce fish consumption, countervailing risks substantively reduce net benefits. If adults reduce their fish consumption, the net public health impact is negative (Cohen et al., 2005).

4.2.3 Fish risk-benefit assessment in France

France conducted a fish risk-benefit assessment using a similar QALY model as in the USA study (Cohen et al., 2005). The benefit of omega-3 fatty acids and the risk of mercury were considered. Results show that increasing fish consumption may have a beneficial impact on health. The increase in fish consumption in pregnant women may have a negative impact due to mercury (Guevel et al., 2008).

In general, integrated quantitative risk-benefit assessment of food seems a more structured and transparent approach which explicitly shows what is included, missed and assumed in the assessment. This helps the decision maker to understand the logic, interpretation and limitation the assessment at each step of the process. In addition, it enables to compare the healthy life year's loss and gain due to the hazardous and beneficial components of food, which cannot be determined using qualitative assessment.

4.3 Opinion about fish risk-benefit assessment studies

Since fish is somehow a unique food that contains several harmful and beneficial components in one packet, several risk-benefit assessment case studies have been performed on fish. In addition to the above selected case studies on fish, several studies have also been conducted at national and international level (Ponce et al., 2000; IOM, 2007; FAO/WHO, 2010).

Many of fish risk-benefit analysis conclude that the benefit of fish consumption in the general population greatly outweighs the risk. However, special attention should be given to sensitive populations such as pregnant women, infants and neonates.

All the fish risk-benefit assessment studies focus on comparison of nutritional benefits and risks of chemical contaminants. Ideally, an integrated risk-benefit assessment that considers the major nutritional benefits, the risk of pathogens and chemical contaminants would be sought to better estimate the overall health impact. As a result, an optimum risk-benefit management practice can be reached to support decision making. Currently, there are very limited studies that integrate the risk of microbial pathogens in risk-benefit assessment (Havelaar et al., 2000; Berjia et al., 2012). But, to the author's knowledge there is currently no study that holistically integrates the risk of pathogens and chemical contaminants with the benefit of nutrients. The quantitative risk-benefit assessment study on cold-smoked salmon integrates the risk of *Listeria monocyogenes* and the benefit of omega-3 fatty acid (Berjia et al., 2012).

Although a majority of research has shown that the benefit of fish consumption greatly outweighs the risks, it is important to keep in mind that this field of science is just at the incipient levels of determining how to precisely assess the everyday choices we make in our diet and how these ultimately affect our lives (Rosalee et al., 2012). However, integrated risk-benefit assessment of food clarifies the overall health impact of food and food component consumption. It helps to set the best food or food component consumption recommendation by balancing the risk and the benefit. It supports to identify potential health risk mitigation and benefit improvement strategies linked to food consumption, so optimum health gain can be attained. It provides a broader

overview of public health statistics related to food consumption as it combines incidence, mortality, severity and duration of health effect, unlike the classical risk assessment, which often measures only incidence.

Chapter 5

Other issues in risk-benefit assessment of food

5. Other issues in risk benefit assessment of food

Risk-benefit assessment of food is a complex multi-disciplinary study. The following section discusses other topics related to risk-benefit assessment of food including data needed, uncertainty analysis, experts needed and consumer perception in risk-benefit assessment food.

5.1 DALY and QALY in integrated risk-benefit assessment

In performing integrated quantitative risk-benefit assessment, one of the challenges is the comparison of positive and negative health effects associated with nutritional, chemical and microbiological components of food using common scale.

Negative health impact due to the exposure to the hazardous components of food is measured in terms of healthy life year's loss. The positive health impact due to the intake of the beneficial components of food is measured in terms of the extra healthy life year's gain or reduction of the negative health impact. For both the positive and negative health impact different dimensions of health (incidence, severity, morbidity, mortality and duration of the disease) together with consumption data are used to measure the health gain and loss (EFSA, 2010; Hoekstra et al., 2012).

In order to integrate the negative and positive health impacts of food consumption to predict the net health effect, a common health metric is used (EFSA, 2010; Hoekstra et al., 2012; Sand et al., 2008). In risk-benefit assessment of food and/or burden of foodborne disease estimation, a common health metric is a measurement expressing the health effect of the hazardous and/or beneficial components in the same unit.

Disability or quality adjusted life years (DALYs or QALYs) are the best known common health metrics used to combine the different dimensions of health outcomes. One DALY can be considered as loss of one healthy life year (Murray and Lopez, 1996). QALY measure the total

number of years of perfect health in a population. A year in perfect health is considered equal to 1.0 QALY (Murray and Lopez, 1996).

In this PhD thesis, several other studies on risk-benefit assessments of food as well as burden of disease studies, DALY is the most frequently used common health metric. Murray and Lopez (1996) first used DALY in a global burden of diseases estimate to measure the population health outcome using the information on mortality and morbidity in the population. According to Murray and Lopez (1996), DALY is calculated as the sum of YLL (years of life lost due to death) and YLD (years lived with disability) in the population. This DALY model predicts only the health outcomes of the hazardous components in terms of health life year's loss at population level. It considers the health loss due to mortality and morbidity. It is suitable to be used in burden of disease estimation studies but not for risk-benefit assessment studies. This DALY model is extended later by Hoekstra et al. (2012) who consider recovery from the disease when quantifying the morbidity. In the Murray and Lopez (1996) model, the duration of the disease after its onset is only considered for those who live with the disease but not for those who eventually die from it. In Hoekstra el al. (2012) model, the duration is considered for those who die due to a disease, lives with disease and recover from disease. This extended model can be used to estimate the health loss and gain due to the hazardous and beneficial components of food at individual level. In Hoekstra et al. (2012) model, the probability of onset of disease (P_{eff}) has to be estimated depending on the intake of compounds for individuals. The relation between intake or exposure and P_{eff} is the dose-response relation that is an essential part of the analyses in this thesis. The Murray and Lopez (1996) model does not consider the $P_{eff.}$ Hence, Murray and Lopez (1996) model cannot be applied to estimate the health loss based on intake of food or food components following the risk-benefit assessment paradigm. The risk-benefit assessment paradigm is the steps that are mirrored from the classical risk assessment approach (EFSA, 2010; Hoekstra et al., 2012). It includes: 1) hazard-positive health effect identification; 2) hazard-positive health effect characterization; 3) exposure assessment; 4) risk-benefit characterization. This paradigm used to estimate intake or exposure based disease probability.

The DALY model according to Hoekstra et al. (2012) describes the disability adjusted life years lost for a specific endpoint (a disease) for an individual of age CA:

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

Where:

$DALY_{CA}$	disability adjusted life years for an individual of age CA
P_{eff}	probability of onset of the disease per year
P_{rec}	probability of recovery from the disease
P_{die}	probability that the disease causes death
YLD_{rec}	mean duration of disease for those who recover (in years)
YLD_{die}	mean duration of disease for those who die (in years)
CA	current age of individual in year at disease onset
LE	life expectancy at the onset of the disease
w	disability weight for disease

This equation refers to the DALY loss for three possible health outcomes of a disease. The DALY loss for an individual who recovers from the disease can be calculated as:

$$DALY = YLD_{rec} * w$$

The DALY loss for an individual who does not recover, but survives with the disease until their normal life expectancy can be calculated as:

$$DALY = (LE – CA) * w$$

The DALY loss for an individual who dies from the disease can be calculated as:

$$YLD_{die} * w + LE – CA - YLD_{die}$$

In addition, the Hoekstra et al. (2012) model can be used to quantify the healthy life years gained or lost at population level when exploring the difference in health impact of changing from a reference intake scenario to an alternative scenario.

$$\Delta DALY = \Sigma DALY_{alt} - \Sigma DALY_{ref}$$

Where, ΔDALY is change in DALY; $\Sigma DALY_{alt}$, summation of DALYs caused by every endpoint of all individuals in the population at the alternative scenario and $\Sigma DALY_{ref}$, summation DALYs caused by every endpoint of all individuals in the population at the reference scenario.

The DALY model according to Murray and Lopez (1996) describes the disability adjusted life years lost at population level:

$$DALY = YLL + YLD$$

$$YLL = N_d * e$$

$$YLD = N_i * d * w$$

Where, N_d is number of deaths at each age, e indicates life expectancy for each age, N_i is number of cases, d is duration of the disease (years) and w is the disability weight of the diseases.

In general the DALY model proposed by Murray and Lopez, (1996) is applicable to burden of disease estimate of food and environmental related hazardous compounds as well as infectious diseases. On the other hand, the DALY model proposed by Hoekstra et al. (2012) is applicable to risk-benefit assessment of food with different scenarios. However, experience shows that it is also applicable for burden of disease estimate study.

The DALY model proposed by Hoekstra et al. (2012) is used in this PhD thesis (PAPER I, MANUSCRIPT I and II).

5.2 Data needed in risk-benefit assessment of food

The quality of the output of the assessment is dependent on the quality of input data used during the assessment. Experiences show that, the complete dataset required to perform comprehensive quantitative risk-benefit assessment is not readily available. For performing a comprehensive quantitative risk-benefit assessment of food, various data are needed such as toxicological, nutritional, microbial, epidemiological and population statistics data.

At the beginning of risk-benefit assessment, data related to food safety (pathogens, their toxins and chemical contaminants) and nutritional composition of the food are necessary in order to identify the hazardous and beneficial components. In addition, epidemiological data with respect to the identified hazardous and beneficial components are required to identify the associated health impacts. The strength of the association between the intake of the hazardous or beneficial components and consequences for human health is important (WHO, 2003). Data obtained in intervention studies in human is strong and convincing, whereas, data from animal studies are relatively weaker, which need extrapolation to human situation (EFSA, 2010).

After the identification of the components and the associated health effects, food consumption data is required to estimate the intake of beneficial and hazardous components of food. The food consumption data are often available in national consumption survey studies and food composition data can be obtained from the national food composition database. If the hazard is a pathogen, the required data includes concentration and prevalence of pathogen in the food and pathogen characteristics related food processing pathway. This is because pathogens in the food can grow, survive and/or inactivated during food processing, handling and storage. If the hazard is chemical contaminant, often concentration data is needed, likewise for nutrients. This is the major difference between microbial exposure assessment and nutrient and chemical contaminant intake assessment. Ideally, nutrients and chemical contaminants intake assessment should also consider the effect of food processing, handling and storage on the concentration. This is because certain nutrients and chemical contaminants can be washed out during washing and eliminated or reduced in concentration during high temperature cooking. Nevertheless, there seem to be

relatively less experience in modeling the intake of nutrients and chemical contaminants following food processing pathway, handling and storage compared to pathogens. Besides, data needed for modeling exposure to most foodborne pathogens in relation to growth, survival and inactivation is relatively available. But, the current available data to model the intake of nutrients and chemical contaminants may be inadequate to perform comprehensive quantitative intake assessment by taking food processing pathway, handling and storage into account.

In order to estimate the probability of disease occurrence or prevention due to the exposure to pathogens or intake of contaminants and nutrients, epidemiological data of the health effects are essential. For pathogens, the data required at this level include specific dose-response parameters of the pathogen, which is can be obtained from outbreak data or human volunteer studies. With regard to pathogens, estimate of incidence can be found based on risk assessment or clinical epidemiological data. For the estimation of number of cases, population statistics data is necessary at this level.

In epidemiological studies, the probability of chemical contaminants and nutrients to cause or prevent diseases are usually presented relative to certain reference intake level, often zero intake or placebo, and expressed as relative risk, odds ratio or hazard ratio. For DALY calculations, these have to be converted to absolute probability of effect (P_{eff}), by using incidence data for each health effect.

In order to estimate the health loss and gain due to the hazardous and beneficial components and to estimate the net health impact, further epidemiological data is crucial. In addition to the absolute probability of each disease, mortality, severity, duration of each disease, population statistics (number of population per age and sex for each disease) as well as life expectancy data are required. Furthermore, the duration of the disease is varied depending on whether the disease leads to recovery, death or survival with the disease until the normal life expectancy. Often, these data are not readily available and usually parameter values are estimated based on some assumption. The duration of the disease for those who survive with the disease until their life

expectancy is normally the difference between life expectancy and current age at the onset of the disease (Hoekstra et al., 2012).

Data on the severity weight of some common diseases can be found in WHO (2008) burden of disease estimate study. But, the severity weight is not available for all diseases; in this case assumptions have to be used. When the severity weight of a disease is not available in literature, the severity weight of another disease with similar symptoms, duration and complications from WHO (2008) severity weight list may be used in consultation with a relevant expert. Severity weight is derived from epidemiological data based on relative health state valuation of a disease (Murray et al., 2002).

Furthermore, data on different probabilities related to a disease are required; these probabilities include probability of death due to a disease, probability of recovery from the disease and probability of survival with the disease. Probabilities of death and survival are usually obtained from national public health statistics or other national epidemiological data. On the contrary, probability of recovery from a disease is often unavailable. Basically, probability of survival is not directly required to estimate DALY. However, it helps to estimate the probability of recovery because all these probabilities sum to one.

In general this section discussed what sorts of data are required to perform a quantitative risk-benefit assessment and how these data can be obtained. In risk assessment, estimation of the intake based probability of disease and number of cases are usually the end of the assessment. But, in a comprehensive integrated quantitative assessment of risks and benefits, further steps and data are required. Due to the variability of source of data, expert judgment is essential in interpreting the results of a particular study intended to be used in risk-benefit assessment of food.

5.3. Uncertainty and sensitivity analysis in risk-benefit assessment of food

Uncertainty is a state of having limited or no information to delineate the existing state and predict future outcome (Cacuci et al., 2005). Usually, when there is limited data or no knowledge of one or more inputs in risk-benefit assessment of food, assumptions or expert opinion are used to fill the data gaps. An uncertainty analysis can be used to describe the set of possible outcomes of the input assumptions in the model (Cacuci et al., 2005). In risk-benefit assessment of food, uncertainty analysis supports decision-making through the quantification of uncertainties in the relevant variables in the model. It determines how likely certain outcomes are when some variables in the model are not exactly known and it helps to identify the most important data gaps and guide future data collections (Cacuci et al., 2005).

Uncertainties are inevitable in risk-benefit assessment of food because of the lack of data. They can be treated qualitatively or quantitatively (EFSA, 2006b). For the qualitative treatment of uncertainty EFSA (2006b) suggests to use scores (like, ++/--) to represent a subjective assessment of the direction and magnitude of the potential influence of each source of uncertainty on the assessment. The quantitative treatment of uncertainty considers deterministic or probabilistic approach. Several sources of uncertainty that frequently affect risk–benefit assessment are listed by Hoekstra et al. (2012). Example of uncertainty that may present in risk-benefit assessment of food include uncertainty in intake data, identification of health effect and population, choice of dose-response models and estimation of mortality rate (Hoekstra et al., 2012). QALIBRA presents a general model for food risk–benefit assessment that quantifies variability and uncertainty in a deterministic or a probabilistic way (Hart et al., 2013).

Sensitivity analysis describes how the variation in the output of a model can be apportioned to different sources of variation in the input (Cacuci et al., 2005). The main purpose of sensitivity analysis is to identify and focus on data and assumptions that have most influence on the final outcome.

In risk-benefit assessment of food, sensitivity analysis helps:

- To identify critical assumptions that most influence the model output.
- To improve the interpretation of the outcome of the assessment.
- To optimize resource allocation
- To support food authorities in making decision related to food consumption.

Even though there are challenges in conducting comprehensive quantitative risk-benefit assessments due to a lack of truly representative data, the use of sensitivity analysis may provide a great advantage in providing essential information for decision making in relation to food consumption.

5.4 Consumer perception in health risk and benefit of food

A final outcome of health risk-benefit assessment of food, used to support food policy decision makers, may be to intervene by a food consumption recommendation in order to improve public health. This implies communication of the risk benefit analysis result to consumers. With respect to food choice and consumption, consumers are usually uncertain about the positive and negative health outcomes of the food they consume. Ideally, consumers want to know the risk and the benefit linked to the food consumption. Still, the consumer perception of the health risks and benefits associated with food consumption is highly variable. Risk and benefit of food is key determinants of food choice and food consumption recommendation setting. Therefore, understanding the consumer perceptions of the risk and benefit connected to food consumption is essential in determining food choice and consumption recommendation setting. The consumer perception about the risk and benefit of food is described in general by Ueland et al. (2012) and specific to fish by Willems et al. (2006).

Food risk and benefit perception is the subjective judgment of consumers about the negative and positive health impact of food consumption. Perceived risks due to the consumption of food are associated to morbidity, mortality and severity of consequence which is feared by the consumer

(Ueland et al., 2012). Benefit perceptions are often associated with food's ability to exert positive consequences. This includes sensory properties (good taste, texture and color), convenience, healthiness aspects and value for money or benefit related to food production in an ethically and environmentally friendly way (van Kleef et al., 2005).

Consumers' benefit perception of foods is more emotionally triggered compared to risk perception (Fischer and Frewer, 2009). The taste, color, texture and other emotional aspects of food benefit is often the result of own experience with the products (Cardello, 2003; Fischer and Frewer, 2009). Investigation on consumer choice of products with health benefits, such as lower energy content, show that consumers invariably choose the version with the taste benefit and not the health benefit (Tepper and Trail, 1998; Urala and Lähteenmäki, 2006; Verbeke, 2006). However, consumers with special health interest may find health benefits of higher importance than taste benefits (Zandstra et al., 2001). Nevertheless, in a real consumption situation, consumers may not coincide with their perceptions of the product (Kalogeras et al., 2012). Real product experience has been found to increase consumer acceptance of products that consumers otherwise have negative perceptions about (Cardello, 2003). The food benefits other than emotional aspect of benefits depend highly on the consumer interest (Ueland et al., 2012).

In general, benefit-risk evaluations of consumers incline towards acceptance of all that is traditional and well-known (benefits), and rejection or suspicion towards anything that is novel and highly processed (risks) irrespective of real risk resent in the food (Ueland et al., 2012). So far, this type of perception based evaluations or benefits and risks are not included in formal risk-benefit assessments of foods, as treated in this thesis.

5.5 Expertise needed in risk-benefit assessment of food

Risk-benefit assessment of food is still under development and there is currently no internationally accepted standard methodology available for use. A reason for that may be the complex and demanding nature of the task and the requirement of experts from different disciplines to collaborate intensively. However, new methods for risk assessments are gradually being

developed (Hoekstra et al., 2008; Fransen et al., 2010; Hoekstra et al., 2012; Hart et al., 2013; MANUSCRIPT I).

In order to develop an integrated quantitative risk-benefit assessment of food, it is crucial to determine what data are required, as explained in section 5.2. This section describes the experts involved in quantitative risk-benefit assessment of food.

Basically, at the beginning of a practical quantitative risk-benefit assessment of food, it is required to have experts from food safety (microbial and toxicological), nutrition and epidemiology. This is because, the first steps in risk-benefit assessment of food is to identify the food or food components, safety and benefit concern. Progressively, experts in exposure assessments of pathogens, chemical contaminants and nutritional components supported by a mathematician are required to estimate the intake of the beneficial and hazardous component of food. Successively, statistician is needed to combine all quantitative data and to estimate the health outcome. Eventually, for presentation of the results and discussion, all stakeholders need to sit together to better interpret the results, explain the different disciplines' point of view and to send the message to the public. If all relevant stakeholders are involved in the assessment, the choice of risk-benefit management measure may be optimal from a public health perspective.

Chapter 6

PAPER 1. Risk-benefit assessment of cold-smoked salmon: microbial risk versus nutritional benefit

European Journal of Food Research & Review
2(2): 49-68, 2012

SCIENCEDOMAIN *international*
www.sciencedomain.org

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

Firew Lemma Berjia[1*], Rikke Andersen[2], Jeljer Hoekstra[3], Morten Poulsen[4] and Maarten Nauta[1]

[1]Division of Epidemiology and Microbial Genomics, The National Food Institute, Technical University of Denmark, Mørkhoj Bygade 19, 2860 Søborg, Denmark.
[2]Division of Nutrition, The National Food Institute, Technical University of Denmark, Mørkhoj Bygade 19, 2860 Søborg, Denmark.
[3]National Institute of for Public Health and the Environment (RIVM) Bilthoven, The Netherlands.
[4]Division of Toxicology and Risk Assessment, The National Food Institute, Technical University of Denmark, Mørkhoj Bygade 19, 2860 Søborg, Denmark.

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.

Research Article

Received 3rd May 2012
Accepted 15th September 2012
Online Ready 18th September 2012

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.*

**Corresponding author: Email: fber@food.dtu.dk;*

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. $\Delta IQ = d*I_{DHA}$ (Cohen et al. 2005a)	d = 1.3, uncertainty interval (0.8-1.8)	ΔIQ, 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. $P_{eff,ep,a(a,s)} = RR_{(s)a} \times P_{eff,\,ep,r\,(a,s)} / RR_{(s)r}$	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. $\log N_t = \log N_0 + \mu(t - \lambda)$	N_0, (see section 2.4), t=14; μ=0.113; λ=0.167 (WHO/FAO, 2004)	N_t, concentration of *Listeria* after storage (CFU/g); N_0, initial concentration (CFU/g); μ, growth rate (log CFU/d); t, storage time (day); λ, lag-time (day). At storage temperature of 5ºC
8. $D_{listeria} = F_{intake} * N_t$		$D_{listeria}$, dose of listeria (CFU/d).
9. $P_{inf} = 1\text{-}e^{-rD listeria}$	$r = 5.85 * 10^{-12}$	P_{inf}, probability of infection and illness of *Listeria; r*, dose-response parameter specific to *Listeria* 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 *Listeria.*
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 *Listeria*.
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 *Listeria.* $P_{c,}$ 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 $8.9*10^{-6}$ for women (20g CSS) and $1*10^{-05}$ for men (23 g CSS. In the alternative scenario (40 g CSS) it is $1.78*10^{-05}$. 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:

$\Delta DALY = \sum DALY_{alt} - \sum DALY_{ref}$

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

DALY represents health loss; therefore, if the estimation of ΔDALY results in a positive value then the change in consumption has an adverse health effect. If the ΔDALY 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,* λ (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			
	Ref	**Alt**	**ΔDALY**	**Ref**	**Alt**	**ΔDALY**	**Sum of 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 ΔDALY depending on theparameters. 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.

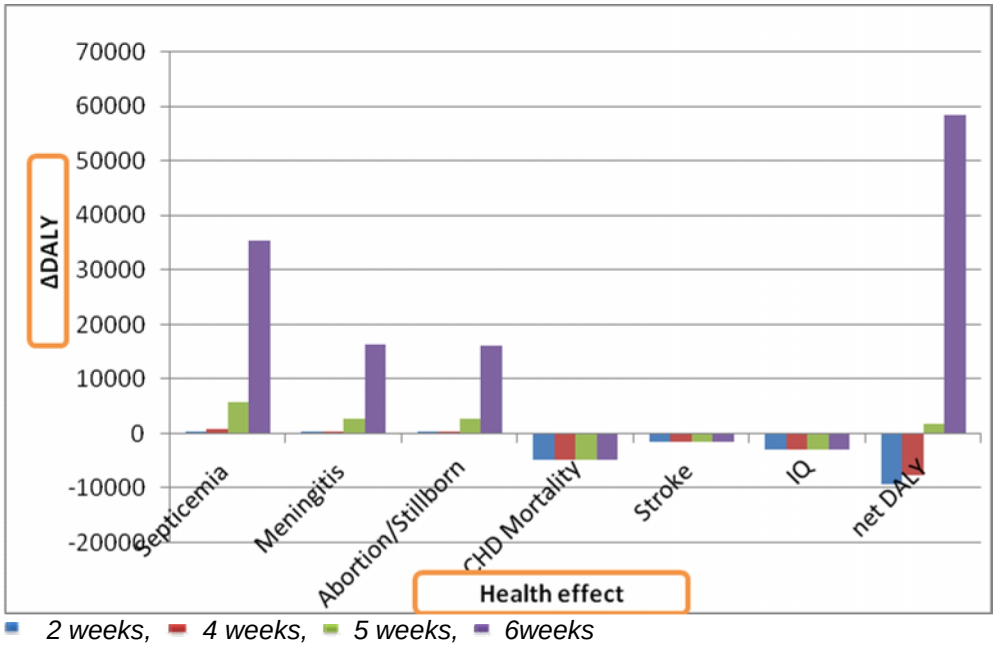


Fig. 1. Sensitivity analysis for the effect of storage time.

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 resulst 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.

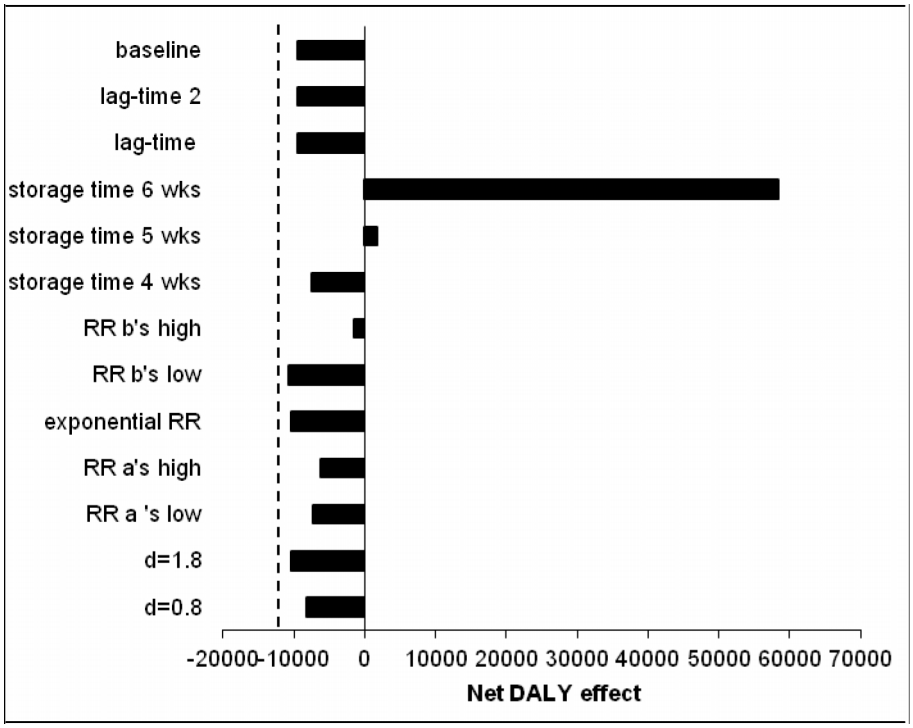


Fig. 2. Result of sensitivity analysis for all selected parameters

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 biovailability 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 listriosis (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, salmonelosis, 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 liseriosis 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.

REFERENCES

Albert, C.M., Hennekens, C.H., O'Donnell, C.J., Ajani, U.A., Carey, V.J., Willett, W.C., Ruskin, J.N., Joann, E., Manson, J.E. (1998). Fish consumption and risk of sudden cardiac death. JAMA., 279, 23–28.

Allerberger, F., Wagner, M. (2010). Listeriosis: a resurgent foodborne infection. Clin. Micro. Infec., 16, 16–23.

Andersen, K.K., Olsen, T.S., Dehlendorff, C., Kammersgaard, L.P. (2009). Hemorrhagic and ischemic strokes compared: stroke severity, mortality and risk factors. Stroke, 40, 2068-2072.

Aouaj, Y., Spanjaard, L., van Leeuwen, N., Dankert, J. (2002). *Listeria monocytogenes* meningitis: serotype distribution and patient characteristics in the Netherlands, 1976-95. Epidemiol Infect., 128, 405-409.

Ascherio, A., Rimm, E.B., Stampfer, M.J., Giovannucci, E.L., Willett, W.C. (1995). Dietary intake of marine n-3 fatty acids, fish intake, and the risk of coronary disease among men. N. Engl. J. Med., 332, 977-982.

Cohen, J.T., Bellinger, D.C., Connor, W.E., Shaywitz, B.A. (2005a). A quantitative analysis of prenatal intake of n-3 polyunsaturated fatty acids and cognitive development. Am. J. Prev. Med., 29, 366 –374.

Cohen, J.T., Bellinger, D.C., Connor, W.E., Kris-Etherton, P.M., Lawrence, R.S., Savitz, D.A., Shaywitz, B.A., Teutsch, S.M., Gray, G.M. (2005b). A quantitative risk-benefit analysis of changes in population fish consumption. Am. J. Prev. Med., 29, 325–334.

Daviglus, M.L., Stamler, J., Orencia, A.J., Dyer, A.R, Liu, K., Greenland, P., Walsh, M.K., Morris, D., Shekelle, R.B. (1997). Fish consumption and the 30-year risk of fatal myocardial infarction. N. Engl. J. Med., 336, 1046-1053.

Denmark Statistics. (2011). http://www.statbank.dk/statbank5a/default.asp?w=1280

Denmark Technical University. (2011). National Food Institute http://www.foodcomp.dk/v7/fcdb_default.asp.

Ekstrøm, C.L., Sørensen, H. (2011). Introduction to statistical data analysis for the life science. CRC Press, USA.

European Food Safety Authority (EFSA). (2010). Guidance on human health risk-benefit assessment of foods. Scientific opinion. EFSA Journal, 8, 1673.

FAO/WHO. (2011). Report of the Joint FAO/WHO expert consultation on the risks and benefits of fish consumption. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization.

Gerner-Smidt, P., Ethelberg, S., Schiellerup, P., Christensen, J.J, Engberg, J., Fussing, V., Jensen, A., Jensen, C., Petersen, A.M., Bruun, B.G. (2005). Invasive listeriosis in Denmark 1994–2003: a review of 299 cases with special emphasis on risk factors for mortality. Clin Microbiol, 11, 618–624.

Gillum, R.F., Mussolino, M.E., Madans, J.H. (1996). The relationship between fish consumption and stroke incidence. The NHANES I epidemiologic follow-up study (National Health and Nutrition Examination Survey). Arch Intern Med, 156, 537-542.

Gladyshev, M.I., Sushchik, N.N., Anishchenko, O.V., Makhutova, O.N., Kalachova, G.S., Gribovskaya, I.V. (2008). Benefit-risk ratio of food fish intake as the source of essential fatty acids vs. heavy metals: A case study of Siberian grayling from the Yenisei River. Food Chemistry, 115, 545-550.

Guevel, M.R., Sirot, V., Volatier, J.L., Leblanc, J.C. (2008). A risk-benefit analysis of French high fish consumption: A QALY approach. Risk Analysis, 28, 37-48.

Hansen, L.T., Røntved, S.D., Huss, H.H. (1998). Microbiological quality and shelf life of cold-smoked salmon from three different processing plants. Food Microbiology, 15, 137-150.

Havelaar, A.H., De Hollander, A.E., Teunis, P.F., Evers, E.G., Van Kranen, H.J., Versteegh, J.F., Koten, V., Joke, E.M., Slob, W. (2000). Balancing the risks and benefits of drinking water disinfection: disability adjusted life-years on the scale. Environ. Health Perspect, 108, 315-321.

He, K., Rimm, E.B., Merchant, A., Rosner, B.A., Stampfer, M.J., Willett, W.C., Ascherio, A. (2002). Fish consumption and risk of stroke in men. JAMA, 288, 3130-3136.

Hoekstra, J., Hart, A., Boobis, A., Claupein, E., Cockburn, A., Hunt, A., Knudsen, I., Richardson, D., Schilter, B., Schutte, K., Torgerson, P.R., Verhagen, H., Watzl, B., Chiodini, A. (2010). BRAFO tiered approach for risk-benefit assessment of foods. Food Chem., Toxico. (Article in press).

Hoekstra, J., Hart, A., Owen, H., Zeilmaker, M., Bokkers, B., Thorgilsson, B., Gunnlaugsdottir, H. (2012). Fish, contaminants and human health: Quantifying and weighing benefits and risks. Food Chem. Toxicol. (Article in press).

Hu, F.B., Bronner, L., Willett, W.C., Stampfer, M.J., Rexrode, K.M., Albert, C.M., Hunter, D., Manson, J.E. (2002). Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. JAMA, 287, 1815-1821.

Ingall, T. (2004). Stroke—incidence, mortality, morbidity and risk. J Insur Med., 36, 143–152.

Iso, H., Rexrode, K.M., Stampfer, M.J., Manson, J.E., Colditz, G.A., Speizer, F.E., Hennekens, C.H., Willett, W.C. (2001). Intake of fish and omega-3 fatty acids and risk of stroke in women. JAMA, 285, 304-312.

Jarvinen, R., Knekt, P., Rissanen, H., Reunanen, A. (2006). Intake of fish and long-chain n-3 fatty acids and the risk of coronary heart mortality in men and women. British Journal of Nutrition, 95, 824–829.

Jensen, A.K., Ethelberg, S., Smith, B., Nielse, E.M., Larsson, J.K., Mølbak, J.K., Christensen, J.J., Kemp, M. (2010). Substantial increase in listeriosis, Denmark 2009. Euro Surveill., 15, 1-4.

Jørgensen, L.V., Huss, H.H. (1998). Prevalence and growth of *Listeria monocytogenes* in naturally contaminated seafood. International Journal of Food Microbiology, 42, 127–131.

Kemmeren, J., Mangen, M-J., van Duynhoven, Y.T., Havelaar, A. (2006). Priority setting of foodborne pathogens. 330080001/2006. National Institute for Public Health and the Environment, the Netherlands (RIVM).

Kromhout, D., Bosschieter, E.B., Coulander, C.L. (1985). The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N. Engl. J. Med., 312, 1205-1209.

Larsson, S.C., Virtamo, J., Wolk, A. (2011). Fish consumption and risk of stroke in Swedish women. Am. J. Clin. Nutr., 93, 487-493.

Lindqvist, R., Westoo, A. (2000). Quantitative risk assessment for *Listeria monocytogenes* in smoked or gravad salmon and rainbow trout in Sweden. Int. J. Fo. Micro., 58, 181–196.

Leroi, F., Joffraud, J.J., Chevalier, F., Cardinal, M. (2001). Research of quality indices for cold-smoked salmon using a stepwise multiple regression of microbiological counts and physico-chemical parameters. J. App. Microbio., 90, 578-587.

Magnússon, S.H., Gunnlaugsdóttir, H., van Loveren, H., Holm, F., Kalogeras, N., Leino, O., Luteijn, J.M., Odekerken, G., Pohjola, M.V., Tijhuis, M.J., Tuomisto, J.T., Ueland, O, White, B.C., Verhagen, H. (2012). State of the art in benefit-risk analysis: Food microbiology. Food. Chem. Toxicol., 50, 33-39.

Mann, J.I., Appleby, P.N., Key, T.J., Thorogood, M. (1997). Dietary determinants of ischaemic heart disease in health conscious individuals. Heart, 78, 450-455.

Mozaffarian, D. (2006). Fish intake, contaminants, and human health: evaluating the risks and the benefits part 1 – health benefits. JAMA, 296, 1885-99.

Mozaffarian, D., Lemaitre, R.N., Kuller, L.H., Burke, G.L., Tracy, R.P., Siscovick, D.S. (2003). Cardiac benefits of fish consumption may depend on the type of fish meal consumed: the cardiovascular health study. Circulation, 107, 1372-1377.

Mozaffarian, D., Longstreth, W.T., Jr, Lemaitre, R.N., Manolio, T.A., Kuller, L.H., Burke, G.L., Siscovick, D.S. (2005). Fish consumption and stroke risk in elderly individuals: the cardiovascular health study. Arch Intern Med, 165, 200-206.

Mylonakis, E., Paliou, M., Hohmann, E.L., Calderwood, S.B., Wing, E.J. (2002). Listeriosis during pregnancy: a case series and review of 222 cases. Medicine (Baltimore), 81, 260-269.

Oomen, C.M., Feskens, E.J., Rasanen, L., Fidanza, F., Nissinen, A.M., Menotti, A., Kok, F.J., Kromhout, D., (2000). Fish consumption and coronary heart disease mortality in Finland, Italy and The Netherlands. Am. J. Epidemiol., 151, 999-1006.

Orencia, A.J., Daviglus, M.L., Dyer, A.R., Shekelle, R.B., Stamler, J. (1996). Fish consumption and stroke in men. 30-year findings of the Chicago Western Electric Study. Stroke, 27, 204-209.

Pielaat, A., Wijnands, L.M., Takumi, K., Nauta, M.J., Van Leusden, F.M. (2005). The fate of *Bacillus cereus* in the gastrointestinal tract. Report, RIVM, Bilthoven, 59.

Pouillot, R., Miconnet, N., Afchain, A.L., Delignette-Muller, M.L., Beaufort, A., Rosso, L., Denis, J.B., Cornu, M. (2007). Quantitative risk assessment of Listeria monocytogenes in French cold-smoked salmon: II. Risk characterization. Society for Risk Analysis, 29, 806-819.

Pedersen, A.N., Fagt, S., Groth, M.V., Christensen, T., Matthiessen, J., Andersen, N.L., Kørup, K., Hartkopp, H., Ygil, H.K., Hinsch, H., Saxholt, E., Trolle, E. (2010). Danskernes kostvaner 2003-2008 Hovedresultater. DTU Fødevareinstituttet, 1-196.

Ross, T (1999). Predictive food microbiology models in the meat industry. Meat and Livestock. Australia, Sydney, Australia, 196.

Saltelli, A., Chan, K., Scott, E.M. (2000). Sensitivity analysis: gauging the worth of scientific models. 1st ed. John Wiley and Sons Chichester, 504.

Smith, B., Kemp, M., Ethelberg, S., Schiellerup P., Bruun, B.G., Gerner-Smidt, P., Christensen, J.J. (2009). *Listeria monocytogenes*: Maternal-foetal infections in Denmark 1994-2005. Scan. J. Infec., 41, 21-25.

Stouthard, M.A.E., Essink-Bot, M.L., Bonsel, G.J., Barendregt, J.J., Kramer, P.G.N., Gunning-Schepers, L.J., van der Maas, P.J. (1997). Department of Public Health Erasmus University Rotterdam.

Wang, M.P., Thomas, G.N., Ho, S.Y., Lai, H.K., Mak, K.H., Lam, T.H. (2011). Fish consumption and mortality in Hong Kong Chinese—the LIMOR study. Ann. Epidemio., 21, 164-169.

WHO. (2008). The global burden of disease: the 2004 update, first ed. WHO, Geneva.

WHO/FAO. (2004). Risk assessment of *Listeria monocytogenes* in ready-to-eat foods. Technical report of microbiological risk assessment series 5, WHO, Geneva.

Young, G., Conquer, J. (2005). Omega-3 fatty acids and neuropsychiatric disorders. Reprod. Nutr. Dev., 45, 1-28.

Zeilmaker, M.J., Hoekstra, J., Jan, C.H., Eijkeren, V., Nynke, J., Hart, A. Kennedy, M., Owen, H. Gunnlaugsdottir, H. (2012). Fish consumption during child bearing age: A quantitative risk–benefit analysis on neurodevelopment. Food Chem. Toxicol. (Article in press).

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, 132)
	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, 135)
	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	1
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.

Chapter 7

MANUSCRIPT I

Finding the optimum scenario in risk-benefit assessment: an example on vitamin D

F. L. Berjia, J. Hoekstra, H. Verhagen, M. Poulsen, R. Andersen, M. Nauta

To be submitted

Journal: European Journal of Nutrition & Food Safety

Finding the optimum scenario in risk-benefit assessment: an example on vitamin D

Authors

F. L. Berjia[1*], J. Hoekstra[2], H. Verhagen[2,5], M. Poulsen[3], R. Andersen[4],M. Nauta[1]

[1]Division of Epidemiology and Risk Modeling, The National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, 2860 Søborg, Denmark.

[2]National Institute for Public Health and the Environment (RIVM), PO Box 1, 3720 BA, Bilthoven, The Netherlands.

[3]Division of Toxicology and Risk Assessment, The National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, 2860 Søborg, Denmark.

[4]Division of Nutrition, The National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, 2860 Søborg, Denmark.

[5]University of Ulster, Northern Ireland Centre for Food and Health (NICHE), Cromore Road, Coleraine, BT52 1SA Northern Ireland

Corresponding author

Firew Lemma Berjia

fber@food.dtu.dk

firew.lemma@yahoo.com

ABSTRACT

Background: In risk-benefit assessment of food and nutrients, several studies so far have focused on comparison of two scenarios to weigh the health effect against each other. One obvious next step is finding the optimum scenario that provides maximum net health gains.

Aim: This paper aims to show a method for finding the optimum scenario that provides maximum net health gains.

Method: A multiple scenario simulation. The method is presented using vitamin D intake in Denmark as an example. In addition to the reference scenario, several alternative scenarios are simulated to detect the scenario that provides maximum net health gains. As a common health metric, Disability Adjusted Life Years (DALY) has been used to project the net health effect by using the QALIBRA (Quality of Life for Benefit Risk Assessment) software.

Results: The method used in the vitamin D example shows that it is feasible to find an optimum scenario that provides maximum net health gain in health risk-benefit assessment of dietary exposure as expressed by serum vitamin D level. With regard to the vitamin D assessment, a considerable health gain is observed due to the reduction of risk of other cause mortality, fall and hip fractures when changing from the reference to the optimum scenario.

Conclusion: The method allowed us to find the optimum serum level in the vitamin D example. Additional case studies are needed to further validate the applicability of the approach to other nutrients or foods, especially with regards to the uncertainty that is usually attending the data.

Keywords: Optimum scenario; vitamin D; risk-benefit assessment; DALY; QALIBRA

1. INTRODUCTION

Risk-benefit assessments of food and nutrients focus on interventions and policies in connection with food consumption and health outcomes [1]. Until recently, the risk and benefit assessments of food have been separate processes with different methods. Due to the increasing interest of estimation of the net health impact of food consumption, development of methods that integrate both the health benefits and risks of food (ingredient) in one go have gained interest.

Currently, there are some methods and approaches on how to perform risk-benefit assessment of food [2, 3, 4, 5, 6, 7]. These methods focus on comparison of two or more scenarios and determine which one of the scenarios prevails over the other from the perspective of public health. When applying these methods, the assessment may stop at an early stage, i.e. before the health effects are integrated in a common health metric. Several case studies have been performed; low calorie sweeteners [8], farmed salmon, soy protein [9], benzo[a]pyrene and heat treatment of milk [10] to validate the applicability of the BRAFO tiered approach [5].

Whereas those studies typically compared two scenarios, one obvious next step is to investigate more than two scenarios and find the optimum scenario to improve public health. This approach was suggested by [11, 12]. This paper aims to show how this can be done by comparing the net health gains of different scenarios, expressed in a common health metric (DALY, disability adjusted life years). The method is presented in a case study on vitamin D intake in Denmark. So far, the health risk and benefit associated with vitamin D have not been integrated using common health metric. Our objective is to illustrate how an optimum scenario can be found. Therefore, several simplifying assumptions are made and the result is a crude estimate of the optimal vit D serum level needed to be achieved for the maximum net health gain in Danish population.

The method focusses on estimating each health effect expressed in DALYs in the reference scenario, followed by the estimation of the health effect of alternative scenarios with changing serum 25(OH)D concentration. The DALY difference between the alternative scenarios and

reference scenario expresses the net health gain. It is computed with the QALIBRA (Quality of Life for Benefit Risk Assessment) tool [4]. Subsequently, the scenario that results in the maximum net health gain is considered the optimum scenario.

2. VITAMIN D

Vitamin D plays an important role in reducing the risk of several diseases [13]. However, the current dietary intake in the population is lower than the recommended intake [14, 15]. Due to the high latitudes, indoor activities and low vitamin D intake, the serum 25(OH)D level is relatively low in most populations in Northern Europe [16]. Hence, it has been suggested to increase the recommended intake [15, 17, 18]. Recently, the Nordic Nutrition Recommendations (NNR) increased the recommended dietary intake from 7.5 to 10 ug/day [15].

Studies suggest that higher 25(OH)D serum levels are associated with beneficial effects, i.e. for reducing the risk of several diseases [13, 19, 20, 21, 22, 23]. On the other hand, some studies report that vitamin D may lead to an adverse effects at both higher and lower levels [24] and when taken excessively [25]. This implies that there will be an intermediate optimal serum 25(OH)D level and finding this level would be imperative to attain the maximum health benefits.

Several studies suggest various optimal serum 25(OH)D levels, needed to reduce the risk of some diseases. Based on the existing data, the suggested optimal serum level needed to reduce the risk of osteoporosis, cardiovascular diseases and colorectal cancer is 75-110 nmol/l [20]. For fracture 75 nmol/l [26] and to lower the risk of mortality 50-60 nmol/l [24] have been suggested.

In this paper, we aimed to establish the optimum serum 25(OH)D level for the Danish population and therefore several alternative scenarios are simulated from which an optimum serum level can be identified using DALY.

2.1 Health effect identification

To identify the endpoints that are associated with vitamin D, we reviewed national and international authoritative reports such as WHO/IARC [27], EFSA [17] and IOM [18] which are up-to-date scientific opinions based on a collection of nutritional and observational studies.

According to WCRF/AIRC and WHO/FAO [28, 29] criteria for the strength of evidences, the most convincing evidence is substantiated by multiple randomized controlled intervention trials of sufficient size, duration and quality in a population representative for the target population showing consistent effects. So far, there is strong scientific evidence for the effect of vitamin D on bone related diseases [18, 19, 21, 22, 30], while the evidence for other endpoints such as cardiovascular diseases and diabetes is conflicting [17, 18, 27].

In this paper, we have considered endpoints that have convincing evidence. These are: hip fracture, other nonvertebral fracture and fall. In addition, we have included an endpoint where the evidence is relatively weak, but the reported quantitative data on the dose-response relationship are particularly suitable for the purpose of our study (total mortality). As the endpoint “total mortality” includes mortality from the other endpoints considered in this assessment, we use the term “other cause mortality” to distinguish this effect from “total mortality”.

2.2 Description of dose-response and selection of population

Various studies on the association between vitamin D and health effects describe the vitamin D dose differently in the dose-response relation. Usually, the dose is represented as 25(OH)D level in nmol/l serum [24] or ng/ml serum [31] and sometimes the dose is represented as intake in IU or µg per day [22]. The serum level of vitamin D gives information about the recent exposure to vitamin D [32] and since we wanted to establish the optimal serum 25(OH)D level, we related the dose to serum level in nmol/l throughout this study. The relation between intake and serum level (nmol/l) is presented in section 2.5.

For fall and fractures, the selected populations are elderly of both sexes because the Randomized Control Trial (RCT) studies included in the dose-response considers elderly of general population [19, 21, 22]. The sub-populations associated to the selected endpoints and the types of study are given in table 1.

Table 1. The selected endpoints and population of interest related to vitamin D intake

Endpoints	**Type of study and population characteristics**	**References**
Fractures (hip and other nonvertebral)	Meta-analysis of RCT*, ≥65 years old, both sexes.	[22]
Fall	Meta-analysis of RCT*, ≥65 years old, both sexes	[19]
Other cause mortality	Cohort study, survey, ≥30 years old, both sexes	[24, 33, 34]

**RCT: Randomized controlled trial*

For other cause mortality, various studies conducted the relation between vitamin D and mortality in different subpopulation [24, 33, 34]. In our study, the target population is both sexes of age ≥30 years old for this endpoint.

2.3 Intake of vitamin D and serum 25(OH)D level

In Denmark, fish is the primary dietary source of vitamin D [14], followed by eggs, milk and meat products. The relative contribution of the various foods for the daily dietary intake of vitamin D in Denmark is shown in table 2 [14].

Table 2. Food type contributing to the daily vitamin D intake of the Danish population

Food type	% of contribution
Fish	43
Meat	29.5
Milk and cheese	13
Eggs	9.5
Butter and margarine	5

The average dietary vitamin D intake is obtained from [14]: 3.8 µg/d for men and 3.1 µg/d for women. These values are transformed to serum levels in nmol/l as explained in section 2.5. As alternative scenarios, we have simulated a series of serum 25(OH)D level: 35, 50, 65, 72, 80, 90, 100, 120 and 166 nmol/l for both sexes and ages. These values were chosen in a process of trial and error, using interpolation to find the optimum.

2.4 Health impact estimation

To estimate the net health effect, the QALIBRA (Quality of Life for Benefit Risk Assessment) software is used [4]. This software run the simulation based on the following DALY equation for each disease [5]. For the net effect, DALYs are summed over every disease.

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

Where:

$DALY_{a,s}$ disability adjusted life years at age group a and sex s

$P_{eff(a,s)}$ probability of onset of the disease at age and sex, per year

P_{rec} probability of recovery from the disease

P_{die} probability that the disease causes death

YLD_{rec} mean duration of disease for those who recover

YLD_{die} mean duration of disease for those who die

CA current age of individual in year of disease onset (years)

$LE_{a,s}$ normal life expectancy (i.e. expected age at death) at age a and for sex s

w disability weight for disease.

Since we are interested in the health effect of the difference of the scenarios, estimating the change in DALY between scenarios is imperative [5].

The ΔDALY between scenarios is calculated using:

$$\Delta DALY = \Sigma DALY_{alt} - \Sigma DALY_{ref} \quad \text{..... (2)}$$

Where, ΔDALY is change in DALY; $\Sigma DALY_{alt}$, summation of DALYs caused by every endpoint of all individuals in the population at the alternative scenario and $\Sigma DALY_{ref}$, summation DALYs caused by every endpoint of all individuals in the population at the reference scenario.

The DALY is summed for the ages and sexes considered for each endpoint, with $N_{a,s}$ the populations size for age and sex in Denmark.

$$\Sigma DALY = \Sigma_{endpoints,} \Sigma_{a,s} N_{a,s} * DALY_{a,s} \quad \text{.....(3)}$$

DALYs represent health loss; therefore, if the estimation of ΔDALY results in a positive value then the change in consumption has an adverse health effect (health loss). If the ΔDALY is negative, then the change in consumption has a beneficial effect [4, 5].

2.5 Conversion of vitamin D intake in 25(OH)D serum level and dose-response

Vitamin D intake has to be related to 25(OH)D serum concentration because our aim is to find an optimum serum level. It also allows a comparison of all endpoints, because some studies

report an association between serum level and disease instead of intake and disease. For the conversion of intake to serum concentration, we used two studies by Cashman et al. [36, 37], that relate the dietary vitamin D intake (μg/d) with serum level (nmol/l) in two age groups. We have chosen the 50 percentile data points from both studies as point estimates of the vitamin D intake level that is needed to achieve the serum level in the study subjects. So, for fall, hip and other nonvertebral fracture the 50 percentile data points for both sexes as reported by Cashman et al. [36] are used, because the target populations for these endpoints are elderly of both sexes. Since the target population for other cause mortality is ≥30 years old of both sexes, the intake in μg/d is related to serum level in nmol/l using the 50 percentile data points of both studies of Cashman et al. [36, 37]. For all conversion from dietary intake in μg/d to serum (S) level in nmol/l serum, the following linear model (eqn. 4) is used.

$$(S, \text{nmol/l}) = a * (\text{vitamin D intake}, \mu g/d) + b \ldots\ldots\ldots (4)$$

The fitted values for a and b are a=2.08 and b=35.2 when both sexes data points are used, i.e. for hip fracture, other nonvertebral fracture and fall [36]; a=1.95 and b=35 when both sexes data points from [36, 37] studies are used, i.e. for other cause mortality.

For different endpoints, this approach may assign different reference scenario intakes in terms of serum levels to men and women. These reference scenario serum levels are derived from different average intakes for men and women (see section 2.3) and eqn. 4. They are 43 nmol/l (fractures and fall) and 42 nmol/l (other cause mortality) for men and 42 nmol/l (fractures and fall); 41 nmol/l (other cause mortality) for women. As a side effect of our approach, this may result in small differences in the assumed reference level as a function of the same intake for different endpoints.

Using this method, intakes are converted to serum concentrations expressed as nmol/l. Subsequently, to determine the relative risk (RR) based on our intake scenarios, the serum level-RR relations are derived from studies that describe vitamin D intake in IU/d for most of

the selected endpoints (table 3). When the doses are given in a range, we have used the mean value. The intake values in the dose-response for fall were reported in ranges and qualitatively as less or greater than 60nmol/l [19]. When given in range, the mean value was used and when given qualitatively, we assumed 55 nmol/l and 87 nmol/l. To relate IU/d to µg/d, we used the standard conversion formula [35]: 1 IU = 0.025 µg.

Table 3. Data (intake, serum level and mean Relative Risks) of each endpoint, used to derive the dose response relations.

The values printed in *italics* in the table are calculated using equation 4.

Endpoints	**IU/d**	**µg/d**	**nmol/l**	**Mean RR**	**95% CI**	**References**
Hip fracture	400*	-	-	1.15	0.88,1.5	[21,22]
	500	12.5	*61*	0.91	0.78, 1.05	
	600	15	*66*	0.82	0.69, 0.97	
	750	18.75	*74*	0.74	0.61, 0.88	
Other nonvertebral fracture	400*	-	-	1.03	0.86,1.24	
	500	12.5	*61*	0.86	0.77, 0.96	
	600	15	*66*	0.8	0.72, 0.89	
	750	18.75	*74*	0.77	0.68, 0.87	
Fall			55	1.35	0.98, 1.84	[19]
	850	21.25	*80*	0.81	0.71, 0.92	
			87	0.77	0.65, 0.9	
Other cause mortality			10	2.13	2.02, 2.24	[24]
			50	1.0	-	
			140	1.42	1.31,1.53	

**Vitamin D dose of ≤400 IU/d is not sufficient for the prevention of fractures [21, 22]. Therefore, data points of ≤400 IU/d were not used for fractures.*

In the studies used for fall, hip and other nonvertebral fracture [19,21,22] the relative risks were determined compared to placebo, so presumably RR= 1 at an intake of 0 IU/d (serum level ≈ 35 nmol/l). This is not included in our analysis because it is not explicitly stated in the papers, and it is indicated that in general vitamin D doses ≤400 IU/d are not sufficient for the prevention of fractures [21, 22]. Also, the fit of the dose response model to the data would not benefit from including values corresponding to this RR=1 in the analysis.

The dose response relations expressing RR as a function of serum level are modeled using a log-linear function for each endpoint separately, except for other cause mortality.

$$\ln(RR) = (S, \text{nmol/l}) * c + d \quad \text{.....(5)}$$

The fitted values (c and d) are given in table 4.

The parameters c, d and the estimated RR for the reference scenario using eqn. 5, are shown in table 3. Equation 5 is applied to calculate the RR for the alternative scenarios.

Table 4. The fitted values and estimated RR's for the reference scenario serum levels per endpoint and sex.

Endpoint	**Fitted values**		**RR-men**	**RR-women**
	c	**d**		
Hip fracture	-0.016	0.85	1.19	1.22
Other nonvertebral fracture	-0.009	0.44	1.04	1.06
Fall	-0.018	1.3	1.67	1.73
*****Total mortality			1.26	1.25

**Calculated by a hazard ratio (HR) using eqn. 10.*

Figure 1 illustrates the serum level (nmol/l)-RR data points that are used to estimate the fitted values shown in table 4, and the fitted dose-response models. The fitted models have RR=1 at different serum levels values above the zero intake and reference intake scenarios, which means that RRs cannot be compared between endpoints.

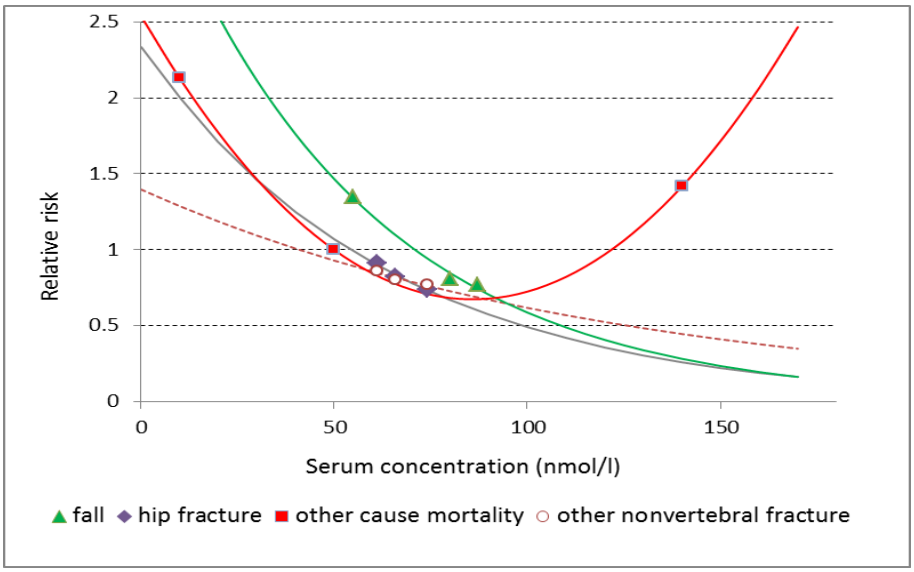


Figure 1. The serum level-RR data points (Table 3) and the fitted lines describing the relation between serum level and relative risk (eqn 5).

The serum level-RR relations for hip fracture, other nonvertebral fracture and fall show the same trend (decreasing risk with increasing serum level). For other cause mortality, the serum level-RR relation shows increasing risk at low and high serum level.

The estimated RR has to be converted to absolute risk for the reference scenario $P_{eff}(S_{ref})$. To this end we have assumed that the current incidence Inc is related to the current intake. Thus,

$$\int_0^\infty P_{eff}(S)\mathrm{p}(\mathrm{S})\mathrm{dS} = \mathrm{Inc/N} \quad \text{..... (6)}$$

However, by assuming that everyone's serum level is the average of the population, we simplified the above equation to:

$$P_{eff(a,s)}(S_{ref}) = Inc_{a,s} / N_{a,s} \text{.....(7)}$$

Where $Inc_{a,s}$ is the incidence of the diseases in the relevant sex and age group, which is obtained from epidemiological studies as described in section 2.6. The variation in incidence per age group and (when relevant) sex is considered for hip fracture and fall. Due to lack of data, only sex variation is considered for other nonvertebral fracture.

To estimate the absolute risk at an alternative scenario $P_{eff}(S_{alt})$, we use the fact that by definition the ratio of absolute risk to relative risk at the reference scenario is similar to the ratio of absolute risk to relative risk at an alternative scenario [38].

$$P_{eff}(S_{ref}) / RR(S_{ref}) = P_{eff}(S_{alt}) / RR(S_{alt}) \text{.....(8)}$$

Then, by rewriting eqn. 8, $P_{eff}(S_{alt})$ becomes:

$$P_{eff}(S_{alt}) = RR(S_{alt}) * P_{eff}(S_{ref}) / RR(S_{ref}) \text{.....(9)}$$

The hazard ratio HR. of total mortality is described by a quadratic function of serum concentration, the reported serum 25(OH)D level S and respective HR data points from [24] are analyzed and the fitted values are given in eqn. 10. In the study, the HR was determined based on a median of three years total mortality [24]. In our study, the estimated HR for the reference as well as alternative serum levels is for one year.

$$HR = 0.0003 * S^2 - 0.0434 * S + 2.54\text{.....} \quad (10)$$

The hazard ratio HR is interpreted as the relative risk RR, and calculated for the reference and alternative scenarios.

To estimate the probability of effect for mortality at the reference scenario ($P_{eff,M}(S_{ref})$), the mortality rate is used. This mortality rate is calculated for the different age groups and sexes separately, data is obtained from [39].

$$P_{eff,M(a,s)}(S_{ref}) = Mortality_{a,s} / N_{a,s} \ldots\ldots\ldots(11)$$

To estimate $P_{eff,M}(I_{alt})$ we use the same definition as eqn. 8. Thus,

$$P_{eff,M}(S_{alt}) = HR(S_{alt}) * P_{eff,M}(S_{ref})HR / HR(S_{ref}) \ldots\ldots\ldots\ldots(12)$$

The above equation gives us total mortality. However, total mortality also includes the mortality from the other endpoints included in our study (fall, hip fracture and other nonvertebral fracture). Hence, taking into account the other endpoints from total mortality, the probability effect for other cause mortality ($P_{eff(other\ cause\ mortality)}$) becomes:

$$P_{eff(other\ cause\ mortality)\ a,s}(S) = P_{eff,M(a,s)}(S) - \sum_j P_{eff(j)a,s}(S) * P_{die(j)a,s}(S) \ldots\ldots\ldots(13)$$

Where $P_{die(i)}$ and $P_{eff(i)}$ are the probability of death due to disease j and the probability of onset of disease j,.

2.6 Parameters for DALY calculation

To estimate the input parameters in QALIBRA, a similar approach as in [38] is used. Mainly Danish national epidemiological data are used and to some extent, international and analogous country epidemiological data were used in case of scarcity of Danish data. Furthermore, in case of data unavailability, assumptions have been made, as explained below. The model population is 1000 individuals with age and sex representative of the Danish population [39].

$P_{eff(a,s),}$ is estimated for all endpoints as explained in section 2.5. The incidences, Inc are obtained from different studies; for hip fracture [40], nonvertebral fracture [41] and fall [42].

$P_{eff,}$ for other cause mortality is described in eqn. 13, mortality rate is obtained from Denmark Statistics [39].

When an individual develops a disease, either the individual recovers from the disease (P_{rec}), dies due to the disease (P_{die}) or survives with the disease (P_{sur}) until the normal life expectancy [4, 5]. $P_{die,}$ is estimated for hip fracture [43], nonvertebral fracture [44] and fall [45]. P_{die}, for other cause mortality by definition is 1.

$P_{sur,}$ is estimated for hip fracture from [46], nonvertebral fracture [44], fall [47] and for other cause mortality is set to 0.

$P_{rec,}$ is calculated for all endpoints using

$$P_{rec} = 1 - (P_{sur} + P_{die}) \text{..... (14)}$$

$YLD_{die,}$ for hip fracture is assumed to be 2 years, the highest mortality period since onset of hip fracture [43] and YLD_{rec} is assumed as the follow up period, 5 years [48]. For the other bone related diseases, it is assumed that YLD_{rec} and YLD_{die} are equal to the values used for hip fracture. For other cause mortality, by definition both parameters are set to 0.

Severity weight w, is obtained from the WHO burden of disease estimation [49]. The summary input parameters are presented in table 5.

Table 5. The summary of input parameters for DALY calculation

Endpoints	Estimated parameters					
	$^{*}P_{sur}$	P_{rec}	YLD_{rec}	P_{die}	YLD_{die}	w
Hip fracture	0.57	0.135	5	0.295	2	0.372
Nonvertebral fracture	0.65	0.23	5	0.122	2	0.18
Fall	0.11	0.78	5	0.11	2	0.2
Other cause mortality	0	0	0	1	0	1

** The P_{sur} is not a typical input parameter required to estimate DALYs in QALIBRA, but it is used to estimate P_{rec}.*

3. RESULTS

Table 6 contains the DALYs simulated with the QALIBRA tool for each endpoint. Our aim was to determine the optimum scenario that provides maximum net health gains in comparison with the reference scenario. The results presented in Table 5 are the differences in DALYs between the alternative scenarios and the reference scenario, for each endpoint. That can be interpreted as the health, expressed in DALYs, one can gain if the serum concentration changes from the reference to the alternative.

It appears that for hip fracture, nonvertebral fracture and fall, the healthy life years increase with increasing serum 25(OH)D level. Among these endpoints, a considerable gain of DALYs is observed primarily from fall prevention when the serum level increases. For other nonvertebral fracture only a slight benefit is observed with increasing serum 25(OH)D level.

Apparently, a substantial gain in healthy life years is achieved with increasing serum 25(OH)D level up to 72 nmol/l from the reduction of the risk of other cause mortality, reaching a maximum benefit of -69 DALY/1000 individuals. However, the benefit gradually decreases when the achieved serum 25(OH)D level at >80 nmol/l. Combined with the other endpoints, the

health benefits gained by the reduction of the risk of other cause mortality is dominant up to the serum level of 72 nmol/l.

Table 6. The estimated median DALYs/1000 individuals for each endpoint.

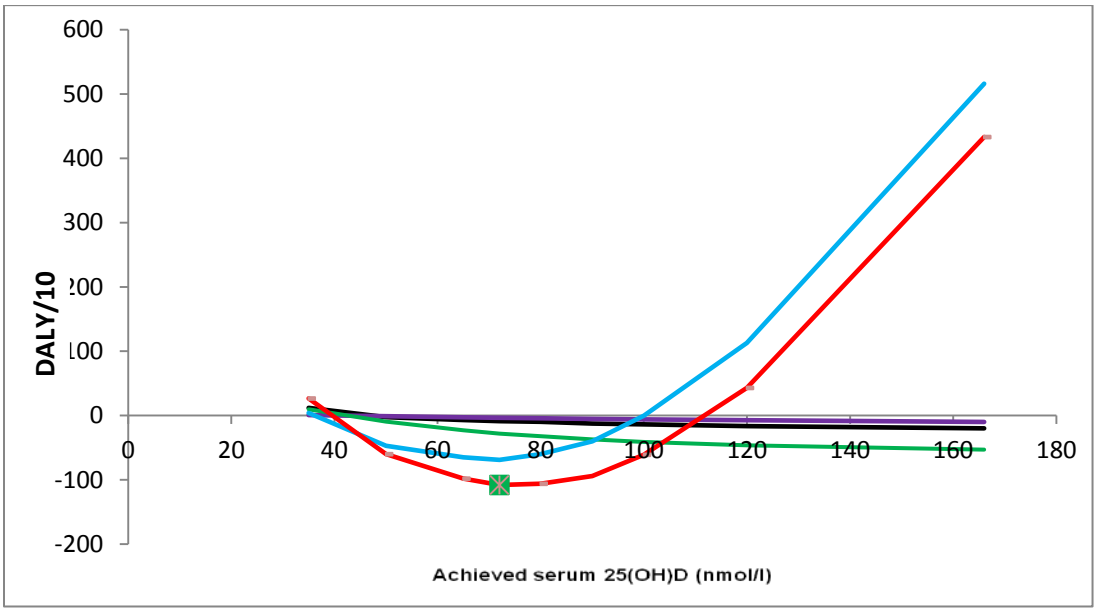
Scenarios (nmol/l)	Hip Fracture	Other nonvertebral Fracture	Fall	Other cause mortality	Net
Ref. Vs. 35	12	0.85	9.7	3.9	26.5
Ref. Vs. 50	-2.6	-1.0	-9.5	-47	-60
Ref. Vs. 65	-7	-2.8	-23	-65	-98
Ref. Vs. 72	**-8.6**	**-3.4**	**-28**	**-69**	**-108**
Ref. Vs. 80	-9.6	-4.2	-32	-60	-106
Ref. Vs. 90	-12.4	-5.2	-37	-40	-94
Ref. Vs. 100	-13.7	-6.0	-41	-0.3	-61
Ref. Vs. 120	-16.4	-7.3	-46.4	113	43
Ref. Vs. 166	-20	-10	-53	516	433

*The DALY of the optimum scenario is printed in **Bold** in table 6.*

Even though the net healthy life year's is gradually reducing after 72 nmol/l, there seems to be a benefit compared with the reference serum concentration up to the 100 nmol/l. At a level of 120, the net benefit shifted to net risk, 43 DALY/1000. Since the DALY was not calculated for the serum level between 100-120 nmol/l, the precise turning point from net benefit to net risk cannot be defined in our assessment. However, it is in the range of serum level of 100-120 nmol/l.

Figure 2 shows the results presented in table 5 in a graph. The maximum net health gains is achieved when serum 25(OH)D level reaches the optimum serum level of 72nmol/l (rectangular green mark on the red line). Note however, that many uncertainties have not been incorporated and that the curves showing the DALYs versus serum level is rather flat.

It is noted that the shape of the curve for the total DALY (red line) in Figure 1 is highly influenced by the DALY of "other cause mortality" (blue line). As it turns out the optimum for just other cause mortality is also the optimum for the combined effect. That is no real surprise, mortality is the most severe health effect (w=1) and affects a wide range of subpopulation groups compared to the other endpoints. Compared with mortality, fall and fractures are relatively small health effects.



– net DALY; – other cause mortality; – fall; – hip fracture; – other nonvertebral fracture

Figure 2. The DALY of each health effect and total DALY.

Table 6 shows the incidences at the reference and the optimum serum levels computed with the QALIBRA tool. The percent of reduction of incidences of fall and hip fracture is relatively higher; accounting for 50% and 36% respectively, when changing from the reference to the optimum scenario.

Table 7. The incidence/1000 person per year at reference optimum scenario and % change of incidences between the scenarios

Scenarios (nmol/l)	Hip Fracture	Other nonvertebral Fracture	Fall	Other cause mortality
*Reference	8.7	5.4	36	54
Optimum	5.6	4	18	40
% of Change	36	26	50	26

**The serum level for the reference scenario varies between 41- 43 nmol/l (see section 2.3).*

Figure 3 shows a scatter plot of the variation in DALYs gained/lost when changing from the reference scenario to the optimum scenario, produced by the QALIBRA tool. Each dot in the graph shows the DALY change for an individual characterized by age (left side plot) and by sex (right side plot).

The left plot shows the variability of DALYs gain/lost with age. The increase in healthy life years gain is observed with increasing age, because elderly people benefit primarily from the prevention of fractures, falls and other cause mortality when the serum 25(OH)D concentration changes from the reference to the optimum scenario. Adults of age 30-64 gain less DALYs compared to the elderly since this age group benefits only from the reduction of other cause mortality as for this age class the other endpoint are not included in the analysis. Also in the young age groups the probability of (other cause) mortality is very low and therefore a reduction in mortality risk has a small effect.

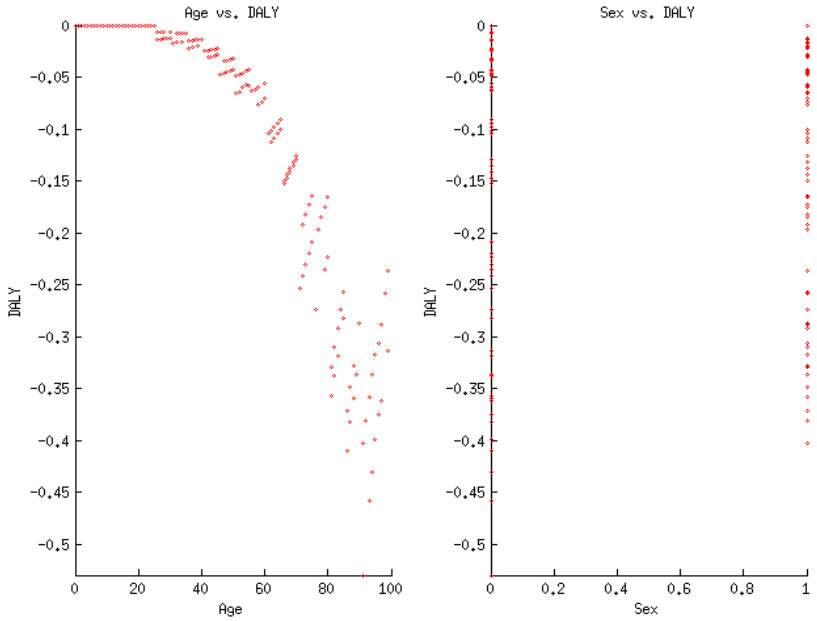


Figure 3. Scatter plot of the DALY change per age and per sex (0 in the x-axis represent women, 1 in the x-axis represent men) at optimum scenario.

The second scatter plot (right plot) shows the variation of DALY in sex, it is noted that age contributes more to the total variation in DALY than sex, because the variation of mortality rate (for other cause mortality) and incidence rate are larger per age class than per sex.

4. DISCUSSION AND CONCLUSION

The case study of vitamin D illustrates a method for finding the optimum scenario that provides maximum net health gains. To show how to find the optimum scenario, the net health impact of the different endpoints for several scenarios were calculated with the QALIBRA software (www.qalibra.eu). The QALIBRA software explicitly uses two scenarios (reference and alternative scenario) and shows the DALY difference of those scenarios. Since our aim was to determine the scenario that provides maximum health gains in comparison with the reference scenario, several alternative scenarios have been simulated in QALIBRA. Then, the difference of the DALY between the alternative and reference scenario that gives the maximum net health

gain is considered the optimum scenario. In the vitamin D example, nine alternative scenarios were compared to the reference scenario to determine the scenario that provides maximum net health gains.

The study is the first to show a comprehensive quantitative assessment of vitamin D on aggregate health effects by expressing them in common health metrics (DALY). In addition to the well-known effect of vitamin D on bone, other effects are reported to be associated with vitamin D. However, so far only strong scientific evidence for a preventive effect of vitamin D on fall and fractures exists [19, 21, 22]. In this assessment, we have considered these endpoints that have strong scientific evidences and another endpoint (total mortality) with less convincing scientific evidences, in order to analyze their contribution to the net health effects. The preferred way to combine convincing and less convincing effects is to incorporate uncertainty. Although the QALIBRA tool allows for the introduction of uncertainty by making a probabilistic assessment, this was not done here. The focus of this paper was to show a method to find the optimum scenario (serum level). For this reason other simplifications have been introduced as well.

The most important finding of this study is showing that it is possible to determine the scenario that provides the optimum public health effect from the cumulative effect of all the endpoints considered. Because the function that describes the relation between serum concentration and net health gain is rather smooth a simple series of increasing serum concentrations readily shows the optimum If the net health effect appears to change quickly with increasing serum levels, then the bisection method or even more sophisticated mathematical methods can be used. Consequently, according to our assessment the simulated scenario that provides the maximum benefit is when serum 25(OH)D level reaches 72 nmol/l. Because the net health effect hardly changes between 65 and 80 nmol/l the optimum would be somewhere between 65 and 80 nmol/l. The result is approximately in accordance with other study [50], although these studies predicted the optimal level for specific endpoints. The result is also in line with another study conducted for multiple diseases [51]. Ideally, men and women would have

different optimal serum levels because of the small difference in response to intake of vitamin D and/or serum level. However, in this assessment the intake and the dose-response difference in men and women are not substantially different (see section 2.3 and 2.5).

It is essential to point out the limitations of the assessment to better interpret the quantitative result. For the reference serum level, we have derived the serum level that results from the mean Danish intake stratified by sex. For the conversion of intake in µg/d to nmol/l, we have used only two studies [36, 37] and ignored the variation between individuals by using the 50 percentile intake-serum relationship only. Note that serum levels do not only depend on intake but that UV exposure contributes a large part of the serum level. However, when we used another study [50] to relate the intake in µg/d to serum 25(OH)D level in nmol/l the results are fairly similar. Also, we have included an endpoint (total mortality) that has relatively weak scientific evidences but dominates the outcome. Moreover, very few data points were available to estimate the relation between the RRs and serum concentration, and extrapolation beyond the range of the available data (see Figure 1) was needed to analyze all the different scenarios. The choice of the log-linear model (eqn 5.) and the decision not to include low intake relative risk values when fitting the model have an impact on the quantitative result. Furthermore, YLD_{die} and YLD_{rec} are assumed to have a fixed value but these values may depend on age, sex and/or vitamin D serum level. Because of all these assumptions and uncertainties, the quantitative result obtained in this assessment should be interpreted with care. When more data will become available in the future, the same methodology can be applied to obtain more precise estimates of the health impact of different intake scenarios.

The change of serum 25(OH)D level from the reference scenario (see section 2.5) to the optimum scenario (72 nmol/l) provides a gain of -108 DALY/1000 Danish individuals. For this -108 DALY net health gain, other cause mortality contributes for more than 64%. The target population for other cause mortality includes a larger fraction of the population compared to the other endpoints. Combined with the maximum severity of the effect (death), this explains why the DALY gain by this endpoint alone accounts for approximately 64% of the net gain. Fall

contributes about 26% for the net gain. The DALY gain from hip and other nonvertebral fracture seem relatively trivial. On the other hand, when the serum level reaches to120 nmol/l and up, the healthy life years loss (due to other cause mortality) prevail over the healthy life years gain due to hip fracture, nonvertebral fracture and fall.

The net DALY in figure 2 is highly influenced by the DALY of the other cause mortality. Studies show a U- or reverse J- shape for the association of vitamin D with mortality [24, 33]. This implies that there is an increasing risk of mortality at high and low serum level. The dose-response study we used in our assessment for the other cause mortality obtained a reverse J-shape association [24]. Both low (10 nmol/l) and high (140 nmol/l) level lead to an increasing risk of mortality and 50-60 nmol/l serum level give the lowest risk of mortality [24]. The fitted DALY curve for this endpoint (equation 10) shows a maximum DALY gain at 72 nmol/l.

Concerning the variation in sex and age, the variation of the DALY in Figure 2 is mostly due to the variation in the incidences and mortality rate variation per age class. For most of the selected endpoints, the difference in incidences and mortality rate (other cause mortality) is larger between age classes than between sexes, (see Figure 2, right plot).

In this study we have used vit D to show a method to find the best scenario, which achieves the maximum net health gain. A similar approach can be used for other foods or food components and biomarkers. As part of determining the best scenario, food processing parameter optimization (e.g. time-temperature, storage time) may also be integrated into the risk-benefit assessment to optimize the health [6].

The vitamin D concentration is the result of food and supplement intake but is also the result of UV-exposure. UV exposure is linked with skin cancer risk. That risk is not directly included in our assessment. A more complete assessment would separate the intake and UV-exposure effects. Furthermore, the development of methods to include aspects of a disease such as disease

recurrence, complete and partial disease remission is needed. Future research may also focus on method development for output validation in relation to the QALIBRA tool.

ACKNOWLEDGEMENT

The first author acknowledges the staff members of the National Institute for Public Health and the Environment (RIVM), The Netherlands, for scientific support and administrative support during a three month research stay.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Briggs DJ. A framework for integrated environmental health impact assessment of systemic risks. Environ. Health. 2008;7,61.
2. EFSA (European Food Safety Authority). Guidance on human health risk-benefit assessment of foods. Scientific opinion. EFSA Journal. 8:1673. Parma, Italy. 2010.
3. Fransen H, Jong N, Hendriksen M, Mengelers M, Castenmiller J, Hoekstra J, et al. A tiered approach for risk-benefit assessment of foods. Risk Analysis. 2010;30(5):808-16.
4. Hart A, Hoekstra J, Owen, H, Kennedy M, Zeilmaker MJ, de Jong N, et al. Qalibra: a general model for food risk-benefit assessment that quantifies variability and uncertainty. Food Chem Toxicol. 2013;54:4-17.
5. Hoekstra J, Hart A, Boobis A, Claupein E, Cockburn A, Hunt A, et al. BRAFO tiered approach for risk-benefit assessment of foods. Food Chem Toxico. 2012;50(4):684-98.
6. Hoekstra J, Verkaik-Kloosterman J, Rompelberg C, van Kranen H, Zeilmaker M, Verhagen H, et al. Integrated risk–benefit analyses: method development with folic acid as example. Food Chem Toxicol. 2008;46(3):893-09.
7. Tijhuis MJ, de Jong N, Pohjola MV, Gunnlaugsdóttir H, Hendriksen M, Hoekstra J, Holm F, Kalogeras N, Leino O, van Leeuwen FX, Luteijn JM, Magnússon SH,Odekerken

G, Rompelberg C, Tuomisto JT, Ueland Ø, White BC, Verhagen H. State of the art in benefit-risk analysis: food and nutrition. Food Chem Toxicol. 2012;50(1):5-25.

8. Verhagen H, Andersen R, Antoine JM, Finglas P, Hoekstra J, Kardinaal A, et al. Application of the BRAFO tiered approach for benefit-risk assessment to case studies on dietary interventions. Food Chem. Toxicol. 2012;50(4):710-23.
9. Watzl B, Gelencsér E, Hoekstra J, Kulling S, Lydeking-Olsen E, Rowland I, et al. Application *of the BRAFO-tiered approach for benefit-risk assessment to case studies on natural foods.* Food Chem. Toxicol. 2012;50(4):699-09.
10. Schütte K, Boeing H, Hart A, Heeschen, W, Reimerdes EH, Santare, D, et al. Application of the BRAFO tiered approach for benefit-risk assessment to case studies on heat processing contaminants. Food Chem Toxicol. 2012;50(4):724-35.
11. Boobis A, Chiodini A, Hoekstra J, Lagiou P, Przyrembel H, Schlatter J, et al. Critical appraisal of the assessment of benefits and risks for foods, 'BRAFO Consensus Working Group'. Food Chem Toxicol. 2013;55:659-75.
12. Hellberg RS, DeWitt CAM, Morrissey MT. Risk-benefit analysis of seafood consumption: A review. Comprehensive reviews in food science and food safety. 2012;11(5):490-517.
13. Holick MF. Vitamin D: A D-lightful solution for good health. J Med Biochem. 2012;31(4): 263-64.
14. Pedersen AN, Fagt S, Groth MV, Christensen T, Matthiessen J, Andersen NL, et al. Danskernes kostvaner 2003-2008 Hovedresultater. DTU Fødevareinstituttet. 2010.
15. Lamberg-Allardt C, Brustad M, Meyer HE, Steingrimsdottir L. VITAMIN D - A systematic literature review for the 5th edition of the Nordic Nutrition Recommendations. Food & Nutrition Research. 2013;57:22671.
16. Grant WB, Cross HS, Garland CF, Gorham ED, Moan J, Peterlik M, et al. Estimated benefit of increased vitamin D status in reducing the economic burden of disease in Western Europe. Pro in Biophy and Mol Bio. 2009;99(2-3):104-13.
17. EFSA (European Food Safety Authority). Scientific Opinion on the Tolerable Upper Intake Level of vitamin D. EFSA Journal, 10:2813. Parma, Italy. 2012.

18. IOM (Institute of Medicine). Dietary Reference intakes for calcium and vitamin D. Washington, D.C. National Academies Press, 2010.
19. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, Orav JE, Stuck AE, Theiler R, et al. Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomized controlled trials. BMJ. 2009;339:3692.
20. Bischoff-Ferrari HA, Shao A, Dawson-Hughes B, Hathcock J, Giovannucci E, Willett WC. Benefit–risk assessment of vitamin D supplementation. Osteoporos Int. 2010;21(7):1121-32.
21. Bischoff-Ferrari HA, Willett WC, Wong JB, Stuck AE, Staehelin HB, Orav EJ et al. Prevention of nonvertebral fractures with oral vitamin D and dose dependency. A meta-analysis of randomized controlled trials. Arch Intern Med. 2009;169(6):551-561.
22. Bischoff-Ferrari HA, Willett WC, Wong WC, Giovannucci E, Dietrich T, Dawson-Hughes B. Fracture prevention with vitamin D supplementation: A meta-analysis of randomized controlled trials. JAMA. 2005;293(18):2257-64.
23. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum levels of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr. 2006;84(1):18-28.
24. Durup HLD, Jørgensen J, Christensen P, Schwarz AM, Heegaard M, Lind B. A reverse J-shaped association of all-cause mortality with serum 25-hydroxyvitamin D in general practice, the CopD Study. J Clin Endocrinol Metab. 2012;97(8):2644-52.
25. Selby PL, Davies M, Marks JS, Mawer, EB. Vitamin D intoxication causes hypercalcaemia by increased bone resorption which responds to pamidronate. Clinical Endocrinology. 1995;43(5):531-36.
26. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. Osteoporosis International. 2005;16(7):713-6.
27. WHO/IARC. Vitamin D and cancer. France. 2008.
28. WCRF/AIRC. Food, nutrition, physical activity and the prevention of cancer: A global perspective. AIRC, Washington. 2007.
29. WHO/FAO. Diet, nutrition and the prevention of chronic diseases. 2003.

30. Fødevareinstituttet. D-vitamin: Opdatering af videnskabelig evidens for sygdomsforebyggelse og anbefalinger. DTU Fødevareinstituttet. Denmark. 2010.
31. Gorham ED, Garland CF, Garland FC, Grant WB, Mohr SB, Lipkin M, et al. Optimal vitamin D status for colorectal cancer prevention a quantitative meta-analysis. Am J Pre Med. 2007;32(3):210-16.
32. Schmidt-Gayk H, Bouillon R, Roth HJ. Measurement of vitamin D and its metabolites (calcidiol and calcitriol) and their clinical significance. Scand J Clin Lab Invest Suppl. 1997;227:35-45.
33. Michaelsson K, Baron JA, Snellman G, Gedeborg R, Byberg L, Sundstrom J, et al. Plasma vitamin D and mortality in older men: a community-based prospective cohort study. Am J Clin Nutr. 2010;92(4):841-8.
34. Ford ES, Zhao G, Tsai J, Li C. Vitamin D and all-cause mortality among adults in USA: findings from the National Health and Nutrition Examination Survey Linked Mortality Study. Int J Epideiolo. 2011;40(4):998-05.
35. RFA. Vitamin D converter. Accessed 04 March 2013. Available: http://www.robert-forbes.com/resources/vitaminconverter.html.
36. Cashman KD, Wallace JMW, Horigan G, Hill TR, Barnes MS, Lucey AL, et al. Estimation of the dietary requirement for vitamin D in free-living adults ≥64 y of age. Am J Clin Nutr. 2009;89(5):1366-74.
37. Cashman KD, Hill TR, Lucey AJ, Taylor N, Seamans KM, Muldowney S, et al. Estimation of the dietary requirement for vitamin D in healthy adults. Am J Clin Nutr. 2008;88(6):1535-42.
38. Berjia FL, Andersen R, Hoekstra J, Poulsen M, Nauta M. Risk-benefit assessment of cold-smoked salmon: microbial risk versus nutritional benefit. EJFRR. 2012;2(2):49-68.
39. Denmark Statistics. Population statistics. Accessed 17 December 2012. Available: http://www.statbank.dk/statbank5a/SelectTable/Omrade0.asp?PLanguage=1 .
40. Nymark T. The Funen county hip fracture study. Incidence, recurrence, mortality, and projection. PhD thesis, Denmark. 2008.

41. Jørgensen L, Jenssen T, Ahmed L, Bjørnerem Å, Joakimsen R, Bjarne K, et al. Albuminuria and Risk of Nonvertebral Fractures. Arch Intern Med. 2007;167(13):1379-85.
42. Høidrup S, Sørensen TIA, Grønbæk M, Schroll M. Incidence and characteristics of falls leading to hospital treatment: a one-year population surveillance study of the Danish population aged 45 years and over. Scand J Pub Heal. 2003;31(1):24-30.
43. Giversen IM. Time trends of mortality after first hip fractures. Osteoporos Int. 2007;18(6):721-32.
44. Huntjens KMB, Kosar S, van Geel TACM, Geusens PP, Willems P, Kessels A, et al. Risk of subsequent fracture and mortality within 5 years after a non-vertebral fracture. Osteoporos Int. 2010;21(12):2075-82.
45. Gribbin J, Hubbard V, Smith C, Gladman J, Lewis S. Incidence and mortality of falls amongst older people in primary care in the United Kingdom. QJM. 2009;102(7):477-83.
46. Foss NB, Kehlet H. Mortality analysis in hip fracture patients: implications for design of future outcome trials. British Journal of Anaesthesia. 2005;94(1):24-9.
47. Dunn JE, Rudberg MA, Fumer SE, Christine K, Cassel CK. Mortality, disability and falls in older persons: The Role of Underlying Disease and Disability. Am J Pub Health. 1992;82(3):395-00.
48. Peterson MG, Cornell CN, Paget SA, Allegrante JP. Five-Year survival in a cohort of hip fracture patients: The predictive role of pre-fracture health status. HSS J. 2008;4(1):43-7.
49. WHO. The global burden of disease: 2004 update. Geneva. 2008.
50. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. Osteoporosis International. 2005;16(7):713-6.
51. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum levels of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr. 2006;84(1):18-28.

Chapter 8

MANUSCRIPT II

Burden of diseases estimates associated to different red meat cooking practices

F. L. Berjia, M. Poulsen, M. Nauta

This chapter is published in a slightly modified version

Food and Chemical Toxicology 66 (2014) 237–244

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Burden of diseases estimates associated to different red meat cooking practices

Firew Lemma Berjia [a,*], Morten Poulsen [b], Maarten Nauta [a]

[a] Division of Epidemiology and Microbial Genomics, The National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, 2860 Søborg, Denmark
[b] Division of Toxicology and Risk Assessment, The National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, 2860 Søborg, Denmark

ARTICLE INFO

Article history:
Received 10 December 2013
Accepted 27 January 2014
Available online 31 January 2014

Keywords:
Cooking practice
Red meat
Cancer
DALY

ABSTRACT

The burden of disease estimate has been performed for diseases attributable to nutritional deficiency, foodborne pathogens, the environment, infection and other factors. However, the burden of disease estimate attributable to different food processing practices has not been investigated before. The aim of this study is to compare the burden of disease estimate attributed to red meat consumption processed using different cooking practices.

The red meat cooking practices were categorized into three: (A) barbecuing/grilling; (B) frying/broiling and (C) roasting/baking. The associated endpoints, affected population, intake and dose–response data are obtained by literature survey. The selected endpoints are four types of cancer: colorectal, prostate, breast and pancreatic. The burden of disease per cooking practice, endpoint, sex and age is estimated in the Danish population, using disability adjusted life years (DALY) as a common health metric.

The results reveal that the consumption of barbecued red meat is associated with the highest disease burden, followed by fried red meat and roasted red meat.

The method used to quantify the difference in disease burden of different cooking practices can help to inform the consumer to make a choice on whether the benefit of a preferred cooking style is worth the associated health loss.

Burden of diseases estimates associated to different red meat cooking practices

Authors

F. L. Berjia[1*], M. Poulsen[2], M. Nauta[1]

[1]Division of Epidemiology and Risk Modeling, The National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, 2860 Søborg, Denmark.

[2]Division of Toxicology and Risk Assessment, The National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, 2860 Søborg, Denmark.

Corresponding author

Firew Lemma Berjia

fber@food.dtu.dk

firew.lemma@yahoo.com

Abstract

The burden of disease estimate has been performed for diseases attributable to nutritional deficiency, foodborne pathogens, the environment, infection and other factors. However, the burden of disease estimate attributable to different food processing practices has not been investigated before. The aim of this study is to compare the burden of disease estimate attributed to red meat consumption processed using different cooking practices.

The red meat cooking practices were categorized into three: A) barbecuing/grilling; B) frying/broiling and C) roasting/baking. The associated endpoints, affected population, intake and dose-response data are obtained by literature survey. The selected endpoints are four types of cancer: colorectal, prostate, breast and pancreatic. The burden of disease per cooking practice, endpoint, sex and age is estimated in the Danish population, using disability adjusted life years (DALY) as a common health metric.

The results reveal that the consumption of barbecued red meat is associated with the highest disease burden, followed by fried red meat and roasted red meat.

The method used to quantify the difference in disease burden of different cooking practices can help to inform the consumer to make a choice on whether the benefit of a preferred cooking style is worth the associated health loss.

Keywords: Cooking practice; red meat; cancer; DALY

* Corresponding author. Tel.: +4551801526
Email address: firew.lemma@yahoo.com (Firew Lemma), maana@food.dtu.dk (Maarten Nauta), morp@food.dtu.dk (Morten Poulsen).

1. Introduction

Food processing adds values to products; processed food products display specific flavor, taste, color or texture, which can be seen as a benefit from a food quality perspective (Heinz and Hautzinger, 2007). In addition, processing, especially cooking at high temperature, may inactivate pathogens in the food (WHO/FAO, 2004) but may also cause formation of hazardous chemical compounds (Badry, 2010). Thus, cooking at high temperature can be both beneficial and detrimental for health, which may leave the consumer with a dilemma when cooking.

Meat and meat products are ubiquitous and consumed cooked worldwide. Meat is cooked in various ways, and the cooking practices often used are braising, stewing, broiling/frying, grilling/barbecuing and roasting/baking. When meat is cooked at high temperatures, several hazardous chemical contaminants such as polyaromatic hydrocarbons (PHAs) and hetrocyclic amines (HCAs) may be formed (Jägerstad & Skog, 2005; Aaslyng et al., 2013). The concentration of these contaminants varies with meat type, temperature and time of cooking, and method of cooking (Knutsen et al., 2007; Badry, 2010; Aaslyng et al., 2013). Some of these chemical contaminants are known to be carcinogenic and may cause substantial health losses (EFSA, 2008; SCF, 2002). To inform consumers on the potential health impact of cooking red meat, it is relevant to quantify these health losses.

Burden of diseases is a quantitative measure of population health outcome using the information on mortality and morbidity (Murray and Lopez, 1996) and in addition recovery in the population (Hoekstra et al., 2012). Knowledge of burden of disease estimates may help to prioritize the major causes of health loss and to evaluate the potential impact of taking action to improve health. Burden of disease estimate has been performed for diseases attributed to nutritional deficiency, foodborne pathogens, environment, infections and other factors (Murray et al., 1996, Murray et al., 2013; Gkogka et al., 2011; Havelaar et al., 2012). However, to our knowledge, the burden of disease estimate attributed to meat cooking practices has not been studied before.

The aim of this study is to compare the burden of disease estimates (expressed in disability adjusted life years, DALY) attributed to different cooking practices used to process red meat. The available data from epidemiological studies where cancer risks (consequential to exposure to PHAs and HCAs) are associated with cooking practices are applied, together with Danish consumption data. A method is developed that allows the estimation of the burden of disease for different cooking practices for different sexes and different age classes, based on these data. The outcome of this study will enable us to inform consumers on the difference between the expected health impacts of different cooking practices and may allow individuals to weigh that against the perceived quality associated to these cooking practices.

2. Material and methods

For the purpose of this study, we have considered three categories of red meat preparation by the consumer: A) barbecuing (BBQ)/grilling (direct heat contact); B) frying/broiling (pan); C) roasting/baking (dry heat, oven).

The health hazards, endpoints and affected populations related to the consumption of cooked red meat are obtained from the literature. In order to estimate the probability of onset of each disease that can arise from the different cooking practices, the relative risks (RR) associated to intake of red meat per cooking practice were identified from literature, stratified and modeled using a linear or a log-linear function. Model validation is performed to select the best fit, using residual analysis and QQ-plot (Ekstrøm and Sørensen, 2011). Next, the RR based on the Danish red meat intake distribution is estimated. Then, the probability of onset of the disease per cooking practice is estimated as a function of the incidence of disease, the frequency of the application of the cooking practices, the probability of intake and the RR for different age classes and sexes.

The burden of disease of each cooking practice per selected endpoints is estimated using the DALY model developed in Hoekstra et al. (2012). For the sake of relative comparison, the

burden of disease for no intake of cooked red meat is also estimated. The analysis is performed in R-statistical software, version 2.15.2 and MS Excel 2010. A detailed description of the method is presented in the following sections.

2.1. Major health hazards associated with cooked meat consumption

When meat is heat treated, deleterious compounds including various mutagens and carcinogens may be formed. The two widely known group of hazardous chemical compounds formed during meat cooking are HCAs and PAHs (Jägerstad and Skog, 2005; Badry 2010; Aaslyng et al., 2013). The formation of these toxicants is primarily linked to the cooking temperature-time relationship and the final concentration in the meat varies with different cooking practices (Badry, 2010).

Even though both HCAs and PAHs are associated with serious health risks, there are only few reports concerning the intake of these components (Aaslyng et al., 2013). The lack of data about the intake of these compounds makes it difficult to make a direct link of these compounds with the cancer risks. However, several epidemiological studies correlate red meat intake cooked in different ways with cancer risks. Hence, in this study we use the epidemiological studies to estimate the burden of disease of eating red meat cooked in various ways.

2.2 Health effect related to cooked red meat consumption

Colorectal cancer is the cancer type most often associated with meat consumption (Probst-Hensch et al., 1997; Sinha et al., 1999; Sinha et al., 2001; Ishibe et al., 2002; Sinha et al., 2005; Wu et al., 2006; Butler et al., 2003; Gunter et al., 2005; Cross et al., 2007) followed by breast cancer (Zheng et al., 1998; Steck et al., 2007), prostate cancer (Cross et al., 2005; Koutros et al., 2008; John et al., 2011) and pancreatic cancer (Anderson et al., 2002; Li et al., 2007;

Stolzenberg-Solomon et al., 2007). In this study we have therefore selected these endpoints. The population is based on studies referred to in table 1.

Table 1

Selected endpoints and population

Selected endpoints	Population	Reference
Colorectal cancer	Both sexes, age 50-71 years	Cross et al., 2007
Prostate cancer	Men, age 40-79 years	John et al., 2011
Pancreatic cancer	Both sexes, age 50-71 years	Stolzenberg-Solomon et al., 2007
Breast cancer	Women, age > 49 years	Steck et al., 2007

2.3. Intake of red meat

To assess the burden of diseases of the different meat cooking practices we have to know the intake of meat. For that purpose we have adopted the red meat consumption distributions by age classes and sexes from Pedersen et al. (2010) and presented in table 2.

Table 2

The red meat intake (g/day) distribution in Denmark by age classes and sexes (Pedersen et al. 2010)

Percentile		1	5	10	25	50	75	90	95	99
Sex	Age									
Men	35-44	38	55	69	100	139	188	261	292	364
	45-54	24	57	67	96	134	177	223	256	282
	55-64	28	60	70	91	124	164	223	259	348
	65-75	16	41	58	81	112	145	178	193	239
Women	35-44	13	27	39	59	83	111	136	158	198
	45-54	7	25	36	53	79	106	140	156	197
	55-64	6	24	37	54	76	103	125	144	183
	65-75	11	18	29	48	70	92	123	147	243
*p(i) (%)		2	6	4	26	24	26	4	6	2

*The probability of intake p(i) gives the fraction of the population that is assumed to have the indicated intake i in each age/sex class. It is assumed to be an interval around the percentiles reported by Pedersen et al. (2010), where the reported percentile is the median value of each interval. This p(i) is required for the model calculations (section 2.4).

The age classes for intake (table 2) were adapted to fit with the selected populations for the endpoints (table 1). When the age class in the intake distribution is 35-44, 45-54, 55-64 and 65-75 years, the age in the selected population (for instance, age 40-79 years, prostate cancer) is then accordingly catagorized as 40-44, 45-54, 55-64 and 65-79 respectively. Furthermore, since the last age class in table 2 is 65-75, we have assumed that persons older than 75 years have the same intake distribution as persons aged 65-75 year.

2.4. Estimating the probability of onset of the diseases

To estimate the probability of onset of the diseases, we did a literature search that particularly focussed on red meat intake by cooking practice in relation with the endpoints we have considered. The relative risk data for different intake levels, endpoints and cooking practices are presented in table 3.

Table 3

Intake-Relative risk (mean, 95%CI) data points per cooking practice and endpoint used in the dose-response modeling

Cooking category	Colorectal cancer			Prostate cancer			Breast cancer			Pancreatic cancer		
	Intake (g/d)	Relative Risk	Ref	Intake (g/d)	Relative Risk	Ref	Intake (g/d)	Relative Risk	Ref	Intake (g/d)	Relative Risk	Ref
Barbecuing/ grilling	0	1	*a*	0	1	*c*	0	1	*f*	0	1	*g*
	3	0.9 (0.6, 1.2)	*a*	3	1 (0.8,1.2)	*c*	12	1.2 (1.0,1.5)	*f*	2	1.4(0.7–2.7)	*g*
	8	0.9 (0.7, 1.3)	*a*	9	1 (0.8,1.4)	*c*	27	1.2 (1.0,1.4)	*f*	7	1.2(0.7–1.9)	*g*
	17	1 (0.7,1.3)	*a*	17	1.1 (0.8,1.5)	*c*	49	1.6 (1.3,1.9)	*f*	35	2.2 (1.4–3.4)	*g*
				*0	1	*d*						
	30	0.9 (0.6, 1.3)	*b*	*0	1.36(0.86,2.16)	*d*						
	0	1	*b*	60	1.26(0.76,2.07)	*d*						
	3	1.58 (0.67-3.71)	*b*	120	1.63(0.99,2.68)	*d*						
	45	1.89 (1.04,3.45)	*b*	0	1	*e*						
				44	1.5(1.03,2.19)	*e*						
				98	1.69(1.19,2.38)	*e*						
				188	1.61 (1.13,2.28)	*e*						

Frying/	0	1	*b*	0	1		0	1	*f*	0	1	*g*
broiling	1	0.55 (0.15-2.03)	*b*	3	0.9 (0.7,1.1)		12	1.0 (0.9,1.3)	*f*	2	1.1(0.6,2)	*g*
	3	0.53 (0.24-1.17)	*b*	7	0.9 (0.6,1.2)		27	1.5 (1.3,1.8)	*f*	8	1.9(1.1,3.3)	*g*
	10	0.69 (0.33-1.47)	*b*	13	1 (0.7,1.4))		49	1.3 (1.1,1.6)	*f*	17	1.6(0.9,2.8)	*g*
	40	0.72 (0.34-1.55)	*b*	0	1	*c*	0	1	*f*	95	1.4 (0.7,2.6)	*g*
	0	1	*b*	3	1.2 (1, 1.5)	*c*	12	1.1 (0.9,1.4)	*f*	0	1	*g*
	18	0.22 (0.07-0.67)	*b*	7	1.2(0.9,1.6)	*c*	27	1.2 (1.0,1.4)	*f*	8	0.9(0.5,1.4)	*g*
				13	1.2 (1, 1.8)	*c*	49	1.3 (1.0,1.4)	*f*	10	0.7(0.4,1.1)	*g*
	0	1	*a*	0	1	*d*				90	0.7(0.4,1.2)	*g*
	2	1.1 (0.8, 1.6)	*a*	0	1.38(0.88,2.16)	*d*						
	7	1.2 (0.8, 1.7)	*a*	60	1.42(0.84,2.39)	*d*						
	13	1.1 (0.7-1.6)	*a*	120	1.41(0.83,2.37)	*d*						
	20	1.3 (0.9-1.9)	*a*	0	1	*d*						
	0	1	*a*	0	1.32 (0.83,2.09)	*d*						
	2	1.1 (0.8,1.6)	*a*	60	1.52 (0.93,2.49)	*d*						
	9	1.3 (0.9-1.9)	*a*	120	1.42 (0.86,2.43)	*d*						
	18	1.5 (1-2.2)	*a*									
	35	2 (1.4,3)	*a*									
Roasting/	0	1	*a*	0	1	*c*	0	1	*f*	0	1	*h*
baking	1.5	1.4 (0.9,2.1)	*a*	1.5	0.7 (0.5,1.1)	*c*	12	1.3 (0.9,1.7)	*f*	1	1.1(0.78, 1.55)	*h*
	4	1 (0.6,1.5)	*a*	3.5	0.8 (0.4,1.4)	*c*	27	1.0 (0.8,1.2)	*f*	2	1.16(0.88, 1.53)	*h*

	6	0.6 (0.3,1)	*a*	6	0.9 (0.5,1.6)	*c*	49	1.1 (0.9,1.3)	*f*	0	1	*h*
				0	1	*d*						
	12	1.1 (0.7, 1.7)	*a*	0	1.4 (0.9,2.18)	*d*				2	0.91 (0.63, 1.34)	*h*
				60	1.38 (0.66,2.9)	*d*				6	0.81 (0.54, 1.2)	*h*
				120	1.55 (0.6,2.58)	*d*						

Ref: References. a: Butler et al. (2003); b: Gunter et al. (2005); c: Joshi et al. (2012); d: John et al. (2011); e: Punnen et al. (2011); f: Fu et al. (2011); g: Anderson et al. (2002); h: Stolzenberg-Solomon et al. (2007).

*The same reference can provide several relative risks for the same intake because the relative risks are estimated for both no intake of red meat (RR = 1) and no intake of barbecued/grilled red meat (RR = 1.36) (John et al., 2011).

The dose-response modeling requires point estimates of mean intake in g/day for the intake categories as applied in the different studies. Generally, these mean intakes are not provided in the selected studies. Therefore, different assumptions have been made when the intakes were not sufficiently quantified to be used for the dose-response modelling in this assessment:

1. For the intakes that are given in interval, for example Butler et al. (2003), we have taken the mean.
2. For the intakes that are described in qualitative terms such as low, medium and high intake (Punnen et al., 2011), we have assumed the 10 percentile, median and 90 percentile of Danish meat consumption (Pedersen et al., 2010; table 2).
3. For the intake given as below median and above median (John et al., 2011), we have assumed 60 and 120 g/day respectively, which is roughly below and above median of Danish red meat intake (Pedersen et al., 2010).
4. For the intakes that are given by quartiles (Fu et al., 2011); Q1, Q2, Q3, Q4, we have assumed no intake, 25%, median and 75% of the Danish intake respectively. The no intake is assumed for Q1 because the relative risk for Q1 is 1.

All the data used for the dose-response modeling are presented in table 3.

Since the prevalence of the selected endpoints at population level in Denmark is less than 10%, we have assumed that the odds ratio (OR) and the hazard ratio (HR) in the Anderson et al. (2012) study are similar to the relative risk (RR) (Cummings, 2009; McNutt et al., 2003).

To estimate the RR based on the intake scenarios, we have analyzed the data using linear, log-linear and exponential model to find the best fit. Model validation is performed using residual analysis and QQ-plot (Ekstrøm and Sørensen, 2011). The selected model for each endpoint per cooking practice and the parameter estimates are given in table 4.

Table 4

Models used to estimate the relative risk based on intake (i) for all endpoints and cooking practices (cp) for the age classes and sexes indicated in table 2.

	Colorectal cancer	Prostate cancer	Pancreatic cancer	Breast cancer
BBQ/ grilling	RR = 0.012 (i) + 0.99	RR=0.004*(i)+1.1	RR= 0.043*(i)+1.08	Ln(RR)= 0.009*(i)+0.015
Frying/ broiling	Ln(RR)= 0.004*(i)-0.12	Ln(RR)= 0.003*(i)+0.09	Ln(RR)= -0.001*(i)+0.1	Ln(RR)= 0.006*(i)+0.0182
Roasting/ baking	Ln(RR)= -0.012*(i)+0.04	RR= 0.005*(i) +0.97	Ln(RR)= -0.04*(i)+0.06	RR= -0.0002*(i)+1.1

The RR per cooking practice (cp) at intake i, RR(i, cp) can be estimated using the equations in table 4 for the different endpoints. As the equations are based on different studies, the RR(i,cp) values have different reference points where RR(i,cp) = 1. To allow a comparison of the RR values, they are rewritten to a relative risk RR^0, so that by definition $RR^0(0,cp) = 1$:

$$RR^0(i, cp) = RR(i, cp)/RR(0, cp) \quad (1)$$

Where RR(0,cp) is relative risk for no intake which is obtained from the equations in table 3, setting i=0.

Disease is associated with absolute risk; therefore, the RR^0 (i, cp) has to be converted to absolute risk ($P_{eff(a,s)}(i,cp)$) for each endpoint (Hoekstra et al., 2013). The incidence rate of a disease due to eating cooked red meat per endpoint is a function of intake, probability of cooking practice, and probability of getting a disease.

$$Inc_{(a,s)ep} = \sum_i p_{(a,s)}(i) * (\sum_{cp} q_{(a,s)}(cp)) * (P_{eff(a,s)ep}(i,cp)) \quad (2)$$

where $Inc_{(a,s)ep}$ is the incidence rate in age group a, for sex s for endpoint ep; $p_{a,s}(i)$ is the probability of intake i in age class a, for sex s; $q_{a,s}$ (cp), is the probability of cooking practice, cp , in

age class a, for sex s; $P_{eff(a,s)ep}(i,cp)$ is the probability of getting the disease with intake i and cooking practice cp, in age class a, for sex s.

However, $q_{(a,s)}$ (cp) is unknown for the different age and sex, so it is assumed to be the same for all age and sex classes and the incidence rate at age a for sex s becomes:

$$Inc_{(a,s)ep} = \sum_i p_{(a,s)}(i) * (\sum_{cp} q(cp))*(P_{eff(a,s)ep}(i, cp)) \quad (3)$$

As for $P_{eff(a,s)ep}(i, cp)$ we know that

$$P_{eff(a,s)ep}(i, cp) = P_{eff(a,s)ep}(0,cp)* RR^0(i, cp)/ RR^0(0,cp) \quad (4)$$

For an individual who does not consume cooked red meat, RR^0 (0, cp) = 1 for each cooking practice and $P_{eff(a,s)ep}(0,cp) = P_{eff(a,s)ep}$ (0) must be the same for all, because the individual does not use any of the cooking practices. Hence,

$$P_{eff(a,s)ep(}i, cp) = P_{eff(a,s)ep}(0)* RR^0(i, cp)) \quad (5)$$

And therefore,

$$Inc_{(a,s)ep} = \sum_i p_{(a,s)}(i) * (\sum_{cp} q(cp) *P_{eff(a,s)ep}(0)* RR^0(i, cp)) \quad (6)$$

$$= P_{eff(a,s)ep}(0) * \sum_i p_{(a,s)}(i) * (\sum_{cp} q(cp)* RR^0(i, cp)) \quad (7)$$

So we can calculate,

$$P_{eff(a,s)ep}(0) = Inc_{(a,s)ep} / (\sum_i p_{(a,s)}(i) * (\sum_{cp} q(cp)* RR^0(i, cp))) \quad (8)$$

$p_{a,s}(i)$ is obtained from the percentile distributions of red meat intake (Pedersen et al., 2010) and given in table 2 as % in class. q(cp) is the probability of cooking practice cp. They have to sum up to

1 and were assumed to be 0.25, 0.5 and 0.25 for barbecuing, frying and roasting respectively. The assumption behind these cooking probabilities is that consumers usually fry meat more frequently than that they roast or barbecue it. The incidence rates for all endpoints are obtained from (Sundhedsstyrelsen, 2004).

Thus, we have estimated the probability of onset of the disease per age and sex for each distribution of intake as presented in table 2. Then, the mean $P_{eff(a,s)ep}(i, cp)$ of the distributions is used to estimate DALY. This has been done for each endpoint per cooking practice.

$$P_{eff(a,s)ep}(cp) = P_{eff\,(a,s)ep}(0)*\sum_i p_{(a,s)}\,(i)* RR^{0}{}_{ep}\,(i, cp) \quad (9)$$

This $P_{eff(a,s)}(cp)$ is the mean probability of disease per person associated with the endpoint ep per year, given the distribution of intakes in Denmark (table 2), for a person of sex s in age class a, given the person is always using cooking practice cp when consuming meat.

2.5 Parameter estimation for DALY calculation

Disability adjusted life years, DALY is a common health metric that combine the different dimension of health outcomes to predict the burden of disease. To estimate the burden of disease of the cooking practices, we have applied the DALY model used in Hoekstra et al. (2012). The DALY is computed for each individual and summed up for each endpoint, in MS Excel 2010. A similar approach as Berjia, et al., (2012) was adopted to estimate all the input parameters.

$$DALY_{(a,s)ep}(cp) = P_{eff(a,s)ep}(cp) * [(P_{rec}*YLD_{rec}*w + P_{die}(YLD_{die}*w+LE_{(a,s)} – CA - YLD_{die}) + (1-P_{die} - P_{rec})*(LE_{(a,s)} - CA)*w] * N_{(a,s)} \quad (10)$$

Where:

$DALY_{(a,s)ep}(cp)$ disability adjusted life years at age and sex for each endpoint per cooking practice

$P_{eff(a,s)ep}(cp)$ probability of onset of the disease (ep) at age and gender, per cooking practice cp

P_{rec} probability of recovery from the disease

P_{die} probability the disease causes death

YLD_{rec} mean duration of disease for those who recover

YLD_{die} mean duration of disease for those who die

CA current age of individual in year of disease onset

$LE_{(a,s)}$ life expectancy for an individual of age at CA

w disability weight for disease.

$N_{(a,s)}$ number of people of age a and sex s in Denmark

$P_{eff(a,s)ep}(i, cp)$ values are calculated for each endpoint as described in section 2.4. (Eqn 9). P_{die} for colorectal, breast and prostate cancer is estimated from (Denmark Statistics, 2013) and for pancreatic cancer P_{die} is obtained from (Sundhedsstyrelsen, 2010).

If someone acquires a disease, either that person survives (P_{sur}) with the disease until the normal life expectancy, recovers from the disease (P_{rec}) or dies due to the disease (P_{die}) before normal life expectancy (Hoekstra et al., 2012). Then, these disease outcomes sum up to one.

$$P_{rec} + P_{sur} + P_{die} = 1 \quad (11)$$

P_{sur} is obtained for colorectal cancer from (Iversen, 2011), prostate cancer (Borre et al., 2011), pancreatic cancer (MDACC, 2013) and for breast cancer (Lietzen et al., 2011). Then, P_{rec} is estimate by 1 - (P_{die} + P_{sur}) for each endpoint. w is obtained from the WHO global burden of disease disability weight estimate (WHO, 2008).

Table 5

Summary of parameter values used for DALY calculation for each endpoint

Endpoints	P_{die}	P_{rec}	P_{sur}	YLD_{die}	YLD_{rec}	w
Colorectal cancer	0.016	0.24	0.74	0.08	5	0.2
Prostate cancer	0.047	0.053	0.9	1	5	0.13
Pancreatic cancer	0.043	0.32	0.64	1	5	0.2
Breast cancer	0.03	0.03	0.94	1	5	0.09

YLD_{die} and YLD_{rec} are assumed to be the minimum and maximum study period of Iversen et al., (2011), Borre et al. (2011), Lietzen et al. (2011) and Cronin-Fenton et al. (2011) for colorectal, prostate, breast and pancreatic cancer respectively. The parameter values used are presented in table 5.

The model is run for individuals that represent the Danish population. The total population sizes for the selected endpoints per sex and age (table 6) and life expectancy are obtained from Denmark Statistics (2013).

Table 6

The total number of population per sex and age $\sum_a N_{a,s}$ for the selected endpoints in Denmark (2013)

	Age (years)	Men	Women
Colorectal cancer	50-71	694902	706881
Prostate cancer	40-79	1187262	
Breast cancer	50-99		1000799
Pancreatic cancer	50-71	694902	706881

The total $DALY_{tot}$ per 100000 for each cooking practice and for each endpoint is calculated as

$$DALY_{tot,ep}(cp) = q(cp) * \sum_{a,s} DALY_{(a,s)\,ep}\,(cp) *100000 / \sum_{a,s} N_{(a,s)ep} \quad (12)$$

To estimate the total health loss per cooking practice, the DALY of all endpoints per cooking practice is summed up.

3. Results

Figure 1 illustrates the total burden of disease estimates of red meat consumption by cooking practice and for no intake of cooked red meat in DALY/100000 population. The population represents different individuals for the different endpoints (table 6) and the results can only be

interpreted for the population of age and sex per endpoints as indicated in table 1, but not for the general population.

It appears that, the consumption of barbecued red meat is the leading cause of healthy life years loss, followed by fried red meat. Relatively, several healthy life years loss can be prevented by eating roasted instead of barbecued or fried red meat.

The "no intake" scenario is calculated using the probability of illness $P_{eff(a,s)ep}(0)$ (eqn. 8) in the DALY calculations (eqns 10 and 12). The results show that the healthy life years lost for roasted red meat are lower than for the no intake scenario, whereas those for barbecued meat is higher and for fried meat slightly higher. This could suggests that consuming roasted red meat is healthier than no intake of red meat, and that barbecued meat and fried meat are less healthy. However, the "no intake" scenario is not directly comparable to the different cooking practices as additional health benefits and risks associated to red meat consumption are not included in the analysis. Furthermore, the impact of that part of the diet, which substitutes the red meat is not included in the calculations.

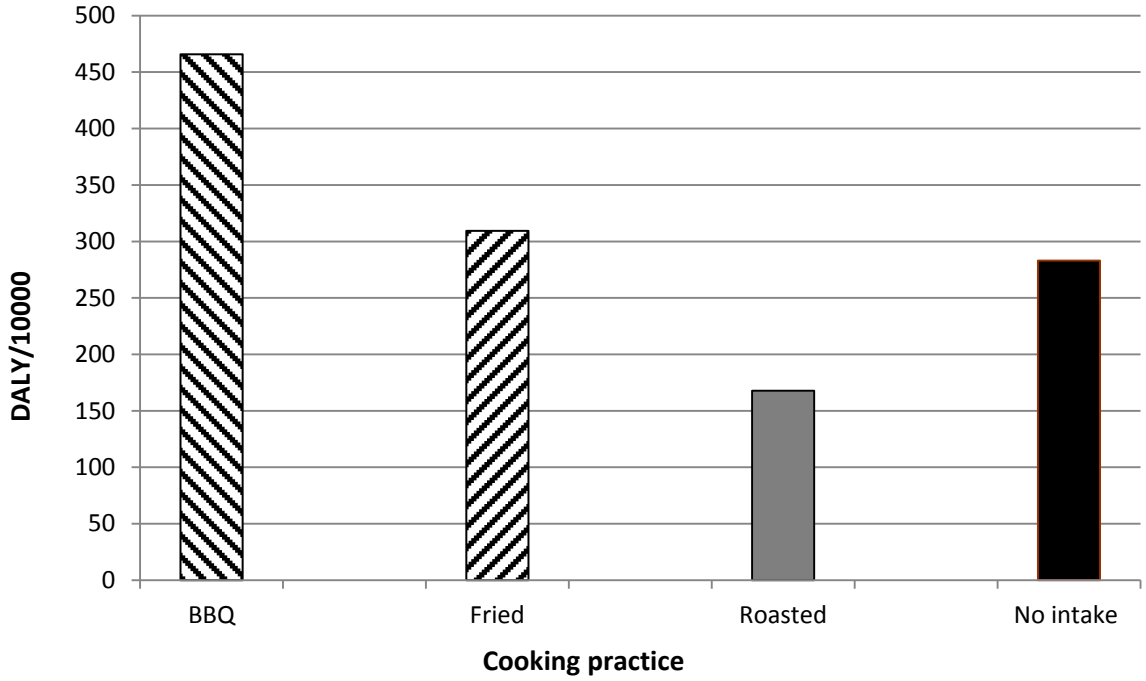


Figure 1. Estimated DALY/100000 population per cooking practice $\sum_{ep} DALY_{tot,ep}(cp)$

Figure 2 demonstrates the variation in DALYs for different sexes and endpoints after intake of red meat prepared by the different cooking practices. Note that, different populations are considered for different endpoints. It can be seen from the figure that colorectal cancer is a major cause of health loss in both men and women. The cooking practice that leads to the largest health loss due to colorectal cancer is barbecuing followed by frying. The loss of DALYs seen for breast cancer in women is primarily caused by barbecuing followed by frying.

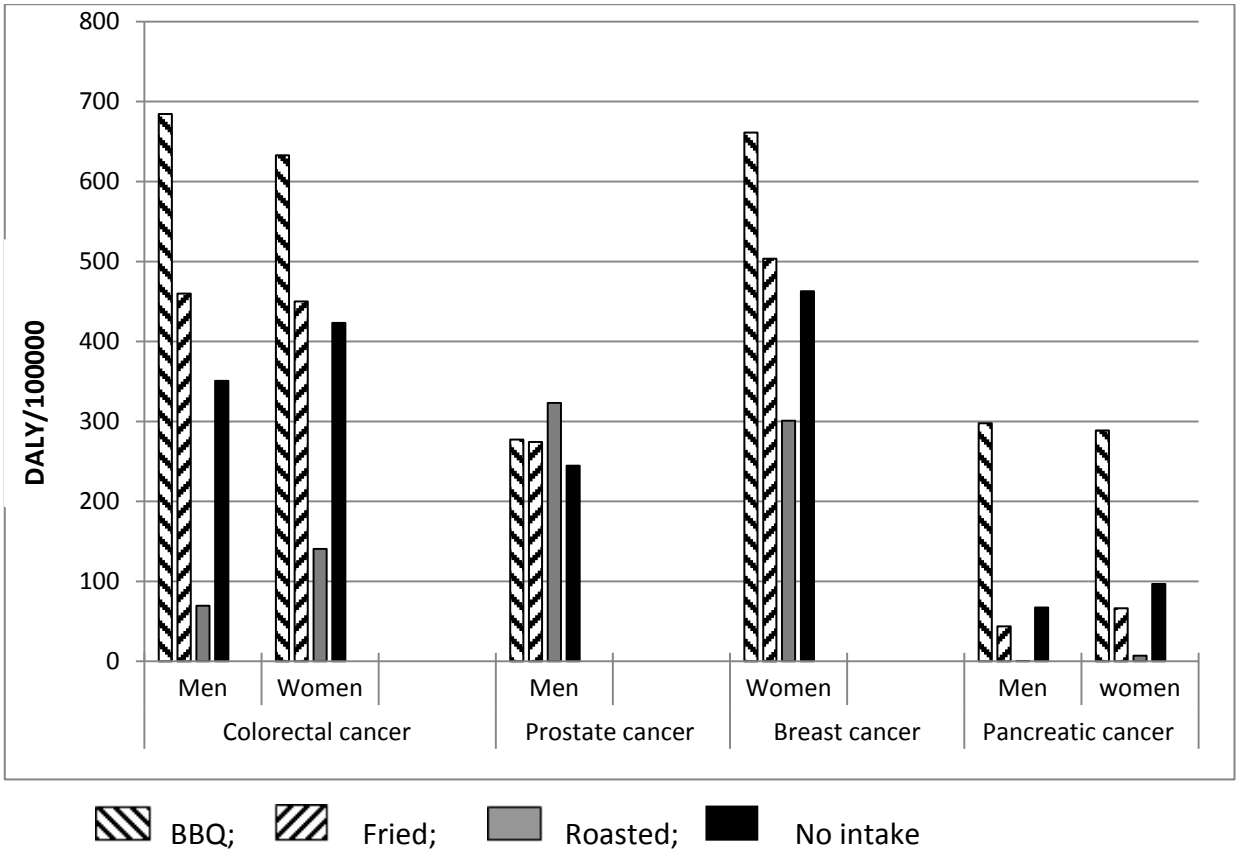


Figure 2. DALYs per endpoint, sex and cooking practice, $\sum_a DALY_{(a,s)ep}(cp)$

The healthy life years loss due to the increase risk of prostate cancer is nearly similar for all cooking practices. Concerning pancreatic cancer, the consumption of barbecued red meat increases the health loss compared to the other cooking practices equally in both sexes. In general, with the exception of prostate cancer, roasting tends to provide a benefit by reducing the healthy life years loss compared to the other cooking practices and compared to no intake of meat.

Usually, individuals decide which cooking practice to apply when cooking meat, based on personal preference. It will be of interest for an individual consumer to know the assessed health burden associated to the choice of a cooking practice. This health burden can be assessed because individual healthy life days loss after a year long consumption of cooked red meat can be predicted with the available data and model for each specific cooking practice. We have performed an example for some selected ages (52, 60 and 67 years old) for both men and women (figure 3). First, the mean DALY is computed for selected age classes (50-54, 55-64 and 65-70 years old) for each endpoint, sex and cooking practice. Then, the mean DALY of all the endpoints per sex and age class are summed. Subsequently, the difference in DALY of the consumption of barbecued and fried is estimated in comparison with consumption of roasted red meat. To estimate the expected individual health loss in days, estimated DALY is divided by 100000 and multiplied with the number of days in a year.

Figure 3 illustrates the predicted healthy life days loss for selected age and sexes for the consumption of barbecued and fried red meat compared to roasted red meat. In general, women have a higher loss of healthy life days due to barbequing than men.

If a woman of age 52 years old consumes barbecued red meat during a year (given the distribution in table 2) instead of roasted red meat, the healthy life days loss can be close to 4 days. Whereas, a man of the same age has a lower loss of healthy life days.

If a man of age 67 years old prefers to consume roasted red meat in stead of barbecued red meat, he prevents the loss of approximately 3 healthy life days/year. Relatively, a women of age 67 years old can benefit more than men by preventing 4.5 healthy life days loss/year, if she consumes roasted red meat instead of barbecued red meat. In general, for the selected age class and sex, consumption of barbecued red meat seems to cause more healthy life days losses than consumption of fried red meat and all give a higher loss than roasted meat.

Figure 3. Individual healthy life loss (days/year) for the consumption of barbecued and fried red meat instead of roasted red meat.

4. Discussion

In this study, we have estimated and compared the burden of disease of red meat prepared by different cooking practices. Several studies suggest that barbecued meat is associated with higher concentrations of HCAs and PAHs, which increase the risk of cancers (Anderson et al., 2002; Knutsen et al., 2007; Badry, 2010; Aaslyng et al., 2013). The results obtained in this study support the notion that consumption of barbecued red meat is a major cause of healthy life year's loss. According to the model prediction, the loss of 298 and 156 DALYs/100000 can be prevented in the selected populations, if roasted red meat is consumed instead barbecued and fried red meat, respectively.

The results obtained in this study reveal that the choice of cooking practice has an impact on health outcome. Referring to Figure 3, regarding the individual healthy life day's loss from all cancers, women seem to benefit more than men, if they consume roasted red meat instead of barbecued red meat. Therefore, attention should also be paid to the gender aspect in the choice of cooking method.

In contrast with the burden of disease studies looking at foodborne pathogens and nutritional deficiency diseases (Gkogka et al., 2011; Havelaar et al., 2012; Murray et al., 2013) and risk-benefit studies of food and food components (Berjia et al., 2012; Hoekstra et al., 2013), this study presents the burden of disease estimate of different cooking practices. In this study we have ignored the burden of disease linked to pathogens. Apparently, in meat products cooked at high temperature, the burden of disease linked to pathogens is likely to be trivial. In addition, the health benefits and risks associated with the nutrients in red meat were not included in this study because the current available data related to the effect of the different cooking practices on the concentration of the nutrients are insufficient to estimate their health outcomes. We assume that as the diet is basically unaltered, and that the nutritional benefits will be highly similar when different cooking practices are compared.

The knowledge of the health loss and gain from different attributions gives an insight to prioritize the major causes of health loss in the population and support risk management decisions on control and prevention of foodborne disease. Usually, statistics on the public health impact of foodborne diseases focus on the burden of illness in a population (incidence), i.e. the incidence of non-fatal illness and of fatal cases (Adak et al., 2002; Scallan et al., 2011). The burden of disease metric DALY integrates incidence, severity, mortality, recovery and duration of a disease (Murray et al.,2002; Hoekstra et al., 2012). Hence, burden of disease estimate is a relatively powerful tool to report comprehensive public health statistics, which facilitates comparison between diseases in terms of healthy life year's loss.

This study compares the health burden related to the cooking practices of red meat, by combining epidemiological data of cancer risks associated with these cooking practices with the Danish red meat consumption data. Epidemiological data to model the dose-response relationship are scarce and not reported uniformly. Most of the dose-response data incorporated (see table 3) in this study are collected through interviews about cooking practices used by populations in different countries. Then, these data were combined with the consumption data to predict the odd ratio/relative risk of the different cancers. The red meat consumption data of these studies are

highly variable. When using the outcome of these studies to estimate burden of disease, several model assumptions had to be made to make the best use of the available data. For instance, the studies incorporated for the dose-response modeling describe the intake qualitatively or quantitatively (see section 2.4) and for some endpoints only a few data points (see table 3) were used to fit the equations for relative risks (Table 4). To make the best use of the available data, we have used different assumptions as a surrogate as described in section 2.4 and 2.5. One of these assumptions is the frequencies in which people in Denmark barbecue, fry and roast their red meat (q(cp)), for which we did not find any data. Hence, the results presented in this paper should be interpreted with care.

Nevertheless, the results give an interesting impression of the impact of different cooking practice on health. Moreover, this paper shows how the impact of cooking practices on burden of disease can be calculated, both at a population level and on an individual level. As shown in Figure 3, the method developed in this paper can offer a tool that estimates the health impact of a preferred cooking practice for an individual of a specific age and sex, compared to an alternative cooking practice. This may provide useful information to the l consumer that can decide whether for her or him personally the perceived benefit of a cooking practice (e.g. better flavor and food quality) outweighs the associated estimated risk of cancer in terms of healthy life days lost.

5. Conclusion

In this study, we estimated and compared in a quantitatively way the burden of disease for different cooking practices, using red meat as a case study. To our knowledge, this study is the first to estimate and compare the burden of disease for different food cooking practices. The result shows that the estimated burden of disease is largest for barbecued meat, followed by fried meat and roasted red meat.

The methodology can provide a tool that can be a help to the consumer in deciding whether the health loss of preferred cooking practices weighs up against its perceived benefit. Even though the

method has been usefully applied in this paper to get quantitative data on the burden of disease, it can be even more improved by using more comprehensive data on the dose-response relations between red meat consumption after the application of a specific cooking practice and cancer risks, the frequency of current cooking practices (q(cp)) and the duration of the diseases. Therefore, the method applied in this assessment can be used as a basis for similar studies in the future.

Future research may focus on the estimation of the burden of disease of the cooking practice of different food products to compare the burden with other foodborne chemical contaminants, pathogens and nutritional deficiency diseases in order to prioritize the major cause of health losses in the population and to take action to mitigate the health losses.

References

1. Aaslyng, M.D., Duedahl-Olesen, L., Jensen, K., Meinert, L. 2013. Content of heterocyclic amines and polycyclic aromatic hydrocarbons in pork, beef and chicken barbecued at home by Danish consumers. Meat Science. 93, 85-91.

2. Adak, G.K., Long, S.M., O'Brien, S.J. 2002. Trends in indigenous foodborne disease and deaths, England and Wales: 1992 to 2000. Gut. 51, 832–841.

3. Anderson, K.E., Mongin, S.J., Sinha, R., Stolzenberg-Solomon, R., Gross, M.D., Ziegler, R.G., Mabie, J.E., Risch, A., Kazin, S.S., Timothy R. Church. T.R. 2012. Pancreatic cancer risk: associations with meat-derived carcinogen Intake in the prostate, lung, colorectal and ovarian cancer screening trial (PLCO) cohort. Mol. carcinog. 51, 128-137.

4. Anderson, K.E., Sinha, R., Kulldorff, M., Gross, M., Lang, N.P., Barber, C., Hanack, L., DiMagno, E., Bliss, R., Kadlubar, F.F. 2002. Meat intake and cooking techniques: associations with pancreatic cancer. Mutation Research. 506-507, 225-231.

5. Badry, N.E.L. 2010. Effect of household cooking methods and some food additives on polyaromatic hydrocarbons (PAHs) formation in chicken meat. World applied science journal. 9, 963-974.

6. Berjia, F.L., Andersen, R., Hoekstra, J., Poulsen, M., Nauta, M. 2012. Risk-benefit assessment of cold-smoked salmon: microbial risk versus nutritional benefit. EJFRR. 2, 49-68.

7. Borre, M., Erichsen, R., Lund, L., Larsen, E.H., Nørgaard, M., Bonde. J., Jacobsen. 2011. Survival of prostate cancer patients in central and northern Denmark, 1998-2009. Clinical Epidemiology. 3, 41-46.

8. Butler, L.M., Sinha, R., Millikan, R.C., Martin, C.F., Newman, B., Gammon, M.D., Ammerman, A.S., Sandler, R.S. 2003. Heterocyclic amines, meat intake, and association with colon cancer in a population-based study. Am. J. Epidemiol. 157, 434-45.

9. Cronin-Fenton, D.P., Erichsen, R., Mortensen, F.V., Dikinis, S., Nørgaard, M., Jacobsen, J. 2011. Pancreatic cancer survival in central and northern Denmark from 1998 through 2009: a population based cohort study. Clinical Epidemiology. 3, 19–25.

10. Cross, A.J., Leitzmann, M.F., Gail, M.H., Hollenbeck, A.R., Schatzkin. A. 2007. A prospective study of red and processed meat intake in relation to cancer risk. PLoS Med. 4, 325.

11. Cross, A.J., Peters, U., Kirsh, V.A., Andriole, G.L., Reding, D., Hayes, R.B., Sinha, R. (2005). A prospective study of meat and meat mutagens and prostate cancer risk. Cancer Res. 65, 11779–11784.

12. Cummings, P. 2009. The relative merits of risk ratios and odds ratios. Arch pediatr adolesc Med. 163, 438-445.

13. Denmark Statistics (2013). http://www.statbank.dk/statbank5a/SelectTable/Omrade0.asp?PLanguage=1.

14. EFSA (2008). Scientific opinion of the Panel on contaminants in the food chain on a request from the European Commission on polycyclic aromatic hydrocarbons in Food. EFSA Journal, 724, 1–114.

15. Ekstrøm, C.L., Sørensen, H. 2011. Introduction to statistical data analysis for the life science. CRC Press, USA.

16. Fu, Z., Deming, S.L., Fair, A.M., Shrubsole, M.J., Wujcik, D.M., Shu, X.O., Kelley, M., Zheng, W. 2011. Well-done meat intake and meat-derived mutagen exposures in relation to breast cancer risk: the Nashville Breast Health Study. Breast Cancer Res. Treat. 129, 919-28.

17. Gkogka, E., Reij, M.W., Havelaar, A.H., Zwietering, M.H., Gorris, L.G.M. 2011. Risk-based estimate of effect of foodborne diseases on public health, Greece. Emerging infectious diseases. 17, 1581-1590.

18. Gunter, M.J., Probst-Hensch, N.M., Cortessis, V.K., Kulldorff, M., Haile, R.W., Sinha, R. 2005. Meat intake, cooking-related mutagens and risk of colorectal adenoma in a sigmoidoscopy-based case-control study. Carcinogenesis. 26, 637—642.

19. Havelaar, A.H., Haagsma, J.A., Mangen, M.J., Kemmeren, J.M., Verhoef, L.P., Vijgen, S.M., Wilson, M., Friesema, I.H., Kortbeek, L.M., van Duynhoven. Y.T., van Pelt, W. 2012. Disease burden of foodborne pathogens in the Netherlands, 2009. Int. J. Food Microbiol. 156, 231-8.

20. Heinz, G., Hautzinger, P. 2007. Meat Processing Technology: For small-to-medium scale producers. FAO report, Bangkok.

21. Hoekstra, J., Hart, A., Boobis, A., Claupein, E., Cockburn, A., Hunt, A., Knudsen, I., Richardson, D., Schilter, B., Schutte, K., Torgerson, P.R., Verhagen, H., Watzl, B., Chiodini, A. 2012. BRAFO tiered approach for risk-benefit assessment of foods. Food Chem. Toxicol. 50, 684-98.

22. Hoekstra, J., Hart, A., Owen, H., Zeilmaker, M., Bokkers, B., Thorgilsson, B., Gunnlaugsdottir, H. 2013. Fish, contaminants and human health: quantifying and weighing benefits and risks. Food Chem. Toxicol. 54, 18-29.

23. Ishibe, N., Sinha, R., Hein, D.W., Kulldorff, M., Strickland, P., Fretland, A.J., Chow, W.H., Kadlubar, F.F., Lang, N.P., Rothman, N. 2002. Genetic polymorphisms in heterocyclic amine metabolism and risk of colorectal adenomas. Pharmacogenetics. 12, 145–150.

24. Iversen, L.H. 2011. Aspects of survival from colorectal cancer in Denmark. Dan. Med. J. 59, 4428.

25. Jägerstad, M., Skog, K. 2005. Genotoxicity of heat-processed foods. Mutation Research. 574, 156–172.

26. John, E.M., Stern, M.C., Sinha, R., Koo, J. 2011. Meat consumption, cooking practices, meat mutagens, and risk of prostate cancer. Nutrition and Cancer. 63, 525-537.

27. Joshi, A.D., Corral, R.,Catsburg, C., Lewinger, J.P., Koo, J., John, E.M., Ingles, S.A., Mariana C.Stern, M.C. 2012. Red meat and poultry, cooking practices, genetic susceptibility and risk of prostate cancer: results from a multiethnic case–control study. Carcinogenesis. 33, 2108–2118.

28. Knutsen, H., Binderup, M.L., Vikse, R., Øvrebø, S. 2007. Vurdering af helserisiko ved konsum av grillet mat. Norges Vitenskapskomiteen for mattrygghet (VKM). In Norwegian, English summary. www.vkm.no.

29. Koutros, S., Cross, A.J., Sandler, D.P., Hoppin, J.A., Ma, X., Zheng, T., Alavanja, M.C., Sinha, R. 2008. Meat and meat mutagens and risk of prostate cancer in the Agricultural Health Study. Cancer Epidemiol. Biomarkers Prev. 17, 80–87.

30. Li, D., Day, R.S., Bondy, M.L., Sinha, R., Nguyen, N.T., Evans, D.B., Abbruzzese, J.L., Hassan, M.M. 2007. Dietary mutagen exposure and risk of pancreatic cancer. Cancer Epidemiol. Biomarkers Prev. 16, 655–661.

31. Lietzen, L.W., Sørensen, G.V., Ording, A.G., Garne, J.P., Christiansen, P., Nørgaard, M., Jacobsen, J. 2011. Survival of women with breast cancer in central and northern Denmark, 1998–2009. Clinical Epidemiology. 3, 35–40.

32. McNutt, L., Wu, C., Xue, X., Hafner, J.P. 2003. Estimating the Relative Risk in Cohort Studies and Clinical Trials of Common Outcomes. Am. J. Epidemiol. 157, 940–943.

33. MDACC (MD Anderson Cancer Center) (2013). Pancreatic cancer survival calculator. University of Texas. http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=pancreascancer.

34. Murray et al., 2013. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 380, 2197–223.

35. Murray, C.J., Lopez, A.D. 1996. The Global Burden of Disease: a comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020. Boston: Harvard School of Public Health on behalf of the World Health Organization and the World Bank.

36. Murray, C.J.L., Lopez, A.D., Mathers, C.D. 2002. Summary Measures of Population Health: Concepts, Ethics, Measurement and Applications. World Health Organization, Geneva.

37. Pedersen, A.N., Fagt, S., Groth, M.V., Christensen, T., Matthiessen, J., Andersen, N.L., Kørup, K., Hartkopp, H., Ygil, H.K., Hinsch, H., Saxholt, E., Trolle, E. 2010. Danskernes kostvaner 2003-2008 Hovedresultater. DTU Fødevareinstituttet.196.

38. Probst-Hensch, N.M., Sinha, R., Longnecker, M.P., Witte, J.S., Ingles, S.A., Frankl, H.D., Lee, E.R., Haile, R.W. 1997. Meat preparation and colorectal adenomas in a large sigmoidoscopy-based case-control study in California (United States). Cancer Causes and Control. 8, 175–183.

39. Punnen, S., Hardin, J., Cheng, I., Klein, E.A., Witte, J.S. 2011 Impact of Meat Consumption, Preparation, and Mutagens on Aggressive Prostate Cancer. PLoS ONE. 6, e27711.

40. Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.L., Griffin, P.M. 2011. Foodborne illness acquired in the United States—major pathogens. Emerging Infectious Diseases 17, 7–15.

41. SCF (2002). Opinion of the Scientific Committee on Food on the risks to human health of polycyclic aromatic hydrocarbons in food. SCF/CS/CNTM/PAH. European Commission Health and Consumer Protection Directorate- General. http://ec.europa.eu/food/food/chemicalsafety/contaminants/out153_en.Pdf.

42. Sinha, R., Chow, W.H., Kulldorff, M., Denobile, J., Butler, J., Garcia-Closas, M., Weil, R., Hoover, R.N., Rothman, N. 1999. Well-done, grilled red meat increases the risk of colorectal adenomas. Cancer Research. 59, 4320–4324.

43. Sinha, R., Kulldorff, M., Chow, W.H., Denobile, J., Rothamn, N. 2001. Dietary intake of heterocyclic amines, meat-derived mutagenic activity, and risk of colorectal adenomas. Cancer Epidemiol. Biomarker and Prev. 10, 559–562.

44. Sinha, R., Peters, U., Cross, A.J., Kulldorff, M., Weissfeld, J.L., Pinsky, P.F., Rothman, N., Hayes, R.B. 2005. Meat, meat cooking methods and preservation, and risk for colorectal adenoma. Cancer Res. 65, 8034–8041.

45. Steck, S.E., Gaudet, M.M., Eng, S.M., Britton, J.A., Teitelbaum, S.L., Neugut, A.I., Santella, R.M., Gammon, M.D. 2007. Cooked Meat and Risk of Breast Cancer—Lifetime versus Recent Dietary Intake. Epidemiology. 18, 373–382.

46. Stolzenberg-Solomon, R.Z., Cross, A.J., Silverman, D.T., Schairer, C., Thompson, F.E., Kipnis, V., Subar, A.F., Hollenbeck, A., Schatzkin, A., Sinha, R. 2007. Meat and meat-mutagen intake and pancreatic cancer risk in the NIH-AARP cohort. Cancer Epidemiol Biomarkers Prev. 16, 2664–2675.

47. Sundhedsstyrelsen (2004). Cancer incidens i Danmark 2000/Cancer Incidences in Denmark 2000. Version: 1. National Board of Health.

48. Sundhedsstyrelsen (2010), Danish Cause of Death in 2009.

49. WHO (2008). The global burden of disease: 2004 update. Geneva: WHO.

50. WHO/FAO (2004). Risk assessment of *Listeria monocytogenes* in ready-to-eat foods.Technical report of microbiological risk assessment series 5, WHO, Geneva.

51. Wu, K., Giovannucci. E., Byrne, C., Platz, E.A., Fuchs, C., Willett, W.C., Sinha, R. 2006. Meat mutagens and risk of distal colon adenoma in a cohort of US men. Cancer Epidemiol. Biomarker and Prev. 15, 1120–1125.

52. Zheng, W., Gustafson, D.R., Sinha, R., Cerhan, J.R., Moore, D., Hong, C.P., Anderson, K.E., Kushi, L.H., Sellers, T.A., Folsom, A.R. 1998. Well-done meat intake and risk of breast cancer. J. Natl. Cancer Inst. 90, 1724–1729.

Chapter 9

Discussion

9. Discussion

The main work performed in this PhD thesis includes: 1) a case study on risk-benefit assessment of cold-smoked salmon (Chapter 6 – Berjia et al., 2012); 2) method development for finding the optimum scenario in risk-benefit assessment: an example on vitamin D (Chapter 7 – MANUSCRIPT I); and 3) Burden of diseases estimates associated to different red meat cooking practices (Chapter 8 – MANUSCRIPT II).

This chapter is divided into four subsections which discuss the main findings of the different studies performed in this PhD-project. In addition, the challenges in performing comprehensive integrated quantitative risk-benefit assessment are discussed explicitly.

9.1 Risk-benefit assessment of cold-smoked salmon: microbial risk versus nutritional benefit

As indicated in chapter 4, several qualitative and quantitative risk-benefit assessment on fish have been conducted both at national and international level. Fish has been a target for several risk-benefit assessment studies; this is because fish contains multiple beneficial and deleterious components that have considerable health effect. Most of these studies have been comparing the beneficial effect of fish as a source of omega-3 fatty acids, particularly EPA and DHA, and the risk of chemical contaminants. However, fish also contains vitamins (for example, A, B-complex, and D) and minerals (such as selenium, iodine, iron, and zinc) that are linked to various health benefits (Delange and Lecomte, 2000; Rayman, 2000; WHO, 2002). Besides the multiple beneficial components, there are several potential health hazards linked to fish consumption, including pathogens, marine toxins, environmental pollutants and heavy metals (Yasumoto and Murata, 1993; Plessi et al., 2001; Storelli et al., 2003; Iwamoto et al., 2010). With regard to risk-benefit assessment of fish, the health benefit of fish intake lies in the reduction of CHD and stroke, as well as improved cognitive development; and the health risk primarily originates from contaminants like methyl mercury, but also dioxin and dioxin-like PCBs have been the focus area (Cohen et al., 2005; Guevel et al., 2008; Hoekstra et al., 2013b).

Even though pathogens may offer a serious risk to human health linked to fish consumption, this can usually be tackled by proper cooking, storage and handling. This is an important difference

compared with chemical contaminants. The adverse health effects of pathogens connected to fish consumption were not integrated in risk-benefit assessment of food so far. Nevertheless, the disease burden linked to pathogens may be considerable if the pathogens that may be present in fish are not overcome by proper cooking, storage and handling. This aspect has been taken into consideration through the study performed in the present thesis where the risk of *Listeria monocytogenes* and the benefit of fish consumption are compared quantitatively (Berjia et al., 2012). The aim of the study was to illustrate how a microbiological hazard can be included in risk-benefit assessment of food and how this may add to the existing risk-benefit assessment tools and methodologies. Two consumption scenarios were considered: a reference scenario (current mean intake in Denmark, 23g/day and 20g/day for man and woman respectively) and an alternative scenario (40g/day for both sexes).

The results of the study indicate that considerable health benefit is obtained through the reduction of CHD mortality and IQ improvement when changing from the reference to the alternative scenario. In Hoekstra et al. (2013b) fish risk-benefit assessment study, the health benefit was higher for stroke than IQ effect, whereas, in Berjia et al. (2012) study the health benefit is higher for IQ than stroke. This difference is related to the fact that salmon is an oily fish that have a more significant effect on IQ compared with other fish species. In addition, in this study the assessment focused on only the intake of cold-smoked salmon, whereas in Hoekstra et al. (2013b) a general fish intake is considered. Among the risk of *L. monocytogenes,* septicemia scores a higher DALY loss followed by abortion and meningitis. In addition to the quantitative result obtained, the study illustrates how microbial hazard can be included in risk-benefit assessment of food.

The sensitivity analysis reveals that the shift of the net public health benefit to net public health risk is observed when cold-smoked salmon is consumed at five weeks of storage and more. This indicates that if food is not properly processed, handled and stored, the microbiological hazard could be a serious public health loss, which may leads to a shift from net health benefit to risk. Hence, integrating microbial hazards (when relevant) into risk-benefit assessment of food may be

important. In addition, the result of the sensitivity study suggests that the health effect of food consumption can be optimized by modulating the food processing, handling and storage.

In this study only one hazardous (*L. monocytogenes*) and one beneficial component (omega-3 fatty acid) were considered. Since the main aim of the study was to show how microbial hazard can be included in risk-benefit assessment, other beneficial component such as vitamin D and hazardous component such as methylmercury were not included in the assessment. The pronounced negative health effect of methylmercury is on prenatal cognitive development. If methylmercury was included in the assessment, the quantitative result of the study may change (the net benefit may reduce slightly), but the balance of the health effect would likely remain the same. This is because the negative health effect of methylmercury is relatively minor compared to the positive health effect (CHD mortality, stroke and IQ) obtained from omega-3 fatty acids (Hoekstra et al., 2013b).

Moreover, other endpoints such as febrile gastroenteritis (due to exposure to *L. Monocytogenes*) that are related to the identified components are not considered in our assessment. We have only considered cold-smoked salmon components and respective endpoints that have a large public health impact, for which suitable quantitative data for the purpose of the study were reported. For instance, it is well known that infection by *L. monocytogenes* leads to febrile gastroenteritis (WHO/FAO, 2004; Allerberger and Wagner, 2010; Jensen et al., 2010) in addition to septicemia, abortion and meningitis. Nevertheless, the incidence and mortality for febrile gastroenteritis are relatively less reported or it is reported as a part of general listeriosis (Allerberger and Wagner, 2010; Jensen et al., 2010).

Compared to several classical QMRA studies, we have considered the specific clinical syndrome of *L. monocytogenes*. In classical QMRA, the assessment usually considers generic clinical syndrome of *L. monocytogenes*, which is listeriosis. But, in this study, we explicitly assessed the health impact of the specific clinical syndrome (septicemia, meningitis and abortion). The advantage of considering the specific clinical syndromes of a disease is that it would take into account the health loss from all the specific clinical syndromes that contribute to the net health effect. In classical QMRA this is may not be important when the probability of the disease and number of

cases are of the greatest interest. For instance, in several QMRAs it is common to find cases of listeriosis, campylobacteriosis and salmonellosis instead of the specific clinical syndromes. If only the generic disease is included in integrated quantitative risk-benefit assessment, it means that the incidence, severity weight, mortality and duration of the specific disease are not included in the assessment. This will lead to miscalculation of the net health impact. Hence, it is crucial to consider the specific disease (when relevant) instead of the generic disease associated with the pathogens in integrate quantitative risk-benefit assessment.

In addition to the specific clinical syndromes, some disease may have sequelae, recurrent effect and other complications; these were not considered in the present study. The present DALY models do not allow the estimation of health effect for sequelae and recurrent disease, but seemingly the model can be extended to take into account these health effects in the future.

In the study, several assumptions are used; many of the assumptions related to the dose-response modelling such as during the conversion of the relative risk to absolute risk for CHD mortality and stroke. Often, national data for the dose-response modeling are scarce. A surrogate from analogous countries or international data had to be used; for instance, the relative risk data for CHD and stroke. Then, to convert the relative risk to probability of onset of the diseases, it was assumed that the current incidences are associated with the current intake, which apparently also depends on other comorbidities. In addition, a few aggregate data have been used to model the dose-response relationship of the hazardous endpoints. However, the results of the sensitivity analysis indicate that apart from the storage time, the analyzed parameters for sensitivity have trivial impact on the baseline result. Still, due to the several assumptions and simplifications considered for the purpose of the study, the quantitative results should be interpreted with care.

The study is a comprehensive integrated risk-benefit assessment that quantified the health loss and gain of the selected hazardous and beneficial components linked to cold-smoked salmon consumption. As explained in chapter 3, there are few approaches on risk-benefit assessments of food (EFSA, 2010; Hoekstra et al., 2012; Fransen et al., 2010). These approaches suggest a stepwise risk-benefit assessment method, where one could stop the assessment at the early steps,

if it is clear that the benefit outweighs the risk or vice versa. This implies that the net health effects in DALY may not be estimated using a common health metric because a common health metric is not applied at the earlier steps in the existing risk-benefit assessment methodologies. The main advantage of performing comprehensive quantitative risk-benefit assessment of food is that, it helps to weigh the quantity of health loss and gain related to the hazardous and beneficial components of food, using a common scale. In addition, together with sensitivity analysis, a comprehensive assessment helps to identify the area where the potential risk can be reduced and the benefit can be maximized, so that it enables an optimization of the net health effect of food consumption, for example by changing the food processing and storage conditions.

In summary, the cold-smoked salmon case study showed how the microbial hazard can be integrated in risk-benefit assessment of food. Besides this, it provides future implications for the improvement of the existing risk-benefit assessment of food method with regard to the importance of consideration of specific endpoints instead of generic endpoints linked to pathogens and extension of the current DALY model to consider recurrent health effects, sequelae and other complications of a disease. In addition, it indicates the importance considering pathogens in risk-benefit assessment of food (when relevant), as pathogens can be a serious health problem in case of improper processing and handling which give a substantial health loss. Moreover, it shows the importance of a thorough quantitative risk-benefit assessment of food, which enables to combine the health effects of the hazardous and beneficial components to determine the net health effect.

9.2 Finding the optimum scenario in risk-benefit assessment: an example on vitamin D

In chapter 7, a method for finding the optimum scenario that provides maximum net health gain in risk-benefit assessment of nutrient is developed, using vitamin D as an example (MANUSCRIPT I). This section discusses this method, its implication to general food risk-benefit assessment and what needs to be considered to find the optimum scenario in holistic risk-benefit assessment of food (nutrients, pathogens and chemical contaminants).

Several recent studies suggest determination of the optimum scenario as a future research issue in health risk-benefit assessment of food (Boobis et al., 2013; Hellberg et al., 2012). Specific to fish consumption, a risk-benefit analysis approach to determine optimal fish consumption that focus on nutrients and chemical contaminants has been proposed by Sirot et al. (2012). This approach optimizes the fish intake based on the condition to attain the recommended nutritional intake and limit the exposure to contaminants based on the condition that the level of contaminants does not exceed the tolerable upper intake level. The approach takes into account the background intakes during optimization (Sirot et al., 2012).

Several nutrients can have dual health effects; both beneficial and detrimental (see section 2.1.3 and 2.3) effects are found at different intake levels. For these types of nutrients it is imperative to determine the intake level where maximum health gain can be attained to improve public health linked to food consumption. Vitamin D is one of the essential nutrients that have dual health effects at high and low intake levels. Compared to the other essential nutrients, there are several convincing evidences that vitamin D prevents against osteoporosis disease in elderly population (IOM, 2010; Bischoff-Ferrari et al., 2009; Bischoff-Ferrari et al., 2005; Fødevareinstituttet, 2010). On the other hand there are emerging possible evidences that vitamin D could increase the risk of total mortality at both low and high intake, which is a very severe health effect that affects a large proportion of the population (Durup et al., 2012; Michaelsson et al., 2010). This implies that determining the optimal vitamin D intake or serum level, that provides maximum benefit and minimum potential risk, can help to prevent considerable public health losses and reduce the economic expenditure spent to prevent the health losses. To the author's knowledge there is currently no internationally agreed method that enables to find the optimum intake or serum level that provides maximum health gain. With respect to vitamin D, there are limited studies that determine the optimal vitamin D intake or serum level needed to prevent some specific health effects (Dawson-Hughes et al., 2005; Bischoff-Ferrari et al., 2006; Durup et al., 2012). In these studies, the optimal vitamin D intake or serum level is determined semi-quantitatively by considering only either the beneficial health effects or detrimental health effects (but not combined), without integrating them in a common health scale like DALY. These aspects have been taken into account in the study performed on MANUSCRIPT I.

In MANUSCRIPT I, a method for finding an optimum scenario in risk-benefit assessment that provides maximum net health gain is developed, using vitamin D as an example. In the method an aggregate of health effects of vitamin D are considered, both beneficial and detrimental. The method is based on multiple scenario simulations. In addition to the reference scenario, several alternative scenarios are simulated to detect the scenario that provides maximum net health gains, using DALY as a health metric and implemented in the QALIBRA software. The study demonstrates how the optimum scenario that provides maximum net health can be determined for a nutrient.

The health effects of vitamin D considered in the assessment encompass endpoints that have convincing evidences are fall, hip and nonvertebral fractures. In addition, an emerging severe health effect of vitamin D (total mortality) is considered. The result reveals that, among the simulated scenarios, the optimum vitamin D serum level that provides maximum net health gain is 72 nmol/l. The healthy life year's gain due to the prevention of total mortality is larger compared to the other endpoints, when the optimum vitamin D serum level is attained. This is because total mortality has a severe health effect, with a severity weight of w = 1, and affects a large proportion of the population, which implies that the achievement of the optimum vitamin D serum level expectedly provides substantial healthy life years gain due to the prevention of total mortality.

The optimum vitamin D serum level that provides maximum net health gain might be different for the different ages and sexes. However, in our assessment the optimum vitamin D serum level was determined for the general population. This is because the mean dietary vitamin D intake of the population was considered in the first place for all ages, separately for men and women. In addition, the mean dietary vitamin D intakes of men (3.8 µg/d) and women (3.1 µg/d) are not substantially different; especially when the intakes in µg/d is related to nmol/l, the differences are even more trivial (which varies between 41-43 depending on the data points used for conversion, see chapter 7. Furthermore, the dose-response relationship for men and women were similar for men and women; due to this the estimated relative risks were not substantially different for men and women for all the endpoints either (see table 4 in chapter 7). As a result, the probabilities of

onset of the diseases (especially for fall and fractures) as well as DALY were comparable for men and women (see figure 3 in chapter 7).

In the study, several necessary assumptions and simplifications had to be made. First, not all the health effects associated to vitamin D are included in the study. There are several studies that suggest the association of vitamin D with different diseases (both beneficial and detrimental) (EFSA, 2010; IOM, 2010; Grant et al., 2009). However, in this study only health effects with convincing evidence, in addition we have included an endpoint where the evidence is relatively weak, but the reported quantitative data on the dose-response relationship are particularly suitable for the purpose of our study (total mortality). In addition, the optimum scenario is determined based on the vitamin D blood serum level in nmol/l. All the intakes given in µg/d and IU/d in the reference intake as well as in the dose-response studies incorporated in this study were related to serum level. By a lack of data, this relation was established based on a few data points only. Moreover, to estimate the relative risk parameters for the dose-response relation, only few data points were available and extrapolation was needed, which is not common. Furthermore, the duration of the endpoints required for DALY estimation are based on a fixed value, which do however vary depending on ages and sexes of the population. Due to all these assumptions and simplifications, the quantitative result should be interpreted with care. Nevertheless, the result obtained in this study is in line with other studies (Dawson-Hughes et al., 2005; Bischoff-Ferrari et al., 2006).

In addition to the result obtained in this study, the assessment suggests what data and methods are required to find the optimum scenario that provides maximum health gain in particular to vitamin D and in general for nutrients that have both beneficial and hazardous health effects. For instance, the data needed to relate the nutrient intake to serum level are scarcely collected, implying that more data are needed. The importance of converting the intake to serum level (when needed) is that it reduces the uncertainty related to the nutrient after ingestion during intake-disease modeling, because etiologically the serum level is closer to the effect of vitamin D than the intake.

Despite the limitations mentioned above, the method seems to be applicable for the determination of an optimum serum or intake level that provides maximum net health gain in nutrient risk-benefit assessment. As a means of finding the optimum scenario in nutrients, only a multiple scenario simulation in comparison with reference level was applied, using a common health metric, DALY. Because the mean vitamin D intake was considered from all dietary sources and converted to serum level, it was not necessary to include food processing parameter optimization to optimize the health effect of vitamin D. If the vitamin D intake from a specific food such as salmon was assessed, other factors that affect vitamin D concentration in salmon processing could be considered to achieve the optimum health effect. For instance, if vitamin D is sensitive to some of the salmon processing pathway, the parameters in the pathway could be identified and enhanced to maximize the net health gain of vitamin D. However, with regard to nutrients, experiences show that often there seem to be insufficient quantitative data to model the intake and health effect of nutrients following food processing pathway. Hence, multiple scenario simulation seems to be the most effective method to optimize the health effect of nutrients.

9.2.1. Health impact optimization in holistic food risk-benefit assessment

Section 9.2 discussed a method used to find an optimum scenario in risk-benefit assessment of a nutrient, with an example on vitamin D. Based on chapter 6, 7 and section 9.2, this section suggests some ideas that may help to optimize the health outcome of food consumption in holistic risk-benefit assessment of food (pathogens, chemical contaminants and nutrients).

The concentration of nutrients, chemical contaminants and pathogens in foods could be affected by the processing pathway involved in the food chain. Hence, it is may be important to identify the processing parameters that affect the concentration. These parameters can then be included in a model that describes the effect of food processing on exposure, like in Berjia et al. (2012). Such a model can then be used to explore where maximum net health gain can be attained.

Depending on the characteristics of the hazardous and beneficial components being assessed, the parameters that affect the concentration of the components need to be identified. Usually, these parameters are associated with food processing pathway such as temperature-time of cooking. However, sometimes it may also be important to consider what happens after ingestion, depending on what is being assessed, the availability of quantitative data and the expected health impact of the component. For instance, some nutrients bioavailability could be improved by processing (Gibson et al., 2006); in this case it is useful to build a quantitative model including the processing, bioavailability and disease probability in order to describe the net health gain. The study by Gibson et al. (2006) shows how the nutrient bioavailability can be improved by different methods and its direct association with reduction of several diseases. For example, bioavailability can be improved by microbial fermentation or soaking that reduces the phytate and polyphenol content of cereals; addition of ascorbic acid-containing fruits to enhance non-haem-Fe absorption; heating to destroy heat-labile anti-nutritional factors such as goitrogens and thiaminases (Gibson et al., 2006). Diet-related issues in foods that affect bioavailability may comprise: the nature of the food matrix; interactions of nutrients and organic components (e.g. phytate, polyphenols, dietary fiber, oxalic acid, protein, fat, and ascorbic acid); pretreatment of food as a result of processing (Gibson et al., 2006).

If a food contains beneficial nutritional components, pathogens and chemical contaminants, processing can affect them in different ways. Conditions like thermal processing and shortening the storage time of the food can limit the growth and survival of the pathogens. Similarly, the benefit of the nutrients could be increased by improving their bioavailability by processing and minimizing nutrient loss during processing. The chemical contaminants that may be formed during processing (e.g. disinfection, fermentation or grilling) e.g. acrylamide and PAHs, can also be minimized by adjusting the processing. Therefore, modulating of the food processing parameters associated to the components may be suitable to optimize the net health impact food consumption. Modulating the food processing parameters may not always necessarily result in the reduction of the health effect of hazardous component of a food; in certain cases it may also cause disease. For instance, when meat is cooked at high temperature, pathogens could be inactivated but at the same time carcinogenic chemical contaminants such as HCAs and PAHs may be formed.

In addition, heat sensitive beneficial nutrients can be destroyed when cooking at high temperature. Hence, modulating food processing parameters should be balanced from the perspective of harmonized health effects of nutrients, chemical contaminants and pathogens in holistic risk-benefit assessment of food. As a result, the net health impact would be optimized.

In addition to food processing, various beneficial substances can be added to optimize the public health effect of food consumption. Enriching the food with a nutrient that has a beneficial impact on the identified endpoints, will increase the benefit; in this case, it is vital to take into consideration the background intake of the nutrient needed to be enriched as well as the potential negative health consequence due to the enrichment (Hoekstra et al., 2008). Other substances may be added to reduce risks. For instance, if the assessment considers pathogens, then the use of organic acid to reduce the concentration of the pathogens can be considered. For example, the addition of antioxidant such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl paraben (PP) helps to control the growth of fungus and synthesis of aflatoxin, which is a potent carcinogen (Passone et al., 2009). Also, concerning chemical contaminants, the addition of calcium chloride, spices and certain food additives can significantly reduce the formation of acrylamide and PAHs during thermal processing of food (Gökmen and Şenyuva, 2009; Badry, 2010). However, when maximizing the benefit through food enrichment and minimizing the risk by addition of antimicrobial or antioxidant or other means, there could be an associated risk that increases as a consequence of the assessment. When there is such an associated risk it should be considered as a part of the assessment.

In broad context, one might consider applying hurdle technology and CCPs (Critical Control points) to minimize the risk of pathogens and chemical contaminants and to improve the quality of food. Hurdle technology is a technique of guaranteeing that pathogens in food products can be eliminated or controlled, so that the food product will be safe and will have extended shelf-life (Leistner and Gould, 2002). Examples of hurdles may include high temperature processing, low temperature during storage, increasing the acidity, lowering the water activity or the addition of preservatives (Leistner, 2000; Alasalvar, et al., 2010). Hurdle technology often focuses on pathogens and shelf-life extension of food. HACCP (Hazard analysis and critical control points), particularly critical control points (CCP), focuses on all hazards that may present in a food

(chemical, biological and physical hazards). CCP is a step in HACCP at which controls can be applied and a hazard can be eradicated or reduced to acceptable levels (Allie, 2004). The application of the combined effect of hurdle technology and CCP (when relevant) help to make food safe, but also improve the food quality, nutritional quality and economic viability of food products by using the synergistic effects of hurdles (Leistner, 1994; Leistner, 2000; Mayes, 1998; Leistner and Gould, 2002; Giovannucci, et al., 2000; Alasalvar, et al., 2010).

Once the health effect is optimized by different methods, alternative scenarios could be simulated in risk-benefit assessment models, in order to determine how much of that food or food component has to be consumed to achieve the optimal health effect.

The practical application of the suggestions mentioned above for the optimization of the health effects of nutrients, chemical contaminants and pathogens in holistic comprehensive integrated quantitative health risk-benefit assessment of food may face certain limitation especially, with respect to the availability of data. The lack of several data for comprehensive health risk-benefit assessment of food has also been encountered in the main studies performed in this PhD thesis (chapter 6, 7 and 8). Nevertheless, it gives an interesting insight of the data needed and helps to guide future data collection.

9.3 Burden of diseases estimates associated to different red meat cooking practices

Compared to the disease attribute to foodborne pathogens and nutritional deficiency, the burden of disease related to chemical contaminants in food is not well investigated. As explained in section 2.1.2, some food processing practices can lead to the formation of chemical contaminants that are deleterious to human health; and to the author's knowledge there is currently no information about the burden of disease connected to food processing practices. Chapter 8 presents a burden of diseases estimates study associated to the different red meat cooking practices (MANUSCRIPT II). In this section, the major findings of MANUSCRIPT II are discussed.

In MANUSCRIPT II, the burden of disease in Denmark due to the consumption of barbecued, fried and roasted red meat was compared. The result obtained in MANUSCRIPT II shows that the consumption of barbecued red meat leads to a larger health loss than the consumption of fried and roasted red meat. Several healthy life years' loss can be reduced in the population, if roasted

red meat is consumed instead of fried and barbecued red meat. The study indicates that the choice of cooking practice has an impact on the prevention of health loss.

In contrast with the burden of disease studies of foodborne pathogens and nutritional deficiency diseases (Gkogka et al., 2011; Havelaar et al., 2012; Murray et al., 2013) and risk-benefit studies of food and food components (Berjia et al., 2012; Hoekstra et al., 2013b), this study presents the burden of disease estimates of different cooking practices. In this study we have ignored the burden of disease linked to pathogens. Apparently, in meat products cooked at high temperature, the burden of disease linked to pathogens is likely to be trivial. In addition, the health benefits and risks associated with the nutrients in red meat were not included in this study because the current available data related to the effect of the different cooking practices on the concentration of the nutrients are insufficient to estimate their health outcomes. As we assume the diets, except for the cooking practice, to be basically unaltered, the nutritional benefits will be highly similar when different cooking practices are compared.

The knowledge of the health loss from different attributions give an insight to prioritize the major causes of health loss in the population and support risk management decisions on control and prevention of foodborne disease. Usually, statistics on the public health impact of foodborne diseases focus on the burden of illness in a population (incidence) (Adak et al., 2002; Scallan et al., 2011). DALY integrates incidence, severity, mortality, recovery and duration of a disease (Murray et al., 2002; Hoekstra et al., 2012). Hence, the burden of disease estimate is a relatively powerful tool to report comprehensive public health statistics, which facilitates comparison between diseases in terms of healthy life year's loss.

A major problem in this study was that the epidemiological data to model the dose-response relationship are scarce and not reported uniformly. In MANUSCRIPT II, most of the dose-response data incorporated is collected through interviews about cooking practices applied and consumption of cooked red meat in different countries. Then, these data were combined with the consumption data to predict the odds ratio or relative risk of the different cancers. The red meat consumption data of these studies are reported differently and are highly variable (see section 2.4

of chapter 8). When using the outcome of these studies to estimate burden of disease based on the Danish red meat consumption data, several model assumptions had to be made to make the best use of the available data. For instance, the studies incorporated for the dose-response modeling describe the intake qualitatively or quantitatively and for some endpoints only a few data points were used to fit the equations to estimate the relative risks. One of the major assumptions was the frequencies in which people in Denmark barbecue, fry and roast red meat, for which we did not find any data. In addition, for the red meat intakes that are given qualitatively in the studies included for the dose-response relationship, we assume current Danish red meat intake as a surrogate. The assumptions considered in the study are more than the ones mentioned in this section (see chapter 8 for details).

Despite the limitations, the results give an interesting impression of the impact of the different cooking practice on health. Moreover, the study shows how the burden of disease of the different cooking practices can be calculated, both at a population level and on an individual level. The method developed used can offer a tool that estimates the health impact of a preferred cooking practice for an individual of a specific age and sex, compared to an alternative cooking practice. The method applied in this study can be used as a basis for similar studies in the future. This may provide useful information to the consumer that can decide whether the perceived benefit of a cooking practice (e.g. better flavor and texture) outweighs the associated estimated risk of cancer in terms of healthy life days lost. In this study, the risk of cancers associated with red meat intake cooked in different ways are considered, the benefit can be seen as the associated perceived food quality (texture, color, flavor) of the different cooking practices. Future study may need to integrate and compare the health effect of the cooking practices with the associated perceived quality.

9.4 Challenges in performing integrated quantitative health risk-benefit assessment of food

With the increasing interest of knowing the net health impact of food consumption and burden of foodborne disease estimate attributed to food, integrating the health impact of nutrients, chemical contaminants and pathogens has become a core issue for public health improvement related to food consumption. Nevertheless, there are still challenges to be addressed when

integrating the different health impacts of food consumption. The main studies performed in this PhD thesis (chapter 6, 7 and 8) support the identification of these challenges, which could be addressed in future risk-benefit assessment studies. This section discusses the major challenges observed in present risk-benefit assessment of food studies.

Concerning the differences in intake or exposure assessment of nutrients, chemical contaminants and pathogens, the exposure to pathogens is frequently modeled by describing a food pathway because pathogens can multiply, survive and be inactivated during food processing. On the other hand, in assessing the intake of nutrients and chemical contaminants the effect of food processing on the concentration of these components is often neglected.

Most risk-benefit assessments of food and burden of foodborne studies, including the studies conducted in this PhD thesis, predict the health outcome only by comparing a limited number of hazardous and beneficial components of food. This is due to the demanding nature of all inclusive comprehensive quantitative risk-benefit assessment.

When modeling the dose-response of nutrients, chemical contaminants and pathogens, different approaches are used. For instance, nutrients like vitamin D are usually modeled in terms of achieved serum concentration or intake and relative risks obtained from epidemiological studies. Using the serum concentration as a dose is expected to give a better prediction of probability of disease than the intake, because, clinically, the disease onset is more closely linked to the serum concentration than intake. Additionally, for nutrients or chemical contaminants that are modeled based on intake, the predicted probability of disease prevention or occurrence would be relatively uncertain due to, for example, not considering the bioavailability. Similarly, modeling of pathogens or their toxins by exposure would be uncertain due to not considering what happens after ingestion. Concerning chemical contaminants, from research ethics point of view, animal studies are often used to investigate the association of chemical contaminants with disease. To use the outcome of animal studies, further extrapolation is required to apply the results to human situation. At the other hand, dose-response models for microbial pathogens are usually based on human outbreak data or volunteer studies, because good animal models are lacking. These models describe the probability of infection or illness as a function of dose at intake. Usually the health effects of pathogens due to food consumption are acute, whereas, the health effects of nutrients

and chemical contaminants are chronic. These differences make it difficult to compare the outcome of the assessment.

The other challenge is integrating the risk and benefit using a common health metric to predict the net health impact of the positive and negative effects associated with consumption of food products or food components. Traditionally, the recommended and tolerable intake limits for nutritional and toxicological component of food have been used as a reference value. However, the application of these reference values results in a separate risk and benefit assessment and are not predictive of the health loss or health gain quantitatively. Currently, there is no internationally agreed robust model for integrating the health outcomes of beneficial and hazardous components; DALY or QALY is often considered a suitable common health metrics. However, there are still challenges when using common health metrics. For instance, it is under discussion whether or not DALY calculation should considers discounting, age weighting, social demographics, health economics, recurrent health effect and other disease complications (Robberstad, 2005; Sassi, 2006; Hart et al., 2013).

One of the major challenges in performing integrated risk-benefit assessment of food and foodborne burden of disease studies is the unavailability of data, which leaves both the assessor and the recipient of the assessment uncertain about the interpretation of the final outcome. Experience shows that, when performing integrated health risk-benefit assessment of food, data scarcity can happen anywhere from exposure assessment to integrating the various health impacts of the different components of food. Usually, parameter values related to the use of common health metrics such as data on a disease linked to probability of recovery, survival, severity and duration are less commonly reported than incidence and mortality rate data.

The genetic variation within the human population in relation to the impact of exposure to hazardous and beneficial components of food and prediction of the associated health effect is not considered in risk-benefit assessment of food. Obtaining specific quantitative estimates of probability of disease occurrence and prevention for specific population groups in relation to genetic background and susceptibility is complex and challenging. There is currently no adequate quantitative data that support the quantification of health effect of hazardous and beneficial components of food linked to genetic variation of the population. However, the emerging “Omics”

research seem to be promising and may help to consider interspecies variability and susceptibility to disease in reaction to exposure to hazardous and intake of beneficial components of food. “Omics” is a suffix indicating “a totality of some sort”, which in biology is used for very large-scale data collection and analysis, i.e. measuring/profiling a large number of variables simultaneously” (Pielaat et al., 2013). It allows to study the mode of action of compounds or to obtain more insight in processes involved in diseases (Dulin et al., 2013). The added value of “omics” studies to the current classical risk assessment of pathogens, chemical contaminants and some nutrients are suggested in Pielaat et al. (2013).

Chapter 10

Conclusions and future perspectives

10. Conclusions and future perspectives

This section presents the overall conclusions of the main studies conducted in this PhD thesis. In addition, providing the challenges in risk-benefit assessment of food methodologies (see section 9.4), future perspectives that may improve the present risk-benefit assessment of foods are suggested.

10.1 Conclusions

This thesis aimed to further develop methods for health risk-benefit assessment of foods and food components. Three separate but related studies were conducted. The first study focuses on integrating microbial risk and nutritional benefits. The second study focuses on further development of methods for risk-benefit assessment of food, together with a study on vitamin D where a method for finding an optimum intake scenario is explored. The third study focuses on burden of disease study linked to red meat cooking practices. Based on these studies, it was concluded that:

- In risk-benefit assessment of food, integrating microbial hazards may be important, because the healthy life years loss associated with pathogens could be substantial, especially when food is not properly processed, stored and handled.

- When integrating microbial hazards into risk-benefit assessment of food, the specific major clinical syndromes that occur as sequelae of infection are more important than the generic endpoints, which are often used in QMRA. For instance, during infection by *L. monocytogenes*, cases of listeriosis are commonly used as an endpoint in QMRA. But, in an integrated risk-benefit assessment of food, the specific endpoints (in case of infection by listeria: septicemia, meningitis and abortion) should be considered as each specific endpoint has its own mortality, incidence, duration and severity.

- Integrated risk-benefit assessment of food combines the health outcomes of nutrients, chemical contaminants and pathogens when relevant. By altering food processing parameters (to minimize hazards and maximize benefit) and scenario simulation, one can optimize the health outcome of consumption of food. Nevertheless, altering food

processing parameters should be balanced from the perceptive of harmonized health effects of nutrients, chemical contaminants and pathogens in holistic risk-benefit assessment of food. As a result, the net health impact would be optimized.

- A comprehensive integrated quantitative risk-benefit assessment of food using common health metric helps to find the optimum scenario that provides maximum net health gains.
- The burden of disease attributable to different aspects of food helps to rank and prioritize the major health loss in the population and supports to identify the potential mitigation strategy to prevent the health loss.
- In the overall health impact evaluation of food, not only the reduction of health hazard and improvement of the health impact of beneficial components help to optimize the health effect, but also the choice of cooking practice substantially contributes for the achievement of maximum net health gain.
- With regard to the choice of cooking practices, often it is the individual preferences to choose the cooking method by considering the associated risk and benefit. Nevertheless, food authorities consider priorities at population level. Thus, the individual and population level interpretation of the result of the assessment would help to make a better decision on the choice of cooking method

The three studies performed in this thesis focus on three specific problems, combined they address the issue of health risk-benefit assessment of food or nutrients and burden of disease associated to cooking practices. To summarize, this PhD thesis 1) illustrates how a microbial hazard can be integrated into health risk-benefit assessment of food and indicates that improper storage of food could shift the balance of net health gain to net health loss. Thus, it suggests that optimizing food processing parameters (time, temperature, storage condition and other) together with several alternative scenario simulations may help to achieve best scenario that provides maximum health gain. 2) A method for finding the optimum scenario that provides maximum net health gain was developed, using vitamin D as an example. The method considers only several scenario simulations. The method seems the most effective approach to attain maximum net health gain with respect of nutrients risk-benefit assessment, especially for nutrients that are not

affected during food processing. For holistic risk-benefit assessment of food, food processing parameter optimization, addition of substances together with several scenario simulations can be considered to optimize the health effect of food consumption as discussed in section 9.2.1. 3) In order to show the health impact of food processing practices, burden of diseases estimates have been performed on a selected food item. The last study introduces new approach to estimate the burden of disease of food cooking practices, with an example on red meat. While we know that certain food processing practices induces the formation of hazardous compounds, their disease burden were not estimated. The last study estimates the burden of disease linked to red meat cooking practices and suggests that the choice of food cooking practice has a substantial impact in the prevention of health loss. The method applied in this study can be used as a basis for similar studies in the future.

The three studies presented in this PhD thesis are performed on specific topics. Together, they indicate that risk-benefit assessment and burden of disease studies can make a substantial contribution to public health by considering food processing parameter optimization, several scenario simulations, the choice of cooking practice and comprehensive integrated quantitative health assessment of food.

10.2 Future perspectives

Despite the progressive improvements in method development in risk-benefit assessment of food during the last decade, there are still challenges that have to be addressed in the future. Section 9.4 explicates the major challenges linked to the existing health risk-benefit assessment of food methods. This section remarks future perspectives that may help to further improve the existing health risk-benefit assessment of methods. To shift the theoretical study of health risk-benefit assessment of food to more tangible practical application, it is crucial to consider the following points:

- Additional holistic risk-benefit assessment case studies that includes nutrients, chemical contaminants and pathogens (when relevant) need to be performed in the future. This would enable us to show the combined effect of food processing parameter optimization and multiple scenario simulations in maximizing the net health gain.

- As shown in PAPER 1, improper storage of CSS may lead to the shift of net health gain to net health loss due to the increased risk of the pathogen. This indicates that improper handling, processing and storage of food may have a major impact on the balance of the overall health outcome. Therefore, when relevant, future health risk-benefit assessment of food should consider integration of food processing into risk-benefit assessment of food.

- In QMRA the effect of food processing on the concentration of pathogens is often taken into account during modeling the exposure assessment. A similar approach may need to be considered to estimate the concentration of chemical contaminants and nutrients during intake assessment. This improves the accuracy of the estimate of the dose exposed.

- It may be important to identify all the major associated hazardous and beneficial components in performing integrated risk-benefit assessment of food. In addition, it may be essential to identify all the associated specific health effects instead of generic health effects including recurrent disease, sequelae and any related complications during DALY calculation. This enables to perform comprehensive integrated risk-benefit assessment of food and reduce the imprecision of net health impact estimation.

- Although different approaches are used when modeling the dose-response relationship of pathogens, chemical contaminants and nutrients, it may be necessary to find a consistent modeling approach in the future. For instance, when considering a fish product which possesses nutritional, chemical contaminants and microbial components; the health impact of vitamin D may be estimated as a function of the serum concentration, whereas the health impact of the chemical contaminants and pathogens are often estimated just from the intake and exposure. The negligence of factors that may affect the concentration after intake or exposure may lead to an error in the estimate of the probability of disease for the pathogens and chemical contaminants. Therefore, it may be relevant to take into account the factors that affect the concentration of the beneficial and hazardous components after intake or exposure.

- Common health metric models to integrate the health risk and benefit of food consumption many need further development with regard to disease complication, recurrence and health economy integration.

- In order to perform more efficient health risk-benefit assessment of food, construction of a structured database that contains information about food, nutrient, chemical contaminant, pathogens and disease epidemiology is needed.

- In order to consider individual genetic variations in response to exposure to nutrients, toxic chemicals and pathogens, the emerging nutrigenomics, toxicogenomics and pathogenomics data may need to be incorporated in future health risk-benefit assessment of food.

- Moreover, consumer perceptions studies need to be performed with regard to the choice of food cooking practices and the associated perceived food quality benefits. This helps to weigh the health effect of the preferred cooking practices against the perceived food quality benefits (better texture, flavor, color, and aroma).

- Furthermore, in the overall health impact evaluation of food, the presentation of the result of an assessment of health effects of the hazardous and beneficial components of food, as well as the health impact of choice of food cooking practice, may be insufficient for the recipients (food authorities and consumers). The associated food and nutritional quality aspects need to be included as well. This is because methods that can be applied to optimize the health effect of food or food components could have an important effect on the food and nutritional quality aspects, which eventually can have an effect on consumer perception. Therefore, future studies need to consider both food quality and food safety during the overall evaluation of food consumption.

- The overall aim of evaluation of health impact of food is to improve public health, which requires extra costs to implement methods that make it possible to attain maximum net health gain. Contrary, if the methods implemented are able to attain maximum net health gain, the cost expenditure for the treatment of population health can reduce considerably. This implies that future study needs to consider economic cost-benefit analysis in health risk-benefit assessment of food.

Reference list

1. Aaslyng, MD., Duedahl-Olesen, L., Jensen, K., Meinert, L. (2013). Content of heterocyclic amines and polycyclic aromatic hydrocarbons in pork, beef and chicken barbecued at home by Danish consumers. Meat Science, 93, 85-91.
2. Alasalvar, C., Miyashita, K., Shahidi, F., Wanasundara, U. (2010). Handbook of seafood quality, safety and health applications. Wiley-Blackwell, 576.
3. Alli, I. (2004). Food quality assurance: principles and practices. CRC press.
4. Anderson, KE., Sinha, R., Kulldorff, M., Gross, M., Lang, NP., Barber, C., Hanack, L., DiMagno, E., Bliss, R., Kadlubar, F.F. (2002). Meat intake and cooking techniques: associations with pancreatic cancer. Mutation Research. 506-507, 225-231.
5. Badry, NEL. (2010). Effect of household cooking methods and some food additives on polyaromatic hydrocarbons (PAHs) formation in chicken meat. World applied science journal, 9, 963-974.
6. Berjia, FL., Andersen, R., Hoekstra, J., Poulsen, M., Nauta, M. (2012). Risk-benefit assessment of cold-smoked salmon: microbial risk versus nutritional benefit. EJFRR, 2, 49-68.
7. Berjia, FL., Hoekstra, J., Verhagen, H., Poulsen, M., Andersen, R., Nauta, M. (2013). Finding the optimum scenario in risk-benefit assessment: an example on vitamin D. (MANUSCRIPT I, on preparation).
8. Berjia, F., Poulsen, M., Nauta, M. (2013). Burden of diseases estimates associated to different red meat cooking practices. (MANUSCRIPT II, on preparation).
9. Bischoff-Ferrari, H. (2010). Health effects of vitamin D. Dermatologic Therapy, 23, 23–30.
10. Bischoff-Ferrari, HA., Shao, A., Dawson-Hughes, B., Hathcock, J., Giovannucci, E., Willett, WC. (2010). Benefit–risk assessment of vitamin D supplementation. Osteoporos Int, 21, 1121-1132.
11. Bolaman, Z., Kadikoylu, G., Yukselen, V., Yavasoglu, I., Barutca, S., Senturk, T. (2003). Oral versus intramuscular cobalamin treatment in megaloblastic anemia: a single-center, prospective, randomized open-label study. Clin Ther, 25, 3124-3134.

12. Boobis, A., Chiodini, A., Hoekstra, J., Lagiou, P., Przyrembel, H., Schlatter, J., Schütte, K., Verhagen, H., Watzl, B. (2013). Critical appraisal of the assessment of benefits and risks for foods, 'BRAFO Consensus Working Group'. Food Chem Toxicol, 55, 659-75.
13. Briggs, DJ. (2008). A framework for integrated environmental health impact assessment of systemic risks. Environ Health, 7, 61.
14. Butler, LM., Sinha, R., Millikan, RC., Martin, CF., Newman, B., Gammon, MD., Ammerman, AS., Sandler, RS. (2003). Heterocyclic amines, meat intake, and association with colon cancer in a population-based study. Am J Epidemiol, 157, 434-445.
15. Cacuci, DG., Ionescu-Bujor, M., Navon, IM. (2005). Sensitivity and Uncertainty Analysis, Volume II: Applications to Large-Scale Systems. Chapman & Hall.
16. Cardello, AV., (2003). Consumer concerns and expectations about novel food processing technologies: effects on product liking. Appetite, 40, 217–233.
17. Cohen, JT., Bellinger, DC., Connor, WE., Kris-Etherton, PM., Lawrence, RS., Savitz, DA., Shaywitz, BA., Teutsch, SM., Gray, GM. (2005). A quantitative risk-benefit analysis of changes in population fish consumption. Am. J. Prev. Med, 29, 325–334.
18. Dawson, D. (2005). Foodborne protozoan parasites. Int J Food Microbiol, 103, 207-227.
19. Dawson-Hughes, B., Heaney, RP., Holick, MF., Lips, P., Meunier, PJ., Vieth, R. (2005). Estimates of optimal vitamin D status. Osteoporosis International, 16, 713-716.
20. Delange, F., Lecomte, P. (2000). Iodine supplementation: benefits outweigh risks. Drug Safety, 22, 89–95.
21. Dulin, D., Lipfert, J., Moolman, MC., Dekker, NH. (2012). Studying genomic processes at the single-molecule level: introducing the tools and applications. Nat Rev Genet, 14, 1, 9-22.
22. EFSA, (2006a). EFSA Science Colloquium 6. Risk-benefit analysis of foods: methods and approaches. Scientific Colloquium Series of the European Food Safety Authority No.6.
23. EFSA, (2006b). Guidance of the Scientific Committee on a request from EFSA related to uncertainties in dietary exposure assessment. The EFSA Journal 438, 1–54.
24. EFSA, (2008). Scientific opinion of the Panel on contaminants in the food chain on a request from the European Commission on polycyclic aromatic hydrocarbons in Food. EFSA Journal, 724, 1–114.

25. EFSA, (2010). Guidance on human health risk-benefit assessment of foods. Scientific opinion. EFSA Journal, 8, 1673.
26. EFSA, (2011). Results on acrylamide levels in food from monitoring years 2007- 2009 and exposure assessment. EFSA Journal, 9, 2133.
27. FAO/WHO, (1995). Application of risk analysis to food standards issues. Recommendations to the Codex Alimentarius Commission (ALINORM 95/9, Appendix 5) WHO, Geneva.
28. FAO/WHO, (2005). Food Safety Risk Analysis PART I. An overview and framework manual. Rome, Italy.
29. FAO/WHO, (2010). Joint FAO/WHO expert consultation on the risk and benefit of fish consumption, Rome, Italy.
30. Fischer, ARH., Frewer, LJ. (2009). Consumer familiarity with foods and the perception of risks and benefits. Food Qual Pref, 20, 576–585.
31. Fødevaredirektoratet (2003). Helhedssyn på fisk og fiskevarer. FødevareRapport, 17, http://www.fdir.dk/.
32. Fransen, H., de Jong, N., Hendriksen, M., Mengelers, M., Castenmiller, J., Hoekstra, J., van Leeuwen, R., Verhagen, H. (2010). A tiered approach for risk-benefit assessment of foods. Risk Anal, 30, 808-816.
33. Gibson, RS., Perlas, L., Hotz, C. (2006). Improving the bioavailability of nutrients in plant foods at the household level. Proc Nutr Soc, 65, 160-168.
34. Giovannucci, D., Satin, M. (2000). Food quality issues: understanding HACCP and other quality management techniques. A Guide to Developing Agricultural Markets and Agro-enterprises, 22.
35. Gkogka, E., Reij, MW., Havelaar, AH., Zwietering, MH., Gorris, LGM. (2011). Risk-based estimate of effect of foodborne diseases on public health, Greece. Emerging infectious diseases, 17, 1581-1590.
36. Gladyshev, MI., Sushchik, NN., Anishchenko, OV., Makhutova, ON., Kalachova, GS., Gribovskaya, IV. (2008). Benefit-risk ratio of food fish intake as the source of essential fatty acids vs. heavy metals: A case study of Siberian grayling from the Yenisei River. Food Chemistry, 115, 545-550.

37. Gökmen, V., Şenyuva, HZ. (2009) Acrylamide in heated foods, in bioactive compounds in foods. (eds J. Gilbert and HZ. Şenyuva), Blackwell Publishing Ltd., Oxford, UK.
38. Gold, MR., Stevenson, D., Fryback, DG. (2002). HALYS and QALYS and DALYS, Oh My: similarities and differences in summary measures of population Health. AnnuRev Public Health, 23, 115-34.
39. Guevel, MR., Sirot, V., Volatier, JL., Leblanc, JC. (2008). A risk-benefit analysis of French high fish consumption: A QALY approach. Risk Analysis, 28, 37-48.
40. Gunter, MJ., Probst-Hensch, NM., Cortessis, VK., Kulldorff, M., Haile, RW., Sinha, R. (2005). Meat intake, cooking-related mutagens and risk of colorectal adenoma in a sigmoidoscopy-based case-control study. Carcinogenesis, 26, 637—642.
41. Hart, A., Hoekstra, J., Owen, H., Kennedy, M., Zeilmaker, MJ., de Jong, N., Gunnlaugsdottir, H. (2013). Qalibra: a general model for food risk-benefit assessment that quantifies variability and uncertainty. Food Chem Toxicol, 54, 4-17.
42. Havelaar, AH., De Hollander, AE., Teunis, PF., Evers, EG., Van Kranen, HJ., Versteegh, JF., Koten, V., Joke, EM., Slob, W. (2000). Balancing the risks and benefits of drinking water disinfection: disability adjusted life-years on the scale. Environ Health Perspect, 108, 315-321.
43. Havelaar, AH., Friesema, IHM., van Pelt, W. (2012). Disease burden of food-related pathogens in the Netherlands, 2010. RIVM Rapport 330331004, 33.
44. Havelaar, AH., Haagsma, JA., Mangen, MJ., Kemmeren, JM., Verhoef, LP., Vijgen, SM., Wilson, M., Friesema, IH., Kortbeek, LM., van Duynhoven. YT., van Pelt, W. (2012). Disease burden of foodborne pathogens in the Netherlands, 2009. Int J Food Microbiol, 156, 231-238.
45. Hellberg, RS., DeWitt, CAM., Morrissey, MT. (2012). Risk-benefit analysis of seafood consumption: A review. Comprehensive Reviews in Food Science and Food Safety, 11, 490–517.
46. Hoekstra, J., Hart, A., Boobis, A., Claupein, E., Cockburn, A., Hunt, A., Knudsen, I., Richardson, D., Schilter, B., Schütte, K., Torgerson, P., Verhagen, H., Watzl, B., Chiodini, A. (2012). BRAFO Tiered approach for benefit-risk assessment of foods. Food Chem. Toxicol, 50, 684–698.

47. Hoekstra, J., Verkaik-Kloosterman, J., Rompelberg, C., van Kranen, H., Zeilmaker, M., Verhagen, H., de Jong, N. (2008). Integrated risk–benefit analyses: method development with folic acid as example. Food Chem. Toxicol, 46, 893–909.
48. Hoekstra, J., Fransen, HP., van Eijkeren, JC., Verkaik-Kloosterman, J., de Jong N., Owen, H., Kennedy, M., Verhagen, H., Hart, A. (2013a). Benefit-risk assessment of plant sterols in margarine: a QALIBRA case study. Food Chem Toxicol, 54, 35-42.
49. Hoekstra, J., Hart, A., Owen, H., Zeilmaker, M., Bokkers, B., Thorgilsson, B., Gunnlaugsdottir, H. (2013b). Fish, contaminants and human health: quantifying and weighing benefits and risks. Food Chem Toxicol, 54, 18-29.
50. IFT, (2004). Bacteria Associated with Foodborne Diseases. Scientific Status Summary.
51. IOM, (1998). Folate. In: Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. National academy press, Washington, D.C.
52. IOM, (2007). Institute of Medicine of the National Academies, Committee on Nutrient Relationships in Seafood: Selections to Balance Benefits and Risks, Nesheim MC, Yaktine AL, editors. Seafood Choices: Balancing benefits and risks. National Academies Press: Washington, D.C.
53. IPCS, (2004). Harmonization Project Document No.1 -IPCS risk assessment terminology. WHO, Geneva.
54. Iwamoto, M., Ayers, T., Mahon, BE., Swerdlow, DL. (2010). Epidemiology of seafood-associated infections in the United States. Clin Microbiol Rev, 23, 399–411.
55. Kalogeras, N., Odekerken-Schröder, G., Pennings, JM., Gunnlaugsdóttir, H., Holm, F., Leino, O., Luteijn, JM., Magnússon, SH., Pohjola, MV., Tijhuis, MJ., Tuomisto, JT., Ueland, Ø., White, BC., Verhagen, H. (2012). State of the art in benefit-risk analysis: economics and marketing-finance. Food Chem Toxicol, 50, 56-66.
56. Kemmeren, JM., Mangen, MJJ., van Duynhoven, YTHP., Havelaar, AH. (2006). Priority setting of foodborne pathogens: disease burden and costs of selected enteric pathogens. RIVM Report 330080001, 127.

57. Kim, DH., Smith-Warner, SA., Hunter, DJ. (2001). Pooled analysis of prospective cohort studies on folate and colorectal cancer. Pooling project of diet and cancer investigators. Am J Epidemiol Suppl, 153, 118.
58. Knutsen, H., Binderup, M.L., Vikse, R., Øvrebø, S. (2007). Vurdering af helserisiko ved konsum av grillet mat. Norges Vitenskapskomiteen for mattrygghet (VKM). In Norwegian, English summary. www.vkm.no.
59. Kohlstadt, I. (2009). Food and nutrients in disease management. 740. CRC Press.
60. Koopmans, M., Duizer, E. (2004) Foodborne viruses: an emerging problem. Int J Food Microbiol, 90, 23-41.
61. Kris-Etherton, PM., William, S., Appel, LJ. (2003). Fish consumption, fish oil, omega-3 fatty acids and cardiovascular disease. American Heart Association, 23, 20-31.
62. Leistner, L. (1994). Further developments in the utilization of hurdle technology for food preservation. Journal of Food Engineering, 22, 421–432.
63. Leistner, L. (2000). Review. Basic aspects of food preservation by hurdle technology. International Journal of Food Microbiology, 55, 181–186.
64. Leistner, L., Gould, GW. (2002). Food Design and Risk Assessment. Food Engineering Series, Springer, 59-64.
65. Livsmedelssäkerhetsverket (2006). Tillgänglig på webbplatsen. http://www.evira.fi/portal/se/.
66. Livsmedelsverket, (2007). Risks and benefits of fish consumption. A risk-benefit analysis based on the occurrence of dioxin/PCB, methyl Mercury, n-3 Fatty Acids and vitamin D in fish. Report 12.
67. Luteijn, JM., White, BC., Gunnlaugsdóttir, H., Holm, F., Kalogeras, N., Leino, O., Magnússon, SH., Odekerken, G., Pohjola, MV., Tijhuis, MJ., Tuomisto, JT., Ueland, Ø., McCarron, PA., Verhagen H. (2012). State of the art in benefit-risk analysis: medicines. Food Chem Toxicol, 50, 26-32.
68. Magnússon, SH., Gunnlaugsdóttir, H., Loveren, Hv., Holm, F., Kalogeras, N., Leino, O., Luteijn, JM., Odekerken, G., Pohjola, MV., Tijhuis, MJ., Tuomisto, JT., Ueland, Ø., White, BC., Verhagen, H. (2012). State of the art in benefit-risk analysis: food microbiology. Food Chem Toxicol, 50, 33-39.

69. Mayes, T. (1998). Risk analysis in HACCP: burden and benefit?. Food control, 9, 171-176.
70. Murray et al., (2013). Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet, 380, 2197–2223.
71. Murray, C.J.L., Salomon, J.A., Mathers, C.D., Lopez, A.D. (2002). Summary Measures of population health. Concepts, Ethics, Measurement and Applications. WHO. Chapter 9. How to derive disability weight.
72. Murray, CJ., Lopez, AD. (1996). The Global Burden of Disease: a comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020. Boston: Harvard School of Public Health on behalf of the World Health Organization and the World Bank.
73. NTP, (1999). Report on carcinogens background document for 2-Amino-3-Methylimidazo[4,5-f]Quinoline (IQ). National Toxicology Program. http://ntp.niehs.nih.gov/ntp/newhomeroc/roc10/IQ.pdf.
74. NTP, (2002). Report on Carcinogens Background Document for Heterocyclic Amines: PhIP, MeIQ and MeIQx. National Toxicology Program. http://ntp.niehs.nih.gov/ntp/newhomeroc/roc11/HCAsPub.pdf.
75. Passone, MA., Ruffino, M., Ponzio, V., Resnik, S., Etcheverry, MG. (2009). Postharvest control of peanut Aspergillus section Flavi populations by a formulation of food-grade antioxidants. International journal of Food Microbiology, 131, 211-217.
76. Pielaat, A., Barker, GC., Hendriksen, P., Hollman, P., Peijnenburg, A., Ter Kuile, BH. (2013). A foresight study on emerging technologies: State of the art of omics technologies and potential applications in food and feed safety. REPORT 1: Review on the state of art of omics technologies in risk assessment related to food and feed safety. EFSA supporting publication, EN-495, 126 pp.
77. PlantLIBRA (PLANT food supplements: Levels of Intake, Benefit and Risk Assessment), (2013). http://www.plantlibra.eu/web/node/44.
78. Plessi, M., Bertelli, D., Monzani, A. (2001). Mercury and selenium content in selected seafood. J Food Comp Anal, 14, 461–467.

79. Pohjola, MV., Leino, O., Kollanus, V., Tuomisto, JT., Gunnlaugsdóttir, H., Holm, F., Kalogeras, N., Luteijn, JM., Magnússon, SH., Odekerken, G., Tijhuis, MJ., Ueland, Ø., White, BC., Verhagen, H. (2012). State of the art in benefit-risk analysis: environmental health. Food Chem Toxicol, 50, 40-55.
80. Ponce, RA., Bartell, SM., Wong, EY., LaFlamme, D., Carrington, C., Lee, RC., Patrick, DL., Faustman, EM., Bolger, M. (2000). Use of Quality-adjusted life year weights with dose-response models for public health decisions: A case study of the risks and benefits of fish consumption. Risk analysis, 20, 529-542.
81. Ray, JG., Meier, C., Vermeulen, MJ., Boss, S., Wyatt, PR., Cole, DE. (2002). Association of neural tube defects and folic acid food fortification in Canada. Lancet, 360, 2047–2048.
82. Rayman MP. 2000. The importance of selenium to human health. Lancet, 356, 233–241.
83. Robberstad, B. (2005). QALYs vs DALYs vs LYs gained: What are the differences, and what difference do they make for health care priority setting? Norsk Epidemiologi, 15, 183-191.
84. SACN/COT, (2004). Scientific Advisory Committee on Nutrition/Committee of Toxicity. Advice on fish consumption: benefits & risks. London, TSO.
85. Sand, S., Becker, W., Darnerud, PO. (2008). Aspects of Risk-Benefit Assessment of Food Consumption. Directions for the future. Nordic Council of Ministers, Copenhagen. www.norden.org.
86. Sanjoaquin, MA., Allen, N., Couto, E., Roddam, AW., Key, TJ. (2004). Folate intake and colorectal cancer risk: a meta-analytical approach. Int J Cancer, 113, 825–828.
87. Sassi, F. (2006). Calculating QALYs, comparing QALY and DALY calculations. Health Policy Plan, 21, 402-8.
88. SCF, (2000). Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Folate Report id. SCF/CS/NUT/UPPLEV/18. European Commission, health & consumer protection directorate-general, Brussels.
89. SCF, (2002). Opinion of the Scientific Committee on Food on the risks to human health of polycyclic aromatic hydrocarbons in food. SCF/CS/CNTM/PAH. European Commission Health and Consumer Protection Directorate- General. http://ec.europa.eu/food/food/chemicalsafety/contaminants/out153_en.Pdf.

90. Schrenk, D. (2012). Chemical contaminants and residues in food. Food Science, Technology and Nutrition No. 235, Woodhead.
91. Schütte, K., Boeing, H., Hart, A., Heeschen, W., Reimerdes, EH., Santare, D. Skog, k., Chiodini. (2012). Application of the BRAFO tiered approach for benefit-risk assessment to case studies on heat processing contaminants. Food Chem Toxicol, 50, 724-735.
92. Sioen, I. (2007). The nutritional-toxicological conflict related to seafood consumption. PhD-thesis Ghent University.
93. Sirot, V., Leblanc, JC., Margaritis, I. (2012). A risk-benefit analysis approach to seafood intake to determine optimal consumption. Br J Nutr, 107, 1812-1822.
94. Storelli, MM., Giacominelli-Stuffler, R., Storelli, A., Marcotrigiano, GO. (2003). Polychlorinated biphenyls in seafood: contamination levels and human dietary exposure. Food Chem, 82, 491–496.
95. Tepper, BJ., Trail, AC. (1998). Taste or health: a study on consumer acceptance of corn chips. Food Qual Pref, 9, 267–272.
96. Tijhuis, MJ., de Jong, N., Pohjola, MV., Gunnlaugsdóttir, H., Hendriksen, M., Hoekstra, J., Holm F., Kalogeras, N., Leino, O., van Leeuwen, FX., Luteijn, JM., Magnússon, SH., Odekerken G., Rompelberg, C., Tuomisto, JT., Ueland, Ø., White, BC., Verhagen, H. (2012). State of the art in benefit-risk analysis: food and nutrition. Food Chem Toxicol, 50, 5-25.
97. Tuomisto, J. (2010). BENERIS: Benefit-risk assessment for food: an iterative value-of-information approach. http://en.opasnet.org/w/Beneris.
98. Udagawa, S, Ishinkin, N., Zasshi, G. (2005). Fungal spoilage of foods and its risk assessment. J med mycol, 46, 11-15.
99. Ueland, Ø., Gunnlaugsdottir, H., Holm, F., Kalogeras, N., Leino, O., Luteijn, JM., Magnússon, SH., Odekerken, G., Pohjola, MV., Tijhuis, MJ., Tuomisto, JT., White, BC., Verhagen, H. (2012). State of the art in benefit-risk analysis: consumer perception. Food Chem Toxicol, 50, 67-76.
100. Urala, N., Lähteenmäki, L., (2006). Hedonic ratings and perceived healthiness in experimental functional food choices. Appetite, 47, 302–314.

101. Vanderveen, JE. (2006). Gap analysis guidelines for assessing acute, chronic, and lifetime exposures to high levels of various nutrients. J Nutr, 136, 514–519.

102. van Kleef, E., van Trijp, H.C.M., Luning, P., 2005. Consumer research in the early stages of new product development: a critical review of methods and techniques. Food Qual. Pref. 16, 181–201.

103. Verbeke, W. (2006). Functional foods: consumer willingness to compromise on taste for health? Food Qual Pref, 17, 126–131.

104. Verhagen, H., Andersen, R., Antoine, JM., Finglas, P., Hoekstra, J., Kardinaal, A., Nordmann, H., Pekcan, G., Pentieva, K., Sanders, TA., van den Berg, H., van Kranen, H., Chiodini, A. (2012). Application of the BRAFO tiered approach for benefit-risk assessment to case studies on dietary interventions. Food Chem. Toxicol, 50, 710-723.

105. Verhagen, H., Tijhuis, MJ., Gunnlaugsdóttir, H., Kalogeras, N., Leino, O., Luteijn, JM., Magnússon, SH., Odekerken, G., Pohjola, MV., Tuomisto, JT., Ueland, Ø., White, BC., Holm, F. (2012). Review. State of the art in benefit-risk analysis: introduction. Food Chem Toxicol, 50, 2-4.

106. VKM, (2006). Vitenskapskomiteen for mattrygghet, Norge. Et helhetssyn på fisk og annensjømat i norsk kosthold. www.vkm.no.

107. Wang, Q., Dong, J., Zhu, Y. (2012). Probiotic supplement reduces risk of necrotizing enterocolitis and mortality in preterm very low-birth-weight infants: an updated meta-analysis of 20 randomized, controlled trials. Journal of Pediatric Surgery, 47, 241-248.

108. Watson, DH. (2001). Food chemical safety - Volume 1: Contaminants. Food Science, Technology and Nutrition, No. 57, Woodhead.

109. Watzl, B., Gelencsér, E., Hoekstra, J., Kulling, S., Lydeking-Olsen, E., Rowland, I., Schilter, B., van Klaveren, J. Chiodini, A. (2012). Application of the BRAFO-tiered approach for benefit-risk assessment to case studies on natural foods. Food Chem. Toxicol, 50,699-609.

110. WHO, (2003). Diet, nutrition and the prevention of chronic diseases. Report of a Joint WHA/FAO Expert Consultation Technical Report Series 916. World Health Organisation, Geneva.

111. WHO. (2002). Reducing risks, promoting healthy life. Available from: http://www.who.int/whr/2002/en/.

112. WHO/IARC, (2010). Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures. Volume 92.

113. Willems, J., Van Camp, J., Verbeke, W., Cooreman, K. (2006). Integrated evaluation of marine food items: nutritional value, safety, and consumer perception. Project CP/56. Belgium.

114. Yasumoto, T., Murata, M. (1993). Marine toxins. Chem Rev, 93, 1897–1909.

115. Zandstra, EH., de Graaf, C., Van Staveren, WA. (2001). Influence of health and taste attitudes on consumption of low- and high-fat foods. Food Qual Pref, 12, 75–82.

National Food Institute
Technical University of Denmark
Mørkhøj Bygade 19
DK - 2860 Søborg

Tel. 35 88 70 00
Fax 35 88 70 01

www.food.dtu.dk

ISBN: 978-87-93109-12-4