Method development in the study of burden of disease of foodborne chemicals

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Preface

This dissertation is written in order to partial fulfil the requirements for obtaining the PhD degree at the Technical University of Denmark (DTU). The work, on which the thesis is based, has been performed at the National Food Institute (DTU Food) in the period from December 2012 to August 2017, including two maternity leaves. The work has been been supervised by main supervisor senior scientist Morten Poulsen, and co-supervisors senior scientist Sara M. Pires and senior scientist Maarten Nauta.

The work is fully funded by the Technical University of Denmark.

The main goal of the thesis is to discuss and develop the methodological framework for estimating the disease burden caused by foodborne chemicals. It is my hope and expectation that this thesis leads to future and more transparent studies of burden of foodborne disease due to chemicals.

Lyngby, August 2017
Lea Sletting Jakobsen

Acknowledgements

First of all, I would like to thank my supervisors for guidance, inspiration and support throughout the entire project. You all have different competences, which has been a great contribution to this interdisciplinary project. But most of all, your doors have been open and you have always been approachable for questions and discussions. I received my PhD-position just 3 months before I gave birth to my first son, and I thank you not least for your support and understanding of the challenge it is to start a family and learning the true meaning of a work-life balance (especially when son number two arrived in 2016).

The National Food Institute underwent halfway through my project a large restructuring, in which our research group on Risk Benefit was formed. I have greatly benefited from becoming a part of this new interdisciplinary research group. I know that we all have had, and still have to find ways to equally contribute to the strategy and future of our group, but I owe great thanks to all my colleagues for providing a good working environment. I especially thank my fellow PhD-students: Johanne, Sofie and Maria. Sharing office with you has made these last intense months of the PhD feel, well, less intense.

I would also like to thank colleagues in the Division for Risk Assessment and Nutrition, who always have been helpful in answering questions about toxicological risk assessment, and especially Sisse Fagt for providing access to food consumption data. Likewise, I thank all the co-authors for their contributions.

An important part of starting a research career is to establish a scientific network and collaboration. I was very fortunate to spend a week at the National Institute of Public Health and the Environment (RIVM) in Bilthoven, The Netherlands with Bas Bokkers. I thank Bas for a very interesting stay and for continuing (long-distance) support and guidance into the world of probabilistic toxicological risk assessment. I also got the opportunity to collaborate with Stylianos Georgiardis from DTU Compute within the

last project of this PhD. I thank Stylianos for spending time and energy on this project. I hope that the collaborations will continue beyond this project.

Last, but not least, I thank you, Frederik, my loving husband, for your support and encouragement throughout the entire project. Together we have started a family and even though life is busy from time to time, I think that we and the kids are doing all right. Also thanks to the grandparents for helping Frederik out with the two little monkeys, when my weekends and nights were spent in front of the computer. August and Vilhelm: here's the book I have been so busy writing this summer. I promise, I will never read it to you as bedtime story.

Summary

Foodborne diseases are caused by pathogenic microorganisms or harmful chemicals and toxins present in the foods we consume. The harmful chemical substances may be added deliberately, be natural occurring toxins or process contaminants, e.g. acrylamide and benzo[a]pyrene which are mentioned in this dissertation. The impact that exposure to unsafe foods has on public health can be expressed in terms of Disability Adjusted Life Years, DALY, which in one metric combine information on disease duration and severity, how many get the disease and how many die from it. One DALY is one year of healthy life lost. In this way, DALYs can be used to compare the impact that foodborne diseases have on public health across hazards.

The World Health Organization estimated in 2015 that foodborne diseases caused 33 million DALYs on a global level. However, most of the diseases accounted for in the study by WHO were caused by pathogenic microorganisms. The disease burden of three chemical hazards was included in the global estimates, and it is acknowledged that the overall burden is underestimated due to the lack of estimates for foodborne chemicals and toxins. Data on disease incidence caused by the foodborne hazard is a prerequisite for the estimation of DALYs. For foodborne pathogens that often cause acute diarrhoeal disease, incidence and mortality may be obtained from disease registries. However, most diseases caused by foodborne chemicals are multicausal and the disease often occurs long time after exposure. Therefore, it is often only theoretically possible by the use of evidence from human observational studies or toxicological studies in experimental animals to allocate a disease case to a given exposure. However, epidemiological evidence is lacking for a vast majority of foodborne chemicals, and derivation of human disease incidence and mortality must be based on toxicological data from animal studies.

The overall aim of this thesis was to develop models to estimate the burden of disease

and apply the DALY methodology for foodborne chemicals, with a special focus on the use of toxicological data.

The thesis consists of three studies which all apply a hazard- and incidence-based approach to estimate the disease burden of chemicals. That is, the disease burden is calculated for all new disease cases caused by a hazard in a given time period. In all three studies, the incidence of cancer caused by dietary exposure to chemical carcinogens is estimated via a risk assessment approach, which makes use of data from the Danish national survey of dietary habits and physical activity, monitoring data on the chemical concentration in foods and dose response relationships from animal carcinogenicity studies.

In paper 1 (*Burden of disease of dietary exposure to acrylamide in Denmark*) it was evaluated how different methodological choices affect the final DALY estimate and an overall model framework consisting of three modules was proposed to estimate the disease burden to foodborne chemicals. We estimated a disease burden of 0.003 and 1.8 DALY/100,000 inhabitants, depending on the methodological choices and assumptions. We concluded that the assumptions and model approaches in the health outcome module, have a higher impact on the final DALY estimates than does the model approaches taken in the DALY module. In the health outcome module, health outcomes included in the estimation are selected and the estimation of disease incidence is performed based on the dose-response relationship from the animal studies.

The selection of hazard-health outcome pairs to account for in the study of foodborne chemicals is a crucial step, which potentially has a large impact on the final DALY estimates. In manuscript 2 (*"The sensitivity of a cohort study on acrylamide and risk of cancer: using a simulation approach to evaluate the likelihood of a significant effect"*) we assessed the potential bias introduced into studies of disease burden of foodborne chemicals, if only the evidence from human observational studies are used as the criteria for the selection of health outcomes. Using the risk of cancer caused by dietary exposure to acrylamide derived in Paper 1, we simulated the probability of detecting a statistical significant relative risk in a cohort study of "standard" design. We concluded that the lack of sensitivity in epidemiological studies should be taken into account when weighting the evidence of a causal effect, and in turn selecting the hazard-health outcome pairs.

DALYs reduce complex information into a single number. For foodborne chemicals the

knowledge base of the information is rarely complete and the uncertainty considerable, when translating the evidence from animal studies into DALY. This uncertainty should be quantified in order to be able to compare DALY estimates across hazards and direct future research. Moreover, information about how the burden of disease is distributed in different groups of the population is important and evaluated by describing the variation between individuals. In manuscript 3 (*"The Burden of disease of benzo[*a*]pyrene in barbecued meat: informing advice for different population groups"*) we developed a model to account for the variability between individuals and quantitatively propagate uncertainty along the analysis. The disease burden we estimated was low: 9.91×10^{-8} DALY per 100,000 inhabitants with a 95% uncertainty interval of $3.11 \times 10^{-8} - 1.49 \times 10^{-7}$. However, it was also estimated that individuals in the population, characterized by gender and weight, can reach an exposure that exceeds a limit that is associated with a high health risk by only consuming few meals of barbecued meat per year. This illustrates the differences in the purpose of disease burden studies and toxicological risk assessment, respectively.

This thesis contributes to the clarification of the impact of the different components of the analysis of the disease burden, when toxicological data from animal studies are applied. This information is important, if disease burden estimates are performed to assist policy-makers to decide where to allocate food-safety resources. In this thesis models were developed on case studies of two genotoxic carcinogens. Future research should extent model development to chemicals of other toxic effects. Especially the models developed in Manuscript 3 should be developed further and applied to other types and combinations of chemicals.

Sammendrag

Fødevarebårne sygdomme kan være forårsaget af sygdomsfremkaldende (patogene) mikroorganismer. Andre årsager til at man kan blive syg af maden, kan f.eks. være forekomst af tilsatte kemiske stoffer, naturligt forekommende giftstoffer eller procesforureninger, stoffer dannet under madens tilberedning, som f.eks. akrylamid og benzo[a]pyren, som omtales i denne afhandling. Fødevarebårne sygdommes påvirkning af folkesundheden, kan udtrykkes ved hjælp af "Disability Adjusted Life Years" (DALY), som i et tal kombinerer information om sygdommes varighed, i hvilken grad sygdomme påvirker helbredet, hvor mange, der får sygdommen og hvor mange, der dør af den. En DALY repræsenterer tabet af ét sundt leveår. På den måde kan DALYs bruges til at sammenligne forskellige fødevarebårne sygdommes påvirkning af folkesundheden på tværs af farer (hazards). I 2015 estimerede Verdenssundhedsorganisationen (WHO) sygdomsbyrden af sygdomsfremkaldende mikroorganismer og toksiske, kemiske stoffer i maden og kom frem til, at den udgjorde 33 millioner DALYs på globalt plan. De fleste af de sygdomme, der blev redegjort for i WHO's beregninger, var imidlertid forårsaget af patogene mikroorganismer. I det globale skøn af sygdomsbyrden blev kun 3 kemiske stoffer medtaget. Det er derfor anerkendt, at den samlede sygdomsbyrde er undervurderet på grund af manglende estimater for kemiske stoffer i maden. For at beregne DALYs er det nødvendigt at kende antallet af nye sygdomstilfælde forårsaget af en given patogen mikroorganisme eller et kemisk stof. For fødevarebårne patogener, der for eksempel kan forårsage akut diarre, er antallet af sygdomstilfælde som regel tilgængelige fra sygdomsregistre. Mange af de sygdomme, der kan forårsages af kemiske stoffer, kan imidlertid have mange forskellige årsager, de er multi-kausale, og ofte opstår sygdommen også først lang tid efter eksponering. Derfor er det i mange tilfælde kun teoretisk muligt ved brug af data fra humane observationsstudier eller toksikologiske studier i forsøgsdyr, at tildele et sygdomstilfælde

til en given eksponering. Epidemiologiske data mangler dog for størstedelen af de kemiske stoffer, vi indtager fra kosten. I stedet for må sygdomsincidensen for mennesker udledes af dyreforsøg. Blandt andet derfor er der kun publiceret få estimater af sygdomsbyrden forårsaget af kemiske stoffer i maden.

Det overordnede mål med dette ph.d-projekt er at udvikle modeller til at estimere sygdomsbyrden udtrykt i DALYs for toksiske, kemiske stoffer i maden med et særligt fokus på, hvordan man kan inddrage toksikologiske data fra dyreforsøg. Afhandlingen består af tre studier, som alle estimerer sygdomsbyrden ved hjælp af en hazard- og incidensbaseret metode. Det vil sige, at sygdomsbyrden beregnes for alle nye sygdomstilfælde, der er forårsaget af en fare (hazard) i en given tidsperiode. I alle tre studier estimeres forekomsten af kræft, forårsaget af eksponering for procesforureningerne via en risikovurderingsmetode. Der er blevet anvendt data fra "Den nationale undersøgelse af danskernes kost og fysiske aktivitet", data fra danske monitorerings programmer for indhold af toksiske, kemiske stoffer i fødevarer, og data om stoffernes dosis-respons-forhold fra relevante dyreforsøg.

Den første artikel ("Burden of disease of dietary exposure to acrylamide in Denmark") undersøgte, hvordan forskellige metodevalg påvirker det endelige DALY estimat for akrylamid. I artiklen foreslås desuden en overordnet modelramme, bestående af tre moduler, til at beregne sygdomsbyrden af kemiske stoffer. Resultatet var, at akrylamid forårsager 0,003 eller 1,8 DALY per 100,000 indbyggere, afhængigt af den valgte metode. En vigtig konklusion var, at de endelige DALY-estimater, var mere påvirket af antagelserne og metoderne anvendt i "Health Outcome-modulet" end modelvalget i "DALY-modulet". I "Health Outcome-modulet" udvælges hvilke sygdomme, der skal medtages i analysen, og sygdomsforekomsten estimeres på baggrund af sammenhængen mellem dosis-respons i dyreforsøgene.

Udvælgelsen af sygdomme, der skal medtages i en sygdomsbyrdeberegning, er et afgørende trin, der har stor indydelse på det endelige DALY estimat. I manuskript 2 ("The sensitivity of a cohort study on acrylamide and risk of cancer: using a simulation approach to evaluate the likelihood of a significant effect") vurderedes den potentielle bias (skævvridning af resultatet) der kan opstå, hvis humane observationsstudier har den højeste prioritet i udvælgelsen af sygdomme. Sandsynligheden for at påvise en statistisk signifikant relativ risiko i et kohortestudie, der følger et standard design blev

simuleret. Kræftrisikoen fra eksponeringen for akrylamid beregnet i den første artikel blev anvendt. Konklusionen var, at følsomheden af et kohortestudie er begrænset, når der kan være mange andre årsager til forekomsten af sygdommen, for eksempel kræft, end den eksponering man undersøger, for eksempel akrylamid. Derfor kan humane observationsstudier ikke stå alene som kriterium for udvælgelsen af sygdomme, der skal medtages i en sygdomsbyrde beregning.

I beregninger af DALYs bliver kompleks information reduceret til en enkel talværdi. Videns grundlaget er ofte ufuldstændigt og usikkerheden derfor betydelig, når man omsætter sygdomme forårsaget af kemiske stoffer til DALYs. Denne usikkerhed bør kvantificeres for at kunne sammenligne DALY estimater på tværs af forskellige sygdomsrisici fra f.eks. mikroorganismer og kemiske stoffer og for bedre at kunne fastslå, hvor videns grundlaget er utilstrækkeligt. Desuden er omfanget af sygdomsbyrden i forskellige grupper af befolkningen en vigtig oplysning og vurderes ved at beskrive forskelle mellem individer i f.eks. køn, alder, vægt og gener samt livsstilsvaner og socioøkonomisk status. I manuskript 3 ("The Burden of disease of benzo[a]pyrene in barbecued meat: informing advice for different population groups") blev der udviklet en model, der tager højde for forskellen mellem individer og kvantificerer usikkerhed forbundet med de enkelte elementer af analysen. Sygdomsbyrden for benzo[a]pyren var lav, $9,91 \times 10^{-8}$ DALY per 100,000 indbyggere med et 95% usikkerheds interval på $3,11 \times 10^{-8}$ – $1,49 \times 10^{-7}$. Beregninger på de samme data viste, at individer i befolkningen, karakteriseret ved køn og vægt, kan opnå en eksponering, der overskrider en sundhedsmæssigt begrundet grænseværdi, ved et indtag af kun få måltider med grillet kød om året. Dette illustrerer forskellen på formålet af sygdomsbyrde studier og toksikologisk risikovurdering.

Denne afhandling bidrager til at belyse betydningen af de enkelte dele, der indgår i den samlede analyse af sygdomsbyrden når toksikologiske data fra dyreforsøg er anvendt for en bestemt type kemiske stoffer, procesforureninger i maden. Denne information er vigtig, fordi det er foreslået, at sygdomsbyrdeestimater kan være et vigtigt redskab og indgå i beslutningsgrundlaget, når myndigheder der varetager fødevaresikkerheden skal vælge hvilke områder, der skal prioriteres og tilføres ressourcer. I denne afhandling blev der udviklet modeller for stoffer, der virker kræftfremkaldende ved en genotoksisk mekanisme. Fremtidig forskning bør udvide modeludviklingen til kemiske stoffer med andre toksiske virkninger. Specielt modellerne udviklet i Manuskript 3 bør afprøves på andre typer og

kombinationer af kemiske stoffer for at undersøge anvendeligheden af modellerne.

Contents

List of Abbreviations

Abbreviation	Description
AA	Acrylamide
ALARA	As low as reasonably achievable
ARfD	Acute reference dose
AI	Attributable incidence
BaP	Benzo[a]pyrene
BMD	Benchmark dose
BMR	Benchmark response
BoD	Burden of disease
BoFD	Burden of foodborne disease
CIP	Cumulative incidence proportion
CVD	Cardiovascular disease
DALY	Disability adjusted life year
DANSDA	Danish survey of diet and physical activity
DW	Disability weight
eBoD	Environmental burden of disease
EF	Extrapolation factor
EFSA	European food safety authority
FBD	Foodborne disease
FERG	Foodborne disease burden epidemiology reference group
FFQ	Food frequency questionnaire

Abbreviation	Description
GBD	Global burden of disease
GBFD	Global burden of foodborne disease
HALE	Health adjusted life expectancy
HBGV	Health based guidance value
IARC	International agency for research on cancer
IHME	Institute for health metrics and evaluation
IPCS	International programme on chemical safety
JECFA	Joint FAO/WHO expert committee on food additives
MOE	Margin of exposure
NOAEL	No observed adverse effects level
PAF	Population attributable fraction
POD	Point of departure
PTWI	Provisional tolerable weekly intake
QALY	Quality adjusted life years
QSAR	Quantitative structure-activity relationship
RR	Relative risk
SF	Safety factor
SMPH	Summary measures of public health
TDI	Tolerable daily intake
UF	Uncertainty factor
WHO	World health organization
YLD	Years lived with disability
YLL	Years of life lost

Definitions

Categorical attribution Approach to estimate disease burden caused by a hazard when a health outcome is identified as caused by the hazard in individual cases

Counterfactual analysis Approach to estimate disease burden caused by a hazard or risk factor by comparing the burden caused by current exposure levels to exposure levels that would be expected under some alternative hypothetical scenario

Bottom-up approach Approach in which incidence of disease is estimated by combining exposure and dose-response data, synonymous to the risk assessment approach

Hazard A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect

Health outcome tree A schematic representation of the disease model in which the health states associated with a disease and the transition between health states

Incidence The number of new cases during a specified time period

Life expectancy table Information on the expectation of years of life of an individual of any age in a population

Morbidity The disability or poor health due to any cause

Prevalence The number of cases out of the total number of people in the study population

Population attributable fraction The proportion of the incidence rate of a given outcome in a given population that is identified as due to a given exposure, consequently that proportion of the incidence rate could be reduced if the causative exposure was eliminated or reduced to a minimum

Relative risk The ratio of risk of disease among the exposed to the risk among the non-exposed

Risk A function of the probability of an adverse health effect and the severity of that

effect, consequential to a hazard in foods

Risk assessment approach Approach to estimate disease burden caused by a hazard by combining exposure and dose-response model, synonymous to the bottom-up approach

Risk factor A variable associated with an increased risk of disease

Top-down approach Approach in which incidence of disease is estimated by use of registered cases or human observational studies, synonymous to categorical attribution and counterfactual analysis

Toxicodynamics Describes the actions of the chemical within the body

Toxicokinetics Describes the fate of a chemical in the body by its absorption, distribution, metabolism and excretion

Chapter 1

Introduction

1.1 The burden of foodborne disease

Foodborne diseases (FBD) are diseases in humans caused by pathogenic microorganisms or harmful chemicals that are present in the foods we consume. Illnesses and deaths caused by FBDs have a high public health impact, and pose a significant socioeconomic burden, both nationally and globally [1]. For policy makers within food safety, the overall goal is to protect the population from unsafe food and thereby promote public health and limit economic losses. When resources are scarce, policy makers need objective evidence in order to prioritize interventions or mitigation strategies with maximal public health impact [2][3]. Incidence, prevalence or cause-specific mortality are all metrics commonly applied to inform on the impact of a disease or the effect of an intervention on public health. However, different hazards and risk factors in foods cause many different diseases of different severity and likelihood to occur; some hazards may cause self-limiting diseases of short duration, others may cause chronic diseases and even death, and some may cause all. Therefore, incidence or mortality alone do not capture the full range of health dimensions of a disease, and are thus not informative enough when the health impact of a given food-hazard or risk factor is to be compared with others.

The Burden of Disease (BoD) is an estimation of the health impact of disease using a summary measure of public health (SMPH), taking into account both mortality and morbidity. Different metrics have been developed for the purpose, e.g. quality adjusted life

years (QALY) [4] and health adjusted life expectancy (HALE)[5]. However the disability adjusted life years (DALY) has been more widely used since its development for the first Global Burden of Disease (GBD) project published in 1996 [6][7]. The DALY expresses the years of life lost to premature death and years lived with a disability of given severity and duration; one DALY is one lost year of healthy life [6]. In the GBD project, DALYs are used to rank diseases nationally and globally. The GBD project, first institutionalized under the World Health Organization (WHO) and then the Institute of Health Metrics and Evaluation (IHME), has since 1996 undergone four comprehensive updates, which makes it possible to observe shifts in disease burden over time, globally and nationally [8][9][10].

The GBD project uses risk factor studies to attribute DALY to selected risk factors including dietary risk factors [7][11][12][13]. However, the risk factor 'unsafe food' is not included, which motivated, by initiative of WHO, the establishment of the Global Burden of Foodborne Disease (GBFD) project in 2006 [2], with the final report published in 2015 [14]. The primary goal was: *"To enable policy-makers to set appropriate, evidence-based priorities in the area of food safety"* [14][1]. The disease burden attributable to 31 foodborne hazards in 2010 was estimated: 17 enteric hazards, 11 parasitic hazards and only 3 chemical hazards and toxins. The total burden of foodborne disease amounted to 33 million DALYs (95% uncertainty interval: 25 million - 46 million DALY), and is thereby comparable to each of the major infectious diseases: HIV/AIDS, malaria and tuberculosis [1].

1.1.1 Why are chemicals a challenge?

At the initiation of the GBFD project, the foodborne disease burden epidemiology reference group (FERG) core group, which led the GBFD, developed a comprehensive universal list of foodborne hazards that could be addressed in the project. From that list, selected hazards were chosen, based on criteria of data availability to estimate incidence and the likely magnitude of the disease burden attributed to the hazard [15][14]. For the chemicals and toxins, the universal list included 11 groups of hazards (column 1 in table 1.1). It was decided that disease burden estimates could be calculated for eight hazards (column 2 in table 1.1), but in the final report of the GBFD project, global estimates for only

three hazards were approved by WHO and included (column 3 in table 1.1). Overall, the GBFD project estimated that 900,000 DALYs were attributed chemicals and toxins, corresponding to 3% of the total foodborne disease burden estimated. But since only three hazards were estimated, it is acknowledged that the disease burden attributed foodborne chemicals is grossly underestimated [16][14].

Table 1.1: Lists of chemical hazards and toxins considered and included by the Global Burden of Foodborne Disease project and amended by World Health Organization

Universal list of hazards	Included hazards	WHO amended hazards
Elemental contaminants (e.g. lead, mercury, cadmium, manganese, arsenic)	Aflatoxin	Aflatoxin
Mycotoxins (e.g. aflatoxins, ochratoxins, fumonisin, trochothocenes)	Arsenic	Cassava cyanide
Food additives (e.g. sulphites, nitrites/nitrates, benzoic acid)	Cadmium	Dioxin
Pesticides (e.g. oorganophosphates, carbamates, DDT, Pyrethins)	Cassava cyanide	(Peanut allergy only for the AMR A, EUR A and WPR A regions, and excluded from [14])
Organic industrial pollutants (e.g. persistent organic pollutants)	Dioxin	
Veterinary drugs/residues (e.g. antibiotics, hormones-but not antimicrobial residues)	Lead	
Seafood toxins (e.g. tetrodotoxin, ciguatera, shellfish toxins, histamines)	Methyl mercury	
Process contaminants (e.g. acrylamide, PAHs, chloropropanol)	Peanut allergens	
Allergens (e.g.peanuts)		
Natural toxicants (e.g. cyanide in cassava, aminoglycosides		
Radionuclide and depleted uranium		

So why was the list of included hazards not more extensive?

Thousands of chemicals harmful to humans occur in foods, both as anthropogenic contamination and as naturally occurring toxins in raw materials; as a result of production processes or migration from for example packaging. The range of health effects caused by these chemicals is wide, including, but not limited to: acute poisonings (e.g. vomiting and diarrhea caused by lectins [17]), impaired cognitive development (e.g. caused by heavy metals [18]), several types of cancers (e.g. caused by mycotoxins [19]), reduced fertility (e.g. casued by bisphenol-A[20]) and immunosuppression (e.g. caused by dioxin [21]). Additionally, one chemical can exert a spectrum of health effects adverse to human health.

To estimate burden of foodborne disease in terms of DALYs, it is necessary to trace disease cases and deaths back to the causative agents. The degree to which this is possible varies for different chemicals depending on the nature of the associated health effects, disease epidemiology and exposure patterns (fig. 1.1, where the grey shaded area represents the hypothetical fraction of cases to be traced back to the causative chemical exposure).

All chemicals exert an effect on the human body upon exposure, and a distinction between adverse and non-adverse effects is a crucial step in chemical risk assessment [22]. Above the red dashed line in fig. 1.1 are the chemically induced health effects that are considered adverse. Obviously, only the adverse health effects contribute to the disease burden of foodborne chemicals.

A vast majority of adverse effects of foodborne chemicals and toxins can only be characterized in toxicological test systems, e.g. in vitro assays and experimental animals, and do not manifest themselves as clinical human cases [3]. For example, a chemically induced morphological change in an animal organ is an adverse effect, but will not necessarily be identified as a human clinical case (bottom, but above red dashed line of pyramid in fig. 1.1). The test systems are primarily used to support evidence for toxicological mode of actions and to establish reference values applied in a regulatory process. The effects are adverse, but rarely identified as human disease endpoints.

For frequent low level chronic exposures to chemicals that might cause various chronic diseases, it is a great challenge to trace back and allocate cases. This is primarily due to long lag times from exposure to the manifestation of symptoms as well as to the multicausality for a wide range of chronic diseases, e.g. physical inactivity, genetics and methyl mercury may all contribute to development of cardiovascular disease (third from top of

the pyramid in fig. 1.1).

If chronic diseases occur in populations exposed to high levels of specific food chemicals or toxins, it may be possible to allocate cases to the causative agents; e.g. aflatoxin induced liver cancer in certain African regions [23][24], and exposure to methyl mercury causing developmental impairments in unborn and young children in populations with a high fish consumption [25] (second from top of the pyramid in fig. 1.1).

Finally, as for foodborne microorganisms, identifiying a disease case due to chemical exposure can (relatively easily) be done for cases of acute toxic effects or when the chemical or toxin are the sole causative agents; e.g. peanut allergy or cyanogenic cassava induced *konzo*, both included in the GBFD project [15](the top of the pyramid in fig. 1.1).

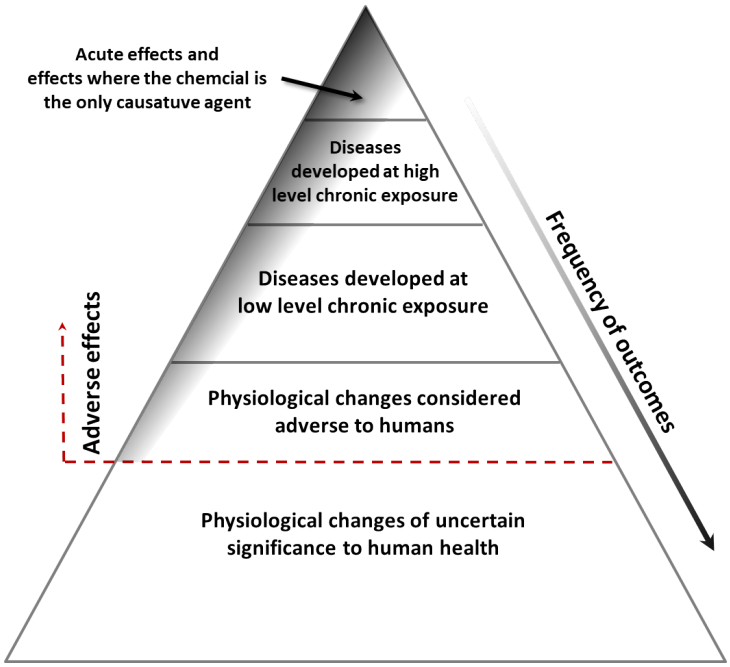

Fig. 1.1: Frequency and detection of health outcomes following exposure to foodborne chemicals, adapted from Hollander (2009) and Pruss-Ustun (2011) [26][3]. From the top and down of the pyramid illustrate an increased frequency of health outcomes. The grey-shaded area indicates the (hypothetical) fraction of the disease cases that with certainty can be attributed to a foodborne chemical as the causative agent. Above the red-dashed line are the health effects that manifest themselves as harmful to humans, below the physiological changes that occur upon chemical exposure but are of uncertain significance to human health.

Surveillance data in the form of registered prevalence or incidence of cases or deaths

ascertained to the causative chemical is the most reliable data. However, as illustrated by fig. 1.1, this type of data is not available or possible to extract for the vast majority of health effects caused by foodborne chemicals. Most health effects associated with chemcial exposures are multi-causal and it may only theoretically be possible to trace back a case to its source. To do this, data can be obtained from human observational studies in terms of e.g. relative risks (RR) or from toxicological test systems e.g. experimental animals. Statistical significant RRs are preferrable but usually only obtained from human observational studies in (sub)populations where disease cases are associated with high exposure levels compared to the controls [3]. Besides, it is not straight forward to extrapolate RRs estimated in one population to another, where distribution of confounders is different than in the study population [27]. If data are derived from experimental animals, the health effects detected are caused solely by the chemical to which the animals are exposed. However, the incidence observed in the animals must be extrapolated to an incidence in humans, which is also not straight forward.

Thus, quantitative estimates of the disease incidence are for many chemical exposures lacking or of varying strenght [3][15][26]. Depending on the strength and origin of the evidence, i.e. epidemiological or toxicological studies, various degrees and sources of uncertainty are associated with the estimates, which, in turn, hampers the reliability of the disease burden estimate and complicates a comparative assessment across hazards.

Further, when incidence data are not available, high quality exposure data is needed, but often also not available. Food consumption surveys (e.g. food frequency questionnaires (FFQ), 4-7 day food diaries, 24-hour dietary recall) and data on chemical concentrations are usually not designed to estimate exposure to chemicals and do not necessarily reflect the foods on the market or take into account processing factors, both by the industry and at consumer level. Biomonitoring data used in exposure assessments are preferable, but are at present not available for many chemical hazards, and pose challenges of attributing exposure to the different sources [28].

Due to these challenges, studies of burden of foodborne chemicals, using DALYs as a common health metric, are of a limited number. Few other studies have been published besides the estimation of burden of foodborne chemicals for the hazards in the GBFD-project [29][30]. The majority of these studies are based on RRs from human observational studies (inorganic arsenic in [29], aflatoxin in [15]), fewer on animal dose response data

(dioxin in [15], acrylamide, PAH, nitrosamines in [30]). If the burden of foodborne disease is to be estimated, more quantitative estimates of the disease burden due to chemical exposures through food must be available. This calls for the need to further explore this area of research, with a specific focus on applying toxicological data to theoretically estimate the disease burden due to foodborne chemicals. It is relevant to investigate the limitations and opportunities of using animal dose response data, with a special focus on increasing transparency in the development of the main indicators, i.e. disease incidence and DALYs.

1.2 Aims and objectives

The overall aim of this thesis was to develop models to estimate the burden of disease and apply the DALY methodology for foodborne chemicals, with a special focus on the use of toxicological data for the purpose. The specific objectives were:

- To investigate how methodological choices and assumptions affect the disease burden estimates of chemical hazards.
- To assess the link between toxicological and epidemiological evidence on the causal relation and size of effect between a hazard and health outcome, and discuss the influence this has on the study on the disease burden of foodborne chemicals.
- To account for variability and uncertainty in the study on burden of disease of foodborne chemicals.
- To discuss the applicability of disease burden studies in risk management.

Two chemical hazards were selected as case studies to address these objectives.

1.3 Outline of the thesis

The preceding **Chapter 1** has introduced the overall concept of burden of foodborne disease, the special challenge that estimating the disease burden due to foodborne chemicals presents and the aim and objectives of this thesis. **Chapter 2** is a background

section, which describe the approaches to estimate the burden of foodborne disease and the framework for performing toxicological risk assessments, respectively. **Chapter 3-5** represent the three manuscripts included in this thesis; **Chapter 3** is a case study on the burden of disease of dietary exposure to AA in Denmark, in which we investigated how different methodological choices affect the final estimates; **Chapter 4** is a simulation of a prospective cohort study on AA and the risk of cancer, which investigates the likelihood of detecting a significant relative risk (RR), and illustrates sources and size of potential bias in BoFD studies; and **Chapter 5** is a case study on the disease burden of Benzo[a]pyrene (BaP) in barbecued meat, including variability in consumer behaviour and individuals' sensitivity to the chemical. Before each manuscript a prelude is given, and after, a broader discussion presented. In **Chapter 6**, the main findings from each manuscript are summarized, an overarching discussion is given with concluding remarks and future perspectives are presented.

Chapter 2

Background

2.1 Approaches for estimating the burden of foodborne disease

As stated in the Introduction, the DALY has become the widely used metric, both in general BoD studies, e.g. the GBD, and in studies of specific risk factors including environmental burden of disease (eBoD) [3][31] and BoFD [1]. Comparability between studies is often hampered by different methodological choices used to estimate DALYs [32][33][34][35]. Which method is applied depends on the question at hand and the origin of the available data. However, even within same question and data frames, methodologies vary, along with how they are defined. In this chapter and in the rest of the thesis, the methodologies (and their definitions) are referred to as described within the methodological framework of the GBFD-project [36]. In the first paper of this thesis, *"Burden of disease of dietary exposure to acrylamide in Denmark"* [37], the reader will notice discrepancies in the definitions applied. However, a unified "language" within the burden of foodborne disease framework is important for its transparency, and these discrepancies will be addressed in the following.

2.1.1 Disability adjusted life years

The DALY is a health gap measure, meaning that it reflects the number of life years lost due to bad health and premature death. It is assumed that each individual in a population is born with the prospect of living a full life of perfect health. However, each individual might during their life experience illnesses of shorter or longer duration and various degrees of severity, and may even die from a disease at an earlier age than would have been expected compared to an expected (reference) age. The time that an individual lives with a disease (morbidity) and the time that an individual dies before the expected age (mortality), is in the DALY combined and expressed as the total loss of healthy life years. The loss of healthy life years may be attributed to different diseases, which again may be caused by different risk factors or hazards, and the loss of healthy life years of a population is estimated by summing over each individual in that population (Fig. 2.1).

2.1.1.1 How to calculate DALYs

The time living with disease is in DALY terminology referred to as the years lived with disability (YLD) and is for a given health outcome in a population calculated by:

$$\mathrm{YLD} = N_{\mathrm{c}} \times t \times \mathrm{DW}, \tag{2.1}$$

where N_{c} is the number of cases of a given health outcome in a defined population, t is the duration in years of the health outcome from disease onset to either remission or death, and DW is the disability weight, which describes the severity of the health outcome on a scale from 0 (perfect health) to 1 (death). Disability weights have been developed for the GBD study [38] and the Dutch Burden of Disease study [39].

The time of death before an expected age is in DALY terminology referred to as the years of life lost (YLL) and is calculated by:

$$\mathrm{YLL} = N_{\mathrm{d}} \times \mathrm{LE}, \tag{2.2}$$

where N_{d} is the number of deaths associated with the health outcome in a defined population and LE is the residual life expectancy at the age of death, thus making YLL age-dependent.

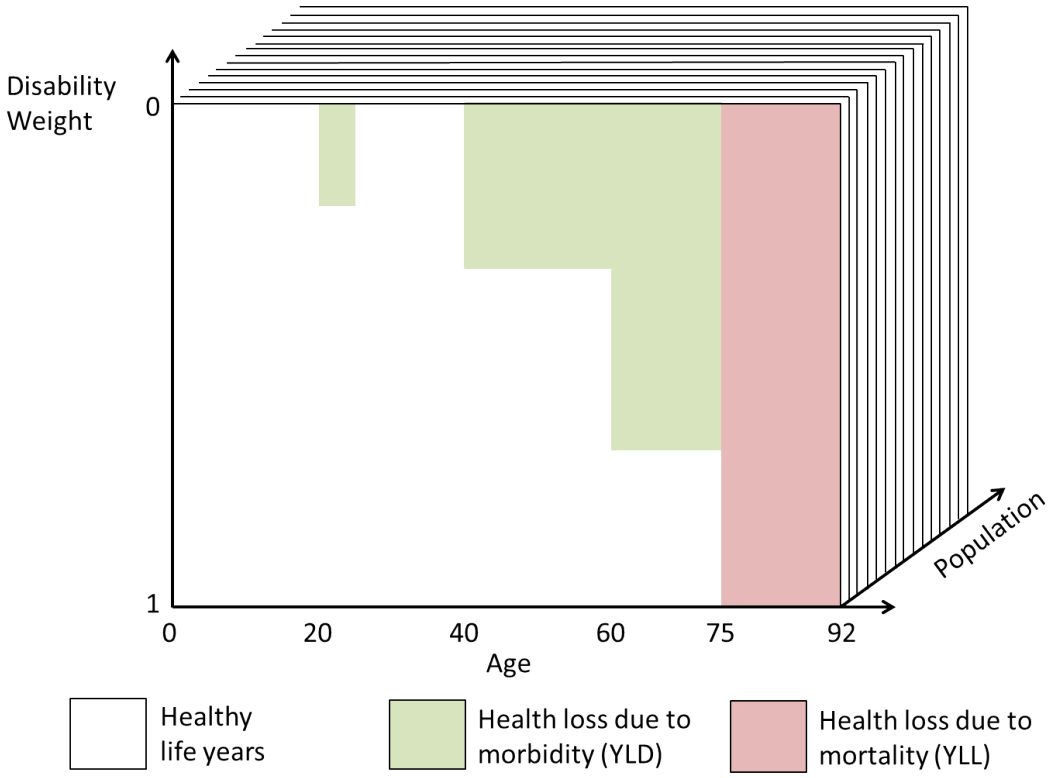

Fig. 2.1: The concept of DALY The x, y dimensions of the graph represent a person's contribution to the loss of health life years in a population (z-dimension). The person is born with a prospect of perfect health and live in this state (disability weight 0) until age 20. For five years he lives with a disease of disability weight 0.2. He regains perfect health until age 40 where he lives 20 years with a disease of disability weight 0.32, which progresses to worse at age 60 (disability weight 0.7), until he dies from the disease at age 75, 17 years before his expected age of death at 92. Thus the total DALY is $0.2 \times 5 + 0.32 \times 20 + 0.7 \times 15 + 17 = 34.9$.

DALYs are the sum of YLDs and YLLs:

$$\text{DALY} = \text{YLD} + \text{YLL}. \tag{2.3}$$

DALY can be presented as the absolute DALY for a given population; presented as relative to the population size, e.g. per 100,000, and thus comparable across different populations; or as DALY per case, which indicates the severity of a given health outcome the individual level [40].

Different social weighting factors can be applied to the general DALY equation (eq. 2.3) [6][40]. In age-weighting, the social value of a healthy life year of young to middle-

aged adult is higher than that of a young child or an elderly individual. It is based on the presumption that young to middle-aged adults contribute more to than depending on society. In discounting, the DALYs lost closer to present time is valued higher than those lost in the future. Discounting of DALYs is based on the fact people seem to prefer a healthy year of life immediately rather than a life lived in the future. Usually a discounting rate of 3% is applied, meaning that a healthy life year gained 10 year from now is worth 24% less than a year gained now [41]. The ethical aspects of age-weighting and discounting (and of the DALY as a concept) has been debated [42][43], and since the 2010 GBD consensus has been that DALY estimates should be presented both with and without the social weight factors [8].

2.1.1.2 Data needs

Every DALY calculation starts with the problem formulation. That is: which disease burden do we want to estimate, in which population and within what time period? The answers to these questions define the type of data needed and the methodologies applied.

To estimate the disease burden of specific health outcomes irrespective of their aetiology, for example the total disease burden of a type of cancer, is referred to as the **outcome-based approach** (Fig. 2.2). In this approach, the total number of cases and deaths of the health outcome is needed. To estimate the burden due to a certain risk factor, for example barbecuing which increases the risk of cancer mediated by exposure to various carcinogenic compounds, is referred to as the **risk factor-based approach**. This approach requires the number of cases and deaths of the health outcome attributed the risk factor. Lastly, to estimate the disease burden caused by a hazard, for example cancer caused by exposure to acrylamide, is referred to as the **the hazard-based approach**. Here the number of cases and deaths attributed the hazard is needed [44] (Fig. 2.2). For all three approaches, a disease model representing all possible health states of the disease(s) accounted for, must be constructed. Additionally, information on duration, the disability weight, and the age of onset of each health state is needed in the YLD calculation; in the YLL calculation, information on the age of death and the life expectancy at age of death of each health state are needed.

Regardless of the approach selected, the number of cases for a health outcome to

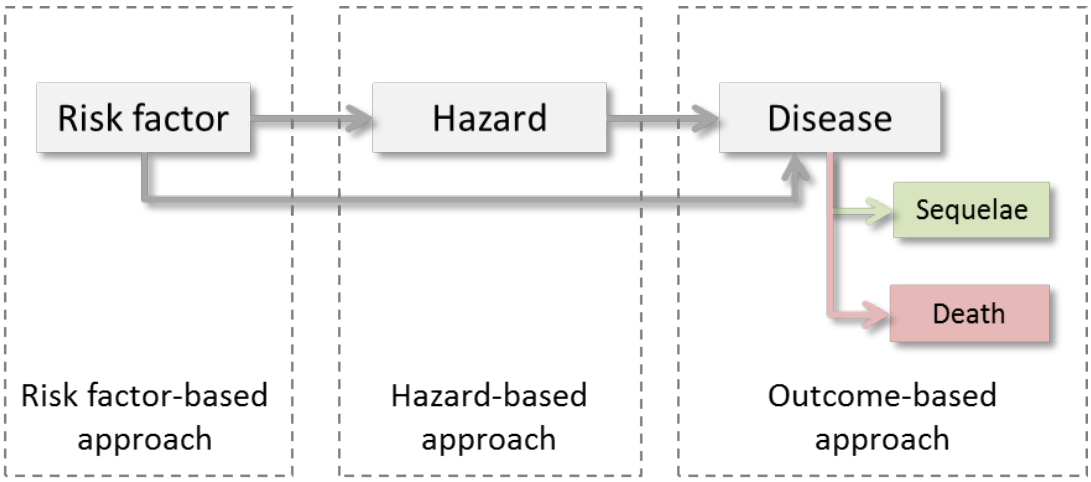

Fig. 2.2: Approaches for estimation of disease burden. A risk factor-based approach estimates number of disease cases due to a given risk factor; a hazard based-approach attributes number of disease cases to a given hazard; a outcome-based approach estimate the number of disease cases irrespective to its aetiology.

calculate YLD must be given either as the incidence or as the prevalence. The incidence refers to the number of new cases per time period, whereas the prevalence is the number of disease cases present at a given point in time. If an incidence based approach is used, the future disease burden is ascribed to the incident cases in the specified year. If a prevalence based approach is used, the disease burden in a given year is the burden experienced by all the individuals living with the disease in that year [6][36][40][45]. As example, if the disease burden of liver cancer is estimated in 2014, then by the incident-based approach the disease burden of liver cancer is estimated as the burden that all patients with liver cancer diagnosed in 2014 will experience in the future years. By the prevalence based approach, the disease burden of 2014 will be the disease burden of all liver cancer patients living with the disease in the various disease stages. If population age structure and disease trends are constant over time, then the two approaches should yield the same result [6][40].

If it is a population-based burden study, the population must be defined, e.g. the Danish population, and often stratified by age and gender. DALYs are then calculated for each stratum and summed for the whole population [40]. Finally, it can be of use to know the disease burden in a given year, e.g. the burden of foodborne disease in 2010

in the Danish population, thus making it possible to compare disease burdens over time [46].

As BoFD is caused by a wide range of hazards, the hazard- and incidence-based approach was adopted in the GBFD-project [36], and likewise in Paper 1 and Manuscript 3 in this thesis. The hazard-based approach is described in detail below.

2.1.2 Hazard-based approaches for foodborne chemicals

The hazard-based approach *"defines the burden of a specific foodborne hazard as that resulting from the health states, i.e., symptoms and sequelae, including death, that are causally related to the concerned hazard transmitted through food and which may become manifest at different time scales or have different severity levels"* [36]. Within the hazard-based approach, the origin of the evidence to inform on the disease incidence has an impact on: i) which disease(s) to account for in the burden estimate, ii) the construction of a health outcome tree serving as the basis for the burden estimation and iii) the methodology applied to calculate or attribute the disease incidences.

2.1.2.1 Selection of health outcomes

Different criteria may be applied in the selection of the health outcomes caused by a given hazard (hazard-health outcome pair) and accounted for in the disease burden [12][36][15] (fig. 2.3). Through structured literature searches, all identified health outcomes, their symptoms and sequelae, are identified [36]. For foodborne chemicals, the structured literature review might be exempt if updated toxicological reviews or opinions are available for the specific chemical from international organizations, e.g. the Joint FAO/WHO Expert Committee on Food Additives (JECFA), International programme on chemical safety (IPCS), International agency for research on cancer (IARC) and the European food safety authority (EFSA). The toxicological reviews offered by the international organizations present thorough evaluations of the hazard-associated risks, by reviewing all available data to be used in a chemical hazard assessment. The data include: human data i.e. case reports, clinical and physiological investigations, volunteer studies, occupational studies, epidemiological studies and meta-analyses; experimental animal (*in vivo*) data; *In vitro data*; and Non-testing data i.e. physico-chemical properties and quanti-

tative structure-activity relationships (QSAR) [47]. If updated toxicological reviews do not exist for the chemical of interest, the structured literature search should include all published literature covering the above data sources.

To be accounted for in the disease burden, the identified hazard-health outcome pair must be evaluated with regard to the strength of evidence of: i) the causal relationship, and ii) the data to estimate the probability of the health outcome to occur in a human population. Regarding i), the strength of evidence of the combined information from several of the above mentioned data sources is evaluated. Effects observed in humans after exposure to the given chemical must be supported by biological evidence on the mode of action of the chemical in the human body from animal and in vitro data. Vice versa, adverse effects of a chemical observed in animal and/or *in* vitro studies are investigated for its human relevance in observational studies, where the findings from the test systems are used to generate a null-hypothesis (i.e. chemical X is not associated with disease Y) to be tested. The combined information is used to grade the strength of the evidence, which is formally done for example for human carcinogens [48] (e.g. Box 1).

Box 1: IARC classification of carcinogens

Group 1: Carcinogenic to humans = sufficient evidence of carcinogenicity in humans.

Group 2A: Probably carcinogenic to humans = limited evidence of carcinogenicity in humans, sufficient evidence of carcinogenicity in animals.

Group 2B: Possibly carcinogenic to humans = limited evidence of carcinogenicity in humans and less than sufficient evidence in experimental animals.

Group 3: Unclassifiable as to carcinogenicity in humans = inadequate evidence in humans and less than sufficient in experimental animals.

Group 4: Probably not carcinogenic in humans = evidence suggesting lack of carcinogenicity in humans and in experimental animals.

Regarding ii), when a hazard-health outcome pair is found relevant for humans, it is assessed whether it is possible to estimate the incidence based on the available data with an acceptable degree of certainty. If a hazard-outcome pair is supported by evidence in the form of surveillance data (rarely the case with chemical exposures) or from high quality human observational studies reporting relative risks (RR) and a dose-response relationship, the task to calculate the incidence is less complicated. However, if this type of data do not exist in the literature or if the RR and dose-response relationship is not useful for extrapolation from one population to

another [12], then the incidence must be calculated from other types of data, which is not necessarily straight forward. To calculate the incidence from animal data is complicated by the fact that the dose response relationships in animals have to be translated to humans by factors that cover the cross-species differences.

A third criterion used to select hazard-outcome pairs, if surveillance data is not collected, is the availability of exposure data [12]. The quality of the data-bases used for assessing the human exposure to foodborne chemicals can vary greatly from chemical to chemical. A fourth criterion is if disability weights are available for the health outcome of interest. If not, disability weights of similar health outcomes may be adopted. The criteria for including a health outcome associated with a hazard to estimate the disease burden connected to that hazard is schematically presented in fig.2.3.

Other subjective criteria have been applied when including health outcomes for a given hazard. If the severity of a potential health effect or the (theoretical) burden is assumed to be low it might be excluded from the estimation [15]. Likewise, exposure levels assumed too low to mediate a certain health outcome may be reason of exclusion. This was the case in the first paper of this thesis, in which only cancer was included as health outcome associated with acrylamide exposure; on the assumption that neurotoxic effects in humans are only linked with occupational exposure, which are substantially higher than exposure levels mediated by foods [49].

2.1.2.2 Incidence attribution for foodborne chemicals

Depending on the data available for calculating incidences, the hazard-based approach is applied by either of the following three methodologies: **categorial attribution** in which incident disease cases are directly identified as caused by the hazard; a **counterfactual analysis** in which the population attributable fraction (PAF) is calculated and multiplied with the total burden estimate for the specific health outcome (i.e. the total burden of cancer in a population, also referred to as the disease envelope); or by a **risk assessment approach** in which the incidence is calculated from the combination of a dose response relationship and exposure data [36] (fig. 2.4). Overall, the approaches applying surveillance data or observational studies are referred to as **top-down approaches**; a risk assessment approach is referred to as a **bottom-up approach**.

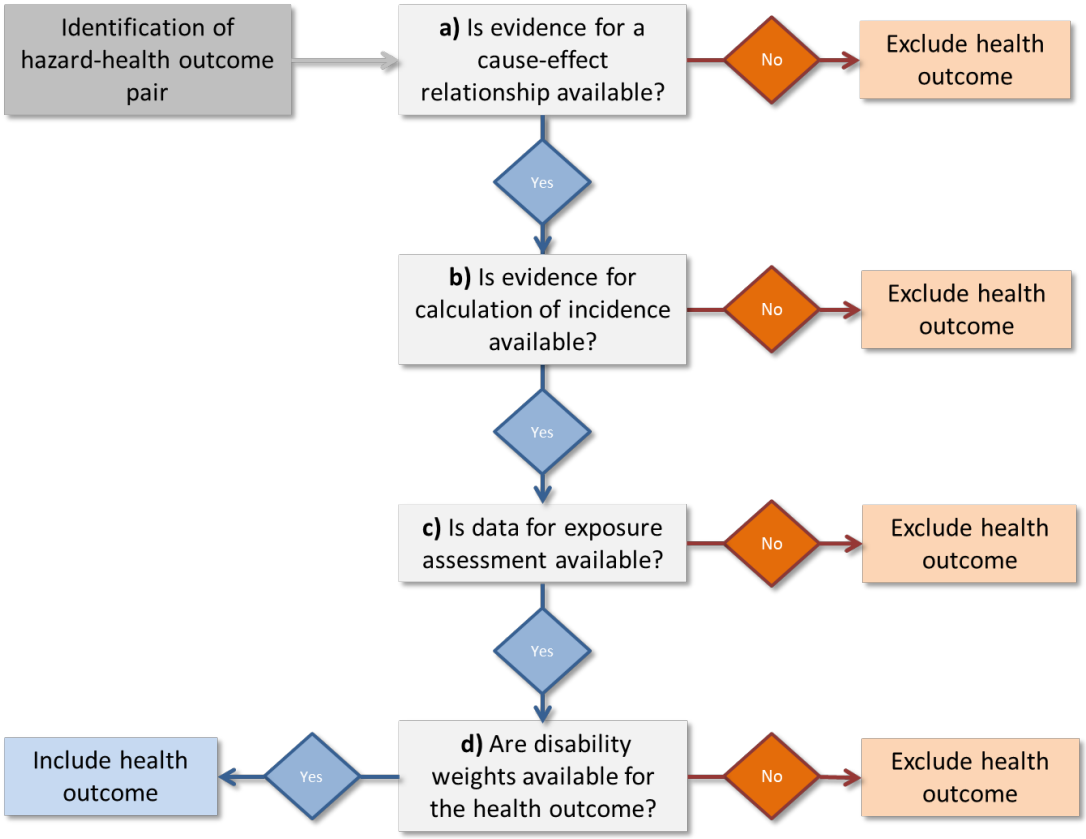

Fig. 2.3: Decision-tree for selection of hazard-health outcomes The identification of hazard-health outcome pairs is done by reviewing the available scientific literature. To include the identified hazard-health outcome pairs depends on at least 4 criteria: a) available evidence for a causal relationship, b) available evidence for incidence estimation, c) available exposure data, d) available disability weights.

For foodborne chemicals, as stated before, it is rarely the case that surveillance data to apply categorical attribution is available. Within categorical attribution, the obtained incidence can be in the form of the direct incidence attributed to the hazard in question (AI) or the incidence of an overall disease envelope (I). In the first, AI is multiplied with the probability of developing the health state (i.e. the transitional model); in the latter, I is multiplied with the attributable proportion (AP) of the hazard in question (top of fig. 2.4).

If RRs from observational studies or dose-response relationships from experimental animal data (or both) are available, either counterfactual analysis or a risk assessment approach can be applied. If a disease envelope for the selected health outcome is available,

the PAF can be estimated from observational studies or animal dose response data. The PAF is calculated from RRs and estimates the proportional reduction in disease if exposure was removed or reduced (hence, a counterfactual analysis).

If an animal dose response relationship is available (risk assessment approach), a "pseudo" PAF is calculated by combining the dose response with exposure data to estimate the attributable incidence (AI), which is divided by the background incidence of the health outcome [31]. Both the PAF and the "pseudo" PAF are multiplied with the disease envelope. If a disease envelope does not exist, an AI is calculated from the PAF multiplied with the background incidence of the health outcome (counterfactual analysis) or from animal dose-response data (risk assessment approach). According to the health outcome tree, the AI may then be multiplied with the probability of developing the specific health states (fig. 2.4).

2.1.2.3 The health outcome tree

For the selected hazard-health outcome pair(s), the various health states and possible transmissions are schematically represented in a health outcome tree (or disease model). This tree serves as a basis for the computation of the disease burden connected to a hazard. With terminology adapted from Devleesschauwer et al. (2015), the outcome tree consist of parent nodes representing either disease incidence, mortality or DALY (seperated into YLD and YLL) rates, and child nodes representing the probabilities of developing a specific disease stage or attributable proportions of the disease. The arrows represent the connection between nodes [36]. Figure 2.5 shows the health outcome tree applied in Paper 1 and Manuscript 3. It describes a non-specific cancer and its various health states following exposure to AA or BaP (same outcome tree for both chemicals, as we assume that a non-specific cancer case following AA exposure do not differ from one following BaP exposure). In this outcome tree, the incidence of a non-specific cancer represents the parent node. Each child node represent the probabilities of moving into each disease stage, and the arrows represent the transitions between disease stages. All green child nodes contribute to YLD, the red contribute to YLL and the grey do not contribute directly to DALYs.

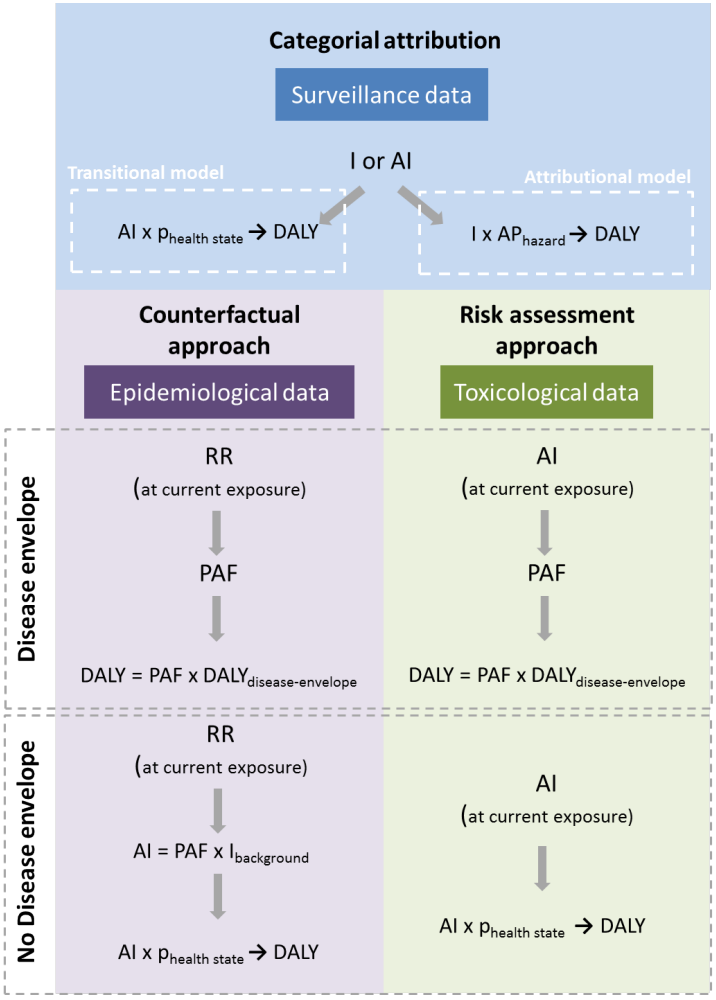

Fig. 2.4: Incidence attribution for foodborne chemicals Overview of the modelling choices within the hazard based approach, depending on the data source, adapted from Hanninen et al. (2014) [31]. Categorical attribution is used if surveillance data is available, either in the form of incidences (I) of the overall health state or as an attributable incidence (AI). If epidemiological data is available, the population attributable fraction (PAF) is derived from the relative risk (RR) estimated for the current exposure for given fractions of the population. Depending on the availability of a disease envelope, disability adjusted life years (DALY) are either calculated by multiplying with the DALY for the disease envelope, or used to calculate the AI. If only toxicological data are available, the AI is estimated from a dose response relationship and either used to calculate the PAF or used directly, if the disease envelope is available or not, respectively.

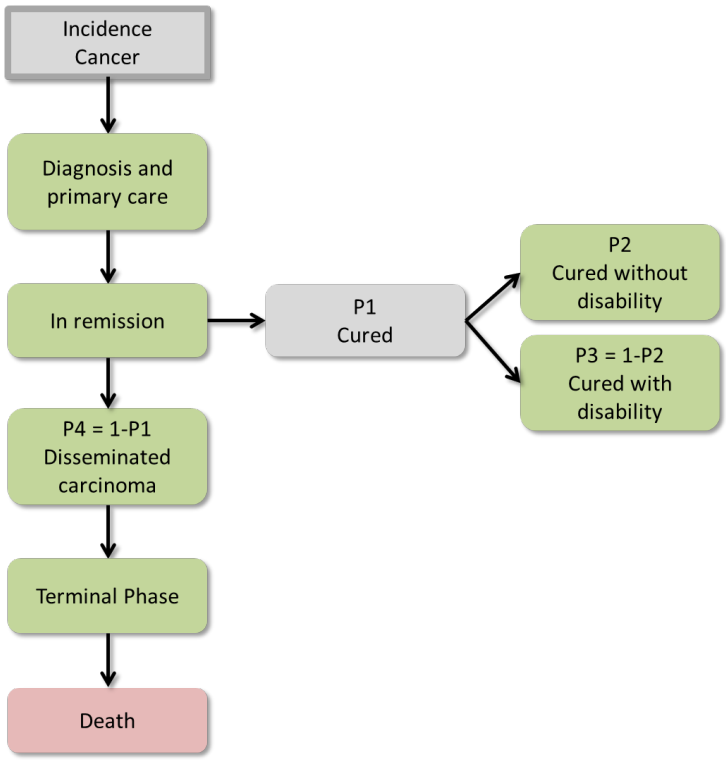

Fig. 2.5: Health outcome tree for cancer caused by acrylamide or benzo[*a*]pyreneThe parent node (rectangle with thick border) represents the incidence of cancer caused by the hazards. Childnodes (rectangles with rounded edges) represent the probability of moving from one disease stage to another. Green childnodes contribute to YLD, red childnodes contribute to YLL and grey childnodes do not contribute to DALYs. P1 = probability of being cured, P2 = probability of being cured without disability, P=3 probability of being cured with disability, P4 = probability of dying from cancer.

2.2 Toxicological risk assessment in a disease burden context

Epidemiological evidence is not available for a vast amount of the harmful chemicals found in foods [50]. Therefore, to estimate the burden of foodborne chemicals, data from animal toxicity studies must be used. Toxicological risk assessment of chemicals is also most often based on information derived from studies on the harmful effect of a chemical in experimental animals. Thus, estimates of burden of foodborne chemicals build on existing methodology applied in toxicological risk assessments of chemicals using animal data, i.e. the risk assessment approach. However, the objectives of toxicological

risk assessment and risk assessments used for burden of disease are different. While a toxcological risk assessment of chemicals provides evidence for policy makers to ensure a high level of protection of human health. The disease burden of foodborne chemicals provide evidence of the current impact that a given exposure to chemical hazards has on human health. Despite the different purposes, the same animal data is applied. In the following, the overall toxicological risk assessment framework is shortly described followed by the risk assessment approach for two categories of chemical hazards, i.e. threshold and non-threshold effects. Lastly, a probabilistic approach to risk assessment of chemicals is presented and proposed as a methodology to be used in disease burden studies of chemicals.

2.2.1 The risk assessment framework

A toxicological risk assessment consist of 4 elements; hazard identification, hazard characterization, exposure assessment and risk characterization (Fig. 2.6). There are discrepancies in the definitions of the elements, originating from the definition of a hazard. The difinition of a chemical hazard according to IPCS and OECD, is "*the inherent property of an agent or situation having the potential to cause adverse effects when an organism, system or (sub)population is exposed to that agent*" [51][52]. Thus, IPCS and OECD define the hazard as the adverse effect that an agent might cause and therefore an agent may present many different hazards. The Codex Alimentarius definition covers hazards of different origin and overall defines hazards as "*a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect*" [53]. As the Codex Alimentarius definition correspond to the definition of the hazard-based approach, I will apply those definitions in this thesis, even though it differs from the traditional chemical definitions.

Hazard identification

Hazard Identification is the first step of a risk assessment in which the chemical hazards capable of causing adverse health effects and which may be present in a particular food or foodgroups are identified [53]. Most often the health outcomes associated with the hazard are also identified. For chemicals, this includes the information on toxicokinetics (i.e. the

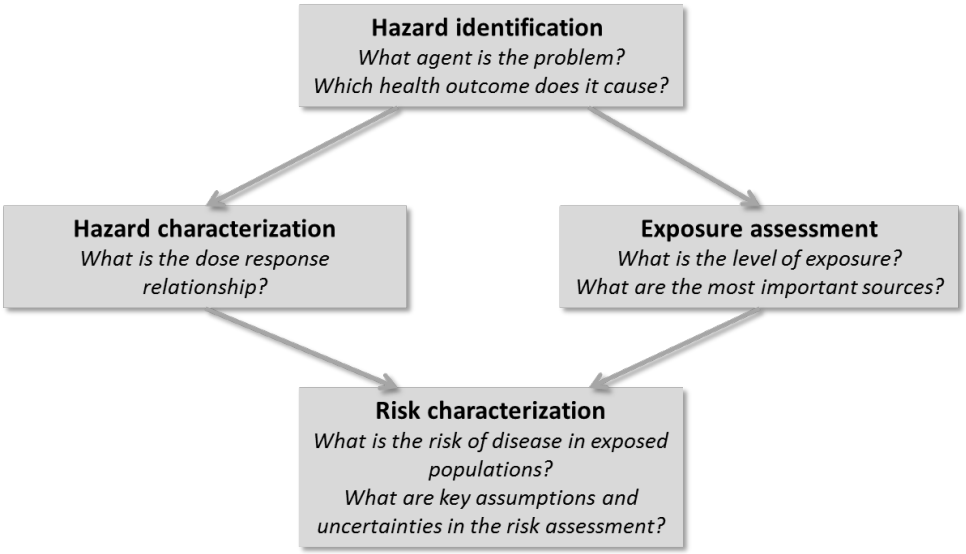

Fig. 2.6: Basic steps of a toxicological risk assessment (adapted from Codex Alimentarius (2016) [53])

absorbtion, distribution, metabolism and excretion of the chemical in the animal/human body) and toxicodynamics (i.e. the mode of action of the chemical i the animal/human body) of the chemical.

Hazard characterization

In this step, the nature of the adverse health effects associated with the chemical hazard present in food are qualitative and/or quantitative evaluated [53]. This includes information on the magnitude of exposure and the related severity or frequency of the adverse health effect provided from a dose-response relationship.

Exposure assessment

In this step, the likely exposure to the chemical via food or other relevant sources is qualitatively or quantitatively evaluated [53]. The exposure assessment of foodborne chemicals takes into account the occurrence and concentration of the chemical in foods as well as the consumption patterns of the foods containing the chemicals.

Risk characterization

In the risk characterization, the information from the 3 previous steps is integrated to qualitatively or quantitatively estimate the risk, i.e. the probability of occurrence and severity of an adverse health effect [53]. The resulting risk estimate is in the form of expected number of diease cases or in the form of an estimate of whether the exposure of a defined population is above or below a level of exposure considered "safe" [54]. In the risk characterization all uncertainties connected to each step in the the risk assessment should be identified.

2.2.1.1 Hazard characterization of threshold effects

Threshold effects are health effects for which it is considered that the effect does not occur below a given dose or concentration [51][52]. Most types of toxic effects are threshold effects, and include effects like neurological, reproductive, developmental and non-genotoxic carcinogenicity [47].

For threshold effects, the aim of the risk assessment is to establish a health-based guidance value (HBGV) and compare it with an estimate of the exposure to make inferences of the population being above or below the guidance value, i.e. at risk of disease or not.

Depending on the chemical, the HBGV is termed differently. For substances intentionally added to foods, i.e. additives, pesticide residues and veterinary drugs, the HBGV is termed the acceptable daily intake (ADI). For contaminants that are unavoidably present in foods, e.g. environmental pollutants like dioxin and heavymetals, the HBGV is termed the tolerable daily intake (TDI) or derivatives there of, i.e. provisional tolerable weekly intake (PTWI). Both ADI and TDI are expressed as the dose of the compound that can be ingested daily over a lifetime without appreciable health risk. For chemicals with effects acute in nature, the HBGV is termed an acute reference dose (ARfD), and expressed as the amount of compound that can be ingested in a period of 24 hours or less, without appreciable health risk [55].

The HBGVs are derived from dose response modelling of data mostly from experimental animal studies. The dose response modelling is performed in the hazard characterization step of the risk assessment, and its purpose is to derive a point of departure (POD) (analogous to a reference point), which divided by extrapolation factors (EF) (analogous

to uncertainty factor (UF) and safety factor (SF)) yields the HBGV:

$$\text{HBGV} = \frac{\text{POD}}{\text{EF}} \tag{2.4}$$

Point of departure

The POD may be represented by the no-observed-adverse-effects-level (NOAEL), which is the highest dose administered to the experimental animals where no treatment related adverse effects are observed. The NOAEL is based on statistical tests comparing the dose groups with the control, biological relevance and expert judgement. The NOAEL is dependent on the doses which have been selected for the animal study and on the statistical power of the study design [56].

To overcome this dependency and to make use of all datapoints from the dose response data, the benchmark dose (BMD) approach was developed. The BMD is the dose level associated with a specified change in response from the background, the benchmark response (BMR), estimated from fitting a model to the dose-response data. The BMR agreed upon is 5% for continuous data (e.g. body weight, enzyme activity, organ weight etc.) and 10% for quantal data (e.g. proportion of animals with tumors in a dose group)[57]. In the BMD approach, the lower level of the 95% one-sided confidence interval around the BMD, i.e. the BMDL, is used as the POD for the HBGV. Thus, the $\text{BMDL}_{5\%}$ is the dose where the change in response is likely to be smaller than 5% [57]. The type of model fitted to the dose response data to derive the BMDL depends on whether the data is continous or quantal. The models that are found appropriate from a biological point of view, to describe the two types of data are implemented in software developed for BMD-modelling, e.g. PROAST [58] or BMDS [59]. The software fits the range of models to the dose response data. The models are evaluated by their goodness of fit to the data, and generally the lowest BMDL obtained from the accepted models is used as the POD [57]. However, to express the uncertainty in the POD it is recommended to apply model averaging for estimation of the BMDL, though this method currently is not implemented in the available software as a default option [57].

Extrapolation factors

When animal data are used for setting the HBGV, the EFs applied cover an interspecies extrapolation (from animal species to humans) and an intraspecies extrapolation (the differences in response human to human). Traditionally, an overall EF of 100 has been applied: an EF of 10 for interspecies differences, assuming that the human is less than 10 times more sensitive than the animal, and an EF of 10 for intraspecies differences, assuming that the most sensitive individual is less than 10 times more sensitive than the typical [60][61]. From the overall EF of 100, adjustments can be made depending on e.g. the quality of data to derive the POD (poor data would require an increased EF), the severity of the effect under consideration (if the effect is reversible, the EF might be decreased), if effects are observed in a subchronic study design but applied to describe chronic effects in humans (EF might be increased to account for this added uncertainty), etc. [55]. Additionally, the default EF of 10×10 can be divided into subfactors, describing the contribution to the overall EFs from toxicokinetic- and toxicodynamic domains of both the inter- and intraspecies extrapolation (Table 2.1)[62].

Table 2.1: Default extrapolation factors applied in toxicological risk assessment of chemicals. Adapted from [55].

	toxicokinetic	toxicodynamic	combined
Interspecies extrapolation	4.0	2.5	10
Intraspecies extrapolation	3.16	3.16	10

Ideally, ADME data for the specific chemical should determine these subfactors, but the data are rarely available. However, in the interspecies toxicodynamic domain, correction for bodysize differences between the experimental animal and the human can be accounted for by allometric scaling [63]:

$$\text{Allometric scaling} = \left(\frac{\text{mean human bodyweight}}{\text{mean animal bodyweight}}\right)^{1-0.7}, \tag{2.5}$$

where the mean of the bodyweights of the human study population is used; the difference in human bodyweight is taken into account in the intra-species extrapolation [64].

2.2.1.2 Hazard characterization of non-threshold effects

Non-threshold effects are health effects that are assumed to occur at any dose or concentration down to zero, and include the health effects: sensitization, mutagenicity, genotoxicity and genotoxic carcinogenicity [65][47].

For non-threshold effects, the aim of the risk assessment is not to establish a HBGV, as any level of exposure could result in an adverse effect. For non-threshold contaminants that are unavoidable in foods, the "as-low-as-reasonable-achievable" (ALARA) principle has been accepted, but for risk management purposes, this principle is not particularly useful as it does not take potency into account. Instead, the margin of exposure (MOE) was developed, which is the ratio between a POD and the estimated human exposure [50][66][67] i.e.:

$$\text{MOE} = \frac{\text{POD}}{\text{human exposure}} \tag{2.6}$$

A MOE of 10,000 or above is considered of low health concern, if the chemical is a genotoxic carcinogen [68]. However, the interpretation of the MOE is in principle analogous to identifying the dose at which the probability of effect is negligible, i.e. a virtual safe dose. For genotoxic carcinogens, a virtually safe dose might be expressed as a dose which results in a lifetime risk of 1 out of 1,000,000 (i.e. 10^{-6}) or 1 out of 100,000 (i.e. 10^{-5}). If the POD used to establish the MOE is the dose reflecting a change in effect of 10%, then the dose corresponding to a 10^{-6} risk is obtained by dividing the POD with 100,000, assuming a linear dose response curve at exposures relevant for humans [55]. Thus, risk assessment of non-threshold effects can also provide a quantitative risk estimate, i.e. what is the risk in a population at different exposure levels [67] [66].

Point of departure

In the MOE approach for genotoxic carcinogens, the BMDL associated with a BMR of 10% above the background response, i.e. BMDL_{10}, is recommended as the POD. The methodology for deriving the BMDL_{10} is the same as applied for threshold effects. If a quantitative risk estimate is derived by linear extrapolation, the extrapolation takes place from a POD, most often the $\text{BMDL}_{10\%}$.

Extrapolation factors

EFs applied for non-threshold effects are applied to account for the uncertainty of: 1) extrapolation from animal to human and human to human and 2) extrapolation to estimate risk for exposures outside (lower than) the range of observation in animal studies. A MOE of 10,000 or above is considered to cover the uncertainties, and thus be protective. To derive a quantitative estimate of the risk requires extrapolation of the animal tumour incidences observed at high doses down to the much lower doses, at which humans are exposed. The tumour incidences estimated at the human relevant doses are greatly influenced by the shape of the dose response relationship, which in principle is unknown. By considering a no-threshold effect for genotoxic carcinogens, it is assumed that the mode of action by which a chemical is causing cancer is stochastic, i.e. the exposure to one single molecule can result in DNA damage in turn resulting i a tumour [50]. In that case, it is assumed that the dose-response relationship at very low exposures is linear. However, several biological arguments infer that the dose-response relationship is in fact sub-linear, as several repair mechanisms take place upon DNA damage, and a linear dose response relationship is in fact a conservative assumption. The inter- and intraspecies EFs applied to derive a quantitative risk estimate are the same as for threshold-effects.

2.2.1.3 Exposure assessment

The exposure assessment of chemicals in foods make use of information on the concentration of the chemical in the foods consumed and information on the amount of food consumed by the population. The general equation for calculating the dietary exposure to foodborne chemicals is the same for chronic and acute effects:

$$\text{Dietary exposure} = \frac{\sum C \times I}{\text{kg bodyweight}}, \tag{2.7}$$

where C is the concentration of the chemical in the food and I is the consumption of the food. The exposure for chemicals is given per kg bodyweight of the consumer, and it can be summed over all foods containing the chemical [28].

For chronic effects, the exposure over long time is estimated and given as an usual exposure per day over a given time period, e.g. a lifetime. For acute exposure assessments,

the exposure over a short period of time, e.g. 24 hours, or even per eating occasion, is estimated [69].

The data source for chemical concentrations depends on the intention of the dietary exposure assessment. If it is intended for substances that are not yet introduced to the market place, regulatory maximal concentrations of the chemicals can be used to estimate a potential worst case exposure assessment [28][70]. If the assessment is intended for chemicals that are already present in the food-supply or for natural or process contaminants, concentration data are obtained from databases representing chemcial concentrations in the foods on the market or at the manufacturer. Databases include chemical concentrations from monitoring and surveillance programs installed by the food authorities targeted to specific foods or sampled at random or from the food manufacturer [28]. For the study of burden of disease of foodborne chemicals, the purpose is to estimate the disease burden connected to the current (or past) foods consumed, and concentration data on the foods on the market and at the point of consumption is required.

The data source for amount of food consumed by individuals in a population may be collected from consumption surveys in the form of e.g. food diaries, FFQs or dietary recalls, performed on an individual or household basis. All of these have the advantage that they provide information on food consumption patterns at the individual level; disadvantages include that people tend to over- and underreport healthy and unhealthy eating, respectively, or otherwise change diet habits from their normal during the survey [70]. In addition, these surveys seldom collect information on the preparation methods of the foods consumed, which is a problem when the purpose is to estimate exposure to for example process contaminants. In Denmark, information on the dietary patterns of the Danish population is assessed in the Danish national survey of dietary habits and physical activity (DANSDA). The survey has been performed 5 times since 1985 with the latest from 2011-2013 [71], and for each cycle a new representative sample of approximately 4000 individuals aged 4-74 Danes is selected. Dietary data are collected by means of a seven day food diary completed by the individuals. The food diary is structured as a typical Danish meal pattern, comprising breakfast, lunch, dinner and three snack meals, and individuals record the types and amounts of foods eaten per meal. Prior to the food recording, information on smoking habits, physical activity, weight and height, educational level and attitudes towards healthy eating habits is collected in face- to-face

interview. After completion of the diaries, total intake of foods is calculated by use of the software system GIES developed at the National Food Institute, which includes standard recipes, information on portion sizes and data from the Danish Food Composition Database (http://frida.fooddata.dk/)[72]. Information on food consumption can also be derived from food supply data informing on the amount of foods produced and changes in stocks to estimate an average consumption of an average individual irrespective of age or gender [70].

If a validated biomarker exist for a chemcial, the exposure assessment may be performed via collection of samples of body fluids or tissue, rather than relaying on equation 2.7, data availability and quality. The biomarker is often the chemical itself or a metabolite and is therefore a direct indication on the individual's exposure level [70]. However, biomarkers also have their limitations; as an example, the biomarker of acrylamide exposure, the acrylamide-haemoglobin (AA-Hb) adduct, is a good indicator for an individual's average exposure to acrylamide, but only for the 4 months that is the lifetime of an erythrocyte [73]. Thus, the AA-Hb does not relay better information on the longterm exposure to acrylamide than does a food diary. Another disadvantage of biomarkers is that it is not possible to distinguish between sources of exposure [70].

2.2.2 Risk characterization vs. disease burden

To summarize: a POD is derived and EFs applied in the hazard characterization to: i) determine a level of exposure, the HBGV, that protects the most sensitive individuals in the population, ii) to estimate the MOE; or iii) as starting point for a quantitative risk estimation. In the risk characterization, the PODs are combined with estimates of current (or expected) population exposure to the chemical, to derive evidence for the (potential) population risk. For this purpose, both POD and EFs are chosen to represent conservative or worst case estimates to take uncertainty into account.

To estimate the burden of disease, a quantitative risk estimate is needed, i.e. the fraction of a population experiencing the health effect. This quantitative risk estimation is not derived in the traditional HBGV approach. In the MOE approach, the quantitative risk estimate is indirectly derived, i.e. a MOE of 25,000 is equivalent to a risk of cancer no higher than 4 cases out of 1 million using $BMDL_{10\%}$ as POD and assuming linear

extrapolation [50][67]. This is similar to deriving risk by extrapolating linearly from incidences in the observable regions in animal studies to lower ranges of human exposures. In disease burden studies, the POD must reflect the dose that separates effect from no effect rather than assuring a margin of safety. EFs are applied to inform on the uncertainty of the extrapolation between species and the variation in the sensitivity among humans, rather than with the purpose to protect the most sensitive individuals.

The results of a risk assessment provide evidence to be used in the risk-management process of securing a high level of protection, e.g. establishing and enforcing maximum limits of harmful chemicals in foods. Indeed, if risk mitigation strategies are effective, the disease burden due to the chemicals should be low.

In some situations, the HBGV and MOE do not present sufficient information to act on. As an example; if a risk assessment concludes that exposure is above the HBGV or MOE is below 10,000, then risk of disease in the popualtion cannot be excluded. If a quantitative estimate of the risk is not available, and at the same time a reduction in exposure is challenging (expensive for producers, practically impossible etc.), no information can be derived regarding the health benefits of reducing exposure contra its costs [74][75][61].

For this purpose, several proabilistic apporaches to toxicological risk assessment have been proposed, where the hazard characterization and/or the exposure assessment has been performed probabilistically, i.e. describing input parameters by probability distributions and combine them by Monte Carlo simulations. In the integrated probabilistic risk assessment (IPRA) methodology proposed by Slob and van der Voet (2007) [74], both the hazard characterization and exposure assessment are described probabilistically and combined to provide estimates of the population risk, i.e. the fraction of a population experiencing a health effect. This approach quantitatively takes into account the variation in individual's exposure and sensitivity to a chemical and the associated uncertainties. Thus, IPRA is a methodology that can be used to derive disease incidence following exposure to most chemicals for which the strenght of evidence allows for a dose response relation, as well as quantify the associated uncertainties, which is necessary for the comparison of disease burden across hazards. The IPRA methodology was applied to estimate the global disease burden of dioxin in the GBFD project [15]. The IPRA methodology is described for non-carcinogens and carcinogens below.

2.2.3 Probabilistic risk assessment of chemicals

In IPRA two distributions are derived: a distribution of the doses above which an effect occur in individuals ("critical effect doses") and a distribution of the dietary exposure of individuals in a population. The two distributions are combined by draws of a random value from each distribution to assess, for a sufficient number of draws, the fraction of individuals for which the exposure exceeds the critical effect dose (fig 2.7)[74][64][76].

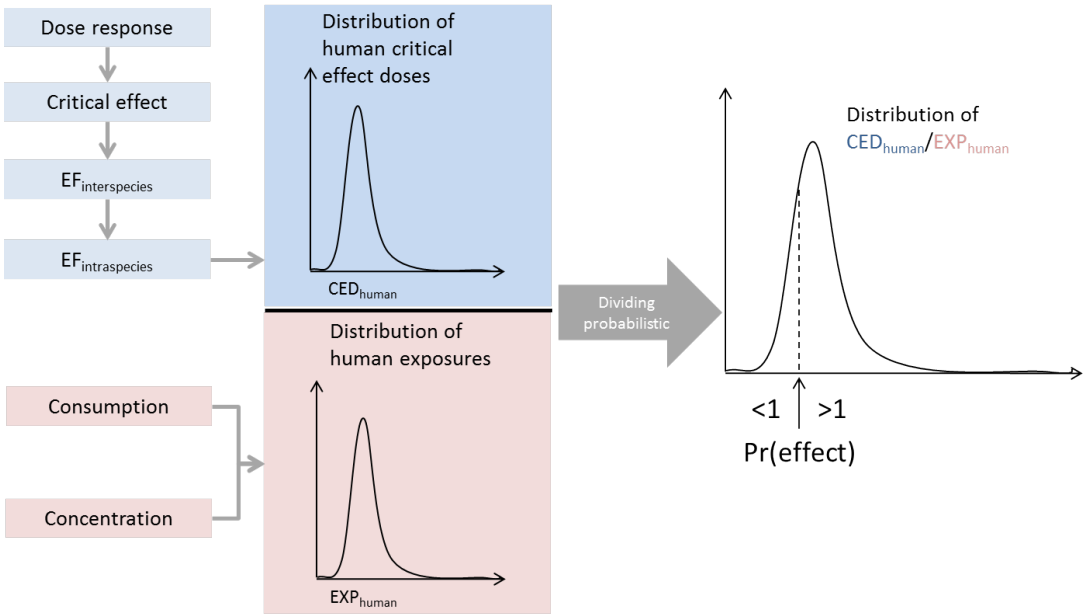

Fig. 2.7: Overview of the integrated probabilistic risk assessment (IPRA) approach The distribution of critical effect doses in humans (CED_{human}) are combined with the distribution of individual exposures (EXP_{human}) to estimate the probability of a critical effect (Pr(effect)) (adapted from van Der Voet (2009) [77])

.

The distribution of critical effect doses reflects that individuals have a different sensitivity to the same chemical, and thus one person might not experience the adverse health effect at a dose where another person does. The distribution of critical effect doses is derived from a POD from animal studies and inter- and intraspecies EFs [74]. However, instead of deterministic worst case estimates, the POD and EFs are described by probability distributions reflecting the plausible range of each factor. In principle, the worst case EFs applied in the traditional deterministic approach should numerically be

similar to the upper bounds of the distributions replacing the EF-point estimates [74]. Likewise, the exposure distribution describes the range of individual exposures in a population. Distributions are combined probabilistically by Monte Carlo simulation, in which the calculation is repeated many times (iterations). In each iteration, a value from each distribution is sampled, where the shape of the distribution determines the frequency of a given value is being sampled. When enough iterations are performed, a probability distribution of the outcome is obtained [78].

Variability and uncertainty

When input parameters in a toxicological risk assessment are applied as point estimates, variability and uncertainty are not distinquished from each other. However, uncertainty and variability are two separate concepts. Variability describes the irreducible variation or heterogeneity of the subjects in a population. If the bodyweight of all individuals in a human population or the concentration of a chemical in all foods eaten is measured, then the distribution of all measurements only reflects the variation in the population. However, it is most often the case that input parameters of the risk assessment are derived from samples that are more or less representative of the population. Therefore, the input parameters do not only represent variation but also uncertainty. Uncertainty can be reduced if more information is gathered about the uncertain parameter [79]. Variability and uncertainty need to be handled separately in second order Monte Carlo simulations [80][81].

In IPRA, variability is taken into account in the exposure and in the intraspecies extrapolation. Uncertainty is accounted for in the exposure assessment, the dose response model, POD and the inter- and intra species extrapolation factors. The distributions describing the inter- and intraspecies extrapolation may be derived specifically for a chemical if data exist. However, generic distributions assumed to cover all chemicals have been derived from historical data and described in the literature [63][77]. The generic distributions are shown in table 2.2.

Thus, the result of the IPRA is an estimate of the fraction of a population experiencing a health effect, i.e. the probability that the exposure exceeds the critical effect dose given the variation of both in the population, and additionally the uncertainty in that estimate.

Table 2.2: Generic distributions for extrapolation factors applied in an integrated probabilistic risk assessment of chemicals. Adapted from Bokkers et al. (2007, 2009), van der Voet et al. (2009) and Slob et al. (2014) [63][64][77][76]

.

	Distributional assumption	Parameter values	Reference
Allometric power	$N(\mu, \sigma)$	$\mu = 0.7$,$\sigma = 0.033$	[76]
Interspecies extrapolation for TKTD	logN(GM, GSD)	GM $= 1$, GSD $= 2$	[63][64]
Intraspecies extrapolation	logN(GM, GSD)	GM $= 1$, GSD $= 3.6$	[75][76]
Uncertainty in intraspecies extrapolation	χ^2	$df = 21$	[77]

IPRA for non-carcinogens

The distribution of critical effect doses are for non carcinogens derived similarly to deriving a HBGV. The POD is derived from the BMD-approach, though the BMR is not 5% by default, by fitting the appropriate models to the animal data. Rather, the POD from animal studies is the dose that separates adverse from non-adverse health effects. For continous effects it must be specified which percent change in the response relative to the response in the controls is considered adverse [64]. For quantal effects, the ED50 can be used as POD, which is the dose where half of the animals under study experience the adverse effect, under the assumption that if all experimental error and variation between animals was removed from the study, the dose response relationship would be a step function separating affected animals from non-affected [64].

The average animal POD, $\text{POD}_{\text{ave,animal}}$ is extrapolated to an average human POD, $\text{PoD}_{\text{ave,human}}$ by:

$$\text{PoD}_{\text{ave,human}} = \frac{\text{POD}_{\text{ave,animal}}}{\text{EF}_{\text{inter}}}, \tag{2.8}$$

where the $\text{POD}_{\text{ave,animal}}$ is described by a distribution which reflects the uncertainty in the dose response model, rather than using the POD derived from the most sensitive model. The model uncertainty is evaluated by bootstrapping of the POD from each accepted model, and the boostrapped PODs are combined in an overall empirical POD distribution [74][63]. Likewise, the EF_{inter} is the product of two parametric distributions: i) allometric scaling (equation 2.5), where the allometric factor (0.7 in equation

2.5) is considered uncertain and described by an normal distribution [74][64],[76]; and ii) uncertainty in the toxicodynamic and toxicokinetic differences between the average experimental animal and the average human [63](Table 2.2). The allometric power is assumed to be uncertain, but in the range of 0.65-0.75. To express this uncertainty, a normal distribution with a mean and standard deviation is defined so that its 5th and 95th percentiles reflect this range. The uncertainty distribution for allometric scaling covers a larger range than the standard (deterministic) scaling factors that are used to adjust by either a caloric requirement or body surface area approach [82]. The remaining TKTD difference after allometric scaling is described probabilistically by a log normal distribution, where the 5th and 95th percentiles are 0.3 and 2.99, respectively [63][64]. For comparison, the default deterministic EF used is 2.5 (table 2.1).

The distribution of the individual critical effect doses in the population, $\mathrm{PoD}_{i,\mathrm{human}}$, is from the POD distribution of the average human derived by:

$$\mathrm{PoD}_{i,\mathrm{human}} = \frac{\mathrm{POD}_{\mathrm{ave,human}}}{\mathrm{EF}_{\mathrm{intra}}}, \tag{2.9}$$

where the $\mathrm{EF}_{\mathrm{intra}}$ in IPRA is a log-normal distribution, with a geometric mean of 1, meaning that 50% of the population is more sensitive and 50% of the population less sensitive, and a geometric standard deviation, which is decided upon depending on an assumption about how much more sensitive the most sensitive individual is compared to the median individual, i.e. it might be assumed that the most sensitive (e.g. 95$\hat{\text{t}}$h percentile individual) is between 5 to 20 times more sensitive than the median individual, which results in a geometric standard deviation of 3.6 [74]. The uncertainty in the geometric standard deviation is then expressed by a Chi-square distribution with 21 degrees of freedom [76].

Finally, the distribution of the critical effect doses in humans is by Monte Carlo simulation divided by the distribution of individual human exposures, i.e. for a sufficient number of iterations, a random value from each distribution is sampled and divided, resulting in a distribution of the ratios between the individual critical effect dose and the individual exposure. The fraction of the simulated population for which the ratio is below 1, equals the fraction of the population experiencing an effect. As described above, in the combination of the probability distributions, the distributions describing variability and uncertainty must be handled separately in second order Monte Carlo simulation. The

result is the fraction of the population experiencing the adverse effect, resulting from the distributions describing variability, and an associated uncertainty interval, resulting from the distributions describing the uncertainty.

IPRA of carcinogens

The majority of carcinogenicity studies in animals report the incidence of tumors in the studied dose groups and are therefore quantal data [64]. The ED50 might be used as POD like quantal data of non-carcinogens (i.e the ED50 is the tolerance dose to exceed in order to get cancer), however, for carcinogens another interpretation of the quantal data is also possible. In this interpretation, it is assumed that the probability of developing cancer is a stochastic process, i.e. it is a matter of chance if a carcinogenic molecule hits the DNA in a gene that is relevant in developing cancer (or other critical events in the cancer process occur). In this case the entire dose response relation informs on an individual's risk of developing cancer at various exposures.

In this case, the IPRA is reversed. Dose response modelling is performed as usual, but the exposure of individuals in the human population, $\mathrm{EXP}_{i,\mathrm{human}}$ is extrapolated to an equivalent animal exposure, $\mathrm{EXP}_{i,\mathrm{animal}}$ by:

$$\mathrm{EXP}_{i,\mathrm{animal}} = \mathrm{EXP}_{i,\mathrm{human}} \times \mathrm{EF}_{\mathrm{inter}} \times \mathrm{EF}_{\mathrm{intra}}, \tag{2.10}$$

and combined with the dose response model. $\mathrm{EF}_{\mathrm{inter}}$ and $\mathrm{EF}_{\mathrm{intra}}$ are the same probability distributions as applied for non-carcinogens. By Monte Carlo simulation the $\mathrm{EXP}_{i,\mathrm{animal}}$ is combined with each of the accepted dose response models and parameter values to estimate the fraction of the population developing cancer, and an estimate of the uncertainty around that estimate [76]. This approach is in IPRA referred to as model extrapolation.

2.2.4 In summary and perspectives

A hazard- and incidence based approach is applied to estimate DALYs for foodborne hazards, including foodborne chemicals. Data from either human observational or toxicological studies are most often used to derive disease incidence, as surveillance data are rarely available for diseases caused by foodborne chemicals. Hazard-health outcome pairs are identified by review of the scientific literature, and the selection is based on at least 4

criteria; available evidence for a causal effect, available evidence for deriving dose response relationship, available evidence for exposure assessment, and available disability weight. In Manuscript 2 (chapter 4), we assess how the criteria for the strength of evidence of a causal effect might influence the selection of hazard-health outcome pairs.

A counterfactual analysis is used if RRs are available. A risk assessment approach is used if a dose response relationship is derived from studies in experimental animals. If disease envelopes are available, PAFs are estimated to attribute DALYs to the foodborne chemical. If disease envelopes are not available, the usual parameters are used to estimate YLD and YLL. For each hazard-health outcome pair, a health outcome tree is designed, in which the various health states and transmissions between them, are schematically presented and the outcome tree informs on how each health state contributes to the overall DALY.

Information on a dose response relationship is for many chemicals only available from studies in experimental animals. A risk assessment approach is applied with methodologies derived from toxicological risk assessment. However, the purpose of toxicological risk assessment differ from the purpose of disease burden studies. In toxicological risk assessments, the population exposure is compared with a HBGV or a MOE is derived, to assess if the population is sufficiently protected. For burden of disease studies, a quantitative risk estimate is needed, i.e. the fraction of a population experiencing the health outcome. A quantitative estimate of the risk posed by a genotoxic carcinogen may be derived by linear extrapolation as a conservative approach. A probabilistic approach can be applied to derive a quantitative risk estimate for both threshold and non-threshold effects, which also allows for propagation of the uncertainty in the exposure assessment, dose response models, and in the extrapolation from the experimental animal to humans.

In Paper 1 (chapter 3) and Manuscript 3 (chapter 5), we estimate the disease burden of dietary exposure to AA and BAP in barbecued meat, respectively. We apply a hazard- and incidence-based approach. The disease incidence in both studies is derived from animal data using a risk assessment approach. In Paper 1, the risk assessment approach is referred to as an exposure-based approach. The exposure assessments in all 3 studies make use of concentration data obtained from the Danish monitoring program of chemicals and, in the case of benzo[a]pyrene, data found in the literature. The consumption data are obtained from DANSDA. In Paper 1, we estimate the incidence of cancer from a linear

extrapolation; in Manuscript 3, we apply the probabilistic model-extrapolation. In Paper 1, we estimate DALY both with and without a disease envelope, in the paper referred to as the indirect and direct approach, respectively; in Manuscript 3, we estimate DALY without a disease envelope.

Chapter 3

Paper I

3.1 Prelude

The first study of the PhD project was undertaken as a case study with the purpose of assessing the applicability of existing BoD methodology to estimate the incidence of disease due to exposure to a foodborne chemical using animal studies. The objectives were to apply the approaches to estimate DALY (identified in chapter 2) and evaluate how the methodological choices affect the final DALY estimate. To estimate the burden of disease of dietary exposure to AA was decided, as AA has received a lot of attention due to its widespread occurrence in a range of commonly consumed food products, and the margin of exposure was estimated to be low and thus considered a health concern. [49].

3.2 Burden of disease of dietary exposure to acrylamide in Denmark

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Burden of disease of dietary exposure to acrylamide in Denmark

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ABSTRACT

Acrylamide (AA) is a process-contaminant that potentially increases the risk of developing cancer in humans. AA is formed during heat treatment of starchy foods and detected in a wide range of commonly consumed products. Increased focus on risk ranking and prioritization of major causes of disease makes it relevant to estimate the impact that exposure to chemical contaminants and other hazards in food have on health. In this study, we estimated the burden of disease (BoD) caused by dietary exposure to AA, using disability adjusted life years (DALY) as health metric.

We applied an exposure-based approach and proposed a model of three components: an exposure, health-outcome, and DALY-module. We estimated BoD using two approaches for estimating cancer risk based on toxicological data and two approaches for estimating DALY.

In Denmark, 1.8 healthy life years per 100.000 inhabitants are lost each year due to exposure to AA through foods, as estimated by the most conservative approach.

This result should be used to inform risk management decisions and for comparison with BoD of other food-borne hazards for prioritizing policies. However, our study shows that careful evaluation of methodological choices and assumptions used in BoD studies is necessary before use in policy making.

1. Introduction

Acrylamide (AA) is a food-process contaminant and classified as a 'probable human carcinogen' (IARC, 1994). Long-term carcinogenicity studies in rats and mice have shown that oral AA exposure may lead to tumors in multiple organs of the rodents (Johnson et al., 1986; Friedman et al., 1995; Beland et al., 2013; FDA, 2012). These findings strongly support mechanistic studies that have shown that AA is a genotoxic carcinogen by its metabolic activation to glycidamide (GA), which is reactive towards DNA and proteins (Fennell et al., 2005; Beland et al., 2013). AA is produced during high temperature processing (>120 °C) of commonly consumed starchy foods, and the relationship between dietary intake of AA and the risk of cancer in humans has been evaluated in several epidemiological studies. A borderline significant increase in risk of cancer of the kidneys (RR = 1.20, CI95: 1.00–1.45), endometrium (RR = 1.23 (1.00–1.51)), and ovaries (RR = 1.39 (0.97–2.00)) was identified in a recent meta-analysis (Pelucchi et al., 2015). Additionally, a significantly increased risk of estrogen receptor-positive breast cancer and mortality has been identified in a study using biomarkers for AA exposure (Olsen et al., 2012; Thonning Olesen et al., 2008). On this basis, EFSA reconfirmed that 'AA in food potentially increases the risk of developing cancer for consumers of all age groups' (EFSA, 2015), and therefore it is of relevance to investigate the contribution of dietary AA to the disease burden of cancer.

Burden of disease (BoD) is the impact that a disease has on society in terms of mortality, morbidity and disability. Several measures have been developed to estimate BoD; one of these is the Disability Adjusted Life Year (DALY), which integrates disease incidence, severity, duration, and mortality (Murray and Lopez, 2013). Estimation of BoD using DALYs is a useful tool to compare the health impact of various diseases, and evaluate the contribution of the risk factor(s) to the disease burden. BoD studies have been conducted for environmental risk factors (e.g. Hanninen et al., 2014), nutritional factors (e.g. Lim et al., 2013), and foodborne pathogens (e.g. Havelaar et al., 2012; Kretzschmar et al., 2012).

For risk ranking purposes and inclusion in risk-benefit

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0278-6915/

assessments, it is of interest to estimate the burden of disease attributed to exposure to chemicals through foods, using DALY as a health metric. The risk due to exposure to the chemical needs to be quantified and expressed as an annual incidence of the given health effect caused by the chemical. The risk quantification can be based on toxicological data, epidemiological data or both, depending on data availability for the chemical.

The aims of this study were to estimate the burden of cancer due to dietary exposure to AA in Denmark in terms of DALYs, and to estimate the contribution of different foods to this disease burden. We applied an exposure-based approach based on toxicological data, and evaluated the impact of different models to quantify the human cancer risk, as well as the impact of different approaches to calculate DALY.

2. Method and materials

To estimate the disease burden, we built a model consisting of three components: an exposure-, health outcome- and DALY module (Fig. 1). In the exposure module we estimated the lifetime mean daily exposure to AA of the Danish population. This estimate was integrated with the health-outcome module, in which the probability of occurrence of the selected health outcomes following exposure to AA was estimated based on dose–response relationships from animal studies. In the third module, we used the probability of occurrence of the health ouctomes, estimates of life expectancy, disease duration and disability weights to calculate the BoD in terms of DALYs. A more detailed description of the model follows.

2.1. Exposure assessment module

We defined exposure as the mean daily intake over a lifetime of μg AA per kg body weight, y, calculated by:

$$y = \frac{\sum_{i=1}^{N_{indi}} \sum_{j=1}^{N_{food}} \frac{I_{ij} C_j}{bw_i}}{N_{indi}} \quad (1)$$

where N_{indi} is the total number of individuals in the model, N_{food} is the total number of food types in the model, I_{ij} is the mean intake over seven days by individual *i* of food *j* in g/day, C_j is the mean concentration of AA in food *j* in μg/kg, and bw_i is the bodyweight of individual *i* in kg.

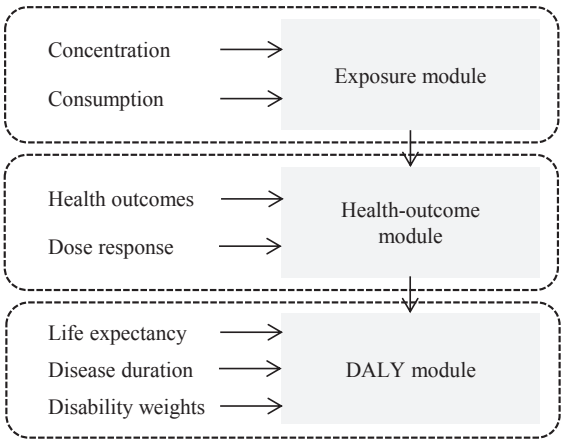

Fig. 1. Framework of model used to estimate the burden of disease of dietary exposure to acrylamide.

2.1.1. Concentration data

Concentrations of AA in various food types have been investigated in Denmark since 2003. Data for the analysis was obtained from Danish surveys on specific food types and from the general monitoring program (Petersen et al., 2013).[1] We used all available concentration data from 2003 to 2013, as no substantial change over time in concentrations in the investigated foods was observed. The food types, mean concentrations and standard deviations are shown in Table 1.

2.1.2. Consumption data

The consumption data were obtained from the Danish National Survey of Diet and Physical Activity, a national-wide, cross-sectional survey in a representative sample of the Danish population (Biltoft-Jensen et al., 2009). Diet is assessed by seven day pre-coded food records, and intake of foods and nutrients estimated by use of the Danish food composition tables (www.foodcomp.dk) and the software system GIES developed at DTU Food. Data applied in this study were collected in 2005–2008 from 2700 individuals. To adjust for potential skewness in the study population, weighting factors constructed on the basis of age, gender and education were applied.

2.2. Health-outcome module

2.2.1. Choice of health effects

The pivotal effects in humans following exposure to AA are neurotoxicity and carcinogenicity (Beland et al., 2013; Burek et al., 1980; Friedman et al., 1995; Johnson et al., 1986). In this study we selected cancer as the health effect to be accounted for in the model, as neurotoxicity is assumed to be caused by higher levels of exposure experienced for example in occupational settings rather than through diet (EFSA, 2015). The cancer types with a (borderline) statistically significant association with AA estimated by Pelucchi et al. (2015), and cancer types identified using biomarkers in exposure assessment of AA by Thonning Olesen et al. (2008) and Olsen et al. (2012) were selected. The following four health outcomes were included in the model: kidney cancer, ovarian cancer, endometrial cancer and breast cancer. In parallel, we accounted for total cancer in the model, recognizing that AA is a multi-site carcinogen (Beland et al., 2013; EFSA, 2015; US EPA, 2010) and assuming that the carcinogenic potency of AA is similarly in all tissues.

2.2.2. Dose-response modeling

We applied two different dose–response models to estimate the slope factor (SF). SF is the slope of a straight line drawn from a dose on the dose–response curve in the observable tumor range (the point of departure (PoD)) to the origin (0,0) in order to estimate effects in the low dose range. SF expresses the increase in the risk of cancer per daily unit of exposure to the carcinogen throughout lifetime (US EPA, 2005). We applied; 1) a model proposed by the US EPA in the toxicological review of AA (US EPA, 2010), and 2) a model proposed by Dybing and Sanner (2003). Both models use extrapolation from the same PoD, but the approach and order of inter- and intra-species extrapolation steps differ. These differences are detailed in Fig. 2.

The data used for the interspecies extrapolation also differ between the models. US EPA used toxicokinetic data based on

[1] Also including the 2011-13 monitoring data, not published in Petersen et al. (2013).

Table 1
Concentration of acrylamide in foods accounted for in the exposure assessment, 2003–2013.

Food group	Food type	Sample size	Mean concentration (μg/kg)	S.D.
Bread	Wheat Bread, fine	20	12.6	11.5
	Wheat Bread, coarse	7	10.2	12.2
	Ryebread	28	9.6	7.5
	Crispbread	22	90.4	52.5
	Crackers/biscuits	24	296.8	238.2
Crisps/Chocolate	Crisps/snacks	76	458.9	418.4
	Chocolate	18	92.2	78.1
	Chocolate spread	2	46.9	32.2
	Nuts[a]	14	61.0	N.A.
	Peanuts	5	28.6	40.1
	Popcorn	7	482.6	434.2
	Pretzels	2	100.0	22.6
	Dried fruits	5	46.3	22.6
Coffee/Cocoa	Coffee/Instant coffee	91	8.1	3.0
Cakes	Cake	18	18.3	58.0
	Pastries	9	5.5	4.8
	Cookies	25	140.4	146.4
	Pancakes	1	15.0	
Potato products	Fried potato incl. French fries	97	512.7	390.4
Convenience food	Burger	3	6.7	6.6
	Spring roll	3	16.7	7.7
	Pita bread	2	3.6	1.6
	Toast	2	3.7	0.6
	Pizza	2	7.8	7.0
Breakfast cereals	Rye mixture	5	31.0	33.9
	Cornflakes, Oat-puffs, Special K	22	74.2	46.7
	Other cereals	32	201.0	241.2
	Oats	14	3.6	4.6
	Musli	30	28.5	41.8
	Rye porridge	2	0.9	0.5

[a] Data extracted from Amrein et al. (2005).

comparable levels of AA and GA in blood between rat and human relative to their respective administered doses (EFSA, 2015; US EPA, 2010), and Dybing & Sanner used allometric scaling by: $(BW_{human}/BW_{rat})^{0,25}$ (Dybing and Sanner, 2003). To express the range of the possible BMD_{10} given the dose–response data, we defined PoD as the upper ($BMDU_{10}$) or lower ($BMDL_{10}$) 95%-confidence bounds (one-sided) of the dose associated with a 10% response (BMD_{10}) adjusted for background. $BMDU_{10}$ and $BMDL_{10}$ were modeled on the statistically significant tumor sites in the National Toxicology Program AA oral carcinogenicity study in F433/N rats (Beland et al., 2013; FDA, 2012). We used PROAST vers. 38.9 for the BMD-modeling. Only the tumor sites with data informative enough to derive a PoD were included, i.e. where the BMDU/BMDL for individual models was small and BMDL among models were similar (EFSA, 2011) (Table 2).

The SF is not specific for each of the cancer sites accounted for in the model. If using the same SF for each of the four cancer sites, the proportion of cases attributed AA would be higher for rarely occurring cancer types (e.g. endometrial cancer) than more common occurring (e.g. breast cancer). This could be interpreted as AA being more potent in the endometrium than in the breast. Under the assumption that the distribution of the cancers caused by AA is the same as the distribution of the reported incidence, we therefore scaled SF, by:

$$SF_{scaled,ep} = SF\ \frac{Inc_{ep}}{Inc_{total}} \tag{2}$$

where, $SF_{scaled,ep}$ is the scaled SF for a given human cancer endpoint, Inc_{ep} is the incidence in Denmark for the same endpoint and Inc_{total} is the total incidence for all cancers in Denmark. $SF_{scaled,ep}$ and cancer incidences in Denmark are given in Table 3.

The SF and $SF_{scaled,ep}$ estimated by the two models are shown in Table 3. The difference between SFs obtained from the two models is constant by a factor of 5.3.

2.2.3. Cancer risk estimation

The cancer risk from dietary AA exposure in a given population, expressed as the annual number of cases (AC), was calculated by multiplying the exposure of that population with the SF for a given cancer endpoint, the population size and divided by the life expectancy of the same population:

$$AC = \frac{N_{pop} \times\ y\ \times SF}{LE_{pop}} \tag{3}$$

where N_{pop} is the size of the exposed population, y is the lifelong mean daily exposure of AA in μg/kg bw, SF is the slope factor, and LE_{pop} the life expectancy of the exposed population.

2.3. DALY model

DALYs are calculated as the sum of Years Lived with Disability (YLD) and Years of Lost Life due to premature death (YLL) due to a given health outcome.

We estimated DALY using two approaches; the direct- and indirect. The direct approach accounted for specific cancer sites and "total cancer", while the indirect accounted for "total cancer" only. Because we have also applied two approaches to model the SF (i.e. the US EPA and the Dybing&Sanner approach), we applied six different modeling combinations to estimate DALYs attributed to the dietary exposure of AA (Fig. 3).

2.3.1. Direct DALY approach

In the direct approach, we used national health statistics and WHO disability weights (dw) to estimate the average DALY per cancer case in Denmark by:

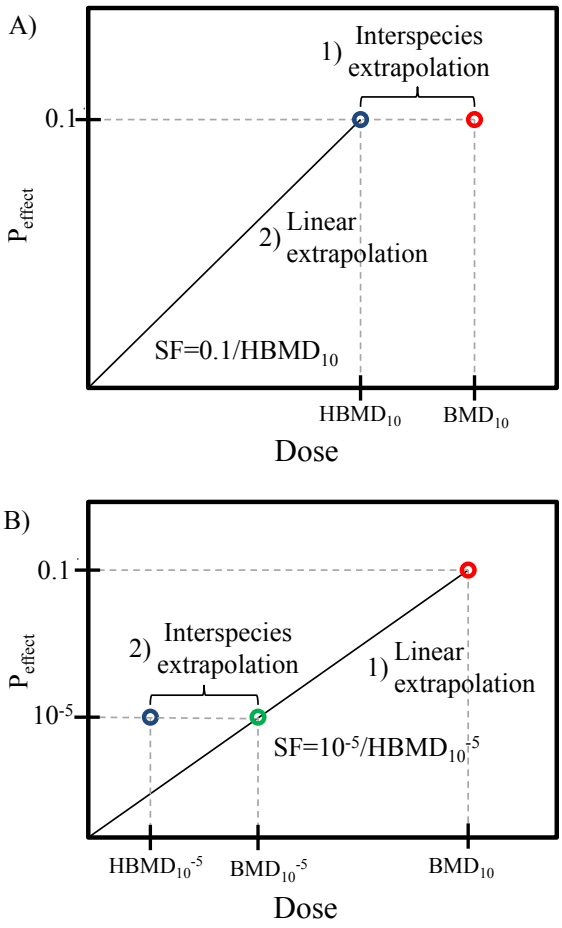

Fig. 2. Overview of the two extrapolation models applied. Red circles: Point of Departure (PoD); Blue circles: Human Benchmark Dose (HBMD). A) US EPA model: interspecies extrapolation is applied to the PoD to obtain a human equivalent PoD ($HBMD_{10}$), from which linear extrapolation is performed. SF is obtained by $0.1/HBMD_{10}$. B) Dybing&Sanner model: PoD is linearly extrapolated to low risk (10^{-5}) animal PoD to which interspecies extrapolation is applied to obtain $HBMD_{10}^{-5}$. By knowing the human dose ($HBMD_{10}^{-5}$) at a risk of 10^{-5}, SF is obtained by $10^{-5}/HBMD_{10}^{-5}$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

$$DALY_{ave/case} = \left(t_f \times dw_f \times p_f\right) + \left(t_{nf} \times dw_{nf} \times p_{nf}\right) + \left(YLL \times p_f\right) \quad (4)$$

where t_f is the duration of disease of fatal cancer in years, dw_f is the disability weight of fatal cancer, p_f is the probability of a cancer being fatal, t_{nf} is the duration of disease of non-fatal cancer in years, dw_{nf} is the disability weight non-fatal cancer, p_{nf} is the probability of a cancer being non-fatal and YLL is the life years lost due to premature death to a fatal cancer. YLD_f and YLD_{nf} are given by the first and second terms in equation (4), respectively. Parameters inserted in equation (4) are shown in Table 4.

DALY attributed to AA per year per cancer type or total cancer was calculated by:

$$DALY_{AA,direct} = AC_{AA,ep} \times DALY_{ave/case} \quad (5)$$

where $AC_{AA,ep}$ is the annual cancer cases per endpoint or total cancer due to AA exposure (eq. (3)).

2.3.2. Indirect DALY approach

In the indirect method the total disease burden for a health effect (i.e. at the population level) as estimated by the Global Burden of Disease (GBD) 2010 project by WHO (2014), (WHO_{BoD}), was multiplied by the fraction of cancer cases caused by AA (AF) out of all cancer cases to estimate the disease burden attributed to AA. Because GBD DALY estimates for each specific cancer type accounted for in this study were not available, we only accounted for total cancer in the indirect DALY approach.

The AF was estimated by:

$$AF = \frac{AC_{AA,ep}}{Inc_{ep}} \quad (6)$$

where $AC_{AA,ep}$ is given by eq. (3) and Inc_{ep} is the actual incidence of cancer.

Thus, DALY attributed to AA per year is given by:

$$DALY_{AA,indirect} = AF \times WHO_{BoD} \quad (7)$$

Again, the two methods differ by a fixed ratio given by:

$$\frac{DALY_{AA,direct}}{DALY_{AA,indirect}} = \frac{DALY_{ave/case} \times Inc_{ep}}{WHO_{BoD}} = 0.5 \quad (8)$$

Parameters used in equations (6) and (7)are given in Table 4.

3. Results

3.1. Exposure assessment

The estimated lifetime daily exposure per kg body-weight of AA through food in the Danish population is 0.36 μg/kg bw/day and 0.27 μg/kg bw/day for males and females, respectively (Table 5). The relative contribution of food groups did not differ considerably between genders, and we estimated that the food group "fried potatoes/French-fries" contributes to nearly half of the total exposure of acrylamide (Fig. 4).

3.2. Burden of disease

The estimated number of cases of specific cancers and DALYs attributed to AA exposure through foods as estimated by the direct DALY approach are given in Table 6. When applying the US EPA

Table 2
$BMDL_{10}$ and $BMDU_{10}$ (mg AA/kg bw/day) for the tumor sites with statistically significant dose–response and informative data to derive a PoD in F433/N rats. The overall lowest and highest $BMDL_{10}$ and $BMDU_{10}$ (highlighted) were used as PoD.

Species	Sex	Tumor	$BMDL_{10}$ (model)	$BMDU_{10}$ (model)
Rat	male	Thyroid gland follicular cell adenoma or carcinoma	**0.887** (Gamma)	3.74 (Log-probit)
Rat	male	Mesothelioma of the epididymis or testes tunica vaginalis	1.21 (Log-probit)	**7.38** (Log-probit)

Table 3
Reported cancer incidence per year in Denmark, SF and scaled SFs ($SF_{scaled,ep}$) i.e. the life time cancer risk per 1 μg AA/kg bw/day, for the two model approaches. Ranges represent the lower and upper bound of the 90% CI around the BMD_{10}.

Cancer site		Incidence[a]	SF model	
			US EPA	Dybing&Sanner
SF	both	36,846	$1.0 \times 10^{-5} - 8.7 \times 10^{-5}$	$5.5 \times 10^{-5} - 4.6 \times 10^{-4}$
	male	18,778		
	female	18,068		
$\mathbf{SF}_{scaled,endometrium}$	female	23	$1.3 \times 10^{-8} - 1.1 \times 10^{-7}$	$7.1 \times 10^{-8} - 5.9 \times 10^{-7}$
$\mathbf{SF}_{scaled,ovaries}$	female	572	$3.3 \times 10^{-7} - 2.8 \times 10^{-6}$	$1.8 \times 10^{-6} - 1.5 \times 10^{-5}$
$\mathbf{SF}_{scaled,breast}$	male	31	$1.7 \times 10^{-8} - 1.4 \times 10^{-7}$	$9.2 \times 10^{-8} - 7.6 \times 10^{-7}$
	female	4910	$2.8 \times 10^{-6} - 2.4 \times 10^{-5}$	$1.5 \times 10^{-5} - 1.3 \times 10^{-4}$
$\mathbf{SF}_{scaled,kidney}$	male	464	$2.6 \times 10^{-7} - 2.1 \times 10^{-6}$	$1.4 \times 10^{-6} - 1.1 \times 10^{-5}$
	female	250	$1.4 \times 10^{-7} - 1.2 \times 10^{-6}$	$7.7 \times 10^{-7} - 6.4 \times 10^{-6}$

[a] Mean cancer incidence per year of 2008–2012 (www.esundhed.dk).

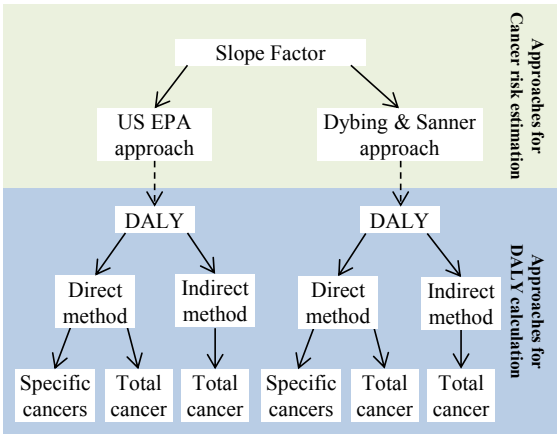

Fig. 3. Overview of the different model approaches applied.

model, we estimated a total of 0.3 annual cancer cases and 1.4 DALY; applying the Dybing&Sanner method, we estimated a total of 1.3 cases and 7.2 DALY (Table 6). We present here only the lower 95% confidence bound i.e. the value estimated with $PoD = BMDL_{10}$.

Table 5
Lifetime daily exposure to AA in Denmark (mean and percentiles).

	AA exposure (μg/kg bw[a]/day)		
	Male	Female	All
Mean	0.36	0.27	0.31
P50	0.26	0.19	0.22
P95	1.01	0.71	0.83
P99	1.50	1.35	1.42

[a] bw: body weight.

Breast cancer in females contributes more than 70% to the total disease burden, which is a consequence of the relatively higher incidence of the considered cancer. Assuming that the current incidence of the specific cancer types (Table 3) is including the cancer risk due to dietary exposure to AA, then 0.005% of the combined incidence for the specific cancer types is attributed to dietary AA if calculated by the US EPA approach. Using the Dybing&Sanner approach, 0.03% is attributed to dietary AA exposure.

Table 7 presents the estimated AA attributed cases and DALYs of total cancer obtained with the direct and indirect approaches. The burden of total cancer attributed to dietary AA estimated by the US EPA-direct DALY approach was 1.7 cases and 9.6 DALYs. Applying the Dybing&Sanner-indirect DALY approach, the estimated burden was more than 8 times higher (90.0 DALYs) (Table 7). The latter

Table 4
Parameters used for DALY calculation by the direct and indirect approaches.

Endpoint	t_n (yrs)[a]	t_{nf} (yrs)[a]	Ave. age of diag.[b]	dw_{nf}[c]	dw_f[c]	p_{nf}[d]	WHO_{DALY}[e]	WHO_{YLL}[e]	WHO_{YLD}[e]	Population[f]	LE[g] (yrs)
Ovarian cancer	3	16	65	0.20	0.47	0.38					
Endometrial cancer	3	14	68	0.20	0.47	0.38					
Breast cancer male	3	6	68	0.32	0.56	0.58					
Breast cancer female	3	19	63	0.12	0.56	0.85					
Kidney cancer male	3	6	64	0.20	0.38	0.57					
Kidney cancer female	3	6	66	0.20	0.38	0.57					
Total cancer male	3	6	67	0.26	0.49	0.58	196,083	190,545	5538		
Total cancer female	3	6	66	0.26	0.49	0.61	177,390	171,203	6187		
Male										2,478,614	78
Female										2,516,681	81.9

[a] Time lived with non-fatal disease (t_{nf}), calculated as the sum of durations of the disease stages: diagnosis and primary therapy, stage after intentionally curative primary therapy, rest of life to expected age of death. t_{nf} for total cancer is assumed on average to be 6 years neglecting rest of life disability after cure. t_f is assumed 3 years for all cancers. Adapted from Australian Burden of Disease and Injury study 1999 (Mathers et al., 1999).
[b] Calculated as the average age of diagnosis weighted by the number of new cancer cases for each age for each cancer, from www.esundhed.dk.
[c] Disability weight, fatal (dw_f) and non-fatal (dw_{nf}) from WHO GBD 2004 Update (WHO, 2004) and Australian Burden of Disease and Injury study (1999) (Mathers et al., 1999).
[d] Obtained from the age-standardized relative survival after 5 years and $p_f = 1 - p_{nf}$ (Engholm et al., 2014).
[e] WHO estimates of DALY (WHO_{DALY}), YLL (WHO_{YLL}) and YLD (WHO_{YLD}) for cancer in Denmark for the year 2012 (WHO, 2014).
[f] Population size of ethnic Danish origin per January 1st 2013, from Statistics Denmark, www.dst.dk.
[g] Life expectancy at birth in Denmark, from Statistics Denmark, www.dst.dk.

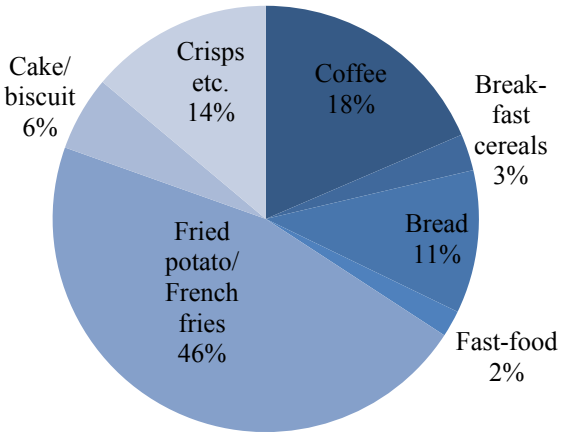

Fig. 4. Contribution of food groups for the mean daily dietary lifetime exposure to AA in Denmark (both genders).

estimate represents 0.02% of the total incidence of cancer in Denmark (Table 3).

Fig. 5 shows the DALYs of total cancer attributed to the specific food groups. Depending on the methods applied, between 0.09 and 0.8 DALY/100,000, could be prevented if the exposure to acrylamide through the food-group that contributes the most to exposure (fried potatoes/French fries) was to be eliminated.

4. Discussion

These are the first estimates of the burden of disease attributed dietary exposure to AA in Denmark. We concluded that elimination of AA through the diet would prevent a health loss of 0.003 DALY/100,000 as estimated by the least conservative approach (using $BMDU_{10}$ as PoD, results not shown), and 1.8 DALY/100,000 as estimated by the most conservative approach (Table 7). We based our estimations on the most recent long-term carcinogenicity study of AA in F433/N rats (Beland et al., 2013; FDA, 2012) and on robust food consumption and food contamination data from Danish official surveys. To our knowledge, the disease burden attributed to AA in foods had been estimated previously only in The Netherlands, in a study that estimated a burden of 2–4 DALY/100,000 (Van Kreijl et al., 2006), a higher estimate than we obtained with either of our methods. This estimate was based on a higher extra annual cases of cancer due to AA (75–130; Konings et al., 2003) and the assumption that all cases are fatal with an average loss of 5 life-years per case (Van Kreijl et al., 2006). Another recent study has estimated the global and regional burden of disease caused by other chemicals in foods and has obtained estimates for the European region in the same range as our results: 0.5 DALY/100,000 for aflatoxin causing liver cancer, and 1 DALY/100,000 for dioxins causing hypothyroidy and decreased sperm count (Gibb et al., 2015).

We developed a BoD model with three components: an exposure module, health-outcome module, and DALY module, and in each of these made assumptions and addressed limitations. In the health-outcome model, we estimated a mean daily dietary exposure to AA through a lifetime in Denmark of 0.36 and 0.27 μg/kg bw/day for males and females, respectively. This is slightly lower

Table 6
Mean incidence per year and disease burden of specific cancers due to AA exposure in Denmark using US EPA and Dybing&Sanner approach and direct DALY[a] calculation. Only estimates using the lower 95% confidence level of BMD ($BMDL_{10}$) as PoD[b] are presented. $DALY_{/case}$ is independent of the dose response models and is estimated from estimates of disease duration, severity and time of onset of disease (eq. (4)); .

Cancer sites	Endometrial	Ovarian	Breast		Kidney		Sum
	Female	Female	Male	Female	Male	Female	
DALY per case	9.0	10.5	4.8	4.6	6.1	6.5	
US EPA							
AC_{AA}[c)]	9.1×10^{-4}	2.3×10^{-2}	1.6×10^{-3}	1.9×10^{-1}	2.4×10^{-2}	9.9×10^{-3}	0.3
DALY total	8.2×10^{-3}	2.4×10^{-1}	7.8×10^{-3}	9.0×10^{-1}	1.5×10^{-1}	6.5×10^{-2}	1.4
DALY/100,000	3.3×10^{-4}	9.4×10^{-3}	3.1×10^{-4}	3.6×10^{-2}	5.9×10^{-3}	2.6×10^{-3}	2.7×10^{-2}
Dybing&Sanner							
AC_{AA}	4.8×10^{-3}	1.2×10^{-1}	8.6×10^{-3}	1.0	1.3×10^{-1}	5.2×10^{-2}	1.3
DALY total	4.3×10^{-2}	1.3	4.1×10^{-2}	4.8	7.8×10^{-1}	3.4×10^{-1}	7.2
DALY/100,000	1.7×10^{-3}	5.0×10^{-2}	1.7×10^{-3}	1.9×10^{-1}	5.9×10^{-3}	1.4×10^{-2}	1.4×10^{-1}

[a] DALY: Disability adjusted life years.
[b] PoD: Point of departure.
[c] AC_{AA}: Annual cases of cancer attributable to exposure to acrylamide through foods.

Table 7
Mean incidence per year and disease burden estimates for total cancer in Denmark using US EPA and Dybing&Sanner approach and the direct and indirect model for DALY calculation. Only estimates using $BMDL_{10}$ as PoD are presented.

Cancer sites	Direct DALY model			Indirect DALY model		
	Male	Female	Sum	Male	Female	Sum
DALY per case	4.7	6.7		10.4	9.8	
US EPA						
AC_{AA}	9.8×10^{-1}	7.1×10^{-1}	1.7	9.8×10^{-1}	7.1×10^{-1}	1.7
DALY total	4.6	4.8	9.6	10.0	7.0	17.0
DALY/100,000	1.9×10^{-1}	1.9×10^{-1}	1.9×10^{-1}	4.1×10^{-1}	2.8×10^{-1}	3.4×10^{-1}
Dybing&Sanner						
AC_{AA}	5.2	3.8	8.9	5.2	3.8	8.9
DALY total	25.0	25.0	50.0	55.0	37.0	90.0
DALY/100,000	9.9×10^{-1}	1.0	1.0	2.2	1.5	1.8

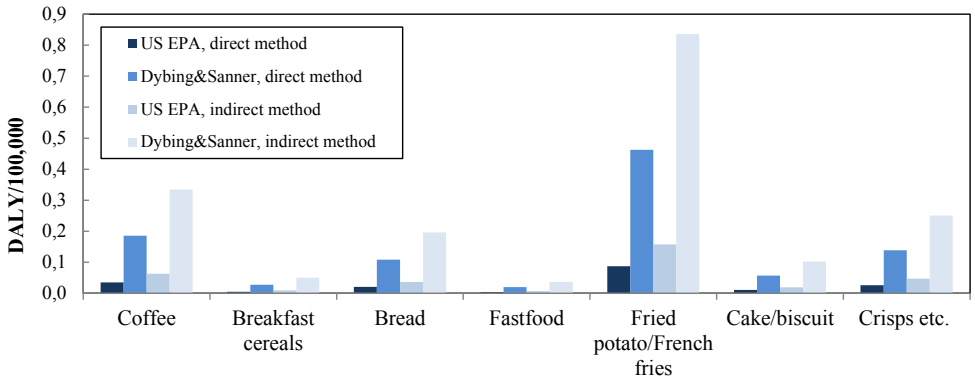

Fig. 5. Attribution of DALYs of total cancer to food groups in Denmark as estimated by different methodological approaches. Only estimates using $BMDL_{10}$ as PoD are presented.

than earlier assessments of AA exposure in the Dutch population, where a mean exposure of 0.48 μg/kg bw/day (median = 0.2 μg/kg bw/day) (Konings et al., 2003) and a median exposure of 0.5 μg/kg bw/day (Boon et al., 2005), were reported. Similarly, in the US the estimated mean AA exposure was 0.44 μg/kg bw/day (Doerge et al., 2008). We found that french-fries/fried potatoes contributed with 45–48% of the total exposure, with coffee (~15%) and chips (14–17%) as the second and third largest contributors. Others reported these food-groups as the largest contributors as well, though fried potato products contributed to a lesser extent (Boon et al., 2005; Doerge et al., 2008; Dybing and Sanner, 2003; Svensson et al., 2003). Overall, estimating exposure to AA through the diet is hampered by large variation in the AA content, both within and between food-types, and between home-prepared and commercial foods (EFSA, 2015, 2012).

We applied two different approaches to estimate the annual extra cancer risk attributable to exposure to AA through foods, and concluded that the Dybing&Sanner approach resulted in risks approximately 5 times larger than the US EPA approach (Table 3). However, these are only two of many approaches, all resulting in different lifetime cancer risk estimates (Chen et al., 2012; Doerge et al., 2008; Dybing and Sanner, 2003; Törnqvist et al., 2008). The highest lifetime risk identified in the literature is 16×10^{-3} (16 out of 1000 individuals) per μg/kg bw/day (Törnqvist et al., 2008), which is around 35 times higher than the highest lifetime risk estimated in our study (4.6×10^{-4}, i.e.4.6 out of 10,000 individuals) per μg/kg bw/day. It is of general agreement that it is preferable to utilize toxicokinetic/toxicodynamic data on specific substances, like AA, in the interspecies extrapolation, which is the case in the US EPA model.

In order to reflect the range of possible BMD_{10} given the dose response data, we calculated risk using both the overall $BMDL_{10}$ and $BMDU_{10}$ of the endpoints showing a statistically significant dose–response relationship, and with data informative enough to derive a PoD (EFSA, 2011). In EFSA (2015), harderian gland tumors in mice are the suggested endpoint for PoD estimations, however, we disregard in this study endpoints in mice, as the toxicokinetic data used in the US EPA approach (US EPA, 2010) relate humans and rats. Mammary gland tumors in rats have likewise been suggested as endpoint for PoD estimations (JECFA, 2010). However, our BMD modeling suggested that the mammary gland tumor data in Beland et al. (2013) was not informative enough to derive a PoD, and we refrained from using restricted benchmark dose models to further treat the data.

Assuming that AA causes cancer through a genotoxic mode of action, we applied linear extrapolation from the observable tumor range to low human dose levels to estimate the SF. This is generally an approach considered conservative, as biological protection mechanisms likely act in the low dose regions, and as a consequence the actual dose–response curve is sub-linear and the risk is overestimated (Boobis et al., 2013; Dybing and Sanner, 2003; O'Brien et al., 2006; US EPA, 2005). Additionally, the approach has been criticized for the large uncertainty of the shape of dose–response relationship at doses relevant for human exposure (Barlow et al., 2006). This implies that our results may likewise be overestimated, or at least that the uncertainty is considerable.

The SF estimates the absolute number of cases that are expected at a certain exposure independent of the background disease rate, as the toxicological tumor data reflect only tumors caused by the given chemical (Hanninen et al., 2014; US EPA, 2005). Therefore, in this study we do not account for other carcinogenic hazards and risk factors. However, validation of the estimated extra cancer cases is very difficult due to the chronicity and multi-causality of cancer, and because exposure can take place a long time before the onset of disease.

Recognizing that AA is a multi-site carcinogen (Beland et al., 2013; EFSA, 2015; US EPA, 2010) and under the assumption that the cancer potency of AA is similar in all tissues, we estimated the disease burden of total cancer. When accounting for total cancer, cancer sites that are not associated with dietary exposures are included, which will lead to an overestimation of the disease burden of total cancer attributed to AA. The assumption that AA has the same potency in all tissues is challenged by animal studies, which show that different tissues vary in sensitivity to AA exposure, ranging from tumors occurring in the low-dose groups to tumor-free tissues (Beland et al., 2013; Friedman et al., 1995; Johnson et al., 1986). To minimize the impact of this assumption, both $BMDL_{10}$ and $BMDU_{10}$ modeled from all statistically significant endpoints were used as PoD, and are therefore believed to cover the sensitivity of the tumor sites identified in the animal studies. On the other hand, the burden calculated for the specific cancers does not account for cancer sites potentially associated with AA that have not been detected in currently available epidemiological studies, which can lead to an underestimation of the burden attributed AA. To improve the estimation of disease burden caused by AA, more research on toxicological data that inform the link of AA exposure to specific human relevant tumor sites/health effects is needed (e.g. QSAR and PBTK models).

Calculating DALYs for total cancer with the indirect approach resulted in approximately twice as large estimates than with the

direct approach. Both methods used the same AA-attributed cancer cases (AC) and health register data for DALY estimations. The main reasons for the discrepancy lie in the parameters used for calculation of $DALY_{/case}$; in the use of average age of death in estimation of YLL in the direct approach and of life-tables by WHO (i.e. indirect); and in the use of prevalence data (direct) or incidence data (indirect) to calculate YLD (WHO, 2013). Even though the WHO estimates are country-specific, the parameters used in the estimates are standardized to be comparable across nations (Schram-Bijkerk et al., 2013; WHO, 2013). In a national BoD study, it is preferable to use original incidence data, as used in the direct DALY approach in the current study. However, choosing one approach over the other will depend on data availability.

We did not include information on time-to-tumor in our DALY calculation, and have disregarded - individuals with a high exposure i.e. the 99th percentile, who might have an earlier disease onset. Time-to-tumor data is rarely available, but should ideally be included in the DALY calculation (Bokkers et al., 2012).

Our estimates of disease burden of AA exposure through foods provide a valuable basis for comparison with disease burden of other food chemicals, environmental chemicals and even of other hazards and risk factors e.g. of biological or nutritional origin. Still, our results shows that all methodological choices and underlying assumptions of a burden of disease model need to be carefully considered when interpreting DALYs and utilizing the estimates for comparison across risk factors.

5. Conclusion

We estimated that exposure to AA through food causes a disease burden of 0.003 DALY/100,000 per year in Denmark as estimated by the least conservative approach, and 1.8 DALY/100,000 as estimated by the most conservative approach, which corresponds to 0.3 (i.e. approximately one case every three years) and 9 annual cases of cancer in the population, respectively. This range reflects the uncertainty of the available dose–response data and the impact of the chosen modeling approach. By developing a method to estimate the burden of foodborne AA, we have paved the way for similar estimations of other chemical hazards, in Denmark and in other countries with representative food-exposure data. Models to estimate the burden of disease of chemicals transmitted through foods would be greatly improved by toxicological data on human health effects following exposure to these chemicals, as well as by the identification and quantification of the sources of uncertainty connected to the model components, including the inter- and intra-species extrapolation steps and the exposure assessment.

Transparency document

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3.3 Discussion

We applied the risk assessment approach (referred to as an exposure based-approach in the paper), to estimate the incidence of cancer caused by dietary exposure to AA. To estimate the disease burden requires integration of data from many different sources. We proposed an overall model-framework consisting of three modules (i.e. the exposure, health outcome and DALY modules), to which each data integration and modelling step can be referred to. Structuring the model in components allows for a transparent overview of the different data needs and modelling steps in the development of the indicators of interest (i.e. the exposure estimates, the population risk estimates and the final DALY estimate), as well as the assumptions and modelling choices applied within each module. Furthermore, the impact of assumptions on the final estimates can easily be allocated to a specific module, as can the sources of uncertainty from input parameters and model assumptions.

We assessed the impact of selection of health outcomes and selection of dose response models on the population risk and on the final DALY estimates in the health outcome module. In the DALY module, we assessed the impact of using, within the risk assessment approach, two different approaches to estimate DALY, as illustrated in fig. 2.4. The impact assessment was done by scenario analysis, i.e. two scenarios for inclusion of health outcomes, two scenarios for dose response modelling and two scenarios for DALY estimation (fig. 3 in the paper). The scenario analyses and the resulting ranges of DALY estimates are not per se describing the uncertainty in the estimates. Rather, they assess how sensitive the overall model is to the input parameters, e.g. is the impact of the different scenarios on the final DALY estimates largest for assumptions in the health outcome module or in the DALY module?

In the health outcome module, the strength of the evidence is evaluated in order to select which health outcomes to account for, and to define a dose response relationship. The selection of the health outcomes to account for is a crucial step in the burden estimation of a given hazard. The total burden incurred by a chemical is of course only reflecting the health outcomes for which the incidence is calculated.

The adverse health effects associated with AA exposure from different sources include, beside cancer, neurotoxicity observed in both experimental animals and humans [83][84],

reproductive impairments observed in experimental animals [85], developmental effects observed in experimental animals and humans [86][87][88] and immunological effects observed in humans [89]. In our study, we excluded neurological effects by the argument that exposure from the diet are of considerable lower magnitude than the exposure experienced in occupational settings, where case reports about neurological effects exist [49]. Reproductive effects have been detected in animal studies, but not reported for humans or found in epidemiological studies. EFSA considered reduced sperm count and testis morphology as critical effects and relevant for humans and identified a NOAEL relevant for reproductive toxicity [49]. In birth-cohort studies, AA exposure of the mother was associated with a low birth weight for gestational age of her child [90][88]. EFSA concluded that based on the two birth-cohort studies available, a cause-effect relationship could not be established, supported by a lack of biological explanation for the association [49]. EFSA did in its 2015 scientific opinion not consider immunotoxic effects [49], however a recent cohort study investigated and found an association between AA exposure and allergy-related outcomes. All of the above mentioned health effects are possibly relevant for humans, however, the evidence available to establish a cause effect relationship is missing, especially relating the developmental (low birth weight) and immunotoxic (allergy related) effects. A low birth weight is an established predictor of adverse health outcomes in adulthood including morbidity and mortality related to cardiovascular diseases (CVD)[91]. CVD accounts for 14% of the total disease burden of all diseases in Denmark in 2015; asthma (in relation to allergy related effects) account for 1% of the total disease burden [92]. The disease burden of CVD and allergy attributed AA may potentially be considerable, and thus excluding the health outcomes potentially underestimates the total disease burden of AA. On the other hand, the disease burden of male infertility (the manifestation of disability in humans from reduced sperm count) in Denmark in 2015, was estimated to account for maximum 0.1% of the disease burden from all causes. This may suggest that including this health effect do not have a major impact on the DALYs attributed AA.

The inclusion of the health effects are likewise depending on the data available for informing a dose response relationship. Bokkers et. al (2009) [64] evaluated the available scientific evidence on non-carcinogenic AA toxicity to calculate the fraction of the population affected via the IPRA methodology, and found the data suitable for BMD-modelling for the effects: neurotoxicity as measured by grip strength [93] and developmental and

systemic effects as measured by number of life pubs per litter and loss of bodyweight [93][86]. As mentioned, neurotoxic effects were excluded based on the expected magnitude of dietary exposure. It was not investigated whether the dose-response data were available for other adverse effects.

The disease burden of dietary AA-induced cancer was evaluated. The strength of evidence on the cause-effect relationship is not completely established and acrylamide is classified as probably carcinogenic to humans [49], as the animal carcinogenicity studies on AA exposure are not supported by convincing evidence in human observational studies. Because of the inconclusiveness in the cohort studies, we assessed the impact on the DALY estimates of accounting for 4 specific types of cancer or the cancer cases attributed to AA out of all different types of cancer. Accounting for total cancer yielded a DALY estimate 7 times higher than accounting only for the 4 specific cancers. As for AA and cancer, the causal evidence between AA and low birth weight and allergy related outcomes is lacking. It would be relevant to investigate how much the disease burden would increase if these outcomes were accounted for.

The strength of evidence that informs the dose response relationship depends on how well the model and its parameters describe the true biological disease models. We assumed that a linear relationship between the probability of developing cancer and a human relevant dose accurately yield the incidence of cancer caused by AA. This assumption is discussed in the paper. We evaluated the range of uncertainty in the POD (by the BMDL and BMDU) given the fitted dose response models and assessed two different models to inform on the toxicodynamic and -kinetic differences between a median human and the experimental animals, i.e. the US EPA [94] and Dybing and Sanner [95] approach. The DALY estimates of the combined most and least conservative combination of scenarios differed by a factor of approximately 43. However, the range of uncertainty might be larger, as the chosen scenarios possibly do not comprehensively describe the true disease models, not least taking into account that the difference between human individual's sensitivity to AA is not described.

All of the points above illustrate that the uncertainty connected to which health outcomes to include, as well as how well the chosen dose-response model describe the disease model is considerable.

In the DALY-module we assessed the impact on the final DALY estimates of using a

disease envelope for the health outcome. The scenarios assessed suggested that estimating the disease burden attributable to AA via the GBD disease envelope for cancer in Denmark, yielded twice as large estimates than estimating DALYs directly from Danish health statistics. It would have been relevant to also assess DALYs via a disease envelope internally estimated in Denmark, had it been available. Litterature reviews of disease burden studies have illustrated that a range of different approaches and parameters have been applied to estimate DALYs [34]. Thus, the two scenarios studied here do not cover the full range of approaches. The chosen methodology should be decided depending on the applicability of the study. If the disease burden for a foodborne hazard is estimated to be representative globally, then the GBD disease envelopes should be used if available; alternatively, health statistics should be standardised across nations. Likewise, if the disease burden is applied in a national context, national health statistics or disease envelopes should be used.

In summary; the scenarios we assessed were chosen mostly based on the available data; i.e. the two scenarios for health outcomes were selected based on evidence from epidemiological studies (i.e. the specific cancers) and on the information of AA being a multi-site carcinogenic (i.e. total cancer); the two scenarios for application of linear extrapolation and extrapolation factors to inform of the dose response relationship were obtained from literature searches; and the two scenarios for DALY computation were decided upon based on the available disease envelopes. Overall, the scenarios assessed in the health outcome module had a considerable larger impact on the final DALY estimates than the scenarios investigated in the DALY module. Even though we can not be sure that the scenarios we have assessed represent the upper and lower bounds of the uncertainty ranges, we argue that the assumptions and model approaches in the health outcome modules have a higher impact on the final DALY estimates than does the assumptions and model approaches taken in the DALY module. Therefore, one can conclude that to optimize confidence in the disease burden estimates on foodborne chemicals, effort should be put into refining the input parameters and model assumptions in the health outcome module, rather than in the DALY module.

Chapter 4

Manuscript II

4.1 Prelude

In Paper 1 we estimated a lifetime extra cancer risk due to dietary exposure to AA of 4.6×10^{-4}, or approximately 9 cancer cases per year in Denmark, which would represent only 0.02% out of the total cancer incidence in Denmark in 2014. At the same time, the collection of human observational studies is inconclusive with regard to the association between AA and the wide range of cancer cites studied [96]. However, evidence from human observational studies are normally given the heaviest weight when causal relationships between a risk factor, or hazards, and a health outcome is established [97]. Therefore we found it relevant to investigate the sensitivity of a prospective cohort study under various study conditions. We assess the potential bias introduced into studies of disease burden of foodborne chemicals if human observational studies are given the highest priority in selection of health outcomes. Also, we inform on the potential bias introduced if epidemiological evidence is used to derive the dose response relationship (the top down approach) compared to using data from studies in experimental animals to derive the dose response relationship (the bottom up approach). Quantitative information on both sources of bias are important to allow for comparability across foodborne disease burdens estimated from different streams of evidence.

4.2 The sensitivity of a cohort study on acrylamide and risk of cancer: using a simulation approach to evaluate the likelihood of a significant effect

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Abstract

Observational human studies such as prospective cohort studies are important tools to establish a causal relationship between exposure to hazards or risk factors and the risk of chronic disease e.g. cancer. However, the statistical strength inherent in the design of epidemiological studies often requires either large effects or large cohorts to show a significant relative risk. In this paper, we perform computer simulations of a prospective cohort study to evaluate under which conditions such a study identifies significant relative risks (RR). We use the case of dietary acrylamide (AA) exposure and the risk of cancer, where the probability of cancer given AA exposure is extrapolated from animal studies, to simulate a prospective cohort study of a realistic design. We apply Monte Carlo simulation to assess the likelihood of detecting a statistical significant RR given the study conditions. Our simulations suggest that in the case of AA, in a feasible cohort study, the likelihood of finding a statistical significant RR is as low as 2.4%. We find that to reach at least 50% probability of detecting a RR of statistical significance, the background probability of cancer should be no larger than six times the probability of disease mediated by the chemical. Observational studies are given a high priority in the evidence of risk of disease and when guidelines are formulated to promote human health, thus the lack of sensitivity should be taken into account. We conclude that simulations of observational studies are a valuable tool to evaluate the sensitivity of a study, both to optimize study design and/or to decide whether to conduct such a study.

Keywords: Monte Carlo simulation, epidemiology, relative risk, acrylamide, prospective cohort study; cancer

Introduction

Chronic diseases such as ischemic heart disease and cancer cause high diseases burdens, not only in Western societies, but also worldwide [1]. To identify effective disease prevention measures, increased focus is given to the causes of these diseases and especially to the associations between these diseases and the diet. However, establishing associations or even causal relationships between dietary risk factors or hazards and health effects is not a trivial task. To investigate these associations, several types of studies are used, including animal studies, mechanistic in vitro/vivo studies and observational studies. Observational studies are often preferred to obtain measures of direct associations in humans. These studies include randomized controlled trials, prospective cohort studies and meta-analyses of the latter, and estimate the risk of disease based on the exposure to a given risk factor or hazard (e.g. smoking or aflatoxins in food) and the occurrence of a specific health outcome, (e.g. lung cancer (smoking) or liver cancer (aflatoxin)). Well performed observational studies are assigned the "heaviest" weight of evidence in the determination or classification of a hazard's or risk factor's effect on human health. For the evidence of a causal relationship to be convincing according to the World Cancer Research Fund (WCRF), it needs to be based on *"evidence from more than one study type*" but *"at least two independent cohort studies"* with "*no substantial unexplained heterogeneity within or between study types or in different populations relating to the presence or absence of an association, or direction of effect*" [2]. Further, convincing causal relationship forms the base on which goals and recommendations to reduce the incidence of cancer are designed. Thus, epidemiological studies are crucial and emphasized in the formulation of food safety policy and dietary guidelines [2]. However, these studies are difficult to perform because the statistical strength inherent in the design of epidemiological studies often requires either very large effects or very large cohorts or study populations to show a significant relative risk

(RR). As scientific evidence for an association between exposure and disease needs to be available for a hazard or risk factor to be regarded as relevant by the health authorities and other parties within food-safety, the lack of power in population studies may impact the extent to which certain foodborne risk factors or hazards are included in mitigation initiatives.

Chemical substances are placed into categories of different likelihood of carcinogenicity in humans on the basis of the strength of the evidence of the causal relationship. Acrylamide (AA) is a process contaminant formed during heat treatment of starchy foods, detected in a wide range of commonly consumed products (e.g. breakfast cereals, coffee, fried potatoes) and categorized as a group 2A carcinogen: probably carcinogenic to humans [3][4]. Animal and mechanistic studies on the toxicological effect of (oral) exposure to AA show clear evidence that AA is carcinogenic through a genotoxic mode of action [5–8]. However, epidemiological studies that investigate the association between dietary exposure to acrylamide and cancer are more ambiguous. The majority of epidemiological studies do not show a statistical significant association between AA and various cancer types e.g [9–11], whereas a few studies show a statistical significance [12,13]. This trend is also observed in a recent meta-analysis [14], where no association was found with most cancer types, except a few where the association was borderline significant.

In a recent study [3], a risk assessment approach using animal data was used to estimate the disease burden caused by dietary exposure to AA in Denmark. The authors assessed several scenarios and found that in the most conservative approach, the disease burden amounted up to 9 annual cancer cases or 1.8 DALY/100.000 attributed dietary AA, or approximately 0,02% of the total cancer incidence in Denmark. With an incidence in this order of magnitude it is unlikely that an epidemiological study, e.g. case-control or prospective cohort study will comprise the power to detect a statistical significant effect, which is also illustrated by the

inconclusiveness of the above mentioned evidence. Likewise, Törnqvist et al. [15] have argued that RRs translated from toxicological data are of magnitudes that will not be detected in epidemiological study designs.

In this study we performed computer simulations of a prospective cohort study to evaluate under which conditions such a study would identify significant cancer risks. We tested the hypothesis that epidemiological studies are sensitive enough to detect statistically significant associations between hazards, for which toxicological risk assessments suggest low incidence of a given health effect, using the case of dietary AA exposure and the risk of cancer. A set of simulations of the cohort study yields a distribution of RRs that might be obtained from these studies. This distribution provides the likelihood of getting a RR that is statistically significant larger than 1 and thus the likelihood of reaching the conclusion that AA is a significant risk factor. We investigated which variables might influence the significance of the association, as well as which variables might be optimized to improve the sensitivity of the epidemiological study.

Method and Materials

In prospective cohort studies, information of exposure to a hazard of initially healthy individuals is collected when individuals enter the cohort. The individuals are then followed for several years to collect information on eventual diagnosis of disease, i.e. cancer, to allow for comparison of disease incidence between individuals of low and high exposure. Usually, the exposure-disease association is modeled by comparing disease incidence in fixed categories of exposure, i.e. quartiles. In a simulated cohort study, we are able to study perfect study conditions including a complete follow-up and exact knowledge on the characteristics of individuals, e.g. level of exposure. Thus, we defined a cohort study considering perfect knowledge of the study population and a realistic set up. We simulated the cohort study with a closed

population of 30,000 persons with an individual entry-age into the cohort of 40-64 years, and a follow-up period of 10 years. We assumed that the age of individuals entering was evenly distributed across the cohort and that each individual entering the cohort did not have a cancer diagnosis prior to entry. All competing illnesses and causes of death were ignored. The outcome of the cohort study was an estimate of the RR between individuals in groups of high and low AA exposure (i.e. the first and fourth quartile), based on incidence proportions (IP). An overview of the simulation strategy of the epidemiological study is given in Fig 1.

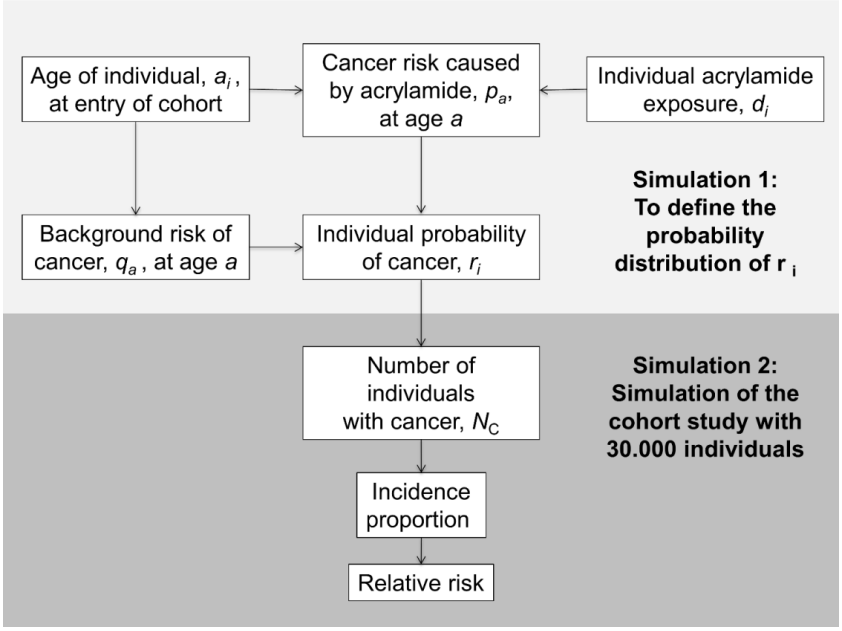

Fig 1. Overview of the simulation model.
Strategy for the simulation of a prospective cohort study on the association between dietary acrylamide exposure and the risk of cancer.

Simulation model

We hypothesized that an individual's probability, r_i, of developing cancer within the 10 year follow-up period depended partly on the risk, p, associated with the dietary exposure to AA and partly on the background cancer risk, q, from other hazards and

risk factors. The background cancer risk, *q*, was calculated by the cumulative incidence proportion (CIP), i.e. the proportion of the population that gets cancer within a specified time-period, using cancer incidences and population size from 2013 published by the Danish Cancer Registry and Statistics Denmark (Table 1).

Table 1 Cancer incidence and population size in Denmark in 2013, calculated incidence rate (IR) per year per 100,000 inhabitants and cumulated incidence proportion (CIP) for each 10-year age-groups.

Age	40-49	45-54	50-59	55-64	60-69	65-74
Incidence (/yr)[a)]	2,350	3,676	5,254	7,541	10,967	12,286
Population size[b)]	714,133	714,133	654,474	631,747	633,924	541,363
IR (/100.000/yr)	329	515	803	1,194	1,730	2,269
CIP	0.032	0.050	0.077	0.112	0.159	0.203

[a] The Cancer Register, www.esundhed.dk/sundhedsregistre/CAR/Sider/Cancerregisteret.aspx
[b] Statistics Denmark, www.statistikbanken.dk/FOLK2

CIP for a time-period of 10 years was calculated for the age groups shown in Table 1, by:

$$CIP_{ag} = 1 - \exp(-IR_{ag} \cdot \Delta t) \qquad \text{(Eq 1)},$$

where IR_{ag} is the incidence rate of age group, *ag*, per year per 100,000 and Δt is the follow-up time (i.e. 10 years) [16]. The risk of cancer increases with age and the 10-year background cancer risk, q_a, of a person with age a (a = 40-64 years) when entering the cohort, was given by the best fit of a (pragmatically chosen) 2nd degree polynomial trend-line fitted to the CIPs calculated for each age group (see Fig 2A).

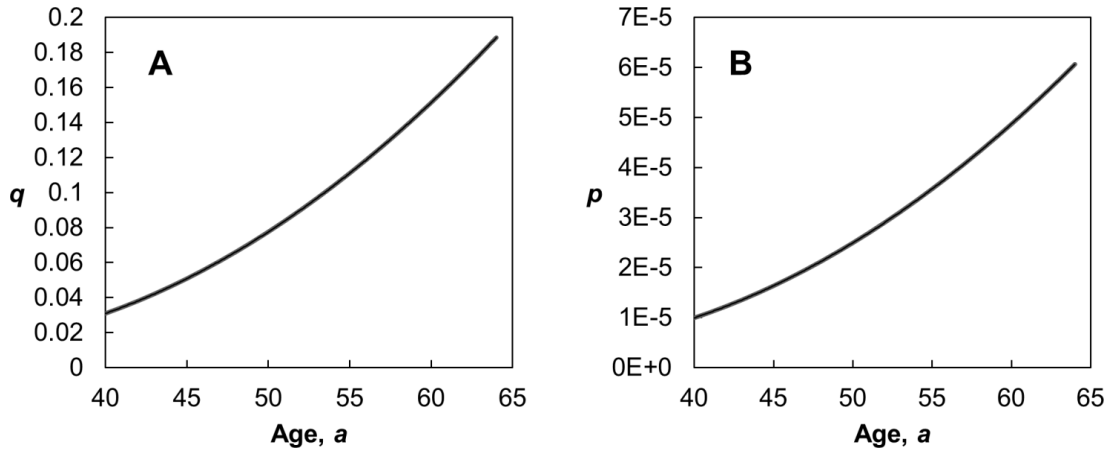

Fig. 2 Cancer risk and age
A) 10-year background total cancer risk, *q*, as a function of age, *a*; B) 10-year cancer risk caused by dietary exposure to acrylamide, *p*, as a function of age, *a*.

For example, $CIP_{40\text{-}49}$ gives the probability of a person of age 40 getting cancer within the next 10 years of his/her life and by the fit of the 2nd degree polynomial to the 10 year risk of each of the age groups (Table 1), the 10-year background risk of cancer given age *a*, was given by:

$$q_a = 0.00014\, a^2 - 0.0076\, a + 0.12 \qquad \text{(Eq 2)}.$$

The lifetime risk of cancer caused by dietary exposure to 1 μg AA/kg bw/day throughout a lifetime i.e. the cancer slope factor (SF) for dietary AA, is reported in Jakobsen et al. [3]. This cancer risk model is based on carcinogenicity studies in rats orally exposed to AA. In the study, two different approaches are used to extrapolate the cancer risk in the rat to humans. Here we use the mean SF of the two approaches (Table 2).

Table 2 Input parameters in the baseline simulation model

	Description	Unit	Distributional assumption	Value		Reference
Cohort size	Number of participants entering the cohort		Point estimate	30,000		
Follow-up	Duration of study period	years	Point estimate	10		

time						
SF	Lifetime cancer risk due to acrylamide	$(\mu g/kg\ bw/day)^{-1}$	Point estimate	$1.09x10^{-4}$		[3]
q_l	Background risk of cancer (probability of cancer before age 75)		Point estimate	0.34		[26]
a_i	Age of the individual entering the cohort	years	U(a,b)	a=40	b=64	
d_i	Individual exposure dose of acrylamide	µg/kg bw/day	Lognormal(µ,σ)	µ=0.34	σ=0.39	

To relate the AA lifetime cancer risk, SF, to the AA cancer risk per age, $p_{a,}$ we assumed that the risk of cancer caused by AA follows the same relation with age as the background cancer risk, by:

$$\frac{SF}{q_l} \cdot q_a = p_a \qquad \text{(Eq 3)}$$

where, q_l (=0.34) is the $CIP_{0\text{-}75}$ (Table 2), here assumed to represent the lifetime risk of cancer. The relation was given by:

$$p_a = 4.4 \cdot 10^{-8} a^2 - 2.4 \cdot 10^{-6} a + 43.8 \cdot 10^{-5} \qquad \text{(Eq 4)},$$

and shown in Fig 2 B.

We aimed to simulate the probability r_i that individual i gets cancer, which, as described above, will depend on the individual's age, a_i and exposure to a lifelong mean daily dose of AA, $d_{i.}$ We assumed a uniform distribution for a, i.e. the distribution of age at entry into the cohort is uniform, given by: $a_i \sim U(40, 64)$ (Table 2), where ~represents "is a sample from". The AA dietary exposure in µg AA/kg bw/day of the Danish population is based on the Danish National Survey of Diet and Physical Activity comprising approximately 2500 individuals [3]. A lognormal distribution was fitted to the AA exposure data, using @Risk version 6 (AIC = -950.02), to describe the probability distribution of an individual's exposure to a dose of AA, $d_i \sim$ Lognorm(0.34, 0.39), shown in Fig 3 and Table 2.

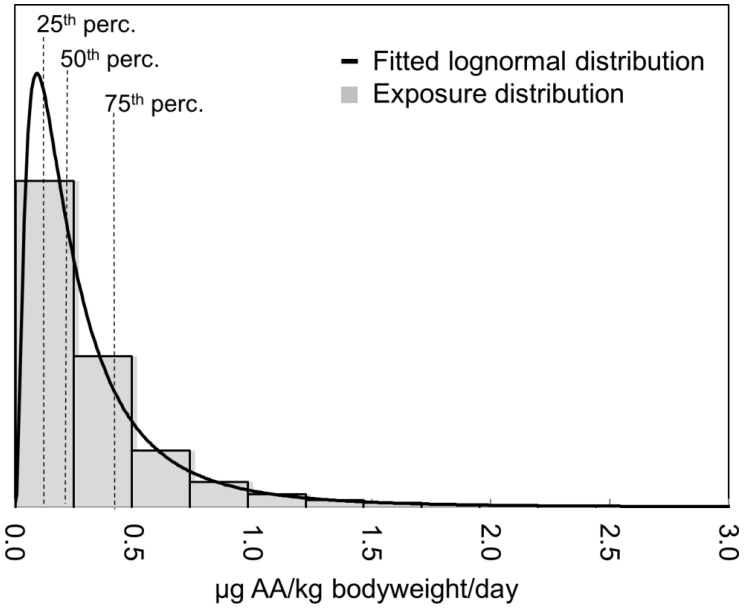

Fig 3. The fit of a lognormal distribution to the Acrylamide exposure.
Fit comparison of the lognormal distribution with mean = 0.34 and standard deviation = 0.39 to the actual AA exposure distribution. Exposure (ug AA/kg bw/day) of the individuals entering the cohort is sampled from the lognormal distribution to obtain the risk of the individual given AA exposure and age. AA = acrylamide.

Simulating a study where four exposure categories are compared, the probability distribution of d_i was split into quartiles, in order to simulate the probability of cancer for individuals in each quartile, *n=1..4*. Let $d_{i,n}$ represent a random sample for the exposure distribution falling in the nth quartile. Then, an individual *i*'s probability of getting cancer within the 10-year follow-up from entering the cohort at age *a* when being in exposure quartile *n*, was given by:

$$r_{i,n} = r(a_i, d_{i,n}) = 1\text{-}(1\text{-}\, p(a_i) \cdot d_{i,n})(1\text{-}q(a_i)) \qquad \text{(Eq 5)}$$

To characterize the exposure distribution, we simulated $r_{i,n}$ for 50,000 individuals *i* per exposure quartile *n* by Monte Carlo technique. The resulting probability distribution was then used to estimate the number of cases in each quartile after the 10-year follow-up period. Acknowledging that r_i is not necessarily linearly related to

the exposure, so that the mean for each $r_{i,n}$ may not be representative, we further split up the probability distributions of $r_{i,n}$ into m = 10 quantiles each, per quartile n, and calculated the median risk $r_{\text{med,n,m}}$ of each quantile. To estimate the number of cases in each quantile m, we sampled from the binomial distribution, Binomial(N_m, $r_{\text{med,n,m}}$), where N_m is the number of individuals in quantile m (= one fourtieth of the total number of participants in the cohort study N_{tot}). The sum of cases from each quantile m equals the total number of cases, $N_{c,n}$, in each quartile n of the exposure distribution, after a 10 year follow-up.

A total of N_{tot} = 30,000 individuals entered the cohort, and we assumed that the participants were evenly distributed in the quartiles of the exposure distribution, hence 7,500 in each (N_m =750 per quantile).

The incidence proportion for each quartile, IP_n, was estimated by:

$$IP_n = \frac{N_{c,n}}{N_{c,n}+N_{nc,n}} \qquad \text{(Eq 6)},$$

where $N_{nc,n}$ is the number of individuals in the n'th quartile with-out cancer after the 10-year follow-up period. RRs were calculated relative to the incidence proportion of the 1st quartile, IP_1, e.g. $RR_4 = \frac{IP_4}{IP_1}$. The 95% confidence interval (CI) of the RRs were calculated and significance tests performed by χ^2-test, to test whether the simulated study would give a statistically significant result.

We simulated the cohort study 100,000 times (100,000 iterations), resulting in the probability distribution of RRs that might be obtained, if the cohort study was conducted in reality.

All input parameters, point estimates and assumed distributions are shown in Table 2. Monte Carlo simulations were performed in @Risk version 6 (Palisade Corporations) as an add-in for Microsoft Excel.

Scenarios

In addition to investigating how often a statistical significant effect is expected with the conditions given (baseline model), we defined different scenarios to investigate: 1) How model parameters influence the significance of an association, and 2) Which parameters can be optimized in a realistic setting to improve the sensitivity of the epidemiological study. To address the first, we investigated different values of the SF and the effect of change in the variation, e.g. the standard deviation (SD) of the exposure distribution. To address the second, we investigated the influence of the number of participants entering the cohort and the length of the follow-up time. Lastly we simulated a combination of a set of realistic scenario values. Table 4 shows the parameter values of each scenario.

Table 4 Parameter values and simulation results of the baseline model and scenarios. Results reported as the probability (%) that the relative risk (RR) >1, that the lower bound (LB) of the 95% confidence interval around RR >1 and that the upper bound (UB) of the 95 % confidence interval around RR<1, of the base scenario and the alternative scenarios. Only simulation results of the 4th vs.1st quartile is presented.

No	Scenario	RR>1	LB>1	UB<1
1	Base scenario	49.64	2.34	2.33
2	*SF*x10	51.14	2.60	2.08
3	*SF*x10^2	65.34	5.68	0.86
4	*SF*x10^3	99.97	96.07	0.00
5	*SF*x10^4	100.00	100.00	0.00
6	$SF_{\text{Tørnqvist}}$	71.82	8.31	0.52
7	SD = 0.5	49.59	2.44	2.27
8	SD = 0.75	49.49	2.42	2.38
9	SD = 1.0	49.39	2.47	2.30
10	SD = 10	49.71	2.41	2.28
11	Cohort sizex2	49.93	2.38	2.28
12	Cohort sizex10	50.21	2.38	2.26
13	Cohort sizex10^2	51.42	2.62	2.16
14	Cohort sizex200	52.24	2.62	2.07
15	Cohort sizex300	52.42	2.77	1.99
16	Cohort sizex10^3	54.80	3.12	1.78
17	Cohort sizex10^5	88.37	21.09	0.07
18	follow-up = 20 years	49.65	2.44	2.31

19	Combination	98.58	57.77	0.00

The SF and the SD of the exposure distribution are parameters that cannot be influenced in the study design; however, the parameters may vary from hazard to hazard. Therefore, in scenario 2-6, we investigated how different (larger) values of SF influence the significance, including a SF reported by Törnqvist et al. [15] (scenario 6), which to our knowledge is the largest SF for AA reported in the literature, and about 150 times the size of the baseline SF. By increasing SF, we increased the probability of getting cancer due to AA. In scenario 7-10, we investigated how an increasing SD of the exposure distribution affects the significance. The larger the SD, the more spread towards low and high end of the distribution, so the difference between the extremes of the exposure distribution is larger.

Parameters that can be modified in the study design to increase its sensitivity include the size of the cohort and the follow-up time. In scenario 11-17 we investigated how an increasing size of the cohort impacted the likelihood of detecting a statistical significant effect. The larger the cohort, the more cancer cases are included to calculate RRs. In scenario 18 we investigated how increasing the follow-up time from 10 years to 20 years affected the significance. A longer follow-up time will increase the probability of cancer (which increases with age), thus include more cancer cases in the study.

Because some of the scenarios investigated are unrealistic (e.g. a cohort size of 3 billion individuals), we chose to investigate a combination of scenarios of larger, but potentially realistic parameter values. In scenario 19 we investigate the combined impact of a cohort size of 300,000, a follow-up of 20 years and the SF reported by Törnqvist et al. [15] (e.g. scenario 6, 12 and 18 combined).

Results

We tested the null hypothesis that there is no difference in the risk of cancer of the individuals in the different quartiles of exposure relative to the lowest quartile, e.g RR = 1. If RR = 1 is included in the 95% confidence interval, we cannot reject the null hypothesis that the risk in the different quartiles are equal. In the 100.000 iterations of the baseline and scenario models, we assessed how often we can expect to reject the null hypothesis, hence we assessed how often the 2.5% lower bound (LB) and 97.5% upper bound (UB) of the 95% confidence interval is larger than 1 (LB > 1) and smaller than 1 (UB < 1), respectively, using the @RiskTarget function.

Baseline model

The mean AA exposure, mean number of cases and mean RR of 100.000 iterations of the baseline-model simulation for each quartile are shown in Table 3. The mean daily exposure in the lowest and highest quartile was 0.07 ug/kg bodyweight and 0.80 ug/kg bodyweight, respectively. The mean RR of the 4^{th} quartile was 1.0014 with associated means of the 2.5% lower- and 97.5% upper bounds of the 95% confidence interval of 0.9080 and 1.1044, respectively. The total mean of cancer cases amounted to 2899.62 among 30,000 study participants during the simulated 10 year follow-up.

Table 3 Mean acrylamide exposure, number of cases and relative risk of 100,000 simulated cohort studies by quartiles of acrylamide exposure (95% confidence intervals represented by the mean of the 2.5 % and 97.5% bounds of 100,000 simulated cohort studies) in the baseline model.

Quartile, *n,* of acrylamide exposure, mean (min-max) in ug/kg/day	Cases	RR(95% CI)
n_1 0.073 (0.002 - 0.118)	724.854	1.00000 (reference)
n_2 0.165 (0.118 - 0.219)	724.874	1.00125 (0.90786-1.10424)
n_3 0.300 (0.219 – 0.408)	724.905	1.00129 (0.90791-1.10429)
n_4 0.804 (0.408- 20.318)	724.988	1.00141 (0.90801-1.10441)

In the simulation of the baseline scenario we found that the probability of finding RR > 1 in a cohort study is 0.495. The probability of finding a statistical significant positive RR (e.g. LB > 1) was 0.023, the same was the probability of finding a significant negative RR (e.g UB < 1) (Table 4). The relation between the RR, LB and UB is shown in Fig 4 by their cumulative probability distributions of the 4th quartile. The median of the RR-distribution is 1 and it is seen that only the right tail of the LB distribution is above 1; likewise the left tail of the UB distribution is below 1.

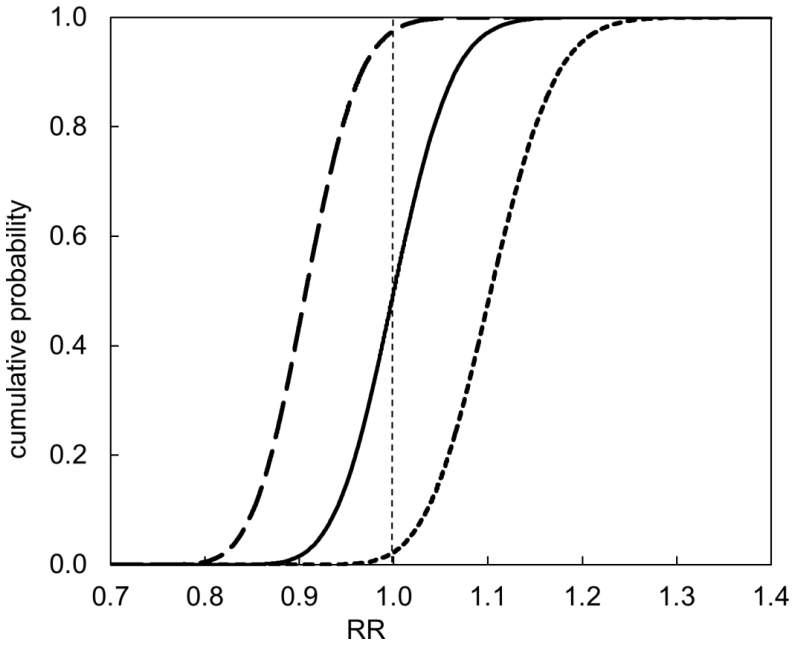

Fig 4. The simulated distributions of RR, LB and UB.
The variation in relative risks (RR - solid curve), 2.5% lower bounds (2.5% LB – dotted curve) and 97.5% upper bounds (97.5% UB- dashed curve) of the of 95% confidence interval around the relative risk of 100,000 iterations of the baseline model.

Scenario Analyses

With increasing SF, the probability that RR > 1 increases, and with SF being 1000-10000 times the size in the base scenario, the probability is 1 (Table 4). With SFx10 the likelihood of RR being statistical significant in either direction is almost equal

(similar to the base scenario); however, with increasing SF the probability of the 2.5% LB > 1 approaches 1 and the probability of the 97.5% UB < 1 approaches 0 (Table 4 and Fig 5). With SF 1000-10000 times higher than the baseline model, one would always expect to detect a statistical significant risk, when performing a cohort study with the baseline characteristics.

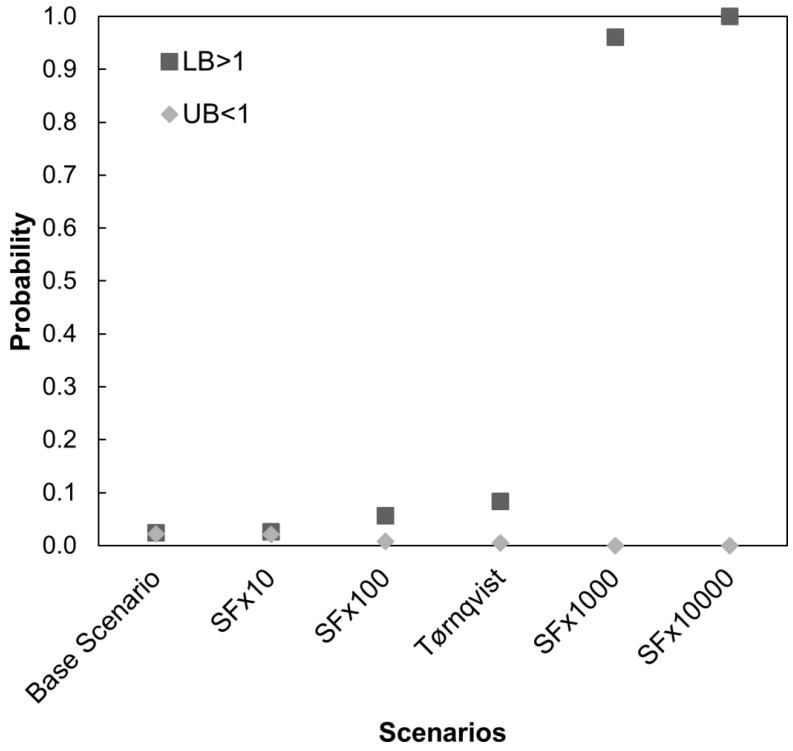

Fig 5. The relation between the likelihood of a statistical significant relative risk and the size of the slope factor

Change in the probability that the lower bound (LB) and upper bound (UB) of the 95% confidence interval around the relative risk is < 1 and > 1, respectively, by scenario of increasing slope factor. Only simulation results for the 4^{th} vs.1^{st} quartile is shown.

The mean of the RR probability distributions is above 1 for $SFx10^2$, $SFx10^3$, $SFx10^4$ and $SF_{törnqvist}$ (Fig 6). However, the mean of the LB probability distribution is above 1 for only $SFx10^3$ and $SFx10^4$ with ~ 4% of the simulated LBs for $SFx10^3$ is below 1 and none for $SFx10^4$ (Fig 6, Table 4).

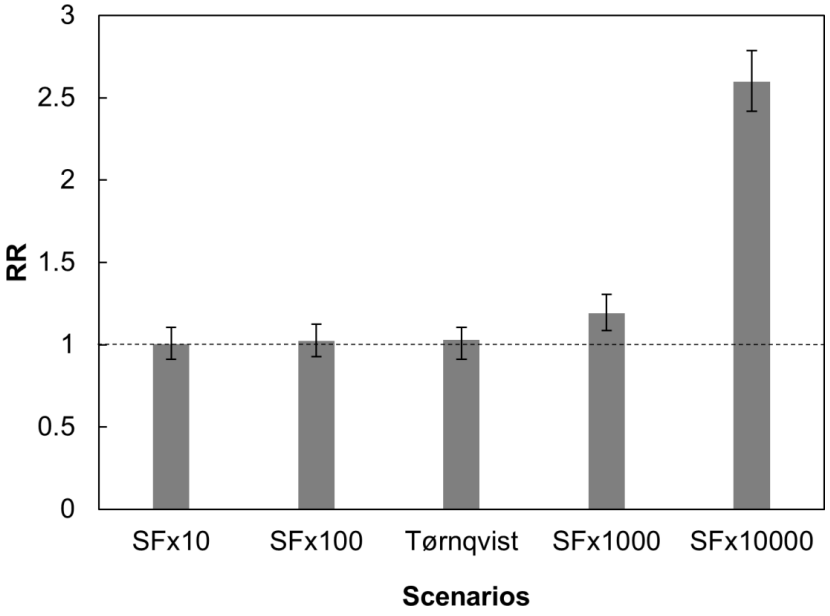

Fig 6. The simulated relative risk by scenario of increasing slope factor.
The mean relative risk of the 100,000 simulations in the baseline models and scenarios of increasing slope factor. Error bars represent the mean of lower- and upper bound probability distributions of the 95% confidence interval around the relative risk. Only simulation results of 4^{th} vs. 1^{st} quartile are shown.

Increasing SD of the exposure distribution from baseline of 0.335 to 10 does not affect the likelihood of detecting a statistical significance in a cohort study (Table 4). Increasing the follow-up time from 10 to 20 years does not affect the likelihood of detecting a statistical significant RR either. Increasing the size of the cohort increased the likelihood of a statistical significant RR, with the most extreme scenario with a cohort size of $3x10^9$ giving a 21% chance of finding a statistical significant RR (Table 4). Fig 7 shows the relation between cohort size, slope factor and the probability of detecting a positive statistical significant RR (LB >1). With increasing SF, the cohort size needed to improve the likelihood of LB>1 decreases. Simulation of the combination of scenario 6, 12 and 18 gave probabilities of RR>1, LB>1 and UB<1 of 0.98, 0.58 and 0.00, respectively.

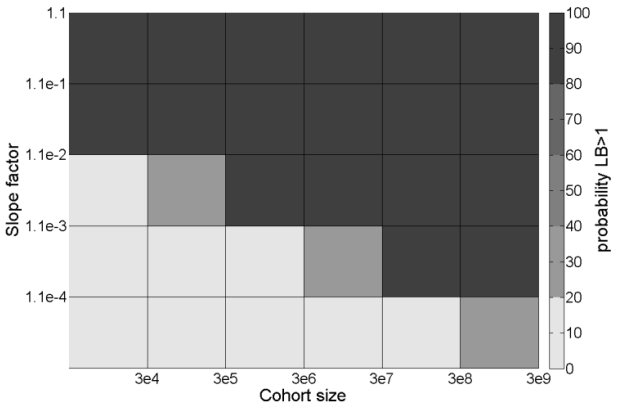

Fig 7. Relationship between slope factor, cohort size and the probability of a significant effect. Contour plot of the relationship between the slope factor, cohort size and the probability (in %) that the 2.5% lower bound (LB) of the 95% confidence interval around the relative risk is above 1.

Discussion

Our simulation results suggest that the chance of finding a statistical significant association between a contaminant with carcinogenic potential of AA in a prospective cohort study is low: 2.3%. In fact, the chance of finding a significant adverse effect of AA in an epidemiological study is the same as of finding a significant protective one. Likewise, the variation in RR shows that it is almost as likely to find a RR above 1 as below. The fact that our simulations do not show RR > 1 exactly 50% of the times is due to RR=1 in some of the iterations. Based on the simulation results it is therefore not surprising that most epidemiological studies do not show a statistical significant association between dietary AA exposure and cancer [13,12,17].

Of the variables investigated, increasing the SF had the largest effect on the statistical significance of the association, where SF times 1,000-10,000 ensure a 100% likelihood of finding a statistical significant RR if performing a cohort study. Intuitively this makes sense, as the size of the SF in the baseline model (1.09 cases out of 10,000) is approximately 3400 times smaller than the lifetime background risk

of cancer, q_l (3400 cases out of 10,000). Only when p_a is approaching the size of q_a, the likelihood of a statistical significant RR is approaching 100%. We applied the SF of $16x10^{-3}$ reported by Törnqvist et al. [15], to our knowledge the highest slope factor reported in the literature, which resulted in a 8.3% likelihood of detecting a significant RR. The authors state that despite their relatively high risk estimate, validation of this estimate in an observational study is unlikely. They argue that this is mainly because AA constitutes a limited range of exposure as the chemical is present in many commonly consumed foods. However, in our simulation we investigated the effect of increasing the range of the exposure by increasing the SD of the exposure distribution. This did not affect the likelihood of finding a statistical significant RR, simply because the background risk of cancer so greatly outweighs the probability of cancer mediated by AA itself.

Neither the SF nor the SD are parameters that can be influenced in a study design. However, if information on the potency of the chemical (i.e. the SF) or risk factor under study and range of exposure is available, computer simulations can be used as a tool to assess the impact of these on the sensitivity of a proposed study design, in advance of performing the study. For example, our simulations show that to obtain a 50% chance of finding a statistical significant RR between the 1^{st} and 4^{th} quartiles of the exposure distribution, the cancer potency of the chemical needs to be at least 500 times larger than the slope factor applied in the base-line model. That is, the background probability of cancer should be no larger than approximately 6 times the probability of cancer arising from the chemical exposure to, with 50% likelihood, detect a statistical significant RR.

On the other hand, both the size of the cohort and the time of follow-up are variable parameters in the study design of an observational study. We investigated the impact of cohort sizes ranging from 30,000 individuals (baseline model) to 3 billion. A cohort size of 3 billion is unrealistic, but was added to illustrate that the cohort size,

in this case, do not greatly improve the likelihood of a statistical significant RR as a cohort of 3 billion yields only 21% chance. Hagmar and Törnqvist (2003) [18] also discussed the cohort size and number of cases needed to detect a statistical significant RR of 1.05 of the association between AA exposure and the risk of cancer. The RR was theoretically extrapolated from animal experiments and an assumption of 18% background risk. Based on exposure estimates from the case-control study by Mucci et al (2003) [19], the authors estimated that 470,000 cancer cases with half the number of controls are needed to detect a significant effect, a number farfetched from what is obtainable in reality. The examples above suggest that the size of the cohort is of limited importance when the potency of a chemical on a given disease is negligible compared to all other causes (the background risk) of the same disease. The relation between the potency of the chemical, the cohort size and the sensitivity of a cohort study is given in Fig 7. This information is valuable to use in the design of a study when theoretical information on the size of the risk posed by a hazard can be derived, i.e. from animal studies.

Increasing the follow-up time to 20 years did not have an impact on the likelihood of a statistical significant relative risk either. Intuitively this makes sense as we assume that the probability of cancer mediated by AA follows the same relation with age as the background probability of cancer, thus the two are proportional to each other. The combination of a follow-up of 20 years, a cohort size of 300,000 and the SF reported by Törnqvist et al., increased the likelihood of a statistical significant RR to 57.7 %. These three scenarios are considered “realistic” in the case of AA

It is relevant to discuss how the study design of our simulated cohort study compares the characteristics of the prospective cohort studies performed in real life. In the baseline model, we simulated a cohort size of 30,000 which resulted in a mean total of 2899.62 cases, with a ratio of cases to number of participants in the cohort of ~10. From the collection of cohort studies included in the meta-analysis of Pelucchi et al.

[14], the size of the cohorts are in the range of 27,111 – 301,113 persons [10,12,20–23]. The ratio of cases to number participants in the cohorts ranged from 16-480. The studies, where the number of cohort participants per case is low, are the studies of the association with high-prevalent cancers (i.e. breast and prostate cancer [12,21]). In our study, we simulated the association of dietary AA to total cancer rather than a specific cancer, which is rarely seen in real-life cohorts. This was chosen, as the SF is not estimated for any particular type of cancer. As a case-study, our simulation on AA and total cancer is an example of a highly prevalent type cancer or of a sub-population with higher cancer prevalence. In our model, we simulated the probability of a person getting cancer within his/hers following 10 life-years from age of entry to estimate the prevalence of cancer cases in that time period. This is similar to a closed cohort, but where all participants remain for the length of the study period, which is most likely not feasible in real life. Further, in the real-life cohort studies, RRs are usually calculated on incidence rates using person years, e.g. the number of years that a person is "at risk" in the cohort from entry date until diagnosis, death (or exit by other cause) or closing of the cohort. The follow-up times in real-life cohort studies are therefore reported as either a mean follow-up time or as the duration of the study, thus not entirely comparable to the 10-year follow-up in our model. However, in the studies collected by Pelucchi et al. follow-up times reported range from 10 years to 27 years, which indicate that a 10-year follow-up (and the scenario of 20-year follow-up) both are in the range of study durations in real-life.

One of the important arguments regarding the apparent inconclusiveness of the findings in observational studies of the association between dietary exposures and cancer is that they suffer from misclassification bias which attenuates the RR [24]. Epidemiological studies of the association between cancer and dietary AA is especially prone to misclassification. Estimating exposure of individuals is usually done by food frequency questionnaires (FFQ), however, the AA content of given

foods are highly variable both between brands of the food type and between preparation styles, which FFQs do not necessarily capture. Besides, people's eating patterns change over the course of life (or cohort follow-up time), which neither is captured in a once in time FFQ. However, the advantage of simulating cohort studies is that we simulate "perfect knowledge" on for example the exposure i.e. a cancer case in the fourth quartile is actually a cancer case with that exposure. Another prerequisite for the design of an epidemiological study is that there are sufficient subjects with high exposures [18]. This is also controlled in our simulation model, where the number of individuals in each exposure category is equal, despite the cohort size. Likewise, residual confounding is not influencing the simulation results, as we solely consider the probability of disease given the action of the chemical. Therefore, the simulation results actually yield the maximal expected chance of finding statistical significant result in an actually performed study, as they will always be influenced by misclassification, residual confounding etc.

Our results show that the sensitivity of a prospective cohort study is limited when studying the association between a chemical contaminant and the risk of cancer. Thus, an observational study cannot prove that a contaminant do not pose a health risk; only inform on an upper level of the adverse effect associated with an exposure [25]. We argue that this should be taken into account in the weight of evidence that observational studies have on the classification of carcinogens, and further in the formulation of guidelines and interventions promoting human health. We argue that simulation of observational studies using probabilities of disease derived from for example toxicological data and a relevant exposure distribution is a useful tool to estimate the possible achievable RRs and the likelihood of observing a statistical significant RR, given the study conditions (follow-up time and cohort size), in advance of actually performing the study. In our view, it would be relevant to define a level of the acceptable probability of detecting a statistical significant RR, before it

is considered worth the resources to undertake an observational study. This acceptable probability could then be used in a simulation of the study to optimize the study design and/or decide whether the study should be conducted or not.

Conclusion

Based on our simulation model, we conclude that the sensitivity of a prospective cohort study on the association between acrylamide and the risk of cancer is limited. Our simulation model shows that when the expected potency of a chemical or risk factor is small compared to the risk of the disease from all other causes, the likelihood that an observational study will detect a statistical significant effect is very low. This also accounts for observational studies of very large cohorts or long follow-up time. We argue that this should be taken into account in health policies informed on currently available epidemiological evidence. A simulation model of an observational study is not prone to bias that attenuates the relative risk, and we suggest that simulation of observational studies is a powerful tool to evaluate the sensitivity of given study design before it is conducted, both to optimize the design and to decide on its justification.

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4.3 Discussion

As discussed earlier, the collected weight of evidence of a causal effect of a hazard-health outcome pair has an influence on which health outcomes are accounted for in a disease burden study, which in turn is a crucial determinant of the overall burden estimate. In the risk factor studies of the GBD project [12][13], the risk-outcome pairs are chosen by meeting the World Cancer Research Fund's grades of convincing and probable evidence [97]. In this framework, convincing evidence consists of biological explanations for the causal effect between exposure and disease established from multiple epidemiological studies in different populations. The collected evidence should be substantial and include relevant prospective observational studies and randomized controlled trials (RCTs), all showing consistent results. If the evidence is probable, the findings in epidemiological studies should still be consistent, but the understanding of the biological explanation for the causal effect is incomplete. Thus, the evidence for the causal effect of a risk factor, or hazard, should mainly come from human observational studies.

In this simulation study, we find that the likelihood of detecting a statistically significant effect is small when there are many other causes for the disease. We simulate that the background risk should be no larger than approximately 6 times the probability of developing cancer arising from the hazard to detect a statistical significant RR with 50% likelihood.

Not discussed in the paper is the assumption that the risk of developing cancer from AA is additive to the risk from other factors, as given by equation 5, i.e. that each hazard or factor causing disease is independent of each other. The opposite assumption would be that hazards or factors causing the disease are proportional to each other, i.e. multiplicative[98]. Different assumptions will result in different risk estimates and have an impact on the result of the simulations of the sensitivity of the studies. Another assumption not discussed is that we estimate the background risk by the cumulative incidence proportion (CIP) using the incidence of cancer in Denmark of different age-groups. Assuming that the current cancer incidence also reflects the cancer cases caused by AA, we account for AA cancer cases twice in our simulation. We argue that when the cancer risk from AA is negligible compared to the background risk, this will not impact the simulation results. However, if the risk from the hazard at hand is considerably larger,

then this would impact the likelihood of detecting a significant RR. Thus, in the scenarios where the risk from AA is increased, this may have an impact, and if taken into account, the cohort study would likely be more sensitive as the background risk would decrease.

The findings of our study suggests that in the case of burden of disease of foodborne chemicals, the weight of evidence used to select health outcomes should take into account the (lack of) sensitivity of human observational studies, especially when the background risk of disease is likeli substantial. If relying only on the evidence from human observational studies as criteria for selection of health outcomes, i.e. as the final convincing evidence on a causal effect, a risk of bias is systematically introduced in the disease burden estimates for foodborne chemicals. Epidemiological evidence is not sufficient to exclude the possibility that a chemical hazard poses a risk in a population. The evidence may only inform on the upper level of an adverse effect associated with an exposure [99].

The preferred evidence for risk estimation of hazards is that obtained from human observational studies. Our study suggests why this evidence is not available for many foodborne chemicals. The disease considered apparently have a large impact on whether convincing evidence for a causal relationship exist or not. Cancer has many causes (hazards) and if each hazard is studied one by one, the rest is always more frequent and the evidence for a causal effect is unlikely to be obtained. It will therefore be a disadvantage for the hazards that cause diseases that are multicausal to base the selection of hazard-health outcome pairs on evidence obtained in human observational studies.

Lastly, if epidemiological evidence is available and used to calculate the disease burden by counterfactual analysis (fig. 2.4, with or without a disease envelope available, the simulation approach provides a way to inform on the likely discrepancy of the results obtained from this top-down approach, compared to results of the bottom-up and risk assessment approach.

Chapter 5

Manuscript III

5.1 Prelude

In Paper 1 and Manuscript 2, we investigated how different scenarios or modelling assumptions may impact the magnitude of the final burden estimates. The aim of Manuscript 3 was to develop a method to account for variability in risk between individuals, and to derive the uncertainty bounds around the relevant descriptor of the population risk given this variability. A quantitative estimate of the uncertainty of a burden estimate is important when comparing disease burden across hazards, especially if the methods applied and strength of evidence to inform model parameters are not compatible. In addition, taking the variability into account also allows for health impact estimation of different population sub-groups, determined by different variables e.g. sex and socio-economic status. The supporting material to the Manuscript can be found in the appendix.

5.2 Burden of disease of Benzo[a]pyrene in barbecued meat: informing advice for different population groups

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In preparation

Abstract

Background Consumption of meat prepared by barbecuing is associated with risk of colorectal cancer due to the formation of carcinogenic compounds including Benzo[*a*]pyrene (BaP). Assessment of a population's burden of disease as well as an individual's probability of disease given specific characteristics and consumer behavior may direct food safety strategies and focus available resources to where impact on public health is largest. The aim of this study was to estimate the disease burden of cancer caused by exposure to BaP from barbecued meat in Denmark in terms of disability adjusted life years (DALYs), as well as to estimate the probability of exceeding an exposure considered a health concern, given consumer behavior.

Methods We developed probabilistic models taking into account consumer exposure patterns given age and sex and the variation in individual's sensitivity to BaP, using the Danish dietary consumption survey, monitoring data of chemical concentrations, data on consumer behavior regarding frequency of barbecuing of meat and animal dose response data.

Findings We estimated 1.24×10^{-6} cancer cases per year in Denmark, resulting in a total disease burden of 4.32×10^{-6} DALY (95% uncertainty interval [UI], 1.70×10^{-6}-8.14×10^{-6}). At the same time, we estimated that a man of low bodyweight who consumes barbecued meat 14 times per year over a lifetime has a 50 % probability of exceeding an exposure level considered a health concern.

Interpretation Our study suggests that even at a low disease burden, individuals in a population may exceed levels of exposure to benzo[a]pyrene, considered a health concern. We propose that our model is useful as a tool to assess the disease burden of foodborne chemicals in subgroups of the population, thus guiding intervention strategies and design advice for specific consumer groups.

Keywords: Benzo[*a*]pyrene, meat, probabilistic risk assessment, Burden of Disease, DALY, Monte Carlo simulation.

1. Introduction

Based on an assessment of the available scientific literature, the International Agency for Research on Cancer (IARC) concluded in 2015 that consumption of processed meat increases the risk of cancer in humans [1]. Processing of meat refers to production or cooking practices in which meat has been transformed to enhance organoleptic properties, digestibility and preservation including smoking, curing and various heat treatments. The compounds that are considered responsible for the carcinogenicity of processed meat are formed during these processes [2]. Polycyclic aromatic hydrocarbons (PAHs) constitute a large group of compounds that are formed during incomplete combustion of organic matter. If meat is prepared over open flame (e.g. barbecuing), fat or meat-juice drips onto the hot coals, wood, etc., and PAHs, formed in the smoke, adhere to the surface of the meat [3]. Sixteen PAHs have been found to be genotoxic/mutagenic and/or carcinogenic in toxicological studies [4–6], benzo[*a*]pyrene (BaP) being the most studied PAH and classified as *carcinogenic to humans* (group 1) by a genotoxic mode of action [7].

Food safety strategies aim to limit the population's exposure to harmful substances present in foods, including chemicals such as PAHs. Strategies can include the establishment of legally enforced maximum contamination levels in certain food types, surveillance of contamination in industrial food-processing, and guidance to both industry and the consumers on how to adjust processing practices to limit contamination. Finally, dietary guidelines are issued to motivate the consumers to decrease consumption of foods containing harmful substances.

To limit the population's exposure to BaP and other carcinogenic PAHs from barbecued meats, the European Commission (EC) has implemented official mitigation strategies that include a legally enforced maximum limit of 5 μg/kg in commercial prepared heat treated meat [8], monitoring of concentration of PAH in meat barbecued in restaurants or other commercially settings followed by guidance

on how to adjust processing to decrease contamination. Furthermore, the Danish National food Authority advises the population to limit consumption of barbecued meat and if barbecuing, to avoid charred meat [9].

Assessment of the public health impact of an intervention strategy to limit exposure to harmful chemical substances in food requires quantification of the associated disease burden. This may help direct food safety strategies and focus available resources on initiatives that result in the greatest increase in public health [10]. Likewise, assessment of the probability of disease given specific food preparation practices may direct food-safety strategies to those consumer-groups that are at higher risk due to preparation and consumption practices. Additionally, consumers, who are ultimately their own risk managers, will be able to make more informed choices on food consumption behavior by having access to information on the probability of a harmful effect given their food consumption patterns [11].

In risk assessment of chemicals, deterministic worst-case scenarios are traditionally performed to provide risk managers with the information to act to protect the overall population. These assessments typically estimate risk for an "average individual", building on average consumption patterns and mean values of exposure. We argue that to provide evidence for risk managers to act with precision, the variability between consumers in a population needs to be described, and the impact that this variability has on public health quantified. Our objectives were to i) estimate the overall population burden of disease of cancer caused by exposure to BaP from barbecued meat in Denmark, using disability adjusted life years (DALY) as a common health metric, and ii) estimate the probability of developing cancer in sub-groups of the population with different consumption patterns of barbecued-meats. To address these, we built probabilistic models taking into account the variation in consumer exposure patterns and the variation in individual's sensitivity to the

chemical. The information derived may be used to assess the overall impact of strategies implemented to improve public health.

2. Method and Materials

We built two probabilistic models; i) a population-level model to estimate the burden of disease of cancer due to exposure to BaP through barbecued meat in terms of DALY, and ii) a subgroup model to estimate the probability of developing cancer given consumer differences in frequency of consumption of barbecued meat. In both models, we applied an event-based simulation scheme for the exposure assessment and a model extrapolation approach for the probability of effect. Fig 1 presents an overview of the model structures. Both models are based on the same datasets. Below are first the datasets described followed by description of the two models.

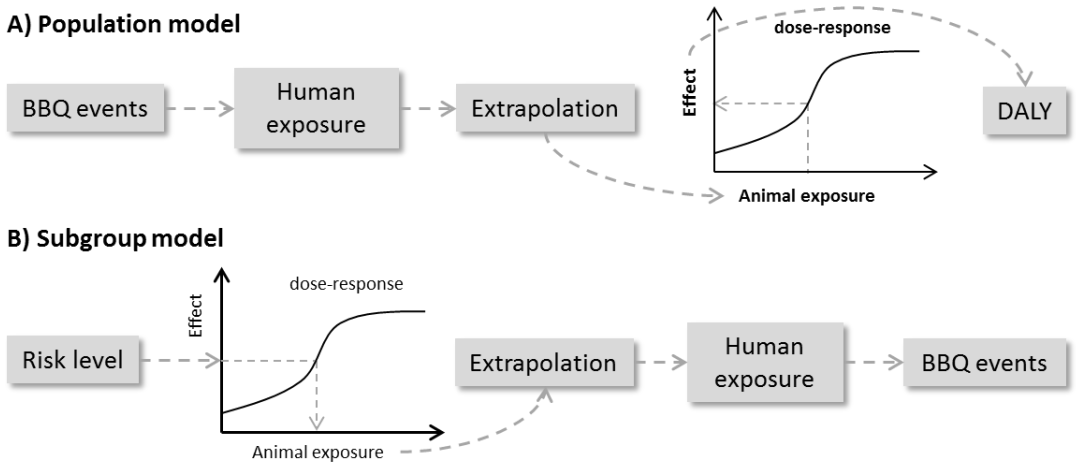

Fig 1. Conceptual overview of the population and subgroup models. In the population model (A) the frequency of consuming barbecued meat was translated into the lifetime exposure to benzo[*a*]pyrene and extrapolated to an equivalent animal exposure which was combined with a dose-response relationship to estimate probability of developing cancer in humans. Based on this, disability adjusted life years were estimated. In the subgroup model (B), the exposure corresponding to a fixed risk level was determined from the same dose-response relationship and extrapolated to an equivalent human exposure, which was compared to the lifetime exposure experienced by an individual consuming barbecued meat and fish with a given frequency.

2.1. Data

2.1.1. Demographics data

To generate the distribution of individual lifetime exposures to BaP from barbecued meat in the population model, the sex and age distribution of the Danish population aged 16-75 years were derived from the official statistics for the 1st quarter of 2017 (S1 Figure) [12].

2.1.2. Consumption data

Both meat and fish consumption was included in the exposure assessment; however, for simplicity we only refer to barbecued meat. Meat consumption data were obtained from the Danish National Survey on Diet and Physical Activity (DANSDA) from 2011-2013 [13], consisting of 7 day food-records from 3,804 individuals, along with sex, age (4-74 years old) and bodyweight for each individual. In our study we considered the food consumption of the adult population, i.e. from 16 years old and up; a total of 1,461 men and 1,572 women. Individual meat consumptions in DANSDA are given for 6 meals per day, but we only considered meat consumptions from dinner-eating occasions as we assumed that consumption of barbecued meat mostly occur for dinner. Each individual in the survey was assigned to a weight-class based on the 33^{rd} and 67^{th} quantiles of the observed bodyweights in the consumption survey (Table 1). For each weight-class, a distinct gamma distribution of meat consumption was described, informed by the consumption in g/meal of each individual (S1 Table). The empirical and fitted censored gamma cumulative density functions of each meat type are shown in Fig 2. To allocate a simulated individual to a weight-class, the relation between age, sex and bodyweight was evaluated based on the data in DANSDA. Different options for the relation were evaluated and the following selected:

$$\text{weight} = \beta_1 * sex + \beta_2 * \ln(age) + \beta_{12} * sex * \ln(age), \quad (1)$$

where $\beta_1 = 35.846$, $\beta_2 = 25.835$ and $\beta_{12} = -13.191$ (adjusted R-squared is 0.969 and residual standard error is 13.764). The weight of an individual was simulated though Eq. (1), with the variance of the error term, ε, in the regression formula estimated by the residual standard error.

Table 1. Classes of bodyweight (in kg).

Weight class*	Man bodyweight in kg (median of weight class)	Woman bodyweight in kg (median of weight class)
Low	< 76 (71)	< 62 (58)
Medium	77 - 87 (82)	63 - 72 (67)
High	> 88 (96)	> 73 (80)

*Weight classes as defined by the 33rd and 67th quantiles of the bodyweight of individuals aged 16-74 in the consumption survey [13]. The median of each weight class is given in parentheses.

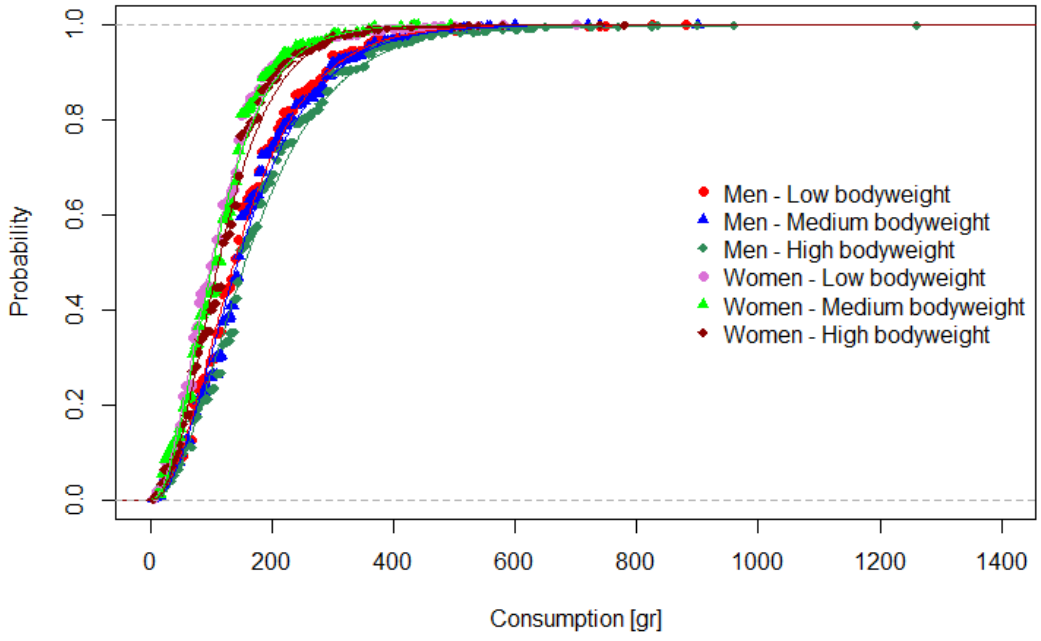

Fig 2. Empirical and theoretical cumulative density functions of meat consumption for each sex and weight class. The marks represent each observation of consumption in g/meal of each sex in each weight class. The lines represent the fitted gamma distributions.

2.1.3. Data on combination of meats consumed

For each barbecue event, we assumed that one individual would eat a maximum of two different meat types. In order to derive the frequency of type of meat consumed if only one meat is consumed (S2 Table) or the possible meat combinations, as well as the partial consumption when two meat types are consumed (S3 Table), the consumption data were coupled with a survey conducted by Coop Denmark A/S in 2013 with 1,009 Danish respondents aged between 15 and 74 years [14].

2.1.4. Data on frequency of barbecuing

We assumed that all individuals in the Danish population who eat meat eat barbecued meat at least once a year. Thus, the fraction of the population never consuming barbecued meat is the same as that of never eating meat, which was estimated to be 4% based on a consumer survey by Coop Denmark A/S [15]. We obtained information on the frequency of consuming barbecued meat from a consumer survey of 76 Danish households, which we translated into an annual number of consumption events [16]. The scenarios for barbecuing behavior that we considered for the Danish population were: 1) less than once per month, i.e. 1-11 times per year; 2) at least once per month but less than once per week, i.e. 12-51 times per year; 3) at least once per week, i.e. 52-365 times per year. The median value for each scenario was selected to represent the scenario. The fraction of the 76 families in each scenario was used to inform on the fraction of the Danish population in the same scenario (Table 2).

Table 2. Frequency of events of consuming barbecued meat per year for fractions of the Danish population derived from consumer surveys in Denmark [15,16].

	Frequency of consuming barbecued meat and fish (events per year)			
	0	9	25	63
Fraction of population	0.04	0.265	0.455	0.24

2.1.5. Concentration data

The concentration of BaP in meat after barbecuing (in µg/kg) was obtained for 407 samples of meat (242 from Denmark obtained from Petersen et al. (2013) [17] and from unpublished monitoring data of commercially barbecued meat from 2012-2015; 136 from UK [18,19]; and 29 from Sweden [20]). Eight meat and fish types were considered: beef (including veal), minced beef (burger patty), pork, pork sausages, lamb, poultry (mainly chicken), fish (mainly salmon) and shellfish. The concentration data from each country were combined for each food type, assuming that the data then reflects the variation in concentration of BaP under different conditions. Values for many samples were below the limit of detection (LOD) of the analytical method applied. After barbecuing all foods will be contaminated with BaP [21]. Therefore, apparent zeros were not regarded as "true zeros", but rather as an expression of a low level of contamination between 0 µg/kg and the LOD. The BaP concentrations for each food type was consequently censored and described by a log-normal distribution (Fig 3) (S4 Table).

2.1.6. Dose-response data

To derive the relationship between exposure to BaP and the risk of developing cancer, we used data on tumor formation in mice orally exposed to either of two coal tar mixtures [22]. The BaP content measured in the coal tar mixtures [23] was used as the dose. The number of all tumor bearing animals (TBA) was used as response variable (S5 Table). Hence, we assumed that the composition of the PAH mixture present in barbecued meat is similar to the composition of PAH in the coal tar mixture, and subsequently that BaP is a surrogate for the total potency of all PAHs present in the coal tar mixtures. We performed dose-response modelling on the data using PROAST version 60.1 [24] developed in R [25]. A set of models were fitted to

the data and their fit was accepted based on the log-likelihoods using the likelihood ratio test.

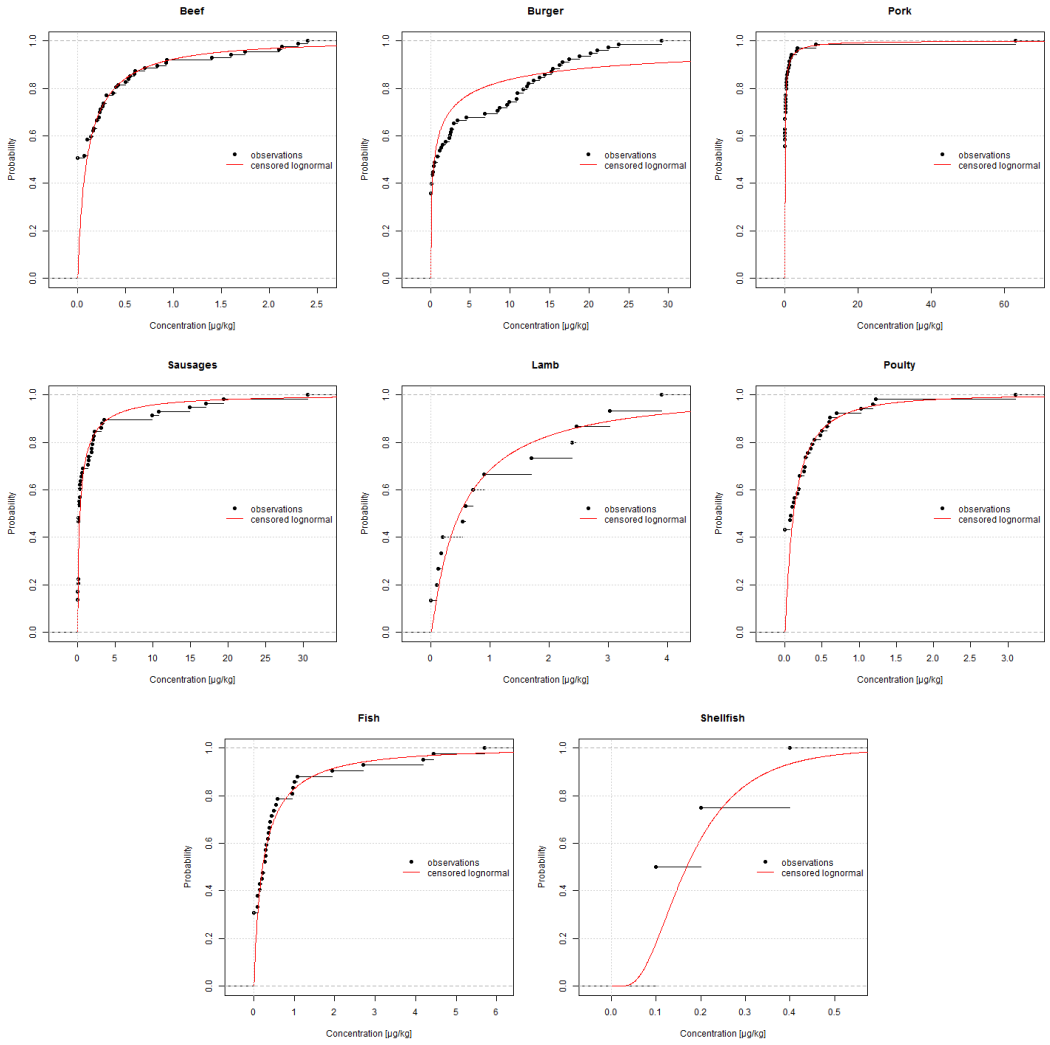

Fig 3. Empirical and theoretical cumulative density functions for concentration of Benzo[*a*]pyrene in each meat type. The black dots in each graph represent the censored concentration of benzo[*a*]pyrene (in µg/kg) of each sample of each meat type. The red lines represent the log normal distributions fitted to the censored concentrations.

We chose the most sensitive model to estimate the extra lifetime risk associated with the lifetime exposure to BaP, i.e. the model yielding the lowest $BMDL_{10}$ (the dose

associated with the lower limit of the 95% confidence interval around the dose at which 10% of the study animals get a tumor), which was the two-stage model (Fig 4):

$$ER_{BaP} = 1 - \left(e^{-\left(\frac{exp_{animal}}{b}\right) - c\left(\frac{exp_{animal}}{b}\right)^2}\right) \qquad (2),$$

where ER_{BaP} is the extra lifetime risk of cancer due to BaP, exp_{BaP} is the animal exposure, b is the potency parameter and c is the shape parameter (Table 3). The values of b and c were obtained and the uncertainty in b and c was propagated by bootstrapping with replacement 1,000 samples in PROAST. In Fig 4, the two stage model is fitted including the background parameter, a, representing the fraction of animals with tumor in the control group. To estimate the extra lifetime risk of cancer from equation 2, the background fraction is omitted.

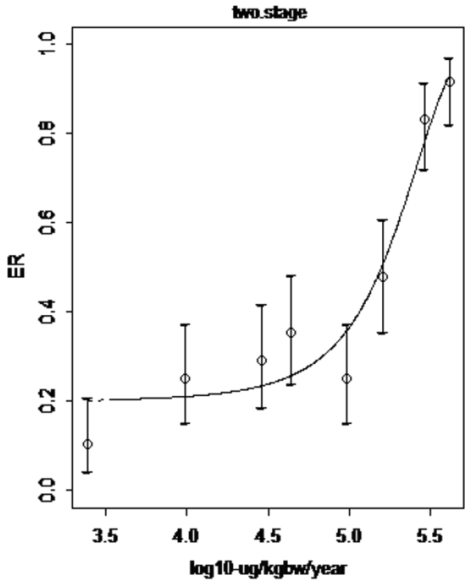

Fig 4. Dose response model. The two stage model fitted in PROAST [24] to the fraction of tumor bearing animals (TBA) per dose group [22,23] to obtain the relation between dose of benzo[*a*]pyrene (ug/kgbw/year) and the extra lifetime risk of cancer (ER_{BaP}). The graph shown is fitted with the background parameter representing the fraction of animals with tumors in the control group.

2.1.7. Extrapolation factors

We used probability distributions to extrapolate between human and animal, i.e. taking the interspecies- and intra species difference between animals and humans into account. The interspecies difference relates to the toxicodynamic and toxicokinetic difference between the experimental animal and the average human, which is uncertain. The interspecies difference is performed in two steps; 1) allometric scaling to account for differences in bodysize ($EF_{inter,allometric}$) by:

$$EF_{\text{inter,allometric}} = \left(\frac{\text{bodyweight}_{\text{human}}}{\text{bodyweight}_{\text{animal}}}\right)^{1-AP} \quad (3),$$

where AP is the allometric power, which is uncertain; and 2) an extrapolation factor ($EF_{inter,TKTD}$) to account for the remaining uncertainty in interspecies difference. The intraspecies extrapolation factor (EF_{intra}) accounts for the variation in sensitivity to the chemical between individuals. Due to lack of substance specific estimates, default probability distributions for AP, $EF_{inter,TKTD}$ and EF_{intra} was applied [26] (table 3).

Table 3. Model parameters and distributional assumptions for the two stage dose response model and extrapolation factors.

	Description	Unit	Distributional assumption	Distribution parameters		Value if point estimate
AP[1]	Uncertainty in the allometric power		Normal(μ,σ)	μ = 0.7	σ = 0.033	0.7[2]
$EF_{inter,TKTD}$[1]	Uncertainty in TKTD difference between human and animal		Lognormal(GM,GSD)	GM =1	GSD=2	1.27[3]
EF_{intra}[1]	Variation in sensitivity to the chemical between individuals		Lognormal(GM,GSD)	GM =1	GSD =3.6	
$GSD_{EFintra}$[1]	Uncertainty in the GSD of EF_{intra}		Chi-squared(df)	df = 21		
bw_{human}	Human bodyweight	kg				70
bw_{animal}	Animal bodyweight	kg				0.03
b	Potency parameter of the two stage model		Bootstrapped			848500
c	Shape parameter of the two stage model		Bootstrapped			8.299

[1]Default distributions used, when no chemical specific information is available [26].
[2]Mean of the AP normal distribution
[3]Mean of the $EF_{inter,TKTD}$ lognormal distribution

2.1.8. Data for DALY calculation

To estimate DALYs, we developed a disease model consisting of 4 stages of cancer [27,28] and used data on the duration and DWs of each cancer state, mortality estimates, and the life expectancy in the population (Table 4). The Danish population in 2015 of individuals above the age of 15 was 4,697,060. We used the life table of the WHO frontier life expectancy [29]

Table 4. Parameters for DALY calculation for non-specific cancer.

Cancer stages[1]	Age of diagnosis (years)	DW[3]			Mortality[4]	Treatment proportion[5]	Duration (years)[6]	Age at death
		mean	min	max				
Diagnosis and primary care	67.38[2]	0.288	0.193	0.339		1	1	
In remission	68.38		0.18	0.47		1	5	
Disseminated carcinoma	73.38	0.451	0.307	0.6		1	1	
Terminal phase	74.38	0.54	0.377	0.687	0.385	1	0.083	74.46

[1]Cancer disease stages accounted for in the DALY calculation adapted from [30] and [28].
[2]Age of diagnosis calculated as the weighted average of number of new cancer cases in 2015 per age group in Denmark (http://esundhed.dk/sundhedsregistre/CAR/Sider/Cancerregisteret.aspx). Age of onset of the other cancer stages is derived by adding the duration of each cancer stage the age of onset of diagnosis.
[3]DW: Disability weights for each disease stage obtained from [10] except for "In remission" which is obtained from [30].
[4] Mortality expressed as 1 – the 5 year age-standardized survival rate for 2010-2014 [31]
[5]Assumed that all cancer cases and associated disease stages in Denmark are treated.
[6] Duration of disease stages are assumed to represent the mean duration of each disease stage adapted from Mathers et al. (1998) [30].

2.2. Population model

To estimate the disease burden we applied a model consisting of 3 components: an exposure, a health-outcome and a DALY-module [32]. In the exposure module, we estimated the lifetime exposure to BaP through barbecued meat, taking into account the variation in the consumer behavior and in the concentration of BaP in meat. The

estimated exposure distribution was integrated with the dose-response model to estimate the probability of disease (health-outcome module). In the third module, DALY was calculated using the probability of disease, health statistics and disability weights. A more detailed description of each module is given below.

2.2.1. Exposure module

We estimated the yearly BaP exposure through simulation of barbecue events per individual within the population by:

$$y_i = \frac{1}{w_i}\sum_{b=1}^{B}\sum_{k=1}^{K_b} x_{ik}^{b} C_k \qquad (4),$$

where y_i is the yearly exposure to BaP of individual *i*, b is a BBQ event with values in $\{1, ..., B\}$ and B is the total number of BBQ events per year; K_b is the total number of meats consumed for a BBQ event b; x_{ik}^{b} is the amount of meat k consumed by individual i at event b; C_k is the concentration of BaP in meat k and w_i is the bodyweight of individual *i*. To simulate the Danish population, we generated the sex and age of individuals from the demographics data (S1 Figure). The bodyweight of the individuals was estimated through the regression model (equation 1) and the individuals were assigned to a weight class (Table 1). For each individual, a number of barbecue events among the possible scenarios was simulated (Table 2). For each barbecue event the consumed meat types and their partial consumption (S2 Table and S3 Table) were simulated independently of the individual's weight class, while the total meat consumption was generated from the gamma distribution (Fig 2, S1 Table) defined for the weight class of the individual. The BaP concentration was randomly sampled from the log-normal distribution of the consumed meat types (Fig 3, S4 Table). We simulated the exposure of 10,000 individuals to make up the population exposure distribution. Simulations were performed in R [25] (for simulation details see supporting material section 3.1.).

2.2.2. Health outcome module

Because the carcinogenicity studies in animals showed a wide variety of tumor sites, we chose *total cancer* as the health outcome associated with exposure to BaP [22,33]. To derive the probability of developing cancer over a lifetime due to exposure to BaP through barbecued meat, we applied the model extrapolation approach based on the integrated probabilistic risk assessment (IPRA) methodology for carcinogens proposed by Slob et al. (2011, 2014) [26,34]. In this approach, the human exposure y_i is extrapolated to an equivalent animal exposure by:

$$exp_{\mathrm{i,animal}} = y_i \times EF_{\mathrm{inter,allometric}} \times EF_{\mathrm{inter,TKTD}} \times EF_{\mathrm{intra}} \quad (5),$$

where $exp_{\mathrm{i,animal}}$ is the animal exposure. To simulate the distribution of animal exposure corresponding to the distribution of human exposure, we generated a value from the human exposure distribution and multiplied it with a random value from each of the probability distributions describing the extrapolation factors (Table 3). We then generated a random value from the resulting animal exposure distribution and combined it with the two stage model (equation 2) to derive the distribution of lifetime risk of cancer from BaP, ER_{BaP}, in the Danish population. As the human exposure distribution and EF_{intra} represent variability but $EF_{\mathrm{inter,allometric}}$, $EF_{\mathrm{inter,TKTD}}$ and two stage model parameters, b and c represent uncertainty, two dimensional Monte Carlo simulation was performed using the `mc2d` package in R [35], to separate variability from uncertainty. We simulated with 10,000 iterations in the variability domain and 1000 iterations in the uncertainty domain (for simulation details see supporting material section 3.2.2.).

2.2.3. DALY module

DALYs are the healthy life years lost in a defined population calculated by adding the number of years lived with disability (YLD) and the number of years lost due to

premature death (YLL), i.e. DALY = YLD + YLL. We express DALYs on an annual basis, but express the disease burden that the incident cases in a given year will bear into the future [36,37]. Therefore, we calculated the annual incidence of BaP-associated cancer, by dividing the mean of the population distribution of lifetime risk of cancer, ER_{BaP} with 92, which is the projected frontier life expectancy at birth for the year 2050for both males and females [29]. We applied a probabilistic model to propagate the uncertainty in the parameters, using the DALY Calculator interface developed in R [38], adapting data from the Global Burden of Disease study, national statistics and literature (Table 4).

2.3. Subgroup model

In the subgroup model, we reversed the population model and estimated the lifetime exposure to BaP from barbecued meat that corresponds to a risk level considered acceptable, i.e. the "virtually safe dose" (vsd). As risk level, we chose 1 cancer case out of million (10^{-6}) in a lifetime, which by international organizations is considered the maximum acceptable extra lifetime cancer risk from a specified exposure [5]. We specified the exposure as the lifetime exposure to BaP from barbecued meat. The outcome of the model was an estimate of an individual's probability of exceeding the virtual safe dose, if barbecuing with a given yearly frequency over a lifetime. Uncertainty in the parameters was excluded for practical reasons.

2.3.1. Exposure module

The lifetime exposure of a "typical" individual of each weight class was simulated by the event-based approach (equation 4), but where *B* is the total number of barbecue events in a year but summed over a lifetime of 60 years. For a "typical" individual of each weight class, we simulated the lifetime exposure if barbecuing from 1 to 365 times per year (i.e. 60 – 21,900 times over a lifetime). The median bodyweight of

each weight class (Table 2) was used to represent the body weight of the individual. For a given weight class and each barbecue event, the total meat consumption of the individual was generated from the appropriate Gamma distribution (Fig 2), along with the consumed meat types and their partial consumption. The BaP exposure was calculated after having sampled the BaP concentration from the appropriate log-normal distribution (Fig 3). The lifetime exposure was simulated for 1,000 individuals of each weight class and *B* from 60 – 21,900 (for simulation details see supporting material section 4.1.).

2.3.2. Health outcome module

We estimated the animal exposure corresponding to a 10^{-6} risk, using the two stage dose response model also applied in the population model (equation 2), but solved for $\exp_{animal}$:

$$\exp_{animal} = \sqrt{\left(\frac{b^2}{4c^2}\right) - \left(\frac{b^2}{c}\right) \times \log(1 - \mathrm{ER}_{BaP})} - \frac{b}{2c} \quad (6),$$

where ER_{BaP} is 10^{-6}. The model parameters, *b* and *c* were represented by point estimates (table 3). We then extrapolated $\exp_{animal}$ to an equivalent human exposure, the vsd, by:

$$\mathrm{vsd} = \frac{exp_{\mathrm{animal}}}{\left(EF_{\mathrm{intra}} \cdot EF_{\mathrm{inter,allometric}} \cdot EF_{\mathrm{inter,TKTD}}\right)} \quad (7),$$

where $\mathrm{EF}_{inter,allometric}$ and $\mathrm{EF}_{inter,TKTD}$ are point estimates representing the uncertainty, and EF_{intra} the distribution representing variation (Table 3). Thus, the resulting distribution of vsd reflects the variation of equipotent doses in the human population. For each of the simulated individuals in the exposure module, a random value from the distribution of vsd was sampled and compared with the individual's exposure from barbecuing at a given frequency. The probability of exceeding the virtual safe

dose was estimated based on fraction of the 1000 individuals exceeding the vsd (for simulation details see supporting material section 4.2.).

3. Results

3.1. Population model

The estimated yearly exposure to BaP though consumption of barbecued meat in the Danish population varied substantially between individuals, with a median of 0.065 µg/kg bodyweight per year, and 95th percentile of 0.47 µg/kg bodyweight (Fig 5). The best estimate of the mean lifetime risk of cancer from BaP in barbecued meat, ER_{BaP}, in the Danish population lies within a 95% uncertainty interval of $1.39x10^{-5}$ to $3.93x10^{-4}$ with a median of $6.91x10^{-5}$ (Table 5).

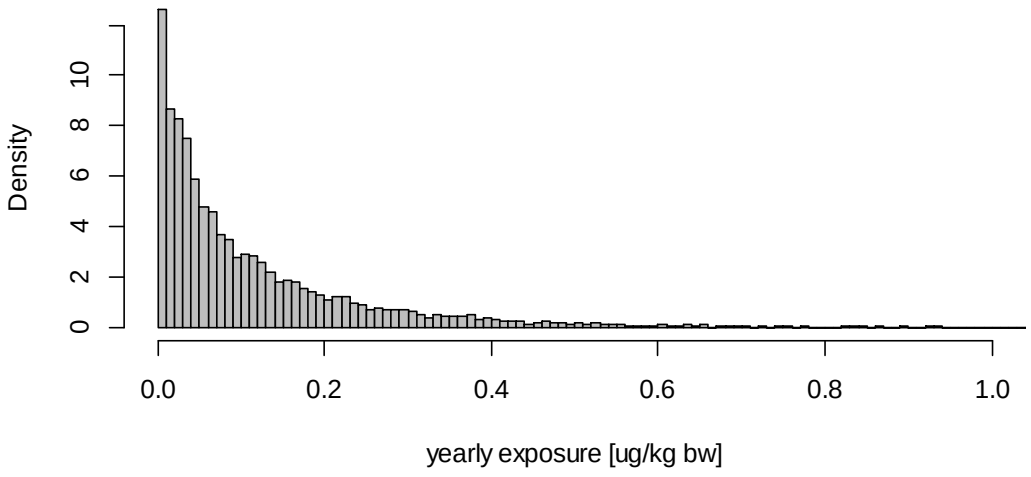

Fig 5. Population exposure to benzo[*a*]pyrene (in µg per kg bodyweight) from barbecued meat in Denmark. These exposure estimates result from the event-based simulation scheme modelling the yearly exposure to BaP through meat consumed at barbecue events.

Table 5. Population lifetime risk of cancer from benzo[*a*]pyrene in barbecued meat.

Uncertainty	Mean lifetime risk	Median lifetime risk
median	$6.91x10^{-05}$	$1.08x10^{-05}$
mean	$1.06x10^{-04}$	$1.55x10^{-05}$
2.50%	$1.39x10^{-05}$	$2.39x10^{-06}$
97.50%	$3.93x10^{-04}$	$5.61x10^{-05}$

Estimates of the mean and median lifetime of cancer in the population presented by the descriptors of the uncertainty distributions: median, mean and 95% uncertainty interval.

The estimated mean annual incidence of cancer cases in Denmark caused by BaP exposure from barbecued meat was $1.24x10^{-6}$, and the disease burden that these cases cause was estimated to be $4.32x10^{-6}$ DALY (Table 6). On average, each cancer case causes 3.49 DALY. 44% of DALYs are life lost due to premature death (YLL), while 56 % are years lived with disease (YLD). The 95% confidence interval describes the uncertainty expressed in the extrapolation factors, EF_{intra}, $EF_{inter,allometric}$, and $EF_{inter,TKTD}$, the parameters of the two stage model, b and c, and in parameters used to calculate DALY.

Table 6. Burden of disease of cancer in Denmark due to benzo[*a*]pyrene exposure from barbecuing of meat.

	Mean	95% CI
Annual Cases[1]	$1.24x10^{-06}$	$2.59x10^{-07}$-$2.76x10^{-06}$
Annual Cases per 100,000	$2.27x10^{-08}$	$4.74x10^{-09}$- $5.05x10^{-08}$
DALY	$4.32x10^{-06}$	$1.70x10^{-06}$-$8.14x10^{-06}$
DALY per 100,000	$9.91x10^{-08}$	$3.11x10^{-08}$-$1.49x10^{-07}$
DALY per case[2]	3.49	
YLL	$1.88x10^{-06}$	$3.95x10^{-07}$-$4.26x10^{-06}$
YLD	$2.44x10^{-06}$	$7.25x10^{-07}$-$5.64x10^{-06}$

Annual number of cases of cancer and disability adjusted life years (DALY), years of lost life (YLL) and years lived with disability (YLD) caused by benzo[*a*]pyrene from barbecued meat in Denmark.

[1]Annual cases reported are the number of cases of diagnosis and primary care (i.e. the first cancer stage in the disease outcome tree of cancer).

[2]Annual cases used to calculate the DALY per case are the number of cases of diagnosis and primary care

(i.e. the first cancer stage in the disease outcome tree of cancer).

3.2. Subgroup model

The lifetime exposure to BaP from barbecued meat depending on a yearly frequency of barbecuing maintained for a lifetime, varied between sexes and weight classes (Fig 6). Both men and women of low bodyweight have a higher lifetime exposure for the same barbecue frequency than individuals of medium and high bodyweight. This is due to a higher consumption of meat per kg bodyweight by the individuals of low bodyweight.

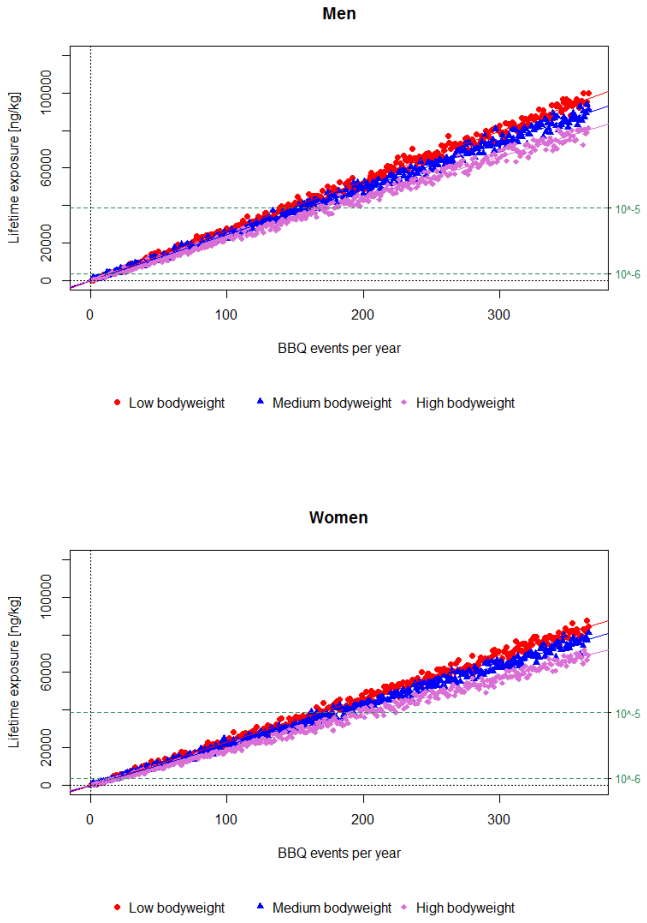

Fig 6. Relation between lifetime exposure, barbecue events per year and risk of cancer. The lifetime exposure to benzo[*a*]pyrene as a function of number of barbecue events per year over a 60 year lifetime given for each sex (A: men, B: women) and bodyweight classes: low = red dot, medium = blue triangle, and high = purple diamonds. The lower horizontal line represents the median of the distribution of virtual safe doses, i.e. the lifetime exposure corresponding to lifetime extra risk of 10^{-6}, and the upper horizontal line represents the median lifetime exposure corresponding to an extra risk of 10^{-5} (distribution not shown).

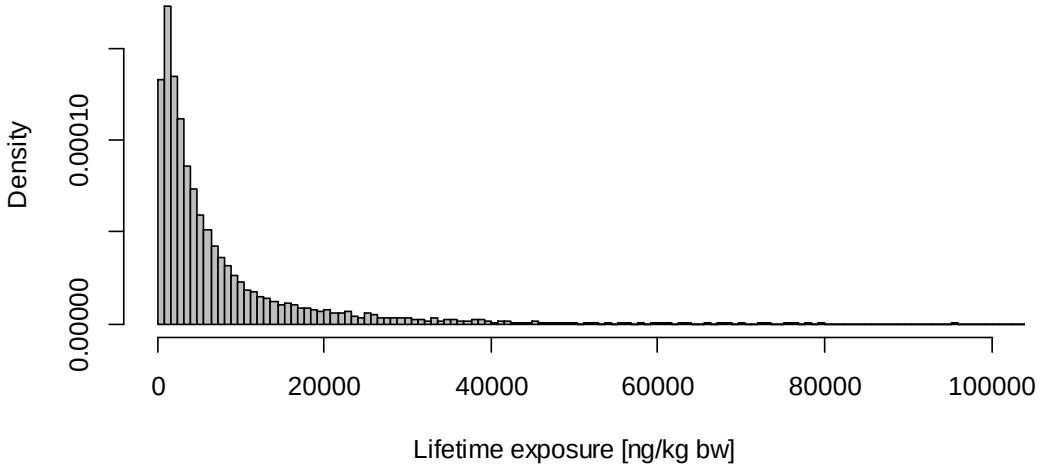

Fig 7. Distribution of virtual doses in the population in (ng/kg bw/lifetime). The distribution reflects the variation, due to differences in the sensitivity to BaP, in the lifetime exposure of individuals in the population corresponding to the same lifetime risk, i.e. 10^{-6}.

The distribution of vsd, i.e. the individual lifetime exposures in ng per kg bodyweight corresponding to a 1 in a million risk is shown in Figure 7. The annual frequency of consuming barbecued meat needed to reach the vsd, i.e. the lower horizontal line in Fig 6 represented by the median of the distribution in Fig 7, is 15 and 17 for low bodyweight men and women, respectively (147 and 169, respectively for a 10^{-5} risk).

For both sexes and all weight groups, one event of consuming barbecued meat per year over a lifetime of 60 years, is associated with the probability to exceed the vsd, i.e. 10^{-6} risk (Fig 8). However, women and men of low bodyweight have a higher probability at lower consumption frequencies compared to the medium and high

weight classes. To exceed the vsd with 100% likelihood, an annual frequency of more than 100 events over a lifetime is required.

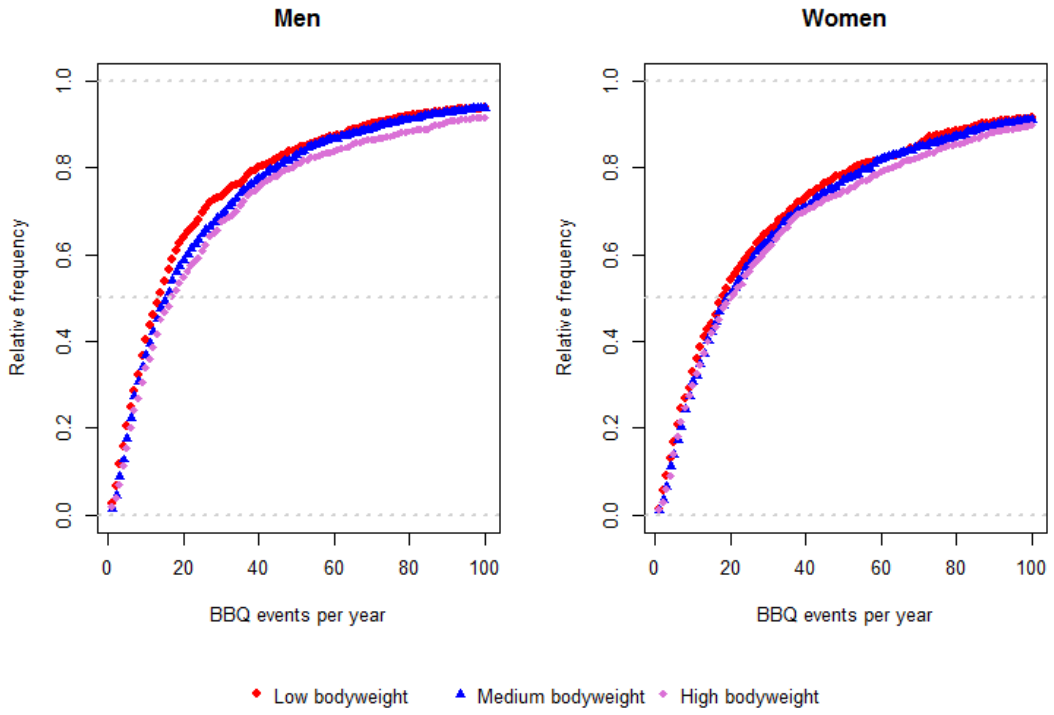

Fig 8. Cumulative probability of exceeding a lifetime exposure corresponding to a fixed lifetime risks per number of barbecue events per year over a lifetime.

In Table 7, the annual number of barbecue events for a 50% likelihood of exceeding the $l0^{-6}$ lifetime cancer risk is presented, for each sex and weight class.

Table 7. Number of barbecue events needed for a 50% probability of exceeding an exposure corresponding to a cancer lifetime risk of 10^{-6}, i.e. the virtual safe dose.

	Men	Women
Low	14	18
Medium	16	20
High	17	20

4. Discussion

We estimated that the disease burden caused by BaP in barbecued meat in the Danish population is low, even considering the large uncertainty interval. This means that the health gains at the population level of removing the exposure to BaP from barbecued meat and fish are negligible. However, our study also shows that the probability of exceeding an exposure considered a health concern might be high for consumers that barbecue frequently.

To our knowledge, this study is the first to present the disease burden due to BaP in barbecued meat. Several exposure and risk assessments of BaP in barbecued meat have been performed, amongst which two Scandinavian studies estimated the yearly exposure to BaP from barbecued meat to be 6.25 ng/kg bodyweight [21] and 2664.5 ng/kg bw/year [39]. These assessments were assuming frequencies of 10 and 30 barbecue events per year, respectively. The high estimate is a worst case estimate; besides from assuming 30 barbecue-events per year, the authors also considered the highest concentrations of BaP found in barbecued meat. We estimated a mean population exposure of 65 ng/kg bodyweight per year, which is comparable to a Middle-Eastern study, which considered also other foods than meat and fish and estimated an exposure of 42 ng/kg bodyweight per year [40]. Each of the three studies reported lifetime cancer risks associated to the exposure of less than 10^{-6}, $7.3x10^{-6}$ and $9.3x10^{-7}$, respectively. These were all deterministic assessments, and the different assumptions applied in the studies' lead to the relatively large variation in both the exposure and risk assessments. We applied a probabilistic approach, i.e. an event-based simulation scheme for the exposure estimation combined with a model extrapolation approach for the hazard characterization adapted from IPRA for carcinogens [26,34]. Our model takes into account the variation in the exposure and sensitivity to BaP in the population, and quantifies the uncertainties associated with the hazard characterization and the parameters for calculating DALYs. This

quantification of uncertainties is an important strength of this approach, since it enables transparent comparisons between the disease burden across hazards [41]. Besides, cancer risk is assumed to vary between individuals, both as a result of different exposure levels and different sensitivity to a chemical, so quantitatively describing this variation allows for the estimation of the fraction of a (sub)population that is subject to a specific risk level [34]. In our study, we applied this to estimate the probability that an individual in a specific subgroup (defined by sex and weight) by a given frequency of barbequing will exceed a lifetime risk of cancer of 10^{-6}.

Other disease burden studies for foodborne chemicals have been conducted in Denmark or at regional and global level. For example, exposure to acrylamide (also a process contaminant) through French fries/fried potatoes is estimated to cause a disease burden of 0.09 DALY/100,000 inhabitants in Denmark [32]; in same order of magnitude, inorganic arsenic in rice was estimated to cause a disease burden of 0.09 DALY/100,000 in the WHO GEMS cluster that Denmark belongs to [42]. Both estimates are the lower bounds, i.e. least conservative estimates, but still considerably larger than the upper bound of our 95% uncertainty interval of 1.49×10^{-7} DALY per 100,000.

Due to lack of data, several assumptions were made, which may influence the representativeness of the input distributions, but are not translated into a quantitative estimate of uncertainty. All assumptions are listed in Table 8, as is the potential impact these assumption may have on the estimations.

Besides the listed assumptions, our study suffered from various limitations. An important limitation of the population study is the data used to inform on the barbecue frequency in the Danish population. This data is only based on information from 76 families and is likely not representative of the total Danish population. For comparison, a Norwegian survey on barbecue behavior including 1,003 participants

reported 26% barbecuing > 17 times/year, 34% barbecuing 6-17 times/year and 27% barbecuing < 6 times per year [39]. If we assume that Danes behave similar to Norwegians, our data seem to overestimate the barbecuing frequency. However, this will not change the conclusion that the disease burden attributed barbecued meat is low.

Table 8. List of assumptions and potential impact on the estimates.

Data source	Assumption	Potential impact on final estimates	Reference
Concentration data	[BaP] is independent on the weight of meat consumed. However, high weight of meat eaten = long barbecue time = high [BaP]	Likely **underestimation** of [BaP] in large portion sizes	
	[BaP] is independent on the fat content. However, high fat content = high [BaP]	Likely **underestimation** of [BaP] in fatty meats	[16,18,20]
	Type of and distance to heating source affect the [BaP], but is unknown.	Unknown if leading to **under- or overestimation** of [BaP]	[16,18,20]
Consumption data	People eat the same amount of meat when barbecuing compared to a non-barbecue eating occasion.	Likely **underestimation** of the meat consumption	[14]
Dose-response data	The total potency of the cumulative effect of all PAHs in the coal-tar mixtures is the same as for BaP	Likely **overestimation** of the potency of BaP	[23]
	Two stage model describe the dose response relationship	Likely **overestimation** in lifetime cancer risk and exposure associated with a 10^{-6} risk	
DALY parameters	Cancer survivors live without disability	Likely **underestimation** of DALYs	[28]

List of assumptions made along the assessment together with their potential impact on the various input parameters and final estimates.

Another important limitation is the lack of information on the dose dependence of the age of onset of disease, i.e. the higher exposure of an individual, the earlier onset of disease [43]. In the DALY module of the population model we assume that the weighted average of the age of diagnosis of cancer is representative of the onset of disease of the cancer cases caused by exposure to BaP from barbecued meat. This leads to an underestimation of the DALY from the contribution from YLL.

Our models suffered from the challenge of combining data from a vast range of sources. This inflicts inconsistency e.g. in the categorization of meat types consumed and meat types sampled for BaP content. Also, this combination of datasets adds to the overall uncertainty of the final estimates.

We only considered BaP in this study. However other carcinogenic PAHs are formed during barbecuing, together with other carcinogenic compounds, and thus the disease burden due to consumption of barbecued meat is likely higher and the frequency of barbecuing to reach a level of health concern likely lower than estimated in our study. Likewise, we did not consider BaP (or other PAH) exposures from other sources, neither other foods nor the environment. If an acceptable lifetime risk of 1 in a million is referring to the aggregate BaP exposure from all sources, it would highly impact our conclusion on the number of barbecue-events per subgroup needed to reach an exposure considered a health concern.

It is important to highlight that our results do not suggest that PAHs are not a health concern. The main sources of BaP and other PAHs from foods in Denmark are cereals, vegetables and milk; the mean exposure from all food sources is 1,413.1 ng/kg bodyweight per year, assuming a 70 kg bodyweight [17]. Smoking and air pollution [44] are other major sources of BaP exposure. If individuals in the subgroups considered in this study are high consumers of the most predominant food sources, smokers or highly exposed to air pollution, this would have a significant impact on the frequency of barbecuing “allowed” (though, it may also imply that the BaP exposure from barbecued meat is of little importance compared to other sources). In quantitative risk assessments of genotoxic carcinogens, the interpretation of the accepted risk level of 10^{-6} is often based on exposure to the chemical from single or small groups of food types, simply due to practical reasons. When referring to a determined risk level, the exposure assumption should be specified [5]. However, an assessment of the aggregate exposure to BaP from other food sources in the

defined subgroups via our proposed event-based simulation approach would greatly improve our study.

The low disease burden to BaP from barbecued meat that this study suggests does not give support to the efforts put into mitigation of exposure by food authorities, even if the concentration of BaP in some meats exceeds the maximum limit (Fig 3). However, there are several reasons why we are cautious to suggest a change in policy priorities. First of all, our study shows that some individuals may experience exposures that exceed a level of health concern. Secondly, health risk perception in the population and risk management go hand in hand. The food authorities cannot disregard substances of potential health concern, even though the population burden is low, as this could compromise consumer trust [45–47].

To our knowledge, no other study has attempted to estimate the probability of exceeding a lifetime exposure considered a health concern given different food consumption and preparation behavior, as well as individual's susceptibility to the effect of an agent. We argue that this information is very useful for consumers that can identify themselves in one of our defined subgroups (on the basis of sex, weight and consumer behavior) and derive the likelihood of being at risk. Best practice for an effective risk communication includes customizing the communication to specific consumer groups taking into account their current behaviors [11,48]. To advise people to limit barbecuing, as is the practice in e.g. Denmark, does not take into account the current behavior of the recipient of the advice. Our approach can be applied to assess the disease burden and risk of other chemical hazards in foods. It allows for individuals to compare and assess their current behavior (in this case barbecuing) and the associated probability of an adverse health effect. Furthermore, in this study we defined subgroups according to sex and bodyweight, determining the amount meat consumed at barbecue events. However, this approach can be adapted to

define subgroups based on other characteristics (e.g. socio-economics status, genetics, etc.) in which mitigation strategies can be more effective to prevent disease.

5. Conclusion

We show that the disease burden due to exposure to BaP through barbecued meat is Denmark is low. Nevertheless, we also show that the probability of exceeding an exposure considered a health concern is high for certain consumer groups for a relatively low annual frequency of consuming barbecued meat. The model suffered from lack of data, especially informing on the frequency of consumption of barbecued meat. However, the developed stochastic model allows for identifying consumer groups who contribute the most to the disease burden. This model can be applied to estimate the health impact of other chemical contaminants in food, and derived information is valuable to direct mitigation strategies to improve public health.

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5.3 Discussion

The first objective of the study was to estimate the disease burden taking into account the variability in the exposure and sensitivity between individuals to the chemical, and to express the uncertainty in the final DALY estimates.

We applied, like in Paper 1, a risk assessment approach to estimate DALYs; however, in this study we applied the integrated probabilistic risk assessment approach for carcinogens as a proof of concept for its utility in the study of burden of disease of foodborne chemicals.

We developed a stochastic model to estimate the population exposure to BaP from consumption of barbecued meat and fish. The resulting distribution reflects the variation in exposure in the Danish population, as it was derived from probability distributions describing the variation in the amount meat consumed and concentration of BaP in the meats. The number of barbecue events per year were simulated from frequency scenarios, as was the type and combinations of meat consumed at an event. At this stage, the uncertainty in the probability distributions of amount meat and chemical concentration was not accounted for, neither was the uncertainty in the frequency scenarios despite the fact that the quality of the data from which they were derived is questionable.

We focused on assessing the uncertainty in the factors used to extrapolate the human exposure distribution to an equivalent animal exposure distribution, which was propagated to the final DALY estimate. As mentioned, in a risk assessment context these extrapolation factors are applied to protect the human population when establishing HBGVs. In the context of disease burden, we apply the factors to express the actual interspecies difference between animals and humans. As this knowledge is uncertain, the distributions applied reflect the range and probability of the plausible values that may describe the species differences in sensitivity to a chemical.
As mice were used in the carcinogenicity study of BaP, the allometric scaling was between an animal bodyweight of 30 g and a human bodyweight of 70 kg. The 5th and 95th percentile of the distribution of the true (but uncertain) scaling factor was 6.43 aand 15.64, respectively. For the other EFs, we applied the generic distributions [63][64] (table 2.2).

In Paper 1, we used a linear extrapolation, i.e. a worst case scenario approach, to derive probability of cancer at human relevant doses. Estimation of the risk at human relevant doses is a major source of uncertainty, and the linear extrapolation method does

not allow for quantification of uncertainty [75][76]. In Manuscript 3, we applied a model extrapolation approach adapted from the IPRA methodology for carcinogens to estimate the uncertainty in the fitted dose response curve at low doses. The uncertainty was propagated by expressing the parameter uncertainty of the selected model. In the dose response modelling, several models were accepted to fit the animal data. We chose the most sensitive model; the two stage model. However, a preferable approach would be to take model uncertainty into account. This was not done due to time constrains, but it would improve the study to include the model uncertainty. The uncertainty of the potency parameter, b, i.e. the shift of the dose response curve along the x-axis, is the second largest contributor to the uncertainty of the mean population risk. Including the model uncertainty would likely increase the range of uncertainty of the potency of the chemical.

In the DALY module, we propagated the uncertainty in the incidence (derived from the health outcome module), disability weights and mortality. The other parameters was kept as point estimates. In the DALY calculator, the indicators of interest (i.e. number of cases, DALY, YLL and YLD) are calculated per disease stage [100]. The number of cases per disease stage do not intuitively make sense, as a cancer patient do not count as a new case when going through each disease stage. However, the DALY of each disease stage represent the total burden that each cancer patient carries. Therefore, when reporting the number of DALY per case, we divided the total DALY with the number of cases diagnosed with cancer. For comparison, the approach to calculate DALYs is similar to the direct approach in Paper 1.

Compared to the deterministic approach applied in Paper 1, the benefit of applying the IPRA methodology to carcinogens is that the uncertainty in the estimated incidence of cancer due to exposure to the chemicals is quantified and thus can be taken into account in the DALY module. Even though we did not carry out a full IPRA as the uncertainty in the exposure estimation and dose-response model was not quantified, we argue that this approach is valuable to inform on the uncertainty originating from the health outcome module of the DALY estimate. An uncertainty analysis inform on how much each quantified uncertainty distribution contribute to the overall credibility interval of the DALY estimate. Unfortunately, the information on the contribution of uncertainty distribution applied prior to the DALY calculation can not, at the moment, be carried along in the

DALY calculator. The uncertainty propagated from each module of the calculation is valuable information, both to direct further initiatives to improve the estimation, but also to make apparent to e.g. risk managers which components of the analysis are the most important limitations to the credibility of the final disease burden estimate.

Notwithstanding the limitations stated above and considering that the upper bound of the 95% confidence interval might be higher, the disease burden of BaP in barbecued meat is low compared to other estimated disease burdens of foodborne chemicals (e.g. Paper 1 and Gibb et al. (2016) [16]), and indeed burden of foodborne pathogens and parasites [1]. With a burden this low, it can be argued that any resources spent on BaP in meat could be better spent to increase public health elsewhere. It can be speculated that the disease burden is low because the present mitigation strategies are effective in ensuring low concentrations in the foods consumed. However, the probability distributions from which the concentrations are sampled, allows for sampling at concentrations higher than the maximum concentration limits, and yet the disease burden is low.

Our stochastic exposure model allows for estimating the contribution of each of the stratified subgroups to the overall disease burden. We did not perform these calculations for the manuscript, since the overall disease burden was very low and the estimation seemed redundant. Besides, each subgroup was characterized by sex, age and bodyweight, which in turn determined the distributions of amount meat consumed per event, but the difference between the distributions of meat consumption of each subgroup was not considerable. However, to proof the concept, case studies should be investigated where other variables, beside age, sex and bodyweight, could be determinants for the consumption of given food types that are vehicles for exposure to harmful chemicals. The identification of variables should be used to inform on whom in the population carry the disease burden and thus direct mitigation strategies.

Finally, manuscript 3 illustrates the schism between disease burden studies and the purpose of toxicological risk assessments. Benzo[a]pyrene is a human carcinogen by a genotoxic mode of action and thus the ALARA principle is of relevance [48]. For regulatory purposes this is not practical and therefore either the MOE of 10000 or above or a risk level of considered "acceptable", i.e. 1 cancer case out of 1 million, are used to evaluate whether an exposure is constituting a health risk higher than accepted. In the population model we estimated a mean lifetime risk higher than the "accepted" lifetime

risk of 1 out of a million cases. And indeed, the subgroup model shows that it is probable that individuals consuming barbecued meat 1 time per year over a lifetime exceed an exposure considered a health concern. In the discussion in the manuscript we consider, why, despite the low disease burden, it is not necessarily straight forward to abolish mitigation strategies to reduce exposure to BaP in barbecued meat and fish. Rather, we argue how the stochastic exposure model allows for informing the individual consumers on how a change in behaviour may adjust the individual's probability of exceeding an exposure considered of health concern.

In summary; the IPRA methodolgy allows for propagating the variability influencing the population risk as well as the uncertainty around the indicators of interest. This is valuable for two reasons: 1) propagation of the uncertainty can direct future research to improve the credibility of the final DALY estimate, but also valuable in order to make the limitations in the knowledge of the true values and models applied in each module transparent for the potential user of the burden estimate. 2) taking variability into account in the stochastic exposure model allows for identifying how disease burden to foodborne chemicals is distributed in subgroups of the population, thus providing evidence for targeting mitigation strategies to the groups that attribute the most to the burden. Finally, the manuscript highlights the schism between the purpose of disease burden studies and toxicological risk assessment. However, even if the disease burden is low, we show that individuals in the population experience exposures that are considered a health concern.

Chapter 6

General discussion

In the following, the main findings of the paper and manuscripts in chapter 3, 4 and 5 are summarized, followed by a general discussion, fitting these findings into the overall context of burden of disease of foodborne chemicals.

6.1 Overview

The main achievements and findings of each study are summarized below:

Paper 1

- We proposed a model framework consisting of three modules, i.e. the exposure, health outcome and DALY-modules, to allow for a structured development of each indicator of interest, and increasing transparency in modelling assumptions and uncertainty.
- The scenarios investigated in the study suggest that the health outcome module attributes more uncertainty to the final DALY estimates than the DALY module, even if the assessed scenarios do not cover the full range of uncertainty.

Manuscript 2

- We developed a simulation approach, which can inform on the possible bias introduced into DALY estimates, for whichever approach is used to estimate the incidence

of disease (i.e. top down or bottom-up).

- The study suggests, that bias might be introduced in the selection of hazard-health outcome pairs if only human observational studies are used as evidence for a cause-effect relationship.

Manuscript 3

- We proved the concept of the applicability of an integrated probabilistic risk assessment approach for the disease burden study of carcinogenic chemicals taking into account variability and uncertainty.

- The study was an illustration of the schism between the purpose of toxicological risk assessment and of disease burden studies and the implication for risk management options.

6.1.1 On the uncertainty in the estimates of disease burden of foodborne chemicals

DALYs reduce complex information into a single number, and every DALY estimate is a result of the input data, model selection and parameters used in the calculation. Rarely the knowledge base is complete and ranges of uncertainty in many elements of the calculation are attached to the DALY estimates [35][101].
In the estimation of the burden of foodborne chemicals by toxicological data from animal studies, the health outcome module contributes considerably to the overall uncertainty in the DALY estimate, as illustrated in the studies included in this thesis. Even though disease burden estimates relying on incidence data from surveillance sources and health registries are also hampered by assumptions and uncertainties, a theoretical estimation of incidence by the use of either animal or human observational studies incur considerable added uncertainty. By use of either source of data, the main issue is connected to that the probability of disease is derived in one population but must be extrapolated to another. Relative risks can be derived based on a cohort in one population where the distribution of exposure, mediators and confounders might not be similar to the population of interest.

The probability of disease derived from animal dose response data must be extrapolated to humans and even between humans. Study designs, experimental error etc. of course add to the uncertainty of how well the data describe the true disease models. If the quality of the data available for a given foodborne chemical is found inadequate, the disease burden may not be estimated and left unknown.

It is therefore relevant to identify an acceptable level of the quality of the data that a DALY estimate is based on. That level is difficult to quantify or standardize among hazards, both chemical and microbial. Nonetheless it has an impact; both on the assessment of the disease burden of a single hazard (i.e. which health outcomes are accounted for) and on an overall estimate of the burden of foodborne disease (i.e. the burden of foodborne disease is only as large as the hazard-health outcome pairs included). If for example the criteria for the quality of the knowledge base is strict, many hazard-outcome pairs might be excluded. If many hazard-outcome pairs are excluded, the impression on hazards ranked by health impact might be distorted. In the end, the risk assessors should not decide on which hazard-outcome pairs should be included in final DALY estimates (and eventually in a hazard ranking). Rather they should supply risk managers with a full account of the database for arriving at the estimates [35].

Knol et al. (2009) identified the need for a structured approach to identify and communicate the uncertainties associated with environmental burden of disease assessments [35]. They suggest a typology, adapted from existing uncertainty typologies, to be used to increase the transparency of how indicators are derived. Our proposed model framework consisting of the three modules presented in Paper 1 combined with the typology adapted from Knol et al. 2009 [35] provides a structured approach facilitating transparency. Tabel 6.1 presents an overview of the components of the analysis, the data sources and characteristics of the associated uncertainty in each module of an hazard-incidence based risk assessment approach.

Table 6.1: Overview of the components of the analysis of disease burden of foodborne chemicals via the risk assessment approach, and their associated data sources and uncertainties

Module	Component of analysis	Data sources used	Source of uncertainty	Expression of uncertainty
Exposure module	Exposure model	Collected scientific evidence	Model structure uncertainty	Scenarios
	Food consumption	Diaries, Recalls, FFQs	Input data uncertainty	Statistical/ Scenarios
	Chemical concentration	Surveillance programs, monitoring programs	Input data uncertainty	Statistical/ Scenarios
	Biomarkers	Biobank databases	Input data uncertainty	Statistical/ Scenarios
Health outcome module	Health outcome selection	Collected evidence for causality	Contextual uncertainty	Scenarios
	Dose response model	Animal studies, Human observational studies	Model structure uncertainty	Scenarios
		Model parameters, Extrapolation factors, RRs	Parameter uncertainty	Statistical
DALY module	Disease model	Collected evidence for health outcome tree	Model structure uncertainty	Scenarios
	Age of onset of disease	Health registries	Input data uncertainty	Statistical
	Severity weigths	Collected scientific evidence	Paramenter uncertainty	Statistical
	Disease duration	Health registries, collected scientific evidence	Input data uncertainty	Statistical/ Scenarios
	Mortality	Health registries	Input data uncertainty	Statistical
	Life tables	Collected scientific evidence	Paramenter uncertainty	Scenarios
	Disease envelopes	Collected scientific evidence	Paramenter uncertainty	Scenarios

The first three columns in table 6.1, describe the modules in terms of which components of the analysis are in each module (column 2) and the data that might be used in each component (column 3). The next two columns describe the uncertainty associated with each component and the data. Four sources of uncertainty are distinguished; contextual uncertainty, model structure uncertainty, parameter uncertainty and data-input uncertainty (column 4). Each source of uncertainty can be expressed either statistically (e.g. by a 95% uncertainty interval) or by scenarios (e.g. worst or best case scenarios), depending on the component of the analysis and data source (column 5). In addition, a column in the table could be inserted to descripe how each source of uncertainty drives the overall uncertainty expressed either quantitatively or semi-quantitatively [35].

The source of uncertainty refers to where in the assessment the uncertainty originates. Contextual uncertainty originates from how the bounds of the assessment are determined. In terms of the burden of a foodborne chemical hazard, contextual uncertainty is the uncertainty originating from the selection of the health outcomes accounted for. The uncertainty depends on the strength of the evidence of the causal effect, but cannot be evaluated in a statistical manner. Rather, the contextual uncertainty can be evaluated by scenarios, i.e. how does the disease burden of a hazard change depending on which health outcomes are accounted for. The contextual uncertainty was evaluated in Paper 1 by estimating the disease burden for total cancer in one scenario and 4 specific cancers in another. The contextual uncertainty in terms of the overall burden of foodborne disease, stems from the definitions used in the assessment (not shown in table), e.g. if water is considered a food, or if dietary risk factors are within the bounds of foodborne disease. Thus, the contextual uncertainty has a great impact on the comparability between studies on the same hazards or overall categories of disease burden.

Model structure uncertainty is introduced in several of the components of the analysis. It concerns how well the chosen models describe the real causal structures which are modelled. When the disease burden of foodborne chemicals is calculated via the risk assessment approach, model uncertainty is introduced in each of the three modules. In the exposure module, uncertainty is connected to how well a model applied describe the population exposure, e.g. the models describing the lifetime exposure to acrylamide in Paper 1 and Manuscript 3. In the health outcome module, it is uncertain how well the chosen dose response model describe the dose response relationship in the population of

interest. For example, it is uncertain whether a linear extrapolation or the two stage model best describe the dose response relationship between the probability of developing cancer and the exposure to AA and BaP, respectively. Likewise it is uncertain whether it is correct to assume an additive, as done for AA and BaP in this thesis, or a multiplicative risk model. In the DALY module, the overall disease model, or health outcome tree, might be uncertain. It is for example uncertain how well the disease outcome trees applied for cancer in Paper 1 and Manuscript 3, describe the disease stages of cancer cases in the population of interest. The model structure uncertainty may be assessed by scenario-analysis.

Parameter uncertainty describes the uncertainty in the relation between variables, and is introduced in the health outcome module and the DALY module. This uncertainty can usually be described statistically, as done in Manuscript 3 for the two stage model parameters, EFs and disability weights.

Input data uncertainty relates to the quality of the data used, reflecting lack of data and inaccuracy of the measurement methods. Depending on the data available, the input data uncertainty can be evaluated both statistically and by scenarios. Data are used to derive all indicators of a burden estimation, and the quality of the data is an important driver of the quality of the final DALY estimates. The estimation of the uncertainty in the input data and their relative contribution to the overall uncertainty is thus important to inform on which data source is most important to improve.

In Paper 1, we assessed model structure uncertainty by evaluating the sensitivity of different scenarios on the final DALY estimates; in Manuscript 3, we statistically propagated parameter and input data uncertainty. In Manuscript 2, we investigated how the contextual uncertainty is depending on the strength of evidence of the causal effect of a chemical.

To tabulate all components of an analysis, for example as suggested in table 6.1, provides an overview of the complex information going into the DALY metric. Of course, if all time and resources are available, a full account of the uncertainty should be presented, i.e. the final DALY estimate should be presented with uncertainty intervals representing all sources of uncertainty that can be statistically derived. It is however rarely the case that this is possible, and also in that case, an overview an full account of the uncertainty propagated along the analysis is an advantage. Likewise, if the uncertainty in a component

of the analysis can not be described statistically, an account of which components are evaluated by (extreme) scenarios should be given. Lastly, the impact of the uncertainty of each component of the analysis on the final estimate should be described. Information on the components contributing most to the uncertainty is valuable, both to direct research initiatives to improve the estimates, but also for the recipients of the disease burden study to be informed on the greatest limitations of the study.

6.1.2 On the usefulness of burden of disease of foodborne chemicals

The difference between the purpose of a study of the disease burden of a foodborne chemical hazard and toxicological risk assessment of the same chemical was highlighted in chapter 2. The added value of the information obtained from a disease burden study can be illustrated by comparing the answers to the questions that can be posed in either context. A toxicological risk assessment attempts to answer the question at which exposure it is expected that no disease in the population will occur, and further evaluate if the population exceed that exposure or not. The information obtained in a disease burden context can answer a wide variety of questions, accounted for below.

The obvious question is which foodborne chemicals generate the largest impact on public health. The internal ranking of chemical hazards are usually done by comparing hazard ratios, i.e. the ratio between the estimated exposure to a chemical and the highest dose associated with no effect (the HBGV). In a report performed for the Danish environmental protection agency, such hazard ratios were calculated for a selection of chemicals whose effects are found to be hormone disrupting and/or neurotoxic [102]. For several of the chemicals, the hazard ratios were found to be above 1, even by several orders of magnitude. However, in a public health perspective, this information is of limited value, as the severity of each chemical is not taken into account. The results from the report indicated that the exposure to neurotoxic substances are most critical (i.e. the combined hazard ratio is largest for neurotoxic substances). However, the conclusion could be different, if the impact of exposure to neurotoxic versus hormone disrupting chemicals on health, in terms of DALYs, was estimated. Also, several of the chemicals assessed exert both health effects, and a burden estimation would allow for a quantitative evaluation of

the relative impact of the different chemicals across health effects.

Information of studies on the burden of foodborne chemicals can also prove its value in planning and evaluation of implementation of interventions. In a risk assessment approach and a counterfactual analysis, it is inherent, that the disease burden estimated is also what would be prevented if exposure was removed or reduced to a minimum. In both Paper 1 and Manuscript 3 of this thesis, the estimated disease burden is equal to the health gain if exposure was removed. Several strategies to reduce the formation of acrylamide during processing have been investigated, and some also implemented in the industry to reduce the AA content in commercial food products [103]. The health impact of each of these strategies can be evaluated by disease burden estimation. Acknowledging that implementation of these intervention strategies often comes with a cost, both to the manufacturer who needs to allocate resources for the implementation and to the food-authorities who needs to allocate resources to control if the chemical concentration is below regulatory limits, disease burden studies can be used in a cost-effectiveness analysis e.g. by the analysis of the cost of the intervention per unit reduction in DALY. This can of course be applied to all types of chemicals and interventions to assess the cheapest way to achieve a given reduction in DALY [104].

The incidence-based approach assesses the future burden of disease experienced by the incident disease cases in the year of study. However, the estimation of disease burden of foodborne chemicals can also be used to predict future disease burden by assessing different exposure scenarios driven by factors external to food-safety, e.g. the effect of climate change on exposure to foodborne chemicals [105].

All of this highlights why national studies of the burden of disease of foodborne chemicals are advocated, and why further research should be put into improving the estimates by improving the data and models needed. However, as illustrated by this thesis, it is not always straight forward to estimate the disease burden of foodborne chemicals. Additionally, the DALY as a metric should be objective, but subjective value choices regarding the disability weights, and, if applied, age-weighting and time-discounting, restrict the objectiveness of the DALY [101]. Besides, the DALY reflects the disability experienced by the diseased individuals, but not the stress of e.g. the relatives of a cancer patient or a child with severe peanut allergy [106]. This may underestimate the disease burden of the more severe diseases, for example associated with foodborne chemical exposures, com-

pared to the less severe, self-limiting diseases, for example associated with some foodborne pathogens.

Two major disadvantages of the DALY in connection with foodborne chemicals have been identified. One disadvantage is that for many health effects associated with foodborne chemicals, disability weights do not exist. In some cases, disability weights may be extrapolated from diseases considered of similar severity, however, in other cases the chemically induced effect do not manifest itself as a clinical case in humans, why no disability weight can be applied [77]. Another disadvantage is that information on the dose dependence on the age of onset of disease often cannot be derived from studies in experimental animals [107]. In both Paper 1 and manuscript 3, we used the weighted averages of the age of onset of disease from health statistics, ignoring that the highly exposed individuals might get the disease at an earlier age. Referring to table 6.1, the impact of age of onset of disease can be evaluated in a scenario-based manner. To which extent it is the case for foodborne chemicals that neither the severity of effect or the onset of disease can be translated into the human situations is unknown. Owens et al. (2002) evaluated 117 high production organic chemicals through the integrated risk information system (IRIS) database and found no means of estimating the severity of effects or the age of onset of disease in humans for any of the chemicals [108]. This also stress the need to assess the knowledge base chemical by chemical, for estimating the disease burden to foodborne chemcials on a national level.

6.2 Concluding remarks

The work presented in this thesis shows that the challenges of estimating the disease burden of foodborne chemicals can to a certain degree be overcome. The proposed model framework, as well as the probabilistic approach developed for the last case study can be used for other chemical hazards and other populations/countries, thereby allowing for increased evidence on the burden of foodborne chemicals in the near future.

We concluded that estimating the health impact of chemicals in foods is the component of the analyses with highest uncertainty due to the difficulty in linking exposure and development of disease. The uncertainty that the health outcome module inflicts to the overall DALY estimate is a reflection of the impact that the strength of evidence has

on both the selection of health outcomes that are accounted for a given chemical, and the ability to estimate the theoretical incidence of disease. We also concluded that, as an overall approach, the integrated probabilistic risk assessment should be applied to estimate disease burden, as it allows for propagating the uncertainty in the estimation of the incidence in the health outcome module.

Disease burden estimates are essential to inform policy-makers and allocate food-safety resources. However, when the disease burden estimate is presented to policy makers, all inherent uncertainties should be stated transparently; in the end, an estimate of the disease burden with a large concealed uncertainty can wrongly direct political initiatives.

We showed that in an industrialized country like Denmark, where foodborne chemicals are relatively well regulated, the population disease burden due to chemicals may be low. However, we also demonstrated that individuals in the population may experience exposures considered adverse to health depending on food consumption patterns (in this case amount meat eaten per kilogram bodyweight). In this context, it may be relevant to identify subpopulations characterized by factors such as diet, lifestyle and susceptibility to disease that are at higher risk and bear a higher disease burden. Information on the contribution of the disease burden on these subpopulations to the population-level health impact is valuable to direct food-safety initiatives.

6.3 Perspectives

Further research should be directed to the development of methods for estimation of the disease burden of foodborne chemicals. Several components of the models, including the data, can be improved. To address the remaining challenges and large uncertainties in the health outcome model, the assessment of the strength of the evidence should be done chemical by chemical. However, several chemicals lead to the same health effects, and developing a common approach to groups of chemicals will be of great value.

In this PhD project the integrated probabilistic risk assessment was only applied to BaP, a genotoxic carcinogen. The IPRA approach should be applied to more chemicals to prove that it is the best method to quantitatively derive disease incidence via the risk assessment approach for both threshold and non-threshold effects, and that it allows for propagation of the uncertainty in the estimation of the incidence in the health outcome

module.

In this PhD project we identified that the strength of evidence is a strong determinant of which health outcomes to account for in burden estimation. Meeting the World Cancer Research Funds grades of convincing evidence is a questionable approach for foodborne chemicals, and there is a need to define a weight of evidence approach for the study of disease burden of foodborne chemicals. This approach should be designed to take into account that even if evidence is weak, the impact of the chemical might still be considerable (but uncertain). In turn, as a form of uncertainty analysis, this identifies the most important knowledge gaps, and where focus should be in collecting more evidence.

Last but not least, future research should be aimed at developing models to estimate the disease burden of subpopulations characterized in terms of diet, lifestyle, susceptibility to disease etc. Individuals overall dietary patterns determine their level of exposure to a chemical, and it would be relevant to define clusters in the population which are hotspots for exposure and estimate the associated disease burden. This may be done not only for single chemicals, but for several chemicals which exert the same adverse health effects. Machine learning techniques have been developed to identify associations within large clinical data sets, but have to our knowledge not been applied to identify clusters of chemical exposures from food. In Denmark, we can apply the methodology to and across large databases that include information on diet, socio-economic status and even genes, which is a unique opportunity.

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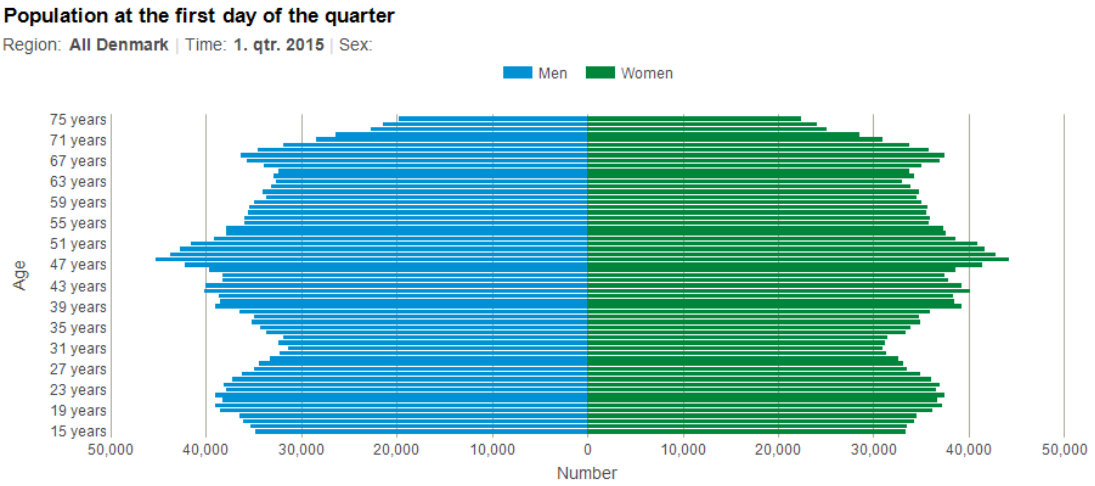
Appendix

Supporting material to manuscript 3

Content

1. Figures



S1 Figure. Demographics of the Danish population. Age and sex distribution of the Danish population in the first quarter of 2015.

2. Tables

S1 Table. Parameters for the gamma distributions of meat consumption.

Weight class	Men	Women
Low	(2.551, 0.016)	(2.483, 0.022)
Medium	(2.549, 0.015)	(2.649, 0.022)
High	(2.513, 0.014)	(2.829, 0.022)

Gamma distributions, Gamma(α, β), with shape parameter α, and rate parameter β, i.e. (α, β) from which the consumption in g/meal of meat is derived.

S2 Table. Probability of type of meat consumed, if one meat per meal is consumed.

Meat type	beef	burger	pork	sausages	lamb	poultry	fish	shellfish
Frequency	0.1285	0.1186	0.1839	0.3448	0.023	0.1609	0.0266	0.0137

S3 Table. Probability of type of meat and fraction of total meat consumed (in parenthesis), if two meats are consumed.

	beef	burger	pork	sausages	lamb	poultry	fish	shellfish
beef		0.0348 (0.5192,0.4808)	0.0561 (0.5906,0.4094)	0.1185 (0.5387,0.4613)	0.0064 (0.6154,0.3846)	0.0484 (0.5495,0.4505)	0.0074 (0.6067,0.3933)	0.0038 (0.6695,0.3305)
burger			0.0515 (0.7282,0.2718)	0.1088 (0.4752,0.5248)	0.0059 (0.5217,0.4783)	0.0444 (0.5750,0.4250)	0.0068 (0.5852,0.4148)	0.0035 (0.8042,0.1958)
pork				0.1745 (0.4989,0.5011)	0.0095 (0.5559,0.4441)	0.0715 (0.4009,0.5991)	0.0110 (0.4565,0.5435)	0.0056 (0.6708,0.3292)
sausages					0.0202 (0.6840,0.3160)	0.1508 (0.5044,0.4956)	0.0234 (0.5116,0.4884)	0.0120 (0.6364,0.3636)
lamb						0.0082 (0.5176,0.4824)	0.0013 (0.5242,0.4758)	0.0006 (0.6772,0.3228)
poultry							0.0095 (0.4476,0.5524)	0.0049 (0.5844,0.4156)
fish								0.0007 (0.6647,0.3353)
shellfish								

S4 Table. Parameters for the log-normal distributions of censored concentration of BaP in barbequed meat.

Meat	samples	<LODs	>LODs	μ	σ
Beef	87	44	43	-2.326	1.626
Burger	78	28	50	-0.783	3.125
Pork	70	39	31	-2.932	2.419
Sausages	58	8	50	-1.141	1.991
Lamb	15	2	13	-0.712	1.484
Poultry	53	23	30	-2.134	1.368
Fish	42	13	29	-1.518	1.599
Shellfish	4	0	4	-1.783	0.575

Censored log-normal distributions with mean, μ, and standard deviation, σ, from which the concentration of BaP in μg/kg in each meat type after barbecuing is derived.

S5 Table. Dose response data.

Coal tar mixture	BaP dose (mg/kg bw/day)	Number of tumor bearing animals	Sample size
1	0	5	48
1	0.027	12	48
1	0.079	14	48
1	0.266	12	48
1	0.789	40	48
2	0.121	17	48
2	0.44	23	48
2	1.12	44	48

Dose response data of BaP in mg/kg bodyweight/day in coal tar mixtures in mice from Culp et al. (1998) [1] as reported by Schneider et al. (2002) [2] and by EFSA (2008) [3]

3. Population model

3.1. Exposure module

3.1.1. Simulation algorithm

Algorithm 2 Population exposure simulation

for each individual $i = 1, \ldots, n$ **do**
  generate a discrete r.v. s_i for the sex of the individual i
  generate a discrete r.v. a_i for the age of the individual i
  estimate the weight w_i of the individual i given s_i and a_i
  assign individual i to a weight class $class_i$ for its sex s_i
  simulate a number of bbq events B_i among the three possible scenarios
  for each event $b \in \{1, \ldots, B_i\}$ **do**
    generate a r.v. t_i^b for the total meat consumption, given the category (s_i, w_i) of the individual
    generate a random vector $\mathbf{m}_i^b$ for the meat types consumed at the event b, independently of the category (s_i, w_i)
    generate a random vector $\mathbf{v}_i^b$ for the fractions of meat types $\mathbf{m}_i^b$
    calculate the vector $\mathbf{x}_i^b$ of meat types $\mathbf{m}_i^b$, i.e. $\mathbf{x}_i^b = t_i^b \mathbf{v}_i^b$
    generate a log-normal random vector $\mathbf{c}_i^b$ for the BaP concentration in the foodcodes $\mathbf{m}_i^b$
  end for
  simulate the BaP intake y_i given the weight w_i
end for

3.2. Health outcome module

3.2.1. Dose response modeling:

- Data from a study on tumor formation in rats based from exposure to two coal tar mixes (Culp et al. 1998).
- Concentrations of BaP in the two coal tar mixes were estimated by Schneider et al. (2002) and exposure to animals expressed as mg BaP/kg bw/day (S7 Table).
- Concentrations are in the dose response modelling expressed as µg BaP/kg bw/year.

Dose response modelling on the data performed in PROAST:

```
bap ,  ER :

model        No.par      log-likelihood          accepted    BMD         BMDL
BMDU

null         1           -262.9      --          NA          NA          NA

full         8           -198.82     --          NA          NA          NA

two.stage    3           -204.03     yes         57300       28100       92700
```

```
log.logist   3          -203.89     yes         106000      71100       142000
Weibull      3          -204.3      yes         75500       35700       118000
log.prob     3          -204.02     yes         110000      77500       143000
gamma        3          -204.27     yes         97000       45300       138000
logistic     2          -203.92     yes         48500       42100       55900
LVM_Exp      2          -203.96     yes         47100       41400       53700
LVM_Hill     3          -204.12     yes         89400       50300       129000
no covariate
 BMR: 0.1 extra risk
 constraint: no
P-value GoF: 0.05
```

3.2.2. Population model in mc2d:

exp_{human} $\sim simulated\ exposure\ distribution$

$EF_{intra,GSDunc}$ $\sim \chi^2_{21}$

EF_{intra} $\sim logN\left(\log(1), \log(3.6) \cdot \sqrt{21/EF_{intra,GSDunc}}\right)$

bw_{human} $= 70\ kg$

bw_{animal} $= 0.03\ kg$

AF $\sim N(0.7, 0.033)$

$EF_{inter,allometric}$ $= \left(\frac{bw_{human}}{bw_{animal}}\right)^{1-AF}$

$EF_{inter.TKTD}$ $\sim logN(1,2)$

exp_{animal} $= exp_{human} \cdot EF_{intra} \cdot EF_{inter,allometric} \cdot EF_{inter,TKTD}$

b $\sim distribution\ of\ bootstrapped\ values\ in\ Proast$

c $\sim distribution\ of\ bootstrapped\ values\ in\ Proast$

ER_{BaP} $= 1 - \left(e^{-\left(\frac{exp_{animal}}{b}\right) - c\left(\frac{exp_{animal}}{b}\right)^2}\right)$

3.2.2.1. mc2d model output:

```
exp_human :
       mean   sd Min 2.5%    25%    50%   75% 97.5% Max   nsv Na's
NoUnc 0.184 3.03   0    0 0.0245 0.0643 0.149 0.464 136 10000    0

EF_intra :
       mean    sd      Min   2.5%   25%   50%  75% 97.5%    Max   nsv Na's
median 2.34  4.86 0.006724 0.0776 0.415 1.000 2.41 12.94  149.6 10000    0
```

```
mean   2.59  6.88 0.008658 0.0809 0.413 1.000 2.47 14.76  291.0 10000    0
2.5%   1.58  1.93 0.000772 0.0303 0.298 0.968 1.91  6.49   34.6 10000    0
97.5%  4.98 23.62 0.026277 0.1511 0.527 1.033 3.34 33.05 1527.3 10000    0

EF_intra_unc :
       NoVar
median  20.1
mean    21.2
2.5%    10.9
97.5%   37.7

AF :
       NoVar
median 0.700
mean   0.699
2.5%   0.633
97.5%  0.768

EF_inter_allometric :
       NoVar
median 10.24
mean   10.65
2.5%    6.04
97.5%  17.19

EF_inter_TKTD :
       NoVar
median 0.939
mean   1.188
2.5%   0.260
97.5%  3.397

b :
       NoVar
median 57643
mean   57576
2.5%   25112
97.5%  90652

c :
          NoVar
median 9.01e+00
mean   1.95e+11
2.5%   1.31e-01
97.5%  1.00e+12

exp_animal :
         mean    sd Min 2.5%    25%   50%  75% 97.5%   Max   nsv Na's
median  4.283  81.8   0    0 0.1456 0.560 1.94 18.24  6326 10000    0
mean    5.748 145.5   0    0 0.1918 0.736 2.56 24.32 12719 10000    0
2.5%    0.925  11.8   0    0 0.0361 0.147 0.51  4.33   794 10000    0
97.5%  19.958 648.9   0    0 0.6037 2.291 7.87 80.93 62000 10000    0

ER_BaP
           mean       sd Min 2.5%      25%      50%      75%    97.5%    Max   nsv Na's
median 6.91e-05 0.001163   0    0 2.84e-06 1.08e-05 3.77e-05 3.30e-04 0.0937 10000    0
mean   1.06e-04 0.002153   0    0 4.02e-06 1.55e-05 5.41e-05 5.20e-04 0.1853 10000    0
2.5%   1.39e-05 0.000149   0    0 5.86e-07 2.39e-06 8.38e-06 6.45e-05 0.0107 10000    0
97.5%  3.93e-04 0.009953   0    0 1.39e-05 5.61e-05 1.92e-04 1.98e-03 0.9142 10000    0
```

3.3. DALY module

3.3.1. Output from the DALY calculator

```
> x <- getDALY()
> print(x, digits = 10)

DALY Calculator:  Cancer

             Mean     Median       2.5%      97.5%
DALY   4.2843e-06 4.0850e-06 1.6831e-06 7.9842e-06
YLD    2.4158e-06 2.1473e-06 7.1530e-07 5.5685e-06
YLL    1.8685e-06 1.7006e-06 3.9800e-07 4.2543e-06
cases  2.7712e-06 2.6816e-06 1.1676e-06 4.8616e-06
deaths 1.5400e-07 1.4010e-07 3.2800e-08 3.5050e-07

YLD/DALY = 56%
YLL/DALY = 44%


#Total DALY per 1000
> x <- getDALY()
> print(x, relative = TRUE, digits = 20)

DALY Calculator:  Cancer

Total population:  5461964

               Mean       Median         2.5%        97.5%
DALY   7.883316e-10 7.560989e-10 3.081722e-10 1.460836e-09
YLD    4.445591e-10 3.970259e-10 1.309945e-10 1.010905e-09
YLL    3.437725e-10 3.124775e-10 7.112159e-11 7.801863e-10
cases  5.077396e-10 4.906157e-10 2.126596e-10 9.009855e-10
deaths 2.832434e-11 2.574586e-11 5.859898e-12 6.428164e-11


#DALY for each outcome
> x <- getDALY()
> print(x, outcomes = TRUE, digits = 10)

DALY Calculator:  Cancer

Diagnosis and primary care
             Mean     Median      2.5%      97.5%
DALY   3.4640e-07 3.1340e-07 7.230e-08 7.9510e-07
YLD    3.4640e-07 3.1340e-07 7.230e-08 7.9510e-07
YLL    0.0000e+00 0.0000e+00 0.000e+00 0.0000e+00
cases  1.2336e-06 1.1228e-06 2.584e-07 2.7826e-06
deaths 0.0000e+00 0.0000e+00 0.000e+00 0.0000e+00

In remission
             Mean     Median      2.5%      97.5%
DALY   1.9929e-06 1.7164e-06 3.767e-07 5.1154e-06
YLD    1.9929e-06 1.7164e-06 3.767e-07 5.1154e-06
YLL    0.0000e+00 0.0000e+00 0.000e+00 0.0000e+00
cases  1.2302e-06 1.1128e-06 2.583e-07 2.7937e-06
deaths 0.0000e+00 0.0000e+00 0.000e+00 0.0000e+00
```

```
Disseminated carcinoma
            Mean    Median     2.5%     97.5%
DALY   6.980e-08 6.240e-08 1.43e-08 1.621e-07
YLD    6.980e-08 6.240e-08 1.43e-08 1.621e-07
YLL    0.000e+00 0.000e+00 0.00e+00 0.000e+00
cases  1.544e-07 1.391e-07 3.19e-08 3.514e-07
deaths 0.000e+00 0.000e+00 0.00e+00 0.000e+00

Terminal phase
              Mean     Median      2.5%      97.5%
DALY   1.8808e-06 1.7126e-06 3.978e-07 4.2686e-06
YLD    6.9000e-09 6.2000e-09 1.400e-09 1.5900e-08
YLL    1.8740e-06 1.7062e-06 3.910e-07 4.2633e-06
cases  1.5320e-07 1.3930e-07 3.210e-08 3.4700e-07
deaths 1.5440e-07 1.4060e-07 3.220e-08 3.5130e-07
```

4. Subgroup model

4.1. Exposure module

4.1.1. Simulation algorithm

Algorithm 3 Simulation of exposure vs. the yearly number of events

for each year $i = 1, \ldots, 60$ **do**
  for each event $b \in \{1, \ldots, B_i\}$ **do**
    generate a r.v. t_i^b for the total meat consumption the "typical" individual
    generate a random vector $\mathbf{m}_i^b$ for the meat types consumed at the event b
    generate a random vector $\mathbf{v}_i^b$ for the fractions of meat types $\mathbf{m}_i^b$
    calculate the vector $\mathbf{x}_i^b$ of meat types $\mathbf{m}_i^b$, i.e. $\mathbf{x}_i^b = t_i^b \mathbf{v}_i^b$
    generate a log-normal random vector $\mathbf{c}_i^b$ for the BaP concentration in the foodcodes $\mathbf{m}_i^b$
    calculate the BaP intake y_i^b
  end for
  calculate the yearly BaP intake y_i (devided by the bodymass of the "typical" man or woman)
end for
calculate the lifetime BaP intake y

4.2. Health outcome module

4.2.1. Subgroup model in mc2d:

b = 848500

c = 8.299

$$ER = 10^{-6}$$

$$exp_{animal} = \sqrt{\left(\frac{b^2}{4c^2}\right) - \left(\frac{b^2}{c}\right) * \log(1 - ER)} - \frac{b}{2c}$$

$$EF_{intra} \sim logN(\log(1), \log(3.6))$$

$$bw_{human} = 70\ kg$$

$$bw_{animal} = 0.03\ kg$$

$$AF = 0.7$$

$$EF_{inter,allometric} = \left(\frac{bw_{human}}{bw_{animal}}\right)^{1-AF}$$

$$EF_{inter.TKTD} = 1.27$$

$$exp_{human} = \frac{exp_{animal}}{\left(EF_{intra} \cdot EF_{inter,allometric} \cdot EF_{inter,TKTD}\right)}$$

4.2.2. mc2d model output

EF_intra :

	mean	sd	Min	2.5%	25%	50%	75%	97.5%	Max	nsv	Na's
NoUnc	2.22	4.45	0.00869	0.0809	0.43	0.996	2.37	11.6	154	10000	0

exp_human :

	mean	sd	Min	2.5%	25%	50%	75%	97.5%	Max	nsv	Na's
NoUnc	0.147	0.29	0.000422	0.00564	0.0276	0.0655	0.152	0.807	7.5	10000	0

5. References

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