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Risk assessment of grilled and barbecued food

Espen Mariussen, Jan Alexander, Barbara Alexandra Bukhvalova Lisbeth Dahl, Ann-Karin Hardie Olsen, Helen Engelstad Kvalem, Martin Schlabach, Heidi Amlund, Rita Hannisdal, Anders Ruus, Ingunn Anita Samdal, Helle K Knutsen

Scientific Opinion of the Panel on Contaminants of the Norwegian Scientific Committee for Food and Environment

VKM has assessed the health risk from grilled food consumption and summarized the knowledge on formation of several carcinogenic process contaminants in grilled food. Based on exposure scenarios for polycyclic aromatic hydrocarbons (PAH) in grilled food VKM concludes that the risk is low for most consumers. VKM noted a public health concern for those who often consume fat rich meat that is grilled in a way leading to high PAH formation, i.e., when fat burns on the heat source, food is grilled very well-done on charcoal or particularly campfire. Such conditions may also increase formation of other heat-induced contaminants.

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Preparation of the opinion

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed a project group to draft the opinion. The project group consisted of five VKM members, 1 VKM staff. Three referees commented on and reviewed the draft opinion. The Committee, by the Panel on Contaminants assessed, and approved the final opinion.

Authors of the opinion

The authors have contributed to the opinion in a way that fulfils the authorship principles of VKM (VKM, 2019). The principles reflect the collaborative nature of the work, and the authors have contributed as members of the project group and/or the VKM Panel on Contaminants.

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VKM would like to thank the referees Wim Mennes (retired, RIVM, Netherlands), David Gott (Food Standards Agency, UK) and Peter Fürst (retired, Chemical and Veterinary Analytical Institute, Germany) for their valuable comments through critical review of the draft opinion. VKM emphasises that the referees are not responsible for the content of the final opinion. In accordance with VKM's routines for approval of a risk assessment (VKM, 2018), VKM received their comments before evaluation and approval by VKM Panel on Contaminants and before the opinion was finalised for publication.

Trine Husøy is acknowledged for advice on probabilistic exposure assessment. Sagnik Sengupta (VKM secretariat) is acknowledged for his contributions with compiling the occurrence data on PAH and the data analysis in R. Gro Haarklou Mathisen (VKM secretariat) is acknowledged for her contribution with initiating work on the protocol and kind advice along the process with this report. The Library at the Norwegian Institute of Public Health is acknowledged for the valuable help an insight in the systematic literature search. Inger Therese Lillegaard (VKM secretariat) is acknowledged for her reflections and advice on food consumption and scenarios.

We also express gratitude to all of our European colleagues that responded to our call for data through the EFSA focal point network on occurrence data of process contaminants; Finland, Italia, Poland, Slovenia, Spain, Sweden, Belgium and Hungary.

Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third-party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

Background

It is well-known that heat treatment of food, such as grilling and frying may give rise to unwanted, harmful substances in food, so-called process contaminants. VKM summarized this in the previous risk assessment of grilled food from 2007. In 2022, the Norwegian Food Safety Authority asked VKM to update and summarize the knowledge on formation of processed contaminants in various food by different grilling methods, and to assess the risk this may pose. More specifically the tasks were:

- Identify process contaminants which are formed to a greater extent by grilling than by frying and create an overview of reported amounts of these process contaminants in various types of grilled food.

- Elucidate factors (for example grill type, grill method and food) that are important for the formation of the identified process contaminants in grilled food.

- If possible, based on available information, assess the health risks associated with the consumption of grilled food compared to fried food.

Methods

VKM prepared a protocol, published in February 2023, describing the scope and methods for this risk assessment. New knowledge since 2006 about the relationship between the consumption of grilled food and health outcomes in humans was collected through an umbrella review of systematic summaries on this topic. Information on which contaminants are formed in grilled food, the amount formed, and factors important for their formation were collected using non-systematic literature searches. Characterization of health hazards linked to the contaminants was obtained from assessments carried out by international risk assessment bodies. VKM considered that the identified data were considered sufficient for a quantitative risk assessment of exposure to polycyclic aromatic hydrocarbons (PAH), but not for other contaminants. The PAH exposure was estimated by a probabilistic approach, with two scenarios for the consumption of grilled food. This involved modelling e the content of PAHs in different grilled food items and exposure to PAHs. The total risk from dietary PAH exposure from was described by the margin of exposure (MOE) approach.

Results

Identification of process contaminants

VKM identified several process contaminants formed during grilling. These were PAHs, PAH analogues such as chlorinated-PAHs and nitrated PAHs, polyphenols, heterocyclic aromatic amines (HAAs), 3- monochloropropane-1,2-diol (3-MCPD) and glycidyl esters, acrylamide,

nitrosamines, nitrite, harmful Maillard reaction products, biogenic amines, 7-ketocholesterol, acridine derivatives, anthraquinone (ATQ), pyrazines, advanced glycation end products (AGE). For two groups of compounds, PAHs and HAAs, there was substantial or plausible evidence for higher concentration in grilled meat and fish than in fried food. There was few data available on process contaminants in other grilled food than meat and fish, such as e.g. vegetables and bread.

Concentration data for PAHs in grilled food (mostly meat and meat products) was collected from a total of 81 studies. The highest concentrations of PAHs (BaP and PAH4) were found in campfire grilled sausages. Concentration data for BaP, regardless of grilling method, indicated variability ranging from levels below quantification limits to more than 100 ng/g in fatty pork meat and sausages. Most of the grilled food items had median BaP concentrations below approximately 1 ng/g but varied from 0.1 to 4.1 ng/g across all food items. The occurrence of the different HAAs in grilled food varied greatly, from below detection limits to about 50 ng/g. The concentrations could, however, be up to 240 ng/g for PhIP in very well-done grilled chicken, and with higher levels in meat than in fish.

Factors (for example grill type, grill method and food) that are important for the formation of the identified process contaminants in grilled food.

Main types of heat sources for grilling are electricity, gas, charcoal (lump charcoal, briquettes) and firewood. The type of heat source may influence the content of PAHs and HAAs differently. Other factors that may influence the formation of PAHs and HAAs include type of fuel, direct or indirect grilling, type of meat, distance from the heat source, grilling temperature, and grilling time.

Contamination of food with PAHs may primarily occur through: (1) deposition on the food of fuel-related PAHs in smoke. Electric heating and gas emit no or little PAHs, while charcoal and in particular firewood may contribute significantly to PAH contamination. When using charcoal, the PAH emission is higher in the initial period after lighting the grill. (2) deposition of smoke from incomplete combustion (pyrolysis) of dripping fat onto the heating source or hot surfaces. Dripping fat on the heat source is a significant source of PAHs in grilled food and is related to the fat content of the food. Avoiding fat dripping directly on the heat source and diverting the smoke from the food may reduce PAH deposition on the food. Studies on the impact of marination are conflicting, as marination may both reduce and increase the content of PAHs. (3) over-heating the food resulting in burned surface during cooking. Temperature, proximity to the heat source, frequent flipping of the food, and cooking time are important for avoiding burning the food during grilling.

HAAs are mainly formed in the crust or gravy by heating. Variables that affect the formation are (1) temperature and cooking time. Concentrations of HAAs increase with rising temperature, while regular turning of the meat during cooking reduce the surface temperature and mitigate HAA formation. (2) presence of precursors, such as creatine, reducing sugars and free amino acids in the food. More HAA is formed in lean meat than in fatty meat, fish or mixed products. (3) method of cooking, such as marination, may affect the formation of HAAs. Microwaving beef and chicken prior to charcoal grilling can reduce the content of HAAs.

Health risk associated with consumption of grilled food

The umbrella review of systematic reviews of epidemiological studies published since 2006 did not reveal additional knowledge on the association between consumption of grilled food and health outcomes in humans. Hence, the conclusion drawn in the previous VKM 2007 assessment remains pertinent, suggesting a possible association between intake of well-done fried or grilled meat and cancer in the colon, rectum, prostate, breast, and pancreas. It was beyond the scope of the present assessment to review original studies on the health effects of consumption of grilled food compared to other cooking methods. VKM therefore assessed the risk from exposure to process contaminants formed by grilling.

VKM estimated PAH exposure in grilled food quantitatively based on scenarios of consumption of grilled food also including a background exposure of PAH from the rest of the diet. Due to lack of data, the risk from HAA exposure and other heat-induced contaminants could not be characterised.

The PAH concentration distributions in various grilled food items based on the extracted information from the 81 identified publications were generated from all types of grilling methods, of which more than 60% of the data were from charcoal grilled food. Due to limitations in the database the data did not allow for differentiation among grilling methods. The cumulative concentration distribution in most of the foods showed a sharp increase in the PAH concentrations above the 75 percentiles. The simulated concentration at the 90- and 95-percentile may represent grilling methods known to increase formation of PAHs in the food. The highest concentrations of BaP were found in sausages grilled on campfire, and in hamburgers and fatty pork.

The two grilled food consumption scenarios include only meat and salmon because of lack of occurrence data in other grilled food items, such as vegetables, bread and meat imitate. The scenarios illustrate possible consequences of different food preferences on the intake of PAHs from grilled foods. The scenario plate of 200 g grilled fat-rich meat contained fat rich pork meat, hamburger, sausages, and chicken with skin. The other plate with 200 g lean meat and fish contained beef, lean pork meat, chicken without skin and salmon. Based on concentration distribution of BaP and PAH4 in each food item on the plate the distribution of the contents of BaP and PAH4 on each plate was simulated.

The exposure scenarios based on the two plates included background PAH exposure from the rest of the diet and 1 to 100 servings of each plate per year. Average daily intakes of BaP or PAH4 for one year were calculated.

Risk was characterized by the MOE approach using $BMDL_{10}$s for BaP and PAH4 from the EFSA risk assessment of PAHs in food from 2008 as reference points. VKM considers that exposure to PAH resulting in MOE below 10,000 is of public health concern. For the plate with lean meat and salmon the use of mean, median and 75 percentile exposure to BaP and PAH4 up to 100 servings per year, resulted in MOEs above 10,000. At the 90 percentile and 100 servings the MOEs were approximately 10,000 for BaP and slightly below for PAH4. For the plate with fat rich meat the MOEs for the scenario related exposures remained above

10,000 when consuming grilled fat rich meat with a mean BaP and PAH4 content up to 100 times a year. At concentrations equivalent to the 95 percentile of BaP and PAH4 the MOEs were below 10,000 when consuming grilled fat rich meat approximately 15 and 25 times a year, respectively. Using PAH4 as an indicator of total PAH exposure, instead of BaP, gave approximately the same result as that of BaP, with slightly higher MOEs for the fat rich meat plate and slightly lower for the lean meat and salmon plate. The impact of campfire grilling on exposure estimates and the associated MOEs was examined in a sensitivity analysis. Data on campfire grilled food was only available for food items on the fat rich meat plate. Excluding campfire grilled food from the fat rich plate substantially reduced impact on the mean, median and higher percentiles values for the contents of both PAH4 and BaP on the plate. The resulting MOEs were above 10,000 when consuming more than 100 servings per year of plates with a mean, median and 75 percentile content of BaP and PAH4. At a 95-percentile content of BaP and PAH4 the MOEs were above 10,000 for up to 30 servings per year.

Due to limitations the database did not allow creation of exposure scenarios reflecting various grilling methods. The MOEs calculated from the mean, median and 75 percentile exposure to PAHs likely represent the use of varied grill methods and food not causing substantial PAH formation. This will probably apply to most grilling situations. The MOEs calculated for the 90- and 95-percentile exposures to PAH may represent frequent use of grilling methods known to increase formation of PAHs in the food, such as high temperature grilling with charcoal or even campfire for a longer grill time leading to well done food.

Due to lack of data, the risk from HAA exposure and other heat-induced contaminants could not be characterised. The concentrations of HAAs seem to be higher in grilled meat than in fried meat, particularly when well done. Grilling of food is associated with higher and less controllable surface temperature than frying. As most of the HAAs are genotoxic and carcinogenic in rodents and some have been classified as possible human carcinogens, their formation during grilling may be of concern. Although not precisely known, it is plausible that several other heat-induced contaminants, such as of PAH-analogues, can occur in higher concentrations in grilled food than in fried food, as the mechanism of formation is presumed to be similar to that of the PAHs or due to a presumably higher and less controllable temperature in grilling.

Uncertainty

The uncertainties in this assessment are large. Exposure to PAH was estimated from simulated occurrence data and consumption scenarios. The health risk from exposure to PAHs in consumed grilled food characterised by the MOE approach may both be over- and underestimated. Due to lack of data, the health risk associated with exposure to heat-induced contaminants in grilled food other than PAH could not be assessed. Due to high and less controllable temperature during grilling, it is likely that HAAs and some other heat-induced contaminants may be present in higher concentrations in grilled food than in fried food. Therefore, the total risk associated with the presence of process contaminants in grilled food is likely to be higher than for PAH alone.

Data gaps

The main data gaps related to the health risk assessment of grilled food identified by VKM are the following: 1) systematic reviews on health outcomes related to consumption of grilled food in comparison with other cooking methods are lacking, 2) data on consumption of grilled food, food items, frequency of grilling and grilling method applied are missing, 3) data on occurrence of PAH and other heat-induced contaminants in food prepared by different grilling methods and by other comparable cooking methods are missing, 4) toxicological data on individual HAAs for better hazard characterization considering toxicokinetic differences between rodents and humans are missing, and 5) occurrence data on HAAs in grilled food analysed with controlled and validated analytical methods are missing.

Key words: VKM, polycyclic aromatic hydrocarbons, heterocyclic aromatic amines, grilling, barbecuing, health risk, margin of exposure, process contaminants, carcinogenic, genotoxic, pyrolysis, risk assessment. Norwegian Scientific Committee for Food and Environment, Norwegian Food Safety Authority

Sammendrag på norsk

Bakgrunn

Det er velkjent at varmebehandling av mat, som grilling og steking, kan gi opphav til uønskede, skadelige stoffer i maten, såkalte prosessfremkalte kontaminanter (forurensninger). VKM oppsummerte dette i forrige risikovurdering av grillmat fra 2007. Mattilsynet ba i 2022 VKM om å oppdatere og oppsummere kunnskapen om dannelse av prosessfremkalte kontaminanter i ulike matvarer under grilling med forskjellige metoder, og vurdere risikoen dette kunne utgjøre. Mer spesifikt var oppgaven å:

- identifisere prosessfremkalte kontaminanter som i større grad dannes ved grilling enn ved steking og lage en oversikt over rapporterte mengder av slike kontaminanter i ulike typer grillmat.
- belyse faktorer (for eksempel grilltype, grillmetode og mat) som er viktige for dannelsen av prosessfremkalte kontaminanter i grillmat.
- hvis mulig, og basert på tilgjengelig informasjon, vurdere helserisikoen knyttet til inntak av grillmat sammenlignet med stekt mat.

Metoder

VKM utarbeidet en protokoll som ble publisert i februar 2023 der mål og metoder for denne risikovurderingen er beskrevet. Ny kunnskap om sammenhengen mellom inntak av grillmat og helseutfall hos mennesker ble samlet inn ved en systematisk gjennomgang av systematiske oppsummeringer om dette tema siden 2006. Informasjon om hvilke kontaminanter som dannes i grillmat, mengden som dannes, og faktorer som har betydning for dannelsen av stoffene ble samlet inn ved hjelp av ikke-systematiske litteratursøk. Karakterisering av helsefare knyttet til kontaminantene ble hentet fra vurderinger utført av internasjonale risikovurderingsorganer. På bakgrunn av det identifiserte datagrunnlaget besluttet VKM å gjøre en kvantitativ risikovurdering av polysykliske aromatiske hydrokarboner (PAH), men ikke for de andre kontaminantene. PAH-eksponeringen ble estimert ved en probabilistisk tilnærming av to scenarier for konsum av grillmat der innhold av PAH i forskjellig grillmat og eksponering for PAH ble modellert. Den totale risikoen fra PAH-eksponering ble beskrevet ved å beregne såkalt eksponeringsmargin (MOE).

Resultater

Identifisering av prosessfremkalte kontaminanter

VKM identifiserte flere prosessfremkalte kontaminanter som dannes ved grilling. Disse var: PAH, PAH-analoger som klorerte PAH og nitrerte-PAH, polyfenoler, heterosykliske aromatiske aminer (HAA), 3-monoklorpropan-1,2-diol (3-MCPD) og glycidylestere, akrylamid, nitrosaminer, nitritt, skadelige Maillard-reaksjonsprodukter, biogene aminer, 7-ketokolesterol, akridinderivater, antrakinon (ATQ), pyraziner, avanserte glykeringssluttprodukter (advanced glycation end products - AGE). For to grupper av forbindelser, PAH og HAA, var det betydelige eller gode holdepunkter for at det er høyere konsentrasjon i grillet kjøtt og fisk enn i stekt mat. Det var lite data om prosessfremkalte kontaminanter i annen grillmat enn kjøtt og fisk, som f.eks. grønnsaker og brød.

Konsentrasjonsdata for PAH i grillmat (for det meste kjøtt og kjøttprodukter) ble samlet inn fra totalt 81 studier. De høyeste konsentrasjonene av PAH (BaP og PAH4) ble funnet i pølser grillet på bål. Uavhengig av grillmetode varierte konsentrasjonene av BaP fra under kvantifiseringsnivå til mer enn 100 ng/g i svinekjøtt og pølser. På tvers av alle matvarer hadde det meste av grillmaten median konsentrasjoner av BaP som lå under ca. 1 ng/g, med en variasjon fra 0,1 til 4,1 ng/g. Forekomsten av de ulike HAA i grillmat varierte sterkt, fra under deteksjonsgrensen til ca. 50 ng/g. Konsentrasjonen kunne imidlertid komme opp i 240 ng/g for PhIP i svært godt stekt grillet kylling, og det er mer i kjøtt enn i fisk.

Faktorer (for eksempel grilltype, grillmetode og mat) som er viktige for dannelsen av de prosessfremkalte kontaminantene i grillmat.

Hovedvarmekilder ved grilling er elektrisitet, gass, trekull (kullbiter, briketter) og ved. Type varmekilde kan påvirke innholdet av PAH og HAA forskjellig. Andre faktorer som kan påvirke dannelsen av PAH og HAA inkluderer type brensel, direkte eller indirekte grilling, type kjøtt, avstand fra varmekilden, grilltemperatur og grilltid.

Forurensning av matvarer med PAH kan primært skje gjennom: (1) avsetning på maten av PAHer i røyk fra brensel der trekull og spesielt ved kan bidra vesentlig til PAH-forurensning, mens elektrisk oppvarming og gass ikke avgir eller avgir lite PAH. Ved bruk av trekull er PAH-utslippet høyere den første tiden etter opptenning av grillen. (2) avsetning av røyk fra ufullstendig forbrenning (pyrolyse) av fett som drypper ned på varmekilden eller varme overflater. Fett som drypper ned på varmekilden er en betydelig kilde til PAH i grillmat og er relatert til fettinnholdet i maten. Ved å unngå fett som drypper direkte på varmekilden og hindre at røyken kommer i kontakt med maten kan man redusere avsetning av PAH på maten. Studier om effekten av marinering er motstridende, ettersom marinering både kan redusere og øke innholdet av PAH. (3) overoppheting av maten som fører til brent overflate under tilberedning. Temperatur, nærhet til varmekilden, hyppig vending av maten og steketid er viktig for å unngå å brenne maten under grilling.

HAA dannes hovedsakelig i stekeskorpen eller stekeskyen ved oppvarming. Forhold som påvirker dannelsen, er (1) temperatur og steketid. Konsentrasjonene av HAA øker med stigende temperatur, mens regelmessig vending av kjøttet under tilberedning reduserer overflatetemperaturen og reduserer dannelsen av HAA. (2) tilstedeværelse i maten av stoffer som inngår i dannelsen som for eksempel kreatin, reduserende sukkere og frie aminosyrer. Mer HAA dannes i magert kjøtt enn i fettrikt kjøtt, fisk eller blandingsprodukter. (3) tilberedningsmetode, for eksempel marinering, kan påvirke dannelsen av HAA. Behandling av biff og kylling i mikrobølgeovn før kullgrilling kan redusere innholdet av HAA.

Helserisiko forbundet med inntak av grillmat

Gjennomgangen av systematiske oversikter av epidemiologiske studier publisert siden 2006 førte ikke til funn av ny kunnskap om sammenhengen mellom inntak av grillmat og helseutfall hos mennesker. Derfor er konklusjonen i den forrige VKM 2007-vurderingen fortsatt relevant. Den antyder at det er en mulig sammenheng mellom inntak av godt stekt eller grillet kjøtt og kreft i tykktarm, endetarm, prostata, bryst og bukspyttkjertel. Det var utenfor rammen av denne vurderingen å gjennomgå originale enkeltstudier om helseeffekter av inntak av grillet mat sammenlignet med andre tilberedningsmetoder. VKM vurderte derfor risikoen ved eksponering for prosessfremkalte kontaminanter dannet ved grilling.

VKM estimerte PAH-eksponering i grillet mat basert på scenarier for inntak av grillmat. I tillegg ble bakgrunnseksponering av PAH fra resten av kostholdet inkludert. På grunn av mangel på data kunne ikke risikoen fra HAA-eksponering og andre varmeinduserte forurensninger karakteriseres.

Fordelingen av PAH-konsentrasjonen i forskjellig grillmat ble modellert basert på informasjon fra de 81 publikasjonene og omfattet alle typer grillmetoder, der data fra kullgrillet mat utgjorde mer enn 60 % av tallmaterialet. På grunn av begrensninger i databasen var det ikke mulig å differensiere mellom grillmetoder. Den kumulative konsentrasjonsfordelingen i de fleste matvarene viste en sterk økning i PAH-konsentrasjonene over 75 persentilene. Den simulerte konsentrasjonen ved 90- og 95-persentilen representerer trolig grillmetoder som er kjent for å øke dannelsen av PAH i maten. De høyeste konsentrasjonene av BaP ble funnet i pølser grillet på bål og i hamburgere og fett svinekjøtt.

De to scenariene for konsum av grillmat omfatter bare kjøtt og laks på grunn av manglende forekomstdata i annen grillmat, som grønnsaker, brød og kjøttimitasjon. Scenariene illustrerer mulige konsekvenser av ulike matpreferanser for inntak av PAH fra grillmat. Scenariotallerkenen med 200 g grillet fettrikt kjøtt inneholdt fett svinekjøtt, hamburger, pølser og kylling med skinn. Den andre tallerkenen med 200 g magert kjøtt og fisk inneholdt storfekjøtt, magert svinekjøtt, kylling uten skinn og laks. Basert på konsentrasjonsfordeling av BaP og PAH4 i hver matvare på tallerkenen ble fordelingen av totalinnholdet av BaP og PAH4 på hver tallerken simulert.

Eksponeringsscenariene omfattet konsum av 1 til 100 porsjoner av hver av de to tallerkenene per år. Bakgrunnseksponering for PAH fra resten av kostholdet ble lag til, og gjennomsnittlig daglig inntak av BaP eller PAH4 i ett år ble beregnet.

Risiko ble karakterisert ved bruk av MOE og $BMDL_{10}$s for BaP og PAH4 fra EFSAs risikovurdering av PAH i mat fra 2008 som referansepunkter. VKM vurderer at eksponering for PAH som resulterer i MOE under 10 000 er en bekymring for folkehelsen. Eksponering for tallerkenen med magert kjøtt og laks ga for gjennomsnittlig, median og 75 persentil innhold av BaP og PAH4 og opptil 100 porsjoner per år MOE over 10 000. Eksponering ved 90 persentilen og 100 porsjoner resulterte i MOE omkring 10 000 for BaP og litt under for PAH4. For scenariet med fettrik kjøttallerken og eksponering for et gjennomsnittlig BaP- og PAH4-innhold var MOE over 10 000 ved et inntak på opptil 100 måltider i året. Ved et innhold tilsvarende 95 persentilen av BaP og PAH4 var MOE-ene under 10 000 ved et inntak av grillmat omkring 15 ganger i året for BaP og 25 ganger i året for PAH4. Bruk av PAH4 som en indikator for total PAH eksponering i stedet for BaP, ga omtrent samme resultat som for BaP, men med litt høyere MOE for tallerkenen med fettrikt kjøtt og litt lavere for den med magert kjøtt og laks. Effekten på eksponeringsestimater og tilhørende MOE av grilling på bål ble undersøkt i en sensitivitetsanalyse. Data for bålgrillet mat var kun tilgjengelig for matvarer med fettrikt kjøtt. Ved å utelukke bålgrillet mat fra den fettrike tallerkenen ble gjennomsnitt, median og høyere persentilverdier for innholdet av både PAH4 og BaP på tallerkenen reduserte betydelig. Det førte til MOE over 10 000 ved inntak av mer enn 100 porsjoner per år av tallerkener med et gjennomsnittlig, median og 75 persentil innhold av BaP og PAH4. Ved et innhold på 95-persentil av BaP og PAH4 var MOE over 10 000 for opptil 30 porsjoner pr. år.

Datamaterialet tillot ikke bruk av eksponeringsscenarier som gjenspeiler ulike grillmetoder. MOE-verdiene som ble beregnet på grunnlag av gjennomsnittlig, median og 75 persentil PAH-eksponering representerer sannsynligvis bruken av varierte grillmetoder og mat som ikke forårsaker betydelig PAH-dannelse. Dette vil nok gjelde de fleste grillsituasjoner. MOE beregnet for 90- og 95-persentil-eksponeringer for PAH kan representere hyppig bruk av grillmetoder som er kjent for å øke forekomsten av PAH i maten, for eksempel høytemperaturgrilling med kull eller med bål og lang grilltid som fører til godt stekt mat.

På grunn av mangel på data var det ikke mulig å karakterisere risikoen fra HAA-eksponering og andre varmeinduserte kontaminanter. Konsentrasjonen av HAA ser ut til å være høyere i grillet kjøtt enn i kjøtt stekt i panne, spesielt når det er godt stekt. Grilling av mat er forbundet med høyere og mindre kontrollerbar overflatetemperatur enn steking i panne. De fleste av HAA-ene er gentoksiske og kreftfremkallende hos gnagere og noen har blitt klassifisert som mulige kreftfremkallende stoffer hos mennesker. Av den grunn kan dannelsen av slike stoffer under grilling være til bekymring. Selv om det ikke er kjent, er det sannsynlig at flere andre varmeinduserte forurensninger, for eksempel PAH-analoger, kan forekomme i høyere konsentrasjoner i grillet mat enn i pannestekt mat, da dannelsesmekanismen antas å være lik den til PAH og på grunn av en antatt høyere og mindre kontrollerbar temperatur ved grilling.

Usikkerhet

Usikkerhetene i denne vurderingen er store. Eksponering for PAH ble estimert fra simulerte forekomstdata og scenarier for konsum. Helserisikoen ved eksponering for PAH i konsumert grillmat karakterisert ved bruk av MOE kan både være over- og underestimert. På grunn av mangel på data kunne ikke helserisikoen knyttet til eksponering for andre varmeinduserte kontaminanter i grillet mat vurderes. Høy og mindre kontrollerbar temperatur under grilling gjør det sannsynlig at HAA og noen andre varmeinduserte forurensninger kan forekomme i høyere konsentrasjoner i grillet mat enn i stekt mat. Derfor er den totale risikoen forbundet med forekomst av prosessfremkalte kontaminanter i grillmat sannsynligvis høyere enn for PAH alene.

Kunnskapsmangler

De viktigste kunnskapsmanglene knyttet til helserisikovurderingen av grillet mat som ble funnet av VKM er følgende: 1) systematiske oversikter om helseutfall knyttet til inntak av grillet mat sammenlignet med andre tilberedningsmetoder mangler, 2) data om inntak av grillet mat , matvarer, frekvenser av grilling og grillmetode som er brukt mangler, 3) data om forekomst av PAH og andre varmeinduserte forurensninger i mat tilberedt ved forskjellige grillmetoder og andre sammenlignbare tilberedningsmetoder mangler, 4) toksikologiske data om individuelle HAA-er for bedre farekarakterisering med tanke på toksikokinetiske forskjeller mellom gnagere og mennesker mangler, og 5) forekomstdata for HAA i grillmat analysert med kontrollerte og validerte analysemetoder mangler.

Abbreviations

BMR	Benchmark response
bw	Body weight
BMDL	Benchmark dose (lower confidence limit)
CWS	Chicken with skin
CWoS	Chicken without skin
EFSA	European Food Safety Authority
HBGV	Health based guidance value
LOD	Limit of detection
LOQ	Limit of quantification
MoBa	The Norwegian Mother, Father and Child cohort study
MOE	Margin of exposure
PBP	Plant based products
RP	Reference point
SD	Standard deviation
SE	Standard error
TDI	Tolerable daily intake
TOR	Terms of reference
TWI	Tolerable weekly intake

Chemical substances

AαC	2-Amino-9H-pyrido[2,3-b]indole
AC	Amino-carbolines
AGE	Advanced glycation end products
AIA	Amino-imidazo-azaarens

ATQ	Anthraquinone
BaA	Benz[a]anthracene
BaP	Benzo[a]pyrene
BcFl	Benzo[c]fluorene
BbF	Benzo[b]fluoranthene
BghiP	Benzo[ghi]perylene
BkF	Benzo[k]fluoranthene
4-CH2OH-8-MeIQx	2-amino-4-hydroxy-methyl-3,8-dimethylimidazo[4,5-f]quinoxaline
Chry	Chrysene
Cl-PAH	Chlorinated polycyclic aromatic hydrocarbon
Cre-P-1	4-amino-1,6-dimethyl-2-methylamino-1*H*,6*H*-pyrrolo[3,4-f]benzimidazole-5,7-dione
DBahA	Dibenzo[a,h]anthracene
DBaiP	Dibenzo[a,i]pyrene
DBalP	Dibenzo[a,l]pyrene
Glu-P-1	2-Amino-6-methyldipyrido[1,2-A:3',2'-D]imidazole Hydrochloride Hydrate
Glu-P-2	2-Aminodipyrido[1,2-A:3',2'-D]imidazole Hydrochloride
HAA	Heterocyclic aromatic amines
Harman	1-Methyl-9*H*-pyrido[3,4-b]indole
IFP	2-amino-(1,6-dimethylfuro[3,2-e]imidazo[4,5-b])pyridine)
IP	Indeno[1,2,3-cd]pyrene
IQ	2-Amino-3-methyl-3H-imidazo[4,5-f]quinoline
IQx	2-Amino-3-methyl-3H-imidazo[4,5-f]quinoxaline
Lys-P-1	3,4- cyclopentenopyrido[3,2-ɑ]carbazole
3-MCPD	3-monochloropropane-1,2-diol
MeAαC	2-amino-3-methyl-9H-pyrido[2,3-b]indole
MeIQ	2-Amino-3,4-dimethyl-3H-imidazo[4,5-f]quinoline

MeIQx	2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline
MHC	5-methylchrysene
Norharman	9*H*-pyrido[3,4-b]indole
O-PAH	Oxygenated polycyclic aromatic hydrocarbon
Orn-P-1	4-amino-6-methyl-1 H-2,5,10,10b-tetraazafluoranthene
PAH	Polycyclic aromatic hydrocarbon
PAH2	The sum of BaP and Chry
PAH4	The sum of BaP, BaA, Chry, and BbF.
PAH8	The sum of BaA, Chry, BbF, BaP, BkF, BghiP, DbahA, and IP.
Phe-P-1	2-amino-5-phenyl-pyridine
PhIP	2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
1,5,6- and 3,5,6-TMIP	1,5,6- and 3,5,6-Trimethyl-1*H*-imidazo[4,5-b]pyridin-2-amine
Trp-P1	3-Amino-1,4-dimethyl-5*H*-pyrido[4,3-b]indole
Trp-P2	3-Amino-1-methyl-5*H*-pyrido[4,3-b]indole

Glossary

Barbecuing	In this assessment barbecuing is defined as grilling. See Grilling.
Contaminants	Contaminants are substances that have not been intentionally added to food. These substances may be present in food as a result of the various stages of its production, packaging, transport or holding. They also might result from environmental contamination.
Fried food	In this report fried food includes food prepared with cooking methods involving high temperatures, that are not defined as grilling (grilling and barbecuing).
Grilling	The term grilling in Norwegian language encompasses both barbecuing and grilling. Both, barbecuing and grilling, are denoted grilling in this report. While barbecuing is known as a low and slow, indirect heat cooking method, grilling involves high temperature and direct heat. In barbecuing the lid is kept closed during cooking allowing a convection process to cook the food, while grilling is without a lid. When barbecuing, there is little need to flip the food to prevent burning, while frequent flipping is necessary when grilling.
Grill habits	In this report, "grill habits" refers to the frequency of grilling, the grill method used (such as charcoal, gas, electric, indirect, or direct grilling), and the types of food grilled, along with the frequency of grilling each specific food type.
Maillard reaction	A complex chemical reaction between amino acids and reducing sugars to create melanoidins, the compounds which give browned food its distinctive flavour. The Maillard reaction may also give rise to some harmful process contaminants, such as acrylamide and heterocyclic amines.
Margin of exposure	The margin of exposure (MOE) is a tool used by risk assessors to consider possible safety concerns arising from the presence in food and feed of chemical substances when they deem it inappropriate or unfeasible to establish a health-based guidance value (HBGV; a ´safety threshold´) such as an Acceptable Daily Intake (ADI) or a Tolerable Daily Intake (TDI). The MOE is the ratio between the human exposure and a reference point (RP) characterizing the hazard, usually a BMDL10
MatPrat	Information office for eggs and meat, financed by meat and egg producers in Norway and subordinated to NORTURA (www.matprat.no) which is a co-operative owned by several thousand Norwegian farmers, and one of the largest producers of foodstuffs in Norway.
Process contaminants	Process contaminants are potentially harmful substances that are formed in food or in food ingredients due to chemical reactions during food processing. Processing methods include fermentation, smoking, drying, refining and high-temperature cooking.
Umbrella review	Systematic review of systematic reviews.

Background as provided by the Norwegian Food Safety Authority

It has been long known that heat treatment of food, such as grilling (grilling and barbecuing) and frying may give rise to unwanted, harmful substances in food, so-called process contaminants. The best-known ones are probably heterocyclic aromatic amines (HAAs) and polycyclic aromatic hydrocarbons (PAHs). In recent times, the spotlight has also been directed at other process contaminants such as acrylamide and furans. Grilling is a common way of preparing food in Norway. In recent years, the grilling season has become longer, the food selection ever wider and sales of various types of grills have increased. Several factors may have changed since VKM's previous assessment of health risks from the consumption of grilled food, which was published in 2007 (VKM, 2007). To be able to give current and relevant advice to consumers and others who sell/offer grilled food, the Norwegian Food Safety Authority needs an up-to-date knowledge about the formation of processed contaminants in various foodstuffs by different grilling methods, and an assessment of the risk this may pose.

Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority asks VKM to do the following:

Identify process contaminants which are formed to a greater extent by grilling than by frying and create an overview of reported amounts of these process contaminants in various types of grilled food.

Elucidate factors (for example grill type, grill method and food) that are important for the formation of the identified process contaminants in grilled food.

If possible, based on available information, assess the health risks associated with the consumption of grilled food compared to fried food.

Clarification of the terms of reference

The assessment is restricted to grilled food and the process contaminants formed by grilling of foods like meat, fish, vegetables, and other food likely to be grilled. In this assessment grilling includes the use of different heat sources like charcoal, wood, gas, campfire, or electricity. Further, devices that intentionally generate smoke (not smoking per se) to give flavours to the food, and more traditional grilling devices with and without a lid are included. The assessment includes health risks related to dietary exposure to process contaminants formed during grilling and excludes exposure that may occur via other exposure routes such as inhalation. Possible health outcomes related to consumption of grilled food in comparison with fried food were considered using studies in humans. The term grilling as used in this report includes barbecuing (see Glossary).

The current assessment does not include undesirable substances originating from incorrect use of food contact materials or incorrect use of the grilling device and heat source.

It is outside the scope of this assessment to evaluate the quality of the various charcoal types. Lump charcoal and briquettes marketed in the EU shall follow the standard described in NS-EN 18 60-2 (VKM, 2007). However, substances potentially generated from the various heat sources that may contaminate the food, either from dripping and burning of fat from the food or smoke from the heat sources, are included in the assessment. Evaluation of potential hazards from fluids used to lighten the charcoal is also outside the scope of this assessment.

1 Introduction

1.1 Harmful substances formed in food during heat treatment such as grilling

The aim of cooking food is to add flavour and taste and make it more easily digestible. Cooking or heat processing ensures microbiological safety. During heating both physical and chemical changes occur that include melting of fat, starch gelatinisation, denaturation of protein and evaporation of water. During browning of food, the Maillard process occurs, in which free amino acids react with reducing sugars forming numerous chemical compounds important for flavour and aroma. However, some of these compounds may be harmful to humans. Heat treatment of food, such as grilling and frying may therefore be a source of unwanted, harmful substances. Such harmful compounds formed in food or food ingredients during all kinds of food processing, including heat treatment, are called process contaminants (see glossary).

The most frequently reported harmful substances formed during grilling of food are polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic amines (HAAs). PAHs are a large group of compounds consisting of two or more fused aromatic rings formed by incomplete combustion of organic material. A common way of formation is incomplete combustion of fat dripping from the food onto the heat source during grilling and formation of PAH-containing smoke that may contaminate the food. Several PAHs are genotoxic carcinogens (EFSA, 2008). HAAs are a group of compounds formed either from high temperature (> 300°C) treatment of amino acids (amino-carbolines, AC) or at lower temperatures from amino acids, creatine and reducing sugars (amino-imidazo-azaarens, AIA) (Mottram et al., 2006). HAAs are strong mutagens and carcinogens (Sugimura et al., 2004) . There is extensive literature about formation of PAHs and HAAs in grilled food and grilling can be a significant source of dietary exposure to PAHs and HAAs (Duedahl-Olesen et al., 2015; Geng et al., 2023).

In addition to PAHs and HAAs, other harmful substances may occur in grilled food due to the heat treatment. These include nitrosamines (formed from nitrite and amines), acrylamide, acrolein, 3-monochloropropan-1,2-diol (3-MCPD), glycidyl esters, anthraquinone (ATQ), pyrazines, 7-ketocholesterol, and PAH-analogues, such as nitrogen and chlorine containing PAHs. Below these compounds are addressed in greater detail and the basis for selection of substances for risk characterization are described.

1.2 Grilling methods and heating sources

In this assessment it was distinguished between the main types of grilling devices by their heat source: electricity, gas and charcoal. In Norway, it is also common to roast sausages on campfires. Moreover, there are two main methods to prepare the food on the grill: direct and indirect grilling. The former is a method using high temperature with the food placed directly above the heat source and short cooking time. The latter is a method using lower temperature with the food placed on the side of the direct heat source, often below a lid. The lower temperature by indirect grilling implies longer cooking time (VKM, 2007).

There are different types of charcoal used for grilling. These include lump charcoal and briquettes. Briquettes are made from compressed charcoal produced from wood by-products with binder and other additives (Jelonek et al., 2020). Some types of briquettes and lump charcoal are mixed with wood chips to add flavour.

The role of grilling methods and heat sources is discussed in Chapter 6, including factors that affect the content of PAHs/HAAs.

1.3 Previous risk assessments of grilled food

1.3.1 VKM 2007

In the previous VKM risk assessment (VKM, 2007), the substances considered to be associated with the highest health risks in grilled food were PAHs and HAAs. VKM 2007 concluded that grilled food contains more PAHs and HAAs than pan-fried, oven-roasted and boiled food, implying that the part of the population consuming high levels of grilled food probably have a higher exposure to PAHs and HAAs than low-consumers. VKM also concluded that grilled food can be a substantial source of PAHs exposure among those who grill often.

Worst-case calculations of exposure to one PAH (BaP, an indicator of PAHs) and one HAA (PhIP) were performed in 2007. For BaP, the daily intake from food, including a high intake of grilled food, was estimated to 11 ng/kg body weight. For PhIP, the intake from grilled and other fried foods was estimated to 27 ng/kg body weight of which 8 ng/kg body weight was from grilled food. These calculations were stated as highly uncertain. As PAHs and HAAs are both genotoxic and carcinogenic, VKM calculated margins of exposure (MOEs) based on worst-case exposure scenario and respective reference points (RP) ($BMDL_{10}$s). For BaP, the MOE was approximately 9,000 and for PhIP, the MOE was 75 000. It was emphasised that the calculated MOEs were associated with high uncertainty.

VKM 2007 concluded on a small to moderate increased risk of cancer in the colon, rectum, prostate, breast, and pancreas based on several epidemiological studies, identified through a non-systematic literature search, suggesting a possible association between intake of well-done fried or grilled meat and cancer. Further, as these cancer forms are prevalent in the western world, a reduction in incidence would have a major impact on the prevalence of these cancer forms. The increased risk of cancer associated with high consumption of well-roasted or grilled meat could only to a limited extent be explained by the exposure to PAHs and HAAs. VKM 2007 concluded, based on the available data, that it was not possible to quantify the risk of exposure to carcinogenic compounds from grilled food separate from the risk from exposure from other sources, such as frying (HAAs) or from contaminated food and air (PAHs).

Collectively, VKM 2007 gave greater weight to the epidemiological studies than to the MOE calculations and reiterated the message that in general exposure to genotoxic and carcinogenic substances should be as low as reasonably possible.

VKM (2007) recommended grilling less often or grilling in a manner forming lower amounts of carcinogenic compounds. The main parameters affecting generation of PAHs identified in the report were temperature, air supply, the distance from the heat source and location of the heat source. Further, it was reported that less PAHs were formed when the heat source is placed above or to the side of the food so that fat cannot drip and ignite. They concluded that less carcinogenic compounds are formed with increasing distance between food and heat source, when less fatty foods are grilled, using clean lava stones in gas grills, and when good air supply is ensured.

The main parameters affecting generation of HAAs identified in the report were temperature and duration of frying/grilling. Less HAAs are formed during frying/grilling at lower temperatures, when the food is turned several times during frying/grilling, when the food is marinated in advance or pre-cooked in the microwave, or when larger pieces of meat are grilled, so that the surface is minimized in relation to the volume.

VKM further concluded in their report that lower levels of both PAHs and HAAs are formed by grilling over embers instead of grilling over an open flame (fire) and by avoiding consumption of gravy from the frying process.

In their assessment of knowledge gaps and research needs they concluded that there was a need for enhanced understanding about both quantities and composition of the barbecued food consumed, as well as the grilling methods commonly used, in Norway. In particular, it was identified a lack of knowledge about consumption of barbecued food among frequent grillers and children. Their conclusion underscores that there is a need for improved data on the content of PAHs and HAAs, and other carcinogenic substances in various types of food, preferably analysed with newer and advanced analytical methods. There was a need for more exact knowledge about the effect of type of fuel and the influence of different grill types on the formation of PAHs, HAAs and other substances. Moreover, they identified a lack of knowledge about concentration of PAHs in food grilled with grill types that are widely used in Norway, in particular grills with lids and disposable grills.

1.3.2 Other than VKM 2007

In a study from Sweden, the authors calculated the mean intake of BaP and PAH4 (Sum of BaA, Chrys, BbF, and BaP) in different food groups in market basket samples which included the most commonly consumed foodstuffs (Abramsson-Zetterberg et al., 2014). In addition, they estimated the intake of BaP and PAH4 from home-grilled chorizo sausages and pork loins, based on a consumption frequency of 10 times per year and a portion size of 150 grams. It was concluded that PAHs in food is of low concern for human health in Sweden and that the additional exposure to PAHs from grilled food was of minor importance.

Duedahl-Olesen and co-workers calculated intake of PAHs from various types of home-grilled and restaurant-grilled food in Denmark (Duedahl-Olesen et al., 2015). The intake of PAH4 was based on average meat intake for Danish adults in the age group 15-75 years in a dietary survey including 2,348 individuals completing a 7-day semi –closed questionnaire in the period 2003 to 2008. The overall assumption was that all meat eaten was barbecued.

With a worst-case scenario assuming daily consumption of barbequed meet using maximum PAH4 concentrations in the restaurant grilled hamburgers, based on the hazard assessment of PAH by EFSA (EFSA, 2008) and an exposure of 48 ng PAH4 per kg bw per day an MOE of 7,080 was calculated. Using the mean concentration of PAH4 analysed in the hamburgers (4.4 ng PAH4 per kg bw per day) an MOE of 77,000 was calculated. It was concluded that with regard to PAH exposure intake of barbequed meat in most instances is of low health concern, but that precautions should be taken to reduce PAHs levels from barbecued meat and home-grilling.

Jakobsen et al. performed a probabilistic approach to assess cancer risk from BaP exposure in grilled food in Denmark. A median yearly exposure to BaP from barbecued food was estimated at 0.07 µg/kg body weight with a 95th percentile of 0.29 µg/kg body weight (Jakobsen et al., 2018). This is equivalent to a daily exposure of 13.3 and 55.5 ng BaP, respectively, per day for a 70 kg individual. An average extra lifetime risk of cancer due to BaP from grilled meat was estimated to be 6.8×10^{-5} (95% uncertainty interval $2.6 \times 10^{-7} - 7.0 \times 10^{-4}$) in the Danish population. The uncertainty in the data was very large, but it was concluded that risk of cancer was not negligible for highly exposed individuals.

2 Methods

A protocol for this risk assessment, describing the scope and methods of this assessment, was published online in advance, February 1, 2023 (VKM, 2023). The deduction of research questions from the terms of reference is summarized in table 1 of the protocol. The target population for this assessment is the Norwegian population.

Heat-induced process contaminants formed, the concentrations in which they occur and the factors that may determine their concentrations in grilled food were identified using published literature. For hazard characterization of the various contaminants, Health Based Guidance Values (HBGVs, e.g., TDIs) or Reference points from previous hazard assessments from EFSA or WHO were used. When no such values were found, it was identified as a knowledge gap.

The exposure was estimated through two scenarios covering different preferences for types of grilled food and use of different grilling methods and devices.

The risk was characterized by comparing the estimated exposures with available health-based guidance values (TDIs/TWIs etc or/and Reference Points for calculation of margin of exposure (MOE)).

New knowledge on the association between consumption of grilled food and health outcomes in humans was collected by conducting an umbrella review, i.e., a systematic review of previously published systematic reviews of epidemiological studies.

The main questions addressed to answer the terms of reference are listed in Table 1.

Table 1. Main questions, addressed to answer the terms of reference.

Terms of reference		**Questions**
1	Identify process contaminants that are formed to a greater extent by grilling compared to frying and give an overview of reported amount of these substances in different types of grilled food.	a. Which process contaminants are formed by grilling? b. Which process contaminants are found in higher concentrations in grilled than in fried food? c. In what concentrations do we find the process contaminants identified in b) in grilled food?
2	Highlight conditions (for example grilling devices and methods, and type of food) that are relevant for the formation of the identified food processing substances by grilling.	d. Which factors alter the formation of process contaminants identified in b)? i. the grill itself (e.g., lid) ii. the food itself (e.g., fat content, protein composition) iii. preparation (e.g., doneness and cooking time, marination) iv.temperature (e.g., heat source)
3	If possible, from available information, evaluate health risks related to consumption of grilled food compared with fried food	e. Is there new knowledge on health risk related to consumption of grilled food and health compared with fried food? f. What are the HBGVs for the process contaminants identified in b)? g. What is the exposure to process contaminants from grilled food in relation to established health-based guidance values or similar?

2.1 Umbrella review of epidemiological studies – systematic literature search

The systematic literature search for systematic reviews was formulated and published as a part of the protocol beforehand (research question e).

The literature searches were performed in the electronic databases from MEDLINE (Ovid), Embase (Ovid), and Web of Science, from inception to search date (02.02.2023), by a skilled research librarian. The search terms and strategy are included in Annex I, "systematic literature search".

The study selection was based on the predefined eligibility criteria published in table 2 in the protocol (VKM, 2023).

Only systematic literature reviews of epidemiological studies on consumption of grilled food and health outcomes in humans was included. The exposure was consumption of grilled food and did not include studies on the exposure to process contaminants per se. The outcomes were included quite widely as any adverse health outcomes.

The identified records were imported into EndNote (Thomson Reuters), duplicates were removed, and the records were imported further into Rayyan (Ouzzani et al., 2016) for the screening of title and abstracts. Screening of records for relevance was performed by pairs of reviewers. To ensure between-reviewer calibration, all conflicts were discussed and clarified in a calibration meeting.

Records selected for full text assessment were evaluated by pairs of reviewers. To ensure between-reviewer calibration, all reviewers participated in a calibration meeting where application of the eligibility criteria was discussed and clarified.

2.1.1 Risk of bias analysis of full text systematic reviews

The full text systematic reviews were then assessed for the risk of bias using risk of bias analysis (ROBIS) (Whiting et al., 2016). Each record (publication) was assessed independently by two members of the project group and disagreements resolved through discussion and when necessary, discussed with a third researcher in the project group.

2.2 Identification of process contaminants in grilled food

To answer the questions related to terms of reference 1 and 2, non-systematic literature searches were performed in Web of Science, PubMed, Google Scholar and Google.

The following search topics were applied in various combinations: grilling, grilled, barbeque/barbecue, fried, fried food, high temperature cooking in combination with food and foodstuff and with more specific foodstuff such as, meat, fish, chicken, poultry, vegetables, plant-based meat alternatives (PBMA) and further combined with contaminants and process contaminants. The search identified a range of substances that were linked to grilling and high temperature cooking. Follow-up searches were performed where specific substances were combined with cooking procedures (grilling, barbecue, fried, high temperature cooking).

The identified substances and substance groups that were connected to grilling of foodstuff were PAHs, chlorinated-PAHs (Cl-PAHs), nitro PAHs, PAH analogues, phenols, HAAs, 3-MCPD (including glycidyl esters), glycidyl esters, acrylamide, nitrosamines, nitrite, Maillard reaction products, biogenic amines, cholesterol oxides, acridine derivatives, anthraquinone, ATQ, pyrazines, and AGE (advanced glycation end products). Several of the substances were named or written differently in the literature, such as the PAHs and HAAs, which could lead to loss of entries if using only one. PAHs were searched for with the spellings "PAH", "PAHs", "polycyclic aromatic hydrocarbons". The HAAs were searched for using the terms "HAA", "HAAs», "heterocyclic aromatic amines" and "heterocyclic amines".

In addition to the scientific papers identified through the search engines, literature was identified from reference lists of reviews and primary literature, and reports from governmental authorities, such as extended reports from EFSA. Additional literature was also found in citation lists of respective publications made available in Google Scholar and Web of Science.

A call for data was made through the EFSA focal point network on occurrence data of process contaminants in grilled food and information/data/feedback from Finland, Italia, Poland, Slovenia, Spain, Sweden, Belgium and Hungary was received.

The literature was sorted and grouped in the Rayyan review tool according to article type (review, primary article, report), substance, foodstuff, grill and cooking method.

Selection of process contaminants to be included for further assessment was based on the identified studies applying expert judgement. The criteria for selecting specific process contaminants for further risk characterization included assessing whether there were enough identified publications to reasonably determine if more of the specific contaminant was formed by grilling compared to frying and whether there were sufficient occurrence data. Additionally, consideration was given to the existence of previous hazard assessments from international food safety organizations, such as EFSA or WHO.

The identification of factors important for the formation of process contaminants in grilled food was done by going through the collected papers which were labelled in Rayyan with factors, such as grill method, fuel type, and type of food and used as a basis/reference for summarizing the factors affecting formation of process contaminants in a narrative way by expert judgement.

Based on the selection criteria above, concentration data of process contaminants in grilled food was only collected from the identified scientific papers for PAHs for which an exposure assessment were conducted as described below.

2.3 Exposure

2.3.1 Consumption of grilled food in Norway

VKM sent a call for data to Norwegian stakeholders that might have insight on grill habits and consumption on grilled food. Information on consumption of grilled food in Norway was obtained from consumer surveys, from stakeholders and others (e.g., data received from The Norwegian Food Safety Authority and MatPrat, which is an information office for eggs and meat, financed by meat and egg producers in Norway and subordinated to NORTURA). Furthermore, unpublished preliminary results were made available to VKM from the national nutritional survey Norkost4 pilot (University of Oslo and Oslo University College).

Background information on habits related to grilling, for example, type of grill (charcoal, gas or electric), type of fuel (charcoal or briquettes) and frequency of grilling, was obtained from the same surveys, MatPrat and Norkost4 pilot.

As there is a large degree of uncertainty in both the amount of grilled food eaten, and the habits related to grilling, the exposure assessment was conducted by creating scenarios using constructed dinner plates.

2.3.1.1 Construction of exposure scenarios

In the Norwegian reference tables for "Weights, measures and portion sizes for foods" the dinner portion sizes for items such as chicken, salmon, burgers, and pork belly are set to 150 g/portion while other food items like lean fish is set to 200 g/portion (Østerholt et al., 2015). Sausages only have an indicated amount in grams per sausage, not per portion. To be on the safer side, and to consider the possibility that a barbeque dinner might make people eat more meat/fish than an average non-grilled dinner, the portion size of total meat/fish on the plates was set to 200 g of prepared food.

Two scenario plates (referred to as "two plates" in the text), were assumed consumed from 1-100 times during the grill season. The compositions of the two scenario plates were intended to reflect different dietary patterns. In addition, it was reasons to believe that one of the plates could contain higher concentrations of PAHs than the other. The "fat-rich plate" consisted of burgers, sausages, fatty pork meat and chicken with skin. The "lean meat and salmon plate" consisted of lean meat like lean pork, chicken without skin, beef and salmon. The composition of food on the plates is shown in Table 2.

Table 2. Composition of the two plates used in the scenarios.

Grilled lean meat and salmon plate	**1 plate (200 g)**	**Percent contribution**
Lean pork	50 g	25%
Chicken without skin	50 g	25%
Beef	50 g	25%
Salmon	50 g	25%
Grilled fat-rich meat plate	**1 plate (200 g)**	**Percent contribution**
Sausages	60 g	30%
Beef patties	60 g	30%
Fatty pork	60 g	30%
Chicken with skin	20 g	10%

2.3.2 Occurrence data on PAHs

2.3.2.1 Extraction of data on concentration of PAHs in grilled food

The papers flagged in Rayyan with data on PAH occurrence were screened and data on the four individual PAHs, Benz[a]anthracene (BaA), Chrysene (Chrys), Benzo[b]fluoranthene (BbF) and Benzo[a]pyrene (BaP), were extracted.

Concentration data on PAHs in grilled food were extracted from all included primary articles and registered in an Excel database. The data was extracted by one researcher and quality checked by at least one other researcher. The articles reporting occurrence data were not subjected to any formal quality assessment. Papers were included when they contained data on one or more of the four above-mentioned PAHs in grilled food. Publications with concentration data were excluded (i) if data were presented only on a dry weight basis and water content in the food was not reported, (ii) if the reported concentration data could not be linked to food type and grill method, (iii) if only the crust of the food was analysed, and not the whole piece or (iv) if it was stated that the food was wrapped in aluminium foil or similar during grilling. This selection led to 81 included publications in the database reporting summarised data on PAHs.

Concentration data (mean, median, standard deviation (SD), standard error (SE), minimum (min) and maximum (max)) of the four individual PAHs and their reported LOD and LOQ in grilled foodstuff were recorded. When available, additional data were extracted from the publications reporting concentration data such as grill method (e.g., electricity, charcoal, campfire, and gas), type of foodstuff (e.g., beef, poultry, sheep, fish, and vegetables) and properties of foodstuff (e.g., fat content and part of animal), cooking method (e.g., marination, temperature, distance from heat source, and cooking time), degree of roasting (i.e., raw, rare, medium or well done), location (e.g., home grilled, restaurant grilled, and grilled in the experiment) and the number of samples analysed. The database is available as supplementary material accessible through VKM website (Annex 2 "Compiled data from papers").

As fat content and food type may influence the occurrence of PAHs in grilled food, the types of foodstuffs were classified into food categories:

Poultry

Poultry was divided in the duck, goose, and chicken categories. The chicken category was further divided into chicken with (CWS) and without skin (CWoS), to separate the fatty skin from the lean meat. All food items with names including skin (e.g., chicken with skin and chicken thigh with skin), plus the pieces of chicken that are normally eaten with skin (e.g., chicken wings, buffalo wings, and chicken drumsticks) were classified as chicken with skin. If fat content was reported, all chicken containing more than 10% fat was sorted under the category "chicken with skin". This left an unsorted category containing food items like whole chicken, chicken meat, chicken mince and a set of dishes. All dishes were looked up using searches on Google.com and, when in doubt, the food items were placed in the category chicken with skin. Duck and goose were not used in the scenario plates, as these are less commonly eaten in Norway. Hence, they were registered as they were and not separated by skin/fat content.

Pork

To distinguish between lean and fatty pork meat, a rough cut-off at 10% fat was used. All samples reported with fat content were sorted into lean and fatty pork meat. For those not reporting fat content, the cuts of pork were divided into lean pork meat (e.g., pork loin and sirloin) and fatty pork meat (e.g., pork neck, pork belly, pork chops, and pork mince/patties) using food composition tables and searches on Google.com.

Beef

Several publications lacked detailed description of the cut of the beef. All meat from cattle was, therefore, categorized as beef except for beef patties (and hamburgers). The beef category included smaller grilled cuts of meat such as in kebabs, larger cuts such as a various beef steaks, T-bone steaks and rib steaks.

Processed meat products

All sausages, independent of name and type of meat, were categorized as sausages. This category included frankfurter sausages, chorizo, sausage made of beef, pork and a mix of meat types. All food items with beef mince made into patties or hamburgers were categorized as beef patties.

Fish

The fish samples were sorted into two exclusive categories: salmon and fish. The fish category contains all other fish species than salmon.

Lamb

All food samples labelled lamb and sheep were marked as lamb.

Vegetables, plant-based products, bread, and other food items

Food items like potato, eggplant, mushrooms, Chinese chives, and green pepper were categorized as vegetables. Plant-based patties and other food items labelled plant based/meat substitutions/vegan/made from plants were categorized as plant-based food.

Samples with bread were categorized as bread.

A heterogenous group was named "other food items", where all unsorted food was placed. This category includes, for example, goat meat and vegetarian patties made from milk protein.

The resulting raw occurrence data are summarised in table 9 in 7.1.1.

2.3.2.2 Simulation of PAH occurrence data in grilled food

To estimate overall distribution of occurrence data of PAHs for each food group, the data from all literature sources were harmonized. Since the articles reported PAH values in various formats, the following rules for how to standardise and harmonise these data were established and used subsequently to simulate the occurrence dataset.

1. If the paper reported the number of samples analysed (n), mean value and SD, the underlying values were assumed to follow the lognormal distribution.

2. If the paper reported n, mean and min/max, but no SD, and more than three samples were analysed (n>3), the value was drawn from a uniform distribution based on min/max.

3. If the paper reported only n and mean, but no SD and no min/max, the mean was assumed to represent n equal values.

4. If the paper reported mean and SD or min/max, but not the number of samples analysed, n was set to 3.

5. If mean was reported, but no SD or min/max, and no n, n was set to 1.

6. When the occurrence was reported for individual samples (or individual sample results could be computed, as, for example, if n=2 and min and max were reported), the individual samples results were used directly.

Simulation was a necessary step in building the occurrence dataset, as much of the data in the literature was only available in the summarized form. The number of simulated observations was n*100, where n is the number of samples summarized by a given mean/standard deviation pair or a value range. To preserve relative prevalence from the literature, each sample was represented by 100 observations. For example, (i) a result from one individual value was registered as 100 identical values, while (ii) a result based on five underlying samples was registered as 500 simulated values. This makes the sampling from the distributions from each study more representative of both the prevalence and the underlying uncertainty.

Several examples of the use of the rules are illustrated in Figure 1. The figure presents the steps involved in building the occurrence dataset from the literature data.

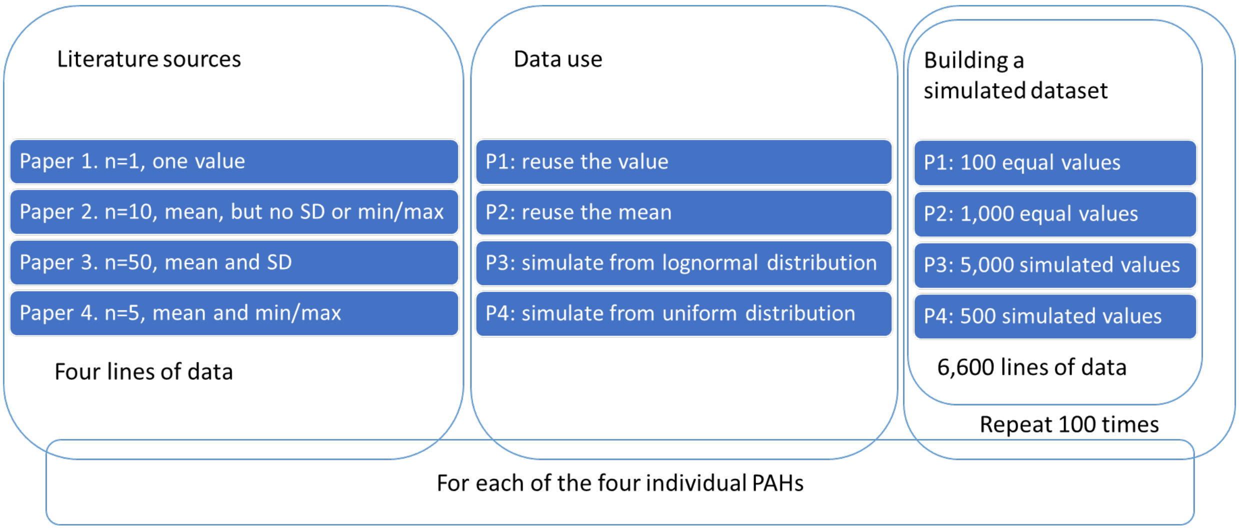

Figure 1. Building occurrence dataset from grilled food PAH concentrations in the literature.

The resulting simulated occurrence datasets for each PAH, in many foods, substantially deviated from standard bell-shaped distributions, as the datasets were often characterized by a combination of high prevalence of low occurrence levels and some high occurrence levels, with few or no observations in-between. For the sake of completeness, it was considered that both distributions were based on the raw simulated dataset and based on the best-fitting lognormal distributions estimated using maximum likelihood (ML).

The simulation process was performed 100 times yielding 100 estimated occurrence distributions for each food and for each PAH. Hence, all distributional parameters (means, standard deviations, and quantiles) were considered in the context of their confidence intervals confirming statistical quality of the estimates.

All analyses described in this subsection and in subsection 2.3.3 were implemented in R and are available as supplementary material accessible through VKM website, Annex 3 "Grill R code".

In addition to the raw data, two approaches were used to assess the uncertainty in the concentrations below the LOQ. In the lower-bound (LB) approach, the concentrations below LOQ were set to 0. In the upper-bound (UB) approach, the concentrations below LOQ were set equal to the LOQ.

As much of the reported data were summary results expressed as means and standard deviations or ranges, some assumptions related to the LOQ in the extracted data had to be made. If the LOQ was not reported, but the LOD was, the LOQ was imputed as 3.3*LOD. If neither LOQ nor LOD was reported, the LOQ was set equal to the mean LOQ for the respective food category.

Results, where means were below the LOQ, were imputed as uniformly-distributed random variables from an interval between 0 and the test (reported) LOQ. Minimums below LOQ were replaced with zeros. Maximums below LOQ were replaced with the LOQ.

The proportion of results below the LOQ, for the different food categories, had to be estimated. The following set of rules were applied when setting the number of non-quantifiable samples for each extracted result:

- If occurrence range was specified and the maximum was below LOQ, all observations were below LOQ.

- If occurrence range was specified and the minimum was above LOQ, all observations were above LOQ.

- If occurrence range was specified and LOQ was in the range, the number of observations below LOQ were set equal to the portion of the range below LOQ.

- If the mean minus three standard deviations was above LOQ, the number of observations below LOQ were set to zero.

- If the mean plus three standard deviations was below LOQ, all observations were considered to be below LOQ.

- If only the mean was available and it was above LOQ, the number of results below LOQ was set to zero, while if the mean was below LOQ, the number of results above LOQ was set to zero.

- If the mean was below LOQ (and none of the previous rules applied), the number of observations below LOQ was set to one.

- In all other cases, the number of observations below LOQ was set to zero.

The mean concentrations in the different foods were quite similar using the LB, unadjusted and UB values (see Appendix I, figure 9, and supplementary material accessible through VKM website, Annex 4 "Grill occurrence summaries"), as the adjustment affected only the leftmost tail of the distribution.

2.3.3 Assessment of exposure to PAH under the two grill scenarios

The exposure to PAHs (BaP and PAH4) from the two scenario plates was calculated using deterministic and probabilistic approaches.

When using the deterministic approach, the overall means for each food item on the plate were summed (see subsection 2.3.3.1). For the probabilistic approach, the random sampling from the simulated occurrence data (described in subsection 2.3.2.2) for each food item resulted in an exposure distribution of PAHs (BaP and PAH4) for each of the two plate types (see subsection 2.3.3.2).

The results with the LB, UB and unadjusted values were quite similar, and all three approaches are available as supplementary material accessible through VKM website, Annex 5 "Grill plates".

Both approaches were applied to two fixed scenario plates described in Table 2. The exposure was assessed for the consumption between one and 100 plates during the year.

2.3.3.1 Deterministic approach

The deterministic exposure approach is based on means of concentrations reported in the literature weighted by the number of the underlying samples in each result. Where means were not available, the mid-points of the reported minimum-maximum ranges for the corresponding samples were used instead. For non-detected means, the value was set at LOQ/2.

2.3.3.2 Probabilistic approach

There are three primary sources of uncertainty/variation in exposure to PAHs: i) different samples have different occurrence levels ii) occurrence distribution is not known with precision, iii) an individual consumer will be exposed to only a limited subset of samples within a reasonable time horizon and consumption frequency. The first two are captured in the simulation process described in 2.3.2.2. The third is captured by randomly sampling from the occurrence distributions estimated in 2.3.2.2.

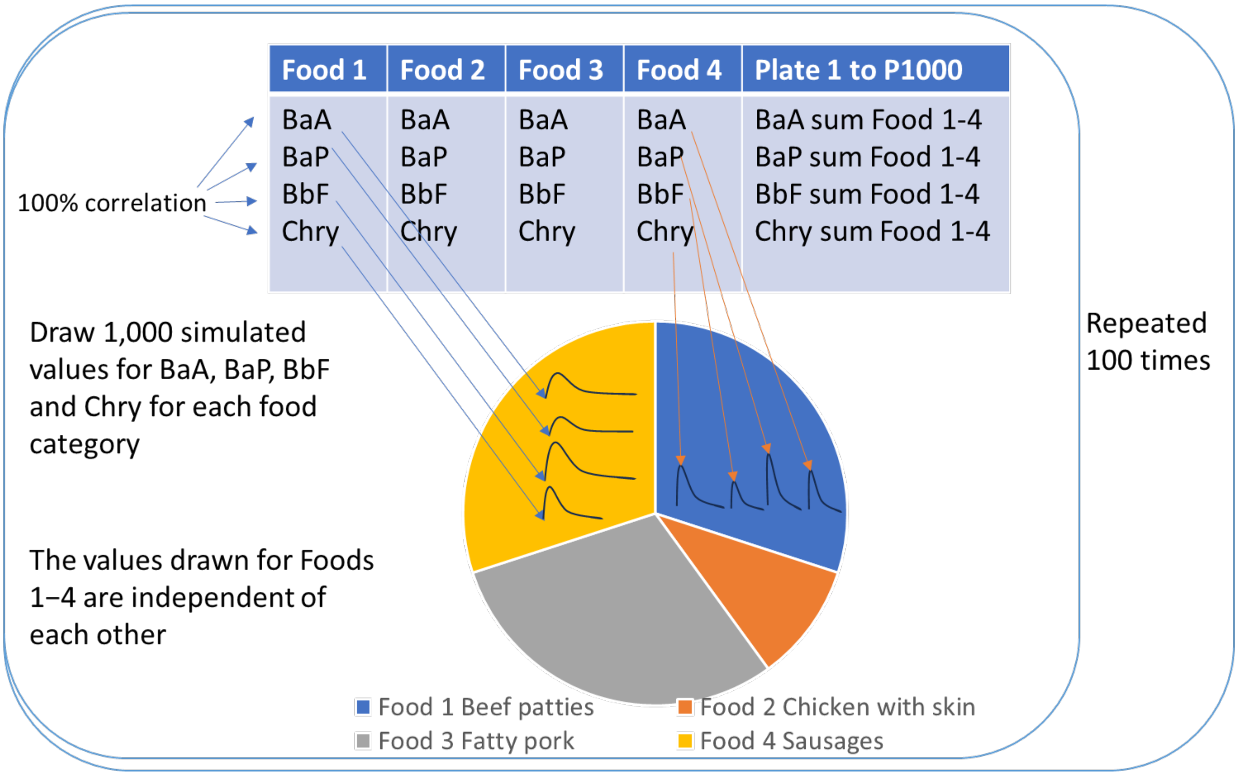

Figure 2. Scenario plate simulation process

In sampling from occurrence distributions, it was used both the best-fitting lognormal and simulated occurrence distributions (see 2.3.2.2 for more details on both). The distributional parameters and the dataset from 2.3.2.2 were reused to preserve comparability between occurrence values and exposure values reported. As a value for PAH4 was not given, it had to be simulated using values for each PAH of PAH4. Given that the four PAHs are correlated, but the data in the literature was too limited to provide good estimates of the correlations, the four PAHs were presumed to have a 100% correlation in this process. The implication of this is that the low percentiles of PAH4 may be biased downward, while the high percentiles may be biased upward. It was sampled 1,000 values (with replacement) from each estimated occurrence distribution. The plate simulation process is summarized graphically in Figure 2.

Technically, it was simulated 1,000 realisations of the cumulative density function (probability percentiles) for each food/simulation pair. As there were eight food types and 100 simulations for all distributions, 800,000 (8*100*1000) uniformly distributed values between zero and one were simulated. The probabilities are then converted to corresponding values for each occurrence distribution of food/simulation/compound triplet. The resulting exposures are then scaled by portion sizes and summarized to the sums of PAHs (if applicable) and plates to estimate exposure distributions.

2.4 Hazard and risk characterization of process contaminants

As PAHs and HAAs are both carcinogenic and genotoxic, it was decided to use the margin of exposure (MOE) approach (EFSA, 2005, 2012) for characterising the risk from their occurrence in grilled food. The MOE approach is used by EFSA in risk assessment of substances in food that are carcinogenic and genotoxic (EFSA, 2005, 2012). The MOE is the ratio between the actual exposure and a reference point (RP) characterizing the hazard, usually a $BMDL_{10}$. The obtained MOE is a measure of potential health risk for a population. The RP is estimated from benchmark modelling of a dose-response function, often derived from animal experiments. The benchmark dose (BMD) corresponds to a benchmark response (BMR). $BMDL_{10}$ is defined as lower confidence interval (95%) of the dose that corresponds to a 10% increased risk of developing cancer (BMR_{10}), corrected for background incidence (EFSA, 2017, 2022). The MOE is then calculated by dividing $BMDL_{10}$ with the daily estimated intake of the substance over a year. According to EFSA (2005), if the RP is derived from an experimental animal study of sufficient quality, an MOE above or equal to 10,000 is regarded as of low concern for public health.

3 Epidemiological studies on grilled food and health outcomes

3.1 Systematic literature search

The search covered all epidemiological systematic reviews on health effects related to grilling between the previous risk assessment published in 2007 until the search date in 2023. The outcome of the selection process in the systematic literature search is illustrated in Figure 3. VKM identified 697 unique publications, of which 595 were excluded based on the predetermined exclusion criteria. The remaining 102 publications were assessed in full text to evaluate whether they were in line with the inclusion criteria. This left only 4 publications (Bandera et al., 2007; Brinkman & Zeegers, 2008; Bylsma & Alexander, 2016; Gamboa-Loira et al., 2022). These final four publications were subjected to risk of bias analyses using the ROBIS-system (Whiting et al., 2016). During the ROBIS assessment, it was discovered that three of the publications were not within the scope of the assessment, as defined in the protocol, and were therefore excluded. The last publication had a high risk of bias and was hence excluded, in accordance with the pre-published protocol. A detailed description of the literature search, results, selection and evaluation including the ROBIS-process is found in Annex 1 "Systematic literature search".

In summary, the systematic search for epidemiological systematic reviews during 2006 until 2023 did not identify any systematic reviews within the assessment scope and of sufficient study quality to be included. The lack of systematic reviews in this field indicates the need for a systematic review on the impact of grilled food on human health. The possible existence of single studies and non-systematic reviews, published after the former VKM report in 2007 was not addressed in this report.

It should be noted that the literature search conducted in the previous 2007 report was not a systematic literature search according to criteria established during recent years. I It was a narrative literature review identifying studies of associations between well done fried or grilled meat and cancer.

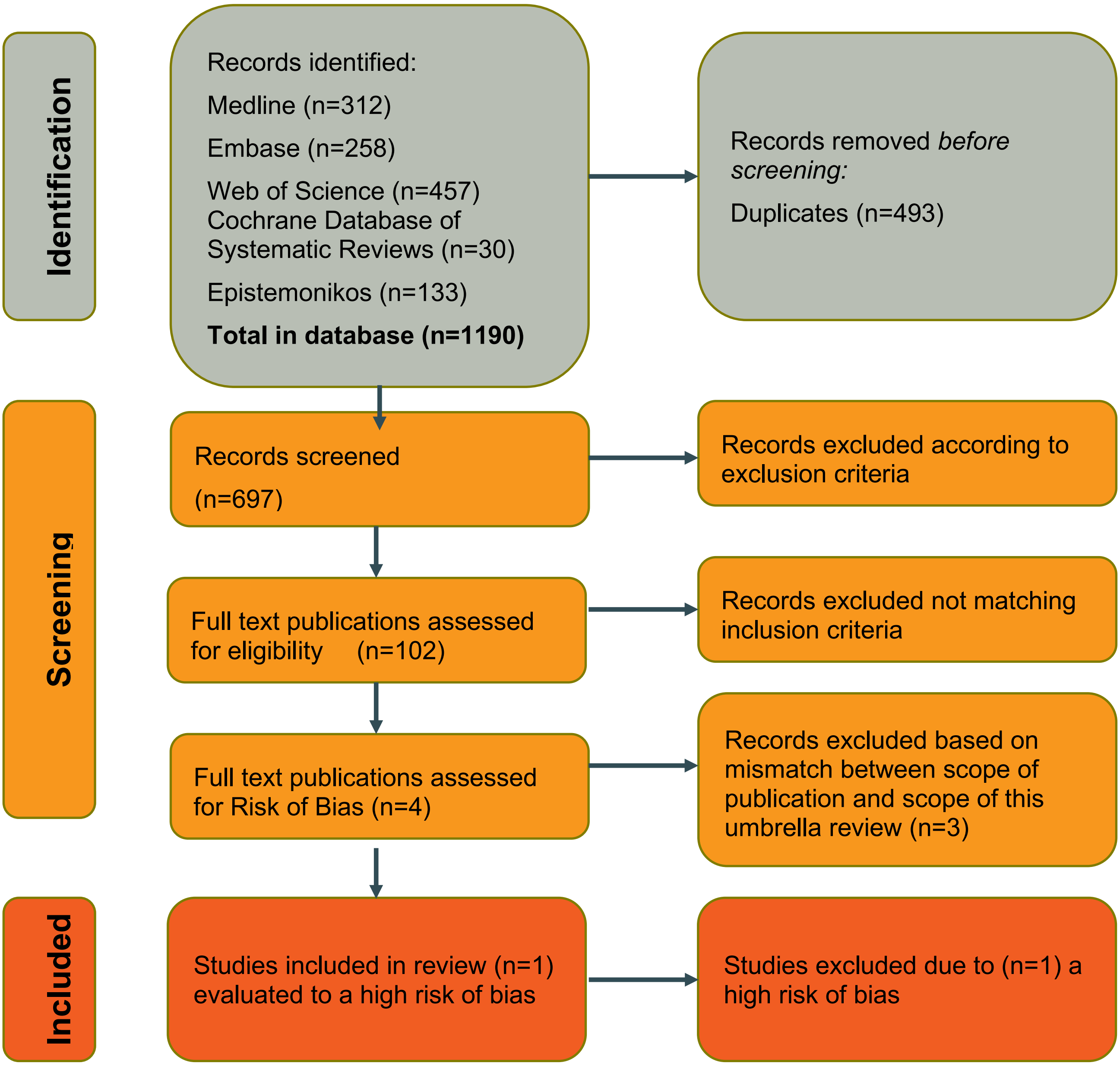

Figure 3. Selection process in the systematic literature search.

4 Heat-induced contaminants identified in grilled food

The identified literature about process contaminants found in grilled food, were uploaded to Rayyan. PAHs and HAAs were by far the most frequently reported potentially harmful substances in grilled food. The literature search also identified other substances that may occur in grilled food. These include nitrosamines, acrylamide, 3-monochloropropan-1,2-diol (3-MCPD), anthraquinone (ATQ), cholesterol oxidation products, glycidyl esters, PAH-analogues (such as nitrogen and chlorine containing PAHs), and substances that are produced by the Maillard reaction. Below is a short description of the substances in grilled food identified from the literature search and an evaluation of whether it was considered if there was enough data to perform an assessment of concentrations in grilled versus fried food. In addition, a few substance groups were identified in connection with grilling, such as polyphenols (Uchida et al., 2017)), advanced glycation end-products (Du et al., 2023; Xu et al., 2024), pyrazines (Garcia-Lomillo et al., 2016; Wall et al., 2019) and biogenic amines (Iko Afé et al., 2021). They are not further described due to a limited amount of published work found on the subjects. The literature search was, however, not performed systematically. Hence, it is possible that some relevant process contaminants have not been identified or that publications about concentrations of process contaminants in grilled food have been missed.

4.1 Acrylamide

Acrylamide is regarded as a carcinogenic substance (classified as probably carcinogenic to humans, group 2A by IARC 1994 (IARC, 1994) and is formed in carbohydrate rich food during conditions of high temperature (above 120 °C) and low moisture content in the Maillard reaction from reducing sugars and amino acids. Acrylamide is primarily found in fried potato and potato crisps because of their high content of asparagine and starch, and in foodstuff such as biscuits and coffee (EFSA, 2015). No studies were found suggesting that formation of acrylamide occurs at a higher rate during grilling than during other high temperature heating methods. In two studies acrylamide was determined in grilled beef and poultry showing concentrations around 50-80 ng/g of acrylamide (Hassan et al., 2010; Kaplan et al., 2009). This is less than found in fried potato products, breakfast cereals and biscuits (EFSA, 2010). In a study by Hanley et al acrylamide was analysed in grilled potato slices and levels ranging from less than 100 to more than 1000 ng/g were found depending on cooking time and pre-treatment of the potato slices (Hanley et al., 2005). The concentrations found were similar to those reported by EFSA (EFSA, 2015). EFSA performed a risk assessment of acrylamide and concluded that the current levels of dietary exposure to acrylamide were not of concern with respect to non-neoplastic effects, such as neurotoxicity. In humans and experimental animals acrylamide is metabolized to glycidamide (GA), which is mutagenic and carcinogenic (EFSA, 2015, 2022). With respect to neoplastic effects based on animal evidence, EFSA concluded that the margins of exposure (MOEs) indicated a public health concern.

In the absence of identified evidence that acrylamide is formed to a larger extent in grilled food than in fried or smoked food, no further assessment of acrylamide in grilled food were performed.

4.2 Anthraquinone (ATQ)

Anthraquinone is an oxidized compound, a quinone, derived from the PAH anthracene. ATQ is classified in group 2B, possibly carcinogenic to humans by IARC 2011 and it is postulated that it can be present in food, after smoking or grilling (Zastrow et al., 2019). Substantial amounts of anthracene have been found in grilled food, particularly in food grilled on wood (Larsson et al., 1983; Zastrow et al., 2019). Zastrow et al (2019) measured maximum level of ATQ, anthracene and PAH4 of 0.5 ng/g, 1.3 ng/g and 6.7 ng/g in grilled frankfurter sausages) indicating a plausible relation between PAH formation and ATQ.

It is plausible that ATQ can be formed in higher concentration in grilled than in fried food. However, the available literature on ATQ in food is very limited and no assessment were, therefore, performed.

4.3 Cholesterol oxides (COPs)

It is suggested that thermal processing of food can induce formation of cholesterol oxidation products (COPs), such as 7-ketocholesterol, which might cause adverse cardiovascular effects. Various cholesterol oxides have, in a few studies, been analysed in grilled food, such as in pork and seafood, and an increased content seems to occur in comparison with that of raw food (Broncano et al., 2009; Freitas et al., 2015; Ohshima et al., 1995; Saldanha et al., 2008; Saldanha & Bragagnolo, 2010). Very little information is available on the significance of the findings of COPs in grilled food and it is not clear if grilling of food may result in more COPs than other heat-treating methods. The identified data on COP in food was limited and therefore no further assessment of COPs in grilled food were performed.

4.4 Heterocyclic aromatic amines (HAAs)

Heterocyclic aromatic amines (HAAs) are a diverse group of aromatic amines that can be formed during heat-treatment of food. HAAs were dealt with in detail, i.e., formation, factors impacting formation, chemical analysis, and toxicology, in the former VKM report from 2007 (VKM, 2007)

The HAAs are classified in two major groups. HAAs formed at temperatures from approximately 100 to 300 °C are categorized as thermic HAAs and amino-imidazo-azaarenes (AIAs), whereas HAAs formed at temperatures higher than 300 °C are categorized as pyrolytic HAAs and amino-carbolines (ACs). This categorization does, however, not imply that the pyrolytic HAAs are not formed in food treated at temperatures below 300 °C but refer to the dominating formation process. The formation of thermic HAAs is linked to the Maillard reaction. These HAAs are generated from the reaction of free amino acids, creatinine, and reducing sugars (Alaejos & Afonso, 2011). The Pyrolytic HAAs are formed during pyrolysis from small fragments of thermally decomposed molecules that condense to form new heterocyclic substances. The thermal and pyrolytic HAAs are also grouped into polar and nonpolar HAAs, respectively. The polar HAAs are grouped into the AIAs including imidazo-quinolines (IQs) and the imidazo-quinoxalines (IQx), whereas the major nonpolar HAAs are various forms of ACs. The most relevant polar HAAs in food are 2-amino-1-methyl-

6-phenylimidazo[4,5-b]-pyridine (PhIP), 2-amino-3-methyl-imidazo[4,5-f]-quinoline (IQ), 2-amino-3,4-dimethyl-imidazo[4,5-f]-quinoline (MeIQ), 2-amino-3-methyl-imidazo[4,5-f]-quinoxaline (IQx), and 2-amino-3,8-dimethyl-imidazo[4,5-f]-quinoxaline (MeIQx). The most relevant nonpolar HAAs are 1-methyl-9H-pyrido[3,4-b]indole (Harman) and 9H-pyrido[3,4-b]indole (Norharman). At least 30 different HAAs are described (Barzegar et al., 2019) and several of these are identified as mutagenic and carcinogenic in experimental animals and classified as possible human carcinogens. IQ is categorized by IARC as probably carcinogenic to humans (Group 2A), whereas PhIP, MeIQ and MeIQx are categorized as possibly carcinogenic to humans (Group 2B) ((IARC, 1993)). Harman and norharman are so-called co-mutagens, which implies that the substances are not mutagens by themself, but in the presence of a mutagen enhances mutagenic activity (Aaslyng et al., 2013). In mammals the HAAs are easily taken up from food and bioactivated by N-hydroxylation followed by esterification of the exocyclic amino group forming ultimate DNA reactive nitrenium ion species and DNA adducts. In rodents, HAAs are rapidly detoxified by ring-oxidation and conjugation, followed by excretion. It should be noted that rats and humans differ quite significantly in their toxicokinetics (biotransformation) of at least PhIP and MeIQx in that humans tend to bioactivate, while rats tend to detoxify these compounds ((Frandsen, 2008; Frandsen et al., 2002)). Hence, humans may be more susceptible to PhIP and MeIQx than rats. HAAs may bind to proteins and have strong affinity to eumelanin and may therefore accumulate in hair, which can be used as an exposure biomarker (Alexander et al., 2002; Bellamri et al., 2021).

4.4.1 Methods for HAAs analysis in food

For several reasons the accurate detection and quantification of HAAs is a difficult analytical task. HAAs show chemical and physical properties close to the surrounding food matrix and display large differences in polarity. In addition, these compounds are not stable and some HAAs might even bind chemically to other matrix components. Therefore, standard analytical extraction and clean-up methods cannot be applied without considerable modifications, testing, and optimizations. The analysis of HAAs follow the fundamental steps: (1) extraction, (2) separation and clean-up, and (3) identification and quantification. For extraction, separation and clean-up solid phase extraction, liquid-liquid extraction, supercritical fluid extraction, pressurized liquid extraction, and microwave-assisted extraction have been described (Barzegar et al., 2019). In addition, miniaturized and enhanced techniques are under development in order to reduce solvent volumes, save time, and to automize the lab work. For identification and quantification, different combinations of liquid chromatography and mass spectroscopy are normally applied (LC-MS, UPLC-HRMS, nanoUPLC-HRMS). Also, methods using molecular imprinted polymers have been used to enrich end analyse HAAs (Frandsen et al., 2002; Sun et al., 2023). Due to the thermo-labile nature of the HAAs gas chromatography is only applicable after derivatization of the HAAs and only some groups have published GC-MS methods.

Few laboratories conduct HAAs analyses. In addition, there are no regular international interlaboratory studies in relevant matrices. In total, the reported results do not show a high level of reliability, and concentrations obtained with different analytical methods and reported by the different research groups should not be compared without additional quality assessment (Alaejos & Afonso, 2011).

4.4.2 Summary

Many studies have investigated the content of HAAs in food and factors that are important for their formation. The most important factors are temperature and cooking time. The formation of AIA requires presence of creatine (i.e., muscle meat) and takes place following evaporation of water and increase in temperature and is associated with doneness. Some of the highest reported levels of HAAs are in grilled food, but it appears that also pan-fried meat can contain similar amount of HAAs as grilled meat (Alaejos & Afonso, 2011). Due to the high temperature that can develop during grilling and increased doneness in comparison with panfrying it is plausible that HAAs, in general, is found in higher concentrations in grilled than in fried food.

There was, however, observed large variations in the reported concentrations of HAAs in grilled food between the laboratories. Based on the abovementioned challenges in HAA analysis, it is imperative to do a thorough quality assessment of the published work to do a proper extraction of the concentration data for exposure assessment. This was not possible within the time frame of the project. To avoid misinterpretations due to possible bias between different analytical methods and groups, VKM decided to use the occurrence data to describe how HAAs vary with grill methods and types of food in a qualitative assessment. The comparison of HAA concentrations and the discussion of factors influencing the formation of HAAs is, therefore, solely based on results from single studies and not by pooled results from a multitude of studies with different data quality.

4.5 3-monochloropropan-1,2-diol (3-MCPD) and glycidyl esters

3-monochloropropanediol (3-MCPD) and its analogue 2-MCPD and glycidyl fatty acid esters in food are contaminants of processed edible oils used as foods or food ingredients. EFSA performed a survey of 3-MCPD in various food showing the highest levels in hydrolysed proteins and in animal- and vegetable fat, and oil products, with approximate concentrations around 1,000 ng/g (EFSA, 2013). Grains and grain-based products, fish and meat had concentrations around 20-50 ng/g. There was identified little information about concentrations of 3-MCPD in food subjected to high temperature cooking such as grilling. In a survey in the UK, grilled beefburgers had a mean level of 24 ng/g 3-MCPD, similar to cured fish and various cereal products (Hamlet et al, 2002). In the same survey it was also shown that various grilled cheese products and grilled bread slices had higher levels of 3-MCPD than the uncooked cheese and bread. A study by Schallschmidt et al., (2012) compared 3-MCPD formation in marinated, salted and unsalted steaks from pork neck prepared on an electric grill, gas grill and charcoal grill. In general, it appeared as the steaks prepared on the electrical grill had the lowest levels of 3-MCPD with contents ranging from less than 1 ng/g to the maximum value of 16 ng/g. The marinated steaks on the gas grill had higher concentrations of 3-MCPD (36-54 ng/g) than the salted and unsalted steaks (<1-11 ng/g). Despite the large variation in content, it appears that the marinated steaks in general had higher levels of 3-MCPD than both salted and unsalted steaks. This may be due to the oil in the marinade. One interesting observation in the study by Schallschmidt et al., was that grilling with a closed lid substantially increased the concentration of 3-MCPD in charcoal cooked marinated steaks (Schallschmidt et al., 2012). The contents of 3-MCPD in charcoal-grilled oil-marinated steaks were 36 and 65 ng/g, whereas oil-marinated steaks prepared with closed lid had concentrations in the range of 282-365 ng/g. It was postulated that the increased level was due to increased influence from smoke and a higher temperature. EFSA performed a risk assessment of 3-MCPD in 2016 with an update in 2018. In the updated 2018 opinion, EFSA established a group TDI of 2 μg/kg bw per day for 3-MCPD and its fatty acid esters (expressed as MCPD equivalents) (EFSA, 2018). It was concluded that the TDI was not exceeded by the adult population, whereas the TDI was slightly exceeded among the high consumers of vegetable fats and oils and cookies, in the younger age groups, and in infants receiving formula only. 3-MCPD and its analogues are primarily contaminants of processed edible oils used as foods or food ingredients and is regarded as a cause of concern. There was found little information about levels of 3-MCPD in food subjected to high temperature cooking such as grilling. It was not identified proper evidence that grilled food contains higher levels of 3-MCPD than similar food items subjected to high temperature cooking. High levels of 3-MCPD and its analogues in food and food marinades is most likely due to their content of processed edible oil. One study indicated that grilling of oil marinated meat with a closed lid may lead to increased levels of 3-MCPD, but this must be confirmed in future studies. Hence, no further assessment of 3-MCPD and its analogues in grilled food was performed.

4.6 Nitrosamines

Nitrosamines are a group of organic compounds that can be found in food due to reactions between nitrosating agents (NO_X) and deprotonated amines. Many nitrosamines are regarded as carcinogenic and genotoxic substances. In a recent risk assessment by EFSA it was concluded that 95% of the population regardless of age group is exposed to nitrosamines (sum of ten different nitrosamines and assuming equal potencies) at a level which implies an MOE less than 10,000. EFSA concluded that the dietary exposure to nitrosamines raises a health concern. The major source of nitrosamines exposure is from food, of which cured meat and meat added nitrite/nitrate as a preserving agent are the most important. There is evidence that cooking by high heat treatment, such as grilling and pan- and deep frying can induce formation of nitrosamines (EFSA, 2023). Some studies have investigated nitrosamine formation in grilled food showing that high temperature grilling may be a risk factor in the formation of various nitrosamines (Al-Kaseem et al., 2014; Lee, 2019; Yuan et al., 2014; Yurchenko & Mölder, 2007). In the study by Kocak et al., (2012) a small increase was observed in nitrosamine formation in meat from lamb with increasing cooking time. Only a few studies have compared formation of nitrosamines in grilled food with other high temperature processing methods. A study by Yurchenko and Molder (2007) observed no major differences in nitrosamine contents between smoked, grilled, or deep-fried Estonian meat products. A similar observation was done by Yuan et al (2015), who compared nitrosamine content in various smoked and grilled Chinese sausages. Al-Kaseem et al., (2014) found the highest nitrosamine contents in fried and smoked meat followed by grilled meat.

Nitrosamines are carcinogenic and genotoxic and can be formed by high temperature cooking. There was not identified substantial evidence that nitrosamines are formed to a larger extent in grilled food compared to fried or smoked food. No further assessment of nitrosamines in grilled food was therefore performed.

4.7 Polycyclic aromatic hydrocarbons (PAHs)

PAHs consist of a range of different compounds made of two or more fused aromatic rings that are formed during incomplete combustion or pyrolysis of organic materials by industrial processes, heating with wood, oil and coal, exhaust from combustion driven vehicles, tobacco smoke and by preparation of food (such as grilling and smoking). Natural sources of PAHs include volcanoes and wildfires (Gorshkov et al., 2021; Kozak et al., 2017). PAHs are always formed as mixtures of a range of substances of which only a few have been subjected to toxicological risk assessment (JECFA, 2006).

Sixteen PAHs were categorized by the EFSA CONTAM Panel (EFSA, 2008) as priority substances due to their potential genotoxicity and/or carcinogenicity in humans. These were BaA, BbF, Benzo[j]fluoranthene (BjF), Benzo[k]fluoranthene (BkF), Benzo[ghi]perylene (BghiP), BaP, Chry, Cyclopenta[cd]pyrene (CPP), Dibenz[a,h]anthracene (DBahA), Dibenzo[a,e]pyrene (DBaeP), Dibenzo[a,h]pyrene (DBahP), Dibenzo[a,i]pyrene (DBaiP), Dibenzo[a,l]pyrene (DBalP), Indeno[1,2,3-cd]pyrene (IP), 5-methylchrysene (MCH) and Benzo[c]fluorene (BcFl). BaP is categorized as carcinogenic to human (Group 1), CPP, DBahA, DBalP are categorised as probably carcinogenic to human (Group 2A) whereas BaA, BbF, Chry, BjF, BkF, DBahP, DBaiP, IP and MCH are categorized as possibly carcinogenic to humans (Group 2B) (IARC, 2010). The 16 priority substances differ somewhat from the sixteen priority PAHs of US-EPA, which, in addition to toxicity, also included as a criterium presence in the environment (Keith, 2015; Zelinkova & Wenzl, 2015). BaP is regarded as the most toxic of the PAHs and often used as a marker of PAHs in food The current legislation in EU uses BaP and PAH4 as indicators for PAH contamination in food (European Commission, 2023).

4.7.1 Methods for PAHs analysis in food

For the analysis of PAHs in food and environmental samples different analytical techniques have been developed during the past 40 years (Bansal et al., 2017). All these methods are based on the following three fundamental steps: (1) extraction, (2) separation and clean-up, and (3) identification and quantification. The extraction techniques for PAHs are selected and optimized based on the characteristics of the food matrices. Earlier, saponification with KOH-methanol, Soxhlet, and liquid-liquid extraction were normally used. In order to reduce solvent consumption, save time, and to automize the lab work, enhanced techniques, such as accelerated solvent extraction, supercritical fluid extraction, and solid-phase extraction have been introduced throughout the recent years. Depending on the type of food matrix and the selected extraction methods different methods for further separation and clean-up are applied. These methods can be either destructive like acid treatment or saponification or non-destructive like solid-phase extraction, column chromatography, and gel permeation chromatography, or liquid-liquid partitioning. Finally, also for identification and quantification several methods are in use either based on gas chromatography combined with mass spectrometry (GC-MS) or liquid chromatography either combined with fluorescence detection (LC-FLD) or mass spectrometry (LC-MS). Very recently, alternative methods like bio-detection (immunoassay and enzymatic assay) and electrochemical (amperometric/voltametric) detection have opened new possibilities with fewer steps of sample preparation. These latter techniques detect PAHs as a group of compounds with common chemical and toxic properties and cannot compete with the classical chromatographic methods, which allow identification of the individual PAH compounds.

Today, an optimized combination of sample preparation, clean-up, and quantification methods results normally in sufficient accuracy and analytical performance for PAH-quantification. Interference between chrysene and triphenylene in the chemical analysis, may however result in an overestimation of chrysene levels in food. Therefore, some caution must be taken when calculating PAH concentrations (EFSA 2008). In an European Interlaboratory study on smoked food the assigned values for most of the PAH compounds showed an expanded uncertainty of 20% or better (Donata et al., 2012). All participating laboratories obtained an instrumental sensitivity (LOQ) for the group of PAH4 sufficient to decide, if measured food was in compliance with the Commission Regulation (EC) No 333/2007 and amendments for PAHs in food.

4.7.2 Summary

There is an extensive literature on PAHs accumulation in grilled food, primarily in various types of grilled meat. Only a few studies have measured PAHs in grilled vegetables or cereal products such as bread. A comprehensive review by Duedahl-Olesen and Ionas summarized concentrations of PAHs in various food grilled by different types of heat sources (Duedahl-Olesen & Ionas, 2022). There is substantial evidence that grilled food contains higher levels of PAHs than similar food items subjected to high temperature cooking such as frying. Occurrence data were extracted from 81 primary studies. PAHs in grilled food was therefore included for further assessment.

4.8 Polycyclic aromatic hydrocarbon (PAH)-analogues

Some studies have shown that various analogues of PAHs, such as acridine derivates (also known as polycyclic aromatic nitrogen hydrocarbons (PANHs) or azaarenes, nitrated PAHs (nitro-PAHs), oxygenated PAHs (O-PAHs) and halogenated PAHs are detected in meat cooked at high temperature. It is reason to believe that these substances are formed in a similar way and concomitant with the PAHs during the cooking process. Reported levels of PANHs have been in the range of <1 ng/g to 3 ng/g (Janoszka, 2010; Rivera et al., 1996; Schlemitz & Pfannhauser, 1996) (Blaszczyk & Janoszka, 2008; Sniezek et al., 2022). Szterk (2015) compared the contents of PANHs in raw, fried and charcoal grilled pork and observed higher concentrations of PANHs in the grilled food (sum PANHs 6-8 ng/g) than in the fried (3-4 ng/g) (Szterk, 2015). No differences in the PANH contents were observed between the raw and fried meat. Qu et al., (2020) compared formation of various nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) in charcoal-grilled and fried food (Qu et al., 2020). In general, it was observed that the concentrations of the nitro-PAHs 1-Nitronaphthalene 1,8-Dinitropyrene and 1-Nitropyrene were higher in grilled food with a concentration range of approximately 1-12 ng/g. Also halogenated PAHs are found in grilled food at low concentrations (< 1 ng/g) (Li & Wu, 2023). Masuda et al (2019) observed higher levels of chlorinated-PAHs (Cl-PAHs) in gas-grilled than charcoal-grilled fish skin and beef rib, whereas the opposite was observed for the PAHs (Masuda et al., 2019). The apparent contradicting findings were explained by the high temperature of the gas flame compared with the lower far-infrared heat from the charcoal bed, which could generate conditions beneficial for Cl-

PAH generation. The Cl-PAH concentrations were low, less than 0.5 ng/g. To compare with Cl-PAH formation, Zastrow et al, (2022) analysed oxygenated-PAHs (O-PAHs) in beef patties and in vegetarian burgers and observed similar formation patterns as for the PAH, with higher levels in the fat rich beef patties and in charcoal grilled burgers compared to gas and electric grilled burgers (Zastrow et al., 2022). The concentration of sum of four O-PAH (benzo[a]anthracene-7,12-dione, 11H-benzo[b]fluorene-11-one, 6H-benzo[cd]pyren-6-one and naphthacene-5,12-dione), which was regarded as the most toxicological relevant, ranged from 0.5-11.1 ng/g, which was in the similar range as for the PAH4.

There are reasons to believe that PAH analogues, such as nitro-PAHs, O-PAHs and halogenated PAHs, can be formed in higher concentrations in grilled food compared with fried food due to the generation of smoke and pyrolysis in the cooking process. The available literature of these substances in food is limited but indicates that the concentrations are low or within the same range as the parent PAHs. No previous hazard characterization was identified for this group of compounds. No further assessment of PAH analogues in grilled food was, therefore, performed.

4.9 Summary of heat-induced contaminants identified in grilled food

Preparing food over charcoal, high temperature and campfire may lead to formation of several heat-induced process contaminants. Out of the substances described above, the ones identified as relevant for grilling of food include PAHs and PAH-analogues, HAAs, nitrosamines, 3-MCPD, acrylamide and anthraquinone. Whereas there is substantial evidence of higher concentrations of PAHs in grilled food compared to fried food, there is only identified plausible evidence for higher concentrations of PAH-analogues, as the mechanism of formation is presumed to be similar to that of the PAHs. HAAs are frequently reported in grilled food. As the temperature may be quite high when grilling, and grilling often implies a higher degree of doneness, it is plausible that HAAs are found in higher concentrations in grilled than in fried food. This may also be the case for other process contaminants, but there is too limited information, with respect to concentration data, to suggest that they occur in higher concentrations in grilled food compared with other types of heat-treated food. Based on the selection criteria above, concentration data of process contaminants in grilled food was only collected from the identified scientific papers for PAHs for which an exposure assessment were conducted as described below.

5 Previous hazard characterization of PAHs and HAAs

5.1 Previous hazard characterization of HAAs

In 2008 EFSA CONTAM Panel (EFSA, 2008) assessed the health risk associated with long-term consumption of PAHs in food. The critical effects of PAHs are carcinogenicity and mutagenicity. BaP is regarded as the most potent carcinogen of the PAHs and often used as a marker of PAHs in food. BaP is frequently found in pan fried, smoked and grilled food. Chrysene is the most frequently detected PAH in food, whereas BaA and BbF are usually found in higher concentrations than BaP (EFSA, 2008). The carcinogenicity was characterized using a study in mice exposed to a coal tar mixture or BaP alone (Culp et al., 1998). EFSA decided to use BaP, PAH2 (sum of BaP and Chry), PAH4 (the sum of BaP, BaA, Chry, and BbF), and PAH8 (sum of BaA, Chry, BbF, BaP, BkF, BghiP, DBahA, and IP) as indicators of total PAH exposure. Dose-response modelling was conducted and the derived $BMDL_{10}$s were as follows: for PAH8 it was 0.49 mg/kg bw per day; for PAH4, 0.34 mg/kg bw per day; for PAH2 0.17 mg/kg bw per day and for benzo[a]pyrene 0.07 mg/kg bw per day.

In the risk characterization EFSA used the $BMDL_{10}$s to calculate MOEs for the dietary exposure. The MOEs for the average consumers were 17,900 for benzo[a]pyrene, 15,900 for PAH2, 17,500 for PAH4 and 17 000 for PAH8. For high level consumers the MOEs were estimated to be close to or less than 10,000, which as described by the EFSA Scientific Committee indicates a potential concern for consumer health and a possible need for risk management action. The relevance for using BaP and PAH4 in the assessment was tested by the EFSA CONTAM Panel (EFSA, 2008). When possible, the EFSA CONTAM Panel concluded that PAH8 or PAH4 are better indicators of PAHs in food than BaP alone. It was, however, concluded that PAH8 did not provide much added value compared to PAH4.

5.2 Previous hazard characterization of HAAs

At least 30 different HAAs are described (Barzegar et al., 2019). Most HAAs are both mutagens and carcinogenic in experimental animals, i.e., mice and rats, and some has been classified as possible human carcinogens. IQ is categorized as probably carcinogenic to humans (Group 2A; (IARC, 1993)) by IARC, whereas PhIP, MeIQ and MeIQx are categorized as possibly carcinogenic to humans (Group 2B) (IARC, 1993). Harman and norharman are so-called co-mutagens (Aaslyng et al., 2013). Whilst IARC has conducted hazard identification of some HAAs, no hazard characterizations of HAAs from international risk assessment bodies such as WHO/ JECFA and EFSA or national food authorities were identified. In a study by O'Brien and co-workers a $BMDL_{10}$ for was derived for PhIP based on a study on colon tumours in rats (O'Brien et al., 2006). The lowest $BMDL_{10}$ was 1.25 mg/kg bw and day using a two-stage dose response model. In a later study (Carthew et al., 2010) based on studies in mice and rats $BMDL_{10}$ values derived for PhIP were 0.48 mg/kg/day for the prostate tumours, 0.74 mg/kg/day for mammary tumours and 2.71 mg/kg/day for colon tumours. As mentioned, due to toxicokinetic differences humans may be more susceptible to PhIP and MeIQx than rats.

6 Factors that may influence the content of PAHs and HAAs in grilled food

6.1 PAHs

The first reports about PAHs in grilled food were published in the 1960s in which Lijinsky & Shubik reported high levels of BaP in the outer part of charcoal grilled beef steaks (Lijinsky & Shubik, 1964). In a follow up experiment Lijinsky and Ross showed that melted fat dripping on the surface of the heat source was an important factor for the formation of PAHs that accumulated on the meat (Lijinsky & Ross, 1967). A range of later studies have investigated the content of PAHs in grilled food and factors that are important for the formation of PAHs in grilled food. These factors include type of fuel, direct or indirect grilling, type of meat, distance from the heat source and grilling temperature, grilling time, construction of the grill to avoid fat dripping directly onto on the heat source and grilling with or without lid. The following pathways for contamination of food with PAHs by grilling food were identified: (1) deposition on the food of fuel-related PAHs through the smoke (both in gas-phase and solid phase), (2) direct pyrolysis of food compounds due to the thermal treatment, and (3) pyrolysis/combustion of dripping fat onto the charcoal, into the flames, or onto other hot surfaces and subsequent transport by smoke onto the food item (Singh et al., 2023).

6.1.1 Type of grill and heat source

The main types of heat sources for grilling are electricity, gas or various types of charcoal and firewood. There are different types of charcoal, of which the most commonly used in Norway are regular lump charcoal and briquettes. Lump charcoal burns relatively fast with a high temperature, whereas briquettes burn slower with a more even temperature. Lump charcoals are made by burning wood at low oxygen. Briquettes are made by compressed charcoal, typically made from sawdust and other wood by-products, often with a binder and other additives (Jelonek et al., 2020). To add some extra smoke flavour to the grilled food it is also common to mix some wood chips with the charcoal.

In comparative studies it is, in general, observed that the PAHs levels in electric and gas grilled food are lower than in charcoal grilled food (Duedahl-Olesen & Ionas, 2022). In a systematic review Ghorbani et al., (2020) performed a meta-analysis showing that charcoal grilled meat had significantly higher concentrations of PAHs than meat subjected to gas grilling (Ghorbani et al., 2020). The difference between charcoal and gas was higher in red meat compared to white meat, which may be due to differences in fat content. Gas grilled food has, however, in some studies been shown to accumulate similar or even higher concentrations of PAHs than charcoal or briquette grilled food.

In an extensive study, Rose et al. compared formation of PAHs in various food items subjected to grilling by electricity, gas, and different types of charcoal, in addition to regular pan frying (Rose et al., 2015). As expected, the food grilled by electricity and pan fried food had very low levels of PAHs. With some exceptions, the differences in PAH levels between gas-, briquettes- and charcoal- grilled food were small. The highest concentrations were found in food grilled with charcoal mixed with wood chips, followed by charcoal-, gas grilled, and briquette-grilled food. In gas-grilled hamburgers the PAH concentrations were in the same range as briquette- and charcoal-grilled hamburgers (Table 3).

High concentrations of PAHs in gas-grilled food may be due to fat dripping directly onto the gas flame leading to pyrolysis and creation of PAH-containing smoke (Lee et al., 2016), see section 6.1.2. The lower concentrations in the briquette-grilled food compared to the charcoal-grilled food as observed by Rose et al (2015), may be attributed to the quality of the coal used in the experiment. It is reason to believe that the charcoal itself can be a major contributor to increased concentrations of some contaminants, such as PAHs, in grilled food (Badyda et al., 2017; Dyremark et al, 1995; Jelonek, Fabiańska, et al., 2021; Lee et al., 2016; Vicente et al., 2018). In a study by Oz , 2021 higher PAH concentrations were measured in briquette-grilled fish comparer to wood charcoal (Oz, 2021) . Use of wood chips together with the charcoal to achieve a more smoky flavour of the food may also contribute to increased levels of contaminants (Oz, 2020). Dyremark et al. measured considerable amounts of PAHs in the smoke emitted from the charcoal, both with and without meat patties on the grill (Dyremark et al, 1995). Badyda et al. measured PAHs in the emission from grills fuelled by gas, lump charcoal, and charcoal briquettes with and without food (Badyda et al., 2017). Highest levels pf PAHs were found in the smoke from the charcoal briquettes, whereas only small amounts of PAHs were emitted from the gas and lump-charcoal. Adding food on the grill increased the concentration of emitted PAHs from both the grills fuelled with lump-charcoal and charcoal briquette. Jelonek et al, (2021) measured the content of PAHs in 31 different commercial brands of charcoals observing large variations in the content of PAHs. The five ringed PAHs, such as BbF and ,BkFand BaP were found in the highest concentrations (Jelonek, Fabiańska, et al., 2021). The concentrations of BaP ranged between non-detectable to 893 ng/g with an average of 72 ng/g. In another study by Jelonek et al. it was shown that most of the tested charcoal products contained impurities exceeding the existing quality standard EN 1860-2:2005, which are directly correlated with the emission of particulate matter (Jelonek, Drobniak, et al., 2021) Lee et al investigated the concentrations of PAHs in various beef and pork meats grilled at different time points after the ignition of the charcoal (Lee et al., 2016). The concentrations were higher in meat grilled in the first period after lighting of the grill than the second and third period after lighting (see section 6.1.2). This implies that the charcoal should achieve a stable combustion before cooking in order to reduce PAHs exposure from the combustion source itself.

The highest concentrations of PAHs have been reported in food grilled on campfires with various types of wood. This is most likely due to smoke from incomplete combustion of the wood. Larsson et al., (1983) measured mean BaP level in sausages of 54 ng/g with max and min concentrations of 6 and 212 ng/g, respectively (Table 4) (Larsson et al., 1983). A study by Wiek and Tkacz (2017) measured mean concentrations of BaP between 12.6 and 18.7 ng/g and 50.8 and 73.5 ng/g of PAH4 in sausages roasted on campfires compared to 1.6 ng/g and 6.7 ng/g in sausages grilled on charcoal (Wiek 2017). Sausages grilled on charcoal mixed with wood chips had a mean concentration of 17 ng/g BaP and 50 ng/g PAH4 compared to 2.3 ng/g and 11 ng/g in sausages grilled on charcoal (Rose et al., 2015). Rey-Salgueiro et al grilled bread in the flame of an oak log fire and measured a concentration of

BaP of approximately 100 ng/g (Rey-Salgueiro et al., 2008). Bread that was wrapped in aluminium foil had a BaP concentration of less than 1 ng/g.

The charcoal and briquettes are primarily lighted by charcoal lighter fluids made of distillate fractions of petroleum. Proper use of the charcoal/briquettes implies that all the lighter fluid are burned before preparing the food. It was not the scope of the current assessment to evaluate potential hazards from the lighter fluids.

Table 3. Summary of the concentrations of BaP and PAH4 in various meats and fatty fish grilled on different heat sources and cooking methods by Rose et al., 2015. Data are presented as mean concentrations (ng/g wet weight) of seven grilled foods with minimum and maximum values in brackets.

	Electric	Gas	Charcoal	Charcoal + wood chips	Briquettes
Sausage					
BaP	< 0.03	0.78 (0.05-2.2)	2.3 (0.27-3.6)	17 (9.9-31)	0.95 (0.26-1.9)
PAH4	<LOD*	2.3 (0.18-6.3)	11 (3.0-16)	50 (28-84)	4.9 (3.2-6.8)
Hamburger					
BaP	< 0.03	11 (4.5-17)	19 (11-29)	17 (11-24)	6.4 (1.9-12)
PAH4	<LOD	28 (12-39)	70 (47-96)	60 (39-79)	26 (9.3-45)
Chicken					
BaP	< 0.02	0.20 (0.06-0.48)	0.35 (0.26-0.57)	0.85 (0.35-1.22)	0.14 (0.07-0.2)
PAH4	<LOD	0.62 (0.15-1.4)	6.1 (4.1-13)	7.9 (4.7-11)	1.7 (0.7-2.4)
Beef steak					
BaP	< 0.03	0.20 (0.07-0.40)	0.46 (0.23-0.92)	0.83 (0.17-2.1)	0.23 (0.16-0.37))
PAH4	<LOD	0.73 (0.30-1.3)	6.0 (3.6-9.8)	5.9 (2.3-11)	2.0 (0.98-3.2)
Pork**					
BaP	< 0.03	0.22 (< 0.09, 0.98)	1.13 (0.36, 1.9)	2.8 (0.25, 3.4)	0.22 (0.21, 0.23)
PAH4	<LOD	1.5 (<LOD, 3.0)	6.3 (4.5, 8.1)	7.8 (2.0, 14)	2.0 (1.8, 2.1)
Lamb**					
BaP	< 0.03	0.39 (0.18, 0.59)	1.5 (0.54, 2.4)	2.8 (2.5, 3.0)	0.45 (0.12, 0.71)
PAH4	<LOD	1.2 (0.58, 1.9)	9.0 (6.9, 11)	10 (9.4, 11)	2.6 (1.4, 3.8)
Salmon					
BaP	< 0.06	0.70 (0.22-1.9)	0.65 (0.38-1.0)	1.8 (0.27-4.5)	0.25 (0.09-0.50)
PAH4	<LOD	2.3 (0.87-6.1)	8.2 (4.9-13)	11 (3.3-24)	2.7 (1.1-4.4)

**LOD for each of the PAH4 ranged from 0.02-0,09, **n = 2*

Table 4. Summary of the concentration of BaP, BaA, Chr, BbF and PAH4 in sausages grilled using different heat sources (Larsson et al., 1983). Data are presented as mean concentrations ng/g wet weight with minimum and maximum values in parentheses.

Larson et al., 1983	BaP	BaA	Chr*	BbF	PAH4
Log fire (n=17)	54.2 (5.7-212)	44.5 (5.7-144)	44.1 (5.5-140)	22.3 (3.1-71.9)	165.1
Log fire embers (n=9)	7.7 (0.6-25.5)	10.8 (1.0-31.1)	15.1 (1.4-48)	6.2 (0.6-20.0)	39.8
Cone** fire (n=7)	17.6 (2.1-30.8)	16.7 (5.0-26.6)	16.8 (6.1-25.6)	8.3 (2.8-12.5)	59.4
Charcoal fire (n=13)	0.3 (nd***-0.3)	0.9 (0.2-3.9)	2.0 (0.4-6.3)	0.6 (nd-1.8)	3.8
Electric oven (n=2)	0.2 (0.1, 0.3)	0.8 (0.6, 1.0)	1.1 (0.9, 1.3)	0.6 (0.6, 0.7)	2.7
Frying pan (n=5)	0.1 (nd-0.2)	0.3 (0.2-0.5)	0.6 (0.3-1.2)	nd	1.0

* Reported as combined with triphenylene which may interfere chrysene in GC-analysis

**Spruce- and pinecones

***Detection limit for individual PAH was reported to be 0.1-0.3 ng/g

6.1.2 Pyrolysis of dripping fat

Pyrolysis is the heating of an organic material in an oxygen deprived environment. Probably more important than pyrolysis on the surface of the meat itself due to high temperature is PAH formation due to pyrolysis of melted fat from the meat dripping on the hot surface of the coal, into the gas flame, or on hot metal surfaces. The volatile PAHs that are formed return with the smoke and adhere on the surface of the meat. Lee et al, (2016) grilled pork and beef on three different grill apparatus of which one grill collected the melted fat in a beaker to avoid dripping on the hot charcoal and one grill diverted the smoke away from the grilled meat (Lee et al., 2016). The sums of PAH4 accumulated on the grilled pork and beef were reduced 48–89% with dripping removed and 41–74% with the smoke removal respectively compared to conventional grilling (Table 5).

Table 5 Summary of concentration of BaP, BaA, Chr, BbF and PAH in beef loin and pork belly grilled with different methods to avoid contact with smoke or dripping of fat (Lee et al., 2016). Data are presented as mean concentrations ng/g wet weight.

Lee et al., 2016	BaP	BaA	Chr	BbF	PAH4
Control - beef loin	3.23	3.62	3.80	6.25	16.91
Grilled just after coal lighting	5.07	6.96	7.14	9.54	28.7
Reduced smoke	1.58	1.17	0.20	1.60	4.55
Reduced dripping	0.78	0.60	0.27	0.81	2.46
Control - pork belly	5.76	3.09	4.15	8.77	21.77
Grilled just after coal lighting	5.99	10.27	7.97	8.93	33.17
Reduced smoke	1.48	1.48	1.31	1.42	5.69
Reduced dripping	0.66	0.40	0.54	0.78	2.38

Gas-grilled food has in some studies been shown to accumulate similar or even higher concentrations of PAH than charcoal or briquette grilled food (Gorji et al., 2016; Rose et al., 2015). This might be explained by huge differences in the quality of the charcoal/briquettes applied and the fact that gas grills are built with significant differences in their internal design, e.g. with or without lava stones or gas flame covers. One would expect to find lower concentrations of PAHs in food from a gas grill, as gas is a clean fuel without PAH impurities. In a gas grill there is a lower chance of pyrolysis of dripping fat directly into the hot gas flame compared to a bed of glowing charcoal. In a study by Farhadian et al., (2010), PAHs concentrations in gas grilled-dishes were found to be much lower when the gas-flame source was placed vertically to food items (Farhadian et al., 2010). It was concluded that the prevention of the fat dripping from meat into the flame was the main reason for the lower concentration of PAHs. However, substantial amounts of PAHs may be observed even in gas grilled meat. In the study by Rose et al., 2015, it was observed PAHs concentrations in gas grilled hamburgers that were higher than in the burgers grilled on briquettes. Reducing the access of air e.g. by grilling with closed lid may lead to incomplete combustion and pyrolysis of melted fat or facilitate PAHs formation from fuel combustion. A closed lid also prevents smoke from escaping, potentially causing smoke to accumulate within the grill, leading to a higher adsorption of PAHs onto the meat. Larsson et al. (1983) grilled meat in a propane flame at a temperature of approximately 600°C with a propane burner with both an open and closed air inlet, and measured BaP levels below 1 ng/g and 15 ng/g in the meat, respectively (Larsson et al., 1983).

6.1.3 Temperature

More heat-induced contaminants like the PAHs and HAAs are often formed with increasing temperature and proximity to the heat source. PAHs can be formed by direct pyrolysis and incomplete combustion of fat, protein, and carbohydrates in the food induced by high temperatures. Nor Hasyimah et al, investigated the formation of PAHs in beef satay grilled with a surface temperature from 150 to 350 ºC on a gas grill (Ahmad Kamal et al., 2018; Nor Hasyimah et al., 2022). The meat was grilled for 7 minutes in total, and the meat grilled at 300 ºC or 350 ºC had a clearly visible black tainted burned surface. The beef grilled at 300 ºC or higher had substantially higher levels of BaP and PAH4 than meat grilled at lower temperature (Table 6). It was, however, not stated in the article if the increased PAHs levels in the beef was due to pyrolytic processes on the surface of the meat or if it was due to smoke from fat dripping on the gas flames.

Table 6 Summary of concentration of BaP, BaA, Chr, BbF and PAH in beef grilled at different temperatures (Nor Hasyimah et al., 2018). Data are presented as mean concentrations (ng/g wet weight) ± standard deviation.

Nor Hasyimah et al., 2018	BaP	BaA	Chr	BbF	PAH4
150ºC	1.0 ± 0.1	0.6 ± 0.03	1.2 ± 0.2	0.4 ± 0.1	3.2
200ºC	3.0 ± 0.8	1.7 ± 0.1	1.9 ± 0.4	0.6 ± 0.01	7.1
250ºC	3.7 ± 0.3	2.7 ± 0.2	5.8 ± 0.3	0.8 ± 0.1	13.0
300ºC	5.5 ± 0.6	3.3 ± 0.2	11.5 ± 0.4	2.1 ± 0.1	22.4
350ºC	18.6 ± 1.2	4.6 ± 0.2	16.9 ± 0.8	3.6 ± 0.03	43.7

Proximity to the heat source seems to be an important factor for the pyrolytic generation of PAHs on the surface of the food. In the study by Rose et al. although inconclusive, proximity to the heat source appeared to be more important than cooking time for the formation of PAHs in the grilled food (Rose et al., 2015).

6.1.4 Type of food

There were relatively few studies, on PAHs in grilled food that reported fat content. It is, however, generally accepted that fat rich food is more prone to generate PAHs than lean food due to pyrolysis of melted fat from the meat. Most studies evaluating the importance of fat content have been performed on grilled hamburgers or beef patties of which all measured the highest concentrations of either sum PAH, BaP, or PAH4 in the patties with the highest fat content (Doremire et al., 1979; Duedahl-Olesen et al., 2015; Lijinsky & Ross, 1967; Maga, 1986). In well done charcoal grilled beef patties, Doremire et al (1979) measured a BaP concentration of 121 ng/g and 16 ng/g in patties with 39% fat and 15% fat respectively. Duedahl-Olesen et al., (2015) measured an average BaP concentration of 3 ng/g in hamburgers with 20.5 % fat and 0.1 ng/g BaP in hamburgers with 15.6 % fat. Several studies on PAHs in grilled chicken show higher concentrations in chicken grilled with skin than without skin (Chiang et al., 2020; Duedahl-Olesen et al., 2015; Gorji et al., 2016).

It is not clear if meat from specific animals is more likely to contain more PAHs than others after grilling. The measured variations in the PAH content reported in most of the published studies are very high and it appears that cooking method and fat content is more important than type of meat. Some grilled food with high fat content, such as sausages, may have lower PAH levels due to ingredients, such as flour, that will stabilize the fat and reduce fat dripping. Sausages are also sealed with a sausage skin that eventually may reduce the spread of melted fat to the source of the heat leading to less PAH formation.

The texture and surface area of the food may also have an impact on the PAH levels in the grilled food. It appears that grilled hamburgers and meat patties may be particularly prone to accumulate high amounts of PAHs (Doremire et al., 1979; Maga, 1986; Rose et al., 2015). In the study by Rose et al., (2015) grilled hamburgers had the highest PAH levels, regardless of heat source (Table 3). It was suggested that the high surface area, the rough surface texture, and relatively high fat content make the patties particularly prone to PAH accumulation. This may also imply that meat with a high surface area to mass ratio may contain higher levels of PAHs as previously shown (El Husseini et al., 2021).

Less information exists for other types of food besides meat. Oz et al investigated PAHs in four charcoal grilled fish species (Oz, 2021). Highest PAH concentrations were found in salmon and shad which had the highest fat content and strengthen the impression that high fat content is an important factor for PAH-formation in grilled food regardless of food type. Rey-Salgueiro et al., 2008 analysed PAHs in bread toasted by different grilling procedures (Rey-Salgueiro et al., 2008). Bread grilled on regular charcoal had a concentration of BaP of approximately 1.5 ng/g, which was attributed to contact with the combustion smoke from the charcoal. Bread has a texture that probably makes it susceptible to PAH accumulation. Bread grilled in the flames of oak wood had a BaP concentration of approximately 100 ng/g, whereas bread that was flame grilled wrapped in aluminium foil or grilled in a gas flame had a BaP concentration of less than 0.5 ng/g. This observation shows that direct contact with the combustion smoke may give rise to very high levels of PAHs in the food and in some food items probably more important than direct pyrolysis of food.

Cheng et al, (2019) grilled vegetables on an electrical grill at a temperature of approximately 300 °C and observed an increase in the PAH levels compared to raw vegetables despite a very low-fat content (Cheng et al., 2019). The mean concentration of BaP in the grilled potatoes was 0.61 ng/g and it was postulated that PAHs were formed by direct pyrolysis of the plant celluloses. The study by Cheng et al., (2019) shows that PAHs also can be formed in other food types than meat and fish and at a level comparable to lean meat, such as chicken filet.

6.1.5 Preparation of the food (e.g., doneness and cooking time, marination)

How the food is prepared is probably an important factor for the contamination of PAHs on the food and involves factors, such as cooking time, doneness, proximity to the heat source and design of the grilling apparatus. One may expect that increased cooking time may lead to higher PAH concentrations in the meat, particular in fat rich food, due to higher risk of more dripping of melted fat on the heat source and higher risk of burning the food. However, to avoid food from burning, increased cooking time will require a longer distance from the heat source or lower temperature, which may be followed by less risk of contamination. Relatively few studies have compared PAH levels in grilled food and doneness and cooking time, and their findings are mixed. In several of the experiments by Rose et al.,

(2015) the PAH levels decreased as a function of cooking time, which was considered a result of variations in the texture within the food items and how the food released fat during the grilling process. In a study by Oz and Yuzer (2016) increased doneness increased PAH concentrations in wire grilled beef steaks, whereas medium done stone grilled beef steek had higher PAH concentration than well done beef steak. Sumer & Oz investigated the effect of direct and indirect grilling and different cooking degrees (Sumer & Oz, 2023). Highest concentrations were detected in the well-done meat samples cooked by the direct grilling method. The lowest concentrations were found in the medium done meat that also was cooked by the direct grilling method, probably due to the shorter cooking time compared to the medium done meat cooked by the indirect method.

Several studies have also investigated how marination affect contamination of PAHs on the food. As the formation of PAHs involves the formation of free radicals, it is hypothesized that radical scavengers or antioxidants will reduce the formation and concentration of PAHs. Marination with ingredients containing high amounts of antioxidants like polyphenols has been reported to decrease concentrations PAHs in grilled food (Singh et al., 2023). In a study by Farhadian et al, different types of commercial and self-made marinades were tested, and meat samples treated with 1.2 % lemon juice had 70 % lower PAH concentrations than non-marinated meat (Farhadian et al., 2011). Also, the addition of sulphur-containing spices like onion and garlic seemed to reduce PAH formation (Wongmaneepratip et al., 2019). However, the identified data on marination shows that it may both decrease and increase PAHs in grilled foods. Some beer marinades are shown to have both a decreasing effect and an increasing effect on the PAH levels in grilled meat depending on the beer type (Viegas et al., 2014; Wang, 2019). Red wine marinades tended to increase PAH concentrations in pork neck steak (Tkacz et al., 2012), whereas wine vinegar tended to decrease the PAH concentrations in charcoal grilled pork loin (Cordeiro et al., 2020). Blank vinegar had no effect on the PAH concentrations. In a study by Nor Hasyimah et al (2022) it was observed an increase in the PAH concentrations in charcoal grilled honey spiced marinated beef satay (Nor Hasyimah et al., 2022).

In summary, marination is shown in several studies to reduce the levels of PAHs in grilled food, maybe because of a protective layer covering the meat or due to high amounts of antioxidants in the marinade. However, marination is also shown in some studies to increase the PAH levels in the grilled meat, which may be due increased pyrolysis of oils from the marinade or marinades that are more sensitive to get burned at high temperatures.

6.2 HAAs

Many studies have been performed to identify factors which affect the formation of HAAs. Variables like temperature, time, and method of cooking significantly affect the content of mutagenic compounds in cooked samples. It has been shown that the concentrations and the number of detected HAAs increase along with raising temperature (Alaejos & Afonso, 2011). Formation seems to increase upon evaporation of water. Different groups of HAAs dominate at different temperatures, the AIAs being formed at lower temperatures (>100-300°C) than the ACs (>300°C). Also longer cooking times and the doneness of the prepared meat are correlated to higher amounts of HAAs (Alaejos & Afonso, 2011). A recent review summarized different mitigation strategies for HAAs, which can be described by following actions: reduce HAA-precursors, optimize cooking and processing conditions, and use of exogenous additives by marination (Geng et al., 2023).

6.2.1 Precursors

It has frequently been shown that the amount of HAAs is strongly correlated with the type of meat or fish. The difference in HAAs concentration detected in different types of food is due to the different amounts of precursors. Precursors are substances commonly found in muscle tissues, such as creatine, reducing sugars, free amino acids, and some dipeptides (Knize et al, 2005; Alaejos & Afonso, 2011; Geng et al., 2023). For example, chicken breast often contains higher amounts of HAAs compared to chicken thighs, probably due to its higher protein level. Compared to fish, cooked meat products have higher levels of HAAs. Protein rich meat with creatine contains larger concentrations of HAAs compared to mixed meat products, such as burgers and sausages (Geng et al., 2023). By carefully choosing food types and ingredients, the formation of HAAs can be reduced. While both groups of HAAs require free amino acids, the AIAs are Maillard reaction products that also dependent on creatine and reducing sugars. Naturally occurring sugars in muscle come from glucose stored as glycogen, glucosamines from connective tissues and mono- and disaccharides. Creatine is mainly found in muscles. ACs form through pyrolytic free radical reactions between amino acids and proteins. It has been shown that transport of water-soluble precursors from the inner parts of the meat may enhance the formation of HAAs (Skog & Jägerstad, 2006). Also, the amount of fat may have an impact as some AIAs seem to occur at higher levels in fried lean beef patties than in fat ones (Skog & Jägerstad, 2006).

6.2.2 Cooking conditions

The levels of all detectable HAAs are shown to increase with increasing temperature (150 to 350° C) in grilled beef (Geng et al., 2023). To reduce formation of HAAs, it has been suggested to limit the thermal treatment of meat to temperatures below 180° C. In addition, processing time influences HAAs formation. By increasing the cooking time by 2.5 min at a temperature of 230 °C the concentration of HAAs increased by 50 % (Utyanov et al., 2020). Advanced cooking methods like infrared grilling or precooking the food with microwave, steaming or use of oven bags before a final short round on the grill showed also lower levels of HAAs (Geng et al., 2023). In an experiment using a double hot plate grill, increasing the temperature from 120 to 280 °C until the internal temperature reached 72 °C, caused a strong increase of HAAs at temperatures ≥260 °C (Polak et al., 2020). Charcoal grilling seems to produce the highest content of HAAs (Han et al., 2023; Jinap et al., 2013; Suleman et al., 2020), possibly due to the difficulties in controlling the temperature.

It is presumed that HAAs are normally formed in the crust of fried meat and fish and lower levels are found in the centre of the prepared product. The formation processes in the meat or fish surface might be reduced by frequently turning the meat during cooking to decrease the surface temperature (Alaejos & Afonso, 2011). As the formation of HAAs is associated with doneness, the appearance of a brown colour may be used as sign for limiting cooking time (Skog & Jägerstad, 2006). In a carefully controlled experiments with charcoal grilling of lamb meat, the HAAs formation was neglectable when grilling time did not exceed 14 minutes at 145 °C, however, the sum of measured HAAs content increased exponentially and reached 246.5 ng/g at 42 min (Han et al., 2023).

Two comparative studies using similar food items prepared by different cooking methods showed higher concentrations for most detectable HAAs when the food was grilled or barbecued compared to pan-frying (Iwasaki et al., 2010; Oz & Yuzer, 2016). Microwaving of beef and chicken prior to charcoal frying reduced total HAAs formation (sum of IQ, MeIQ, MeIQx, DiMeIQx and Phip) significantly, particularly when grilled well-done: 126.6 vs. 81.7ng/g for chicken and 140.7 vs 81.3 ng/g for beef (Jinap et al., 2013).It was suggested that the microwave treatment lead to less transport of precursors to the meat surface.

6.2.3 Marination

Marinating the food before cooking with different kind of plant extracts and phenolic compounds has in several studies been shown to reduce the levels of HAAs in the prepared food (see reviews by (Alaejos & Afonso, 2011; Geng et al., 2023; Olalekan Adeyeye & Ashaolu, 2021)), however the effect is dependent om the type of marinade (Alaejos and Afonso, 2011). Since the formation of HAAs is depending on free radicals and carbonyl compounds, it is presumed that this reduction is related the antioxidant potential of some polyphenols (Geng et al., 2023).

6.3 Summary of factors affecting formation of PAHs and HAAs in grilled food

The major factors affecting the formation of PAHs or HAAs are summarized and combined in Figure 4.

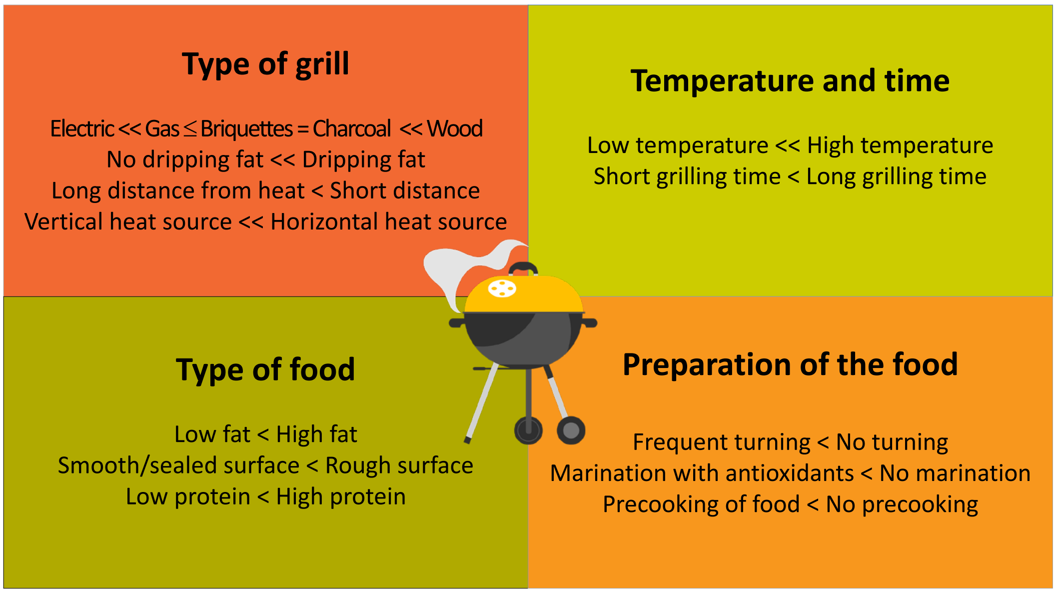

Figure 4. Overview of the factors influencing the formation of PAHs or HAAs (adapted from Duedahl-Olesen and Ionas, 2022).

7 Exposure to process contaminants from grilled food

7.1 Occurrence data

7.1.1 PAH concentrations in grilled food

PAH concentration data from 81 publications were extracted along with necessary metadata and compiled in an excel database (Annex 2 "Compiled data from papers") as described in 2.3.2.1. Of these 81 papers, 53 reported concentration data on all four PAHs included in PAH4. All 81 papers had concentration data on BaP. The occurrence data were mostly presented as mean ± SD, except for a few papers in which only medians were given, and mean and min-max values. Most of the articles reported LOQ and/or LOD.

During proofreading VKM discovered that one eligible publication (Wiek and Tkacz, 2017) on grilled sausages was accidentally left out of the compilation of the data. Unfortunately, it was not possible within the time frame of the project to include these data in the probabilistic modelling of the occurrence data. The possible significance of the missing paper is discussed in 7.1.1.1.

An overall comparison of PAHs generated with the different grill methods based on the extracted data was not made because of lack of information in the studies, and few studies compared PAH formation with different grilling methods. A general weakness in the identified published literature was the lack of detailed description of the food, such as fat content, cut of the meat, size of the grilled piece, and whether it was grilled with or without skin. Several studies also lacked information on the grill method, e.g., whether the food was grilled on charcoal or with gas or cooking time and doneness. PAHs generated from all types of grilling methods were, therefore, included in the data set.

Table 7 shows the number of reported BaP concentrations from the articles by grilling methods. Most data were reported on charcoal-grilled food, which constituted approximately 65% of the data set if briquettes and charcoal + wood chips are included. Although the proportion of the various grill methods used may vary within each food group, this indicates that the concentration data of PAHs were dominated by charcoal-grilled food.

PAHs were predominantly reported in various grilled meat. Fewer concentration data were reported for other types of food, such as grilled vegetables or grilled plant-based alternatives. As discussed in section 6.1, the concentrations of PAHs in grilled food depends on several factors of which fat content, heat source and temperature are the most important. Other factors of importance are food texture and high surface area to volume ratio of the grilled product and cooking time (Duedahl-Olesen & Ionas, 2022; Rose et al., 2015).

Table 7. Overview of number of observations of BaP in various grilled food using different grill methods.

Grill method	Number of observations*	Proportion of the total observations (%)
Charcoal	2354	58
Charcoal + wood chips	72	1.8
Briquettes	199	4.9
Gas	432	11
Electric	257	6.4
Campfire	52	1.3
Other unspecified**	673	17
Sum	4039	100

* Number of observations is the total number of single analyses of BaP that were compiled from the 81 included publications as described in the method chapter.
** Other unspecified include restaurant-grilled and home-grilled food, and use of disposable grills where type of fuel was not reported.

Table 8 shows the number of papers, number of observations, and the % of results < LOQ in the compiled data set. The PAHs concentration data from the studies were of varying completeness and quality. The approach applied for identifying the incidence of non-quantifiable sample results is conservative and the estimate of proportion of results below the LOQ is likely to be below the true proportion. There were only minor differences between LB, UB and simulated concentration values below the LOQ (appendix I, Figure 8). Hence, in the remainder of the report all data shown are the simulated values, including the values below the LOQ.

The occurrence data were very similar for the two methods (deterministic and probabilistic), with a distinct exception of sausages, which had higher mean occurrence with the probabilistic approach. Table 9 and 10 show the mean individual concentrations of the four PAHs estimated with a deterministic and probabilistic methodology for comparison, respectively. Table 11 shows the mean and percentile concentrations for BaP only, in grilled foods estimated by probabilistic methodology. A similar table for PAH4 is not shown as the sum of PAH4 was simulated using the distribution for each of the single compounds, with an assumed 100% correlation. The resulting distribution of the sum of PAH4 is expected to be wider than the true unknown distribution because the low percentiles of PAH4 would be underestimated while the high percentiles would be overestimated.

The data are further presented solely from the probabilistic approach in the main text. However, all results from the deterministic approach are available in the upper panel of Figure 10 (including campfire) and 11 (excluding campfire) in Appendix I and in Annex 4 "Grill occurrence summaries."

In general, the mean concentration data were highly influenced by grilled foods reported with high PAH levels. Most studies however, reported relatively low PAH levels, and median concentrations were less than 1 ng/g BaP for most of the food items. The lowest occurrence was found in plant-based products and vegetables. The highest concentrations of BaP and PAH4 were found in campfire grilled sausages, hamburgers and fatty pork (Figure 5). The high concentration shown in bread is based on only one study in campfire grilled bread and must be interpreted with caution (Rey-Salgueiro et al., 2008). Lean pork had higher relative concentration of chrysene compared to fatty pork (See fig and table 9, and tables in appendix), which implied that sum PAH4 were similar for these two food groups. Figure 5 shows graphically mean concentrations of each of the PAH4 substances and their relative contribution.

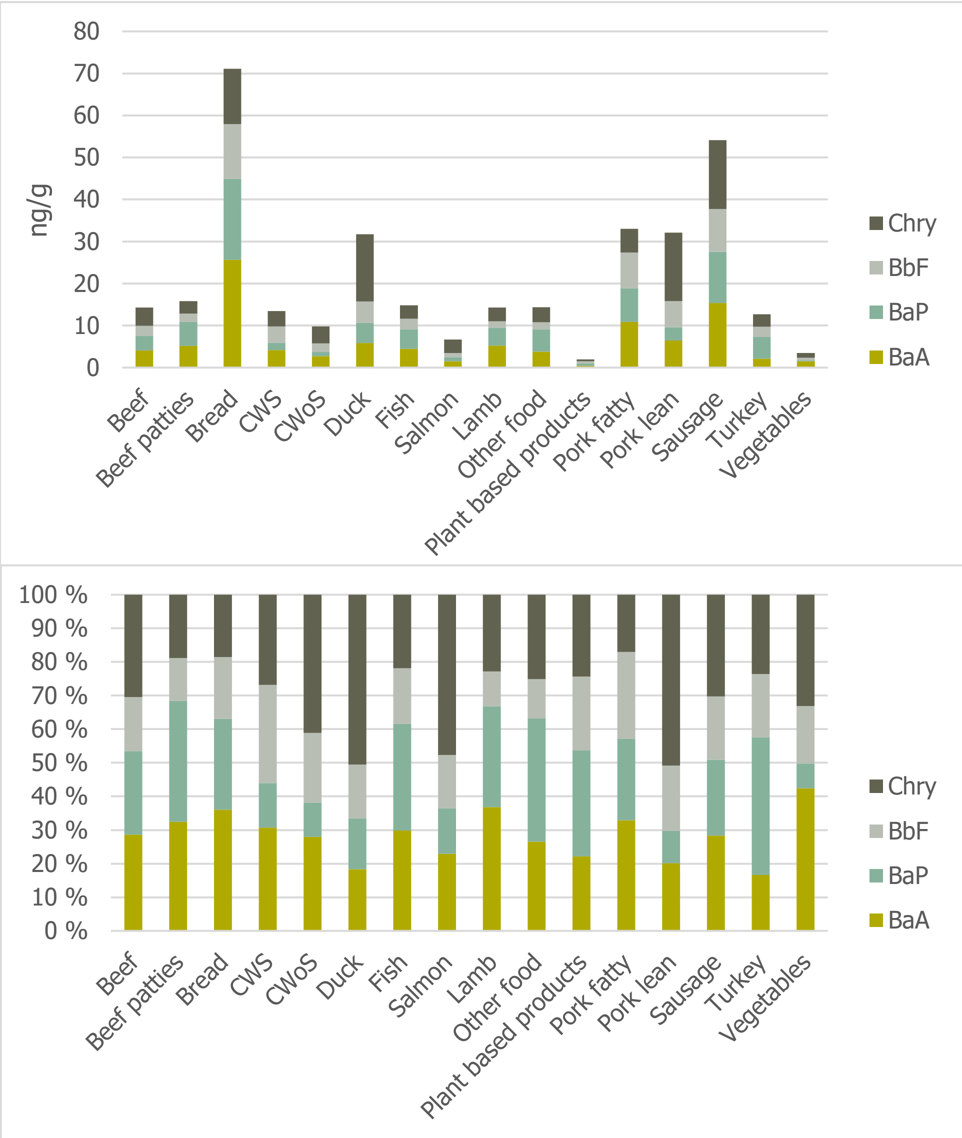

Figure 5. Concentrations in (ng/g) (upper panel) and percent distribution (lower panel) of BaA, BaP, BbF and Chry in 16 food categories.

*Table 8. Overview of number of papers, number of observations and proportion of observations in percent below LOQ for BaA, BaP, BbF and Chry in 16 different food groups *.*

Food name	No. of papers	No. of BaA results	BaA % <LOQ	BaA LOQ	No. of BaP results	BaP % <LOQ	BaP LOQ	No.of BbF results	BbF % <LOQ	BbF LOQ	No. of Chry results	Chry % <LOQ	Chry LOQ
Beef	38	609	13	0.10	961	21	0.25	703	22	0.20	585	20	0.33
Beef patties	17	292	12	0.08	417	21	0.15	255	18	0.19	290	21	0.12
Bread	1	12	25	0.10	12	0	0.07	12	50	0.25	12	50	0.75
Chicken with skin	15	480	12	0.62	656	24	0.27	561	19	0.99	480	18	1.13
Chicken without skin	15	245	10	0.34	370	32	0.26	281	14	0.19	245	9	0.26
Duck	3	19	16	0.26	19	16	0.35	19	37	0.52	19	16	0.56
Fish	9	72	1	0.38	113	21	0.38	90	13	0.38	72	0	0.38
Salmon	5	78	10	0.07	91	14	0.26	78	29	0.31	78	10	0.17
Lamb	12	80	24	0.07	123	39	1.1	80	35	0.08	48	17	0.16
Other food	7	89	22	2.0	140	31	2.0	89	35	2.0	57	19	2.0
Plant based products	2	72	12	0.25	72	10	0.25	72	26	0.19	72	12	0.25
Pork_fatty	23	421	19	0.26	614	30	0.22	415	28	0.28	421	29	0.27
Pork_lean	10	118	11	0.35	181	20	0.34	118	6	0.44	118	4	0.31
Sausage	10	109	24	0.14	184	42	0.22	104	30	0.24	109	16	0.34
Turkey	2	3	0	0.40	15	40	0.20	3	0	0.30	3	0	0.30
Vegetables	3	68	44	0.27	71	48	0.04	68	26	0.15	68	18	0.17

*In this report, BaA, BaP, BbF and Chry was extracted from the individual studies and not the Sum PAH4.

Table 9. Weighted mean PAH concentrations (ng/g) in the deterministic data by food group, for all grilling methods.

Food	BaA	BaP	BbF	Chry	PAH4
Beef	4.1	3.4	2.3	4.3	14
Beef patties	5.1	5.8	2.0	3.0	16
Bread	26	19	13	13	71
Chicken with skin	3.7	1.7	3.7	3.2	12
Chicken without skin	2.2	0.89	1.7	3.3	8.1
Duck	5.8	4.8	5.1	16	32
Fish	4.4	4.7	2.4	3.3	15
Salmon	1.5	0.90	1.1	3.2	6.6
Lamb	5.2	4.2	1.5	3.3	14
Other food	3.8	5.3	1.7	3.6	14
Plant based products	0.44	0.62	0.43	0.48	2.0
Pork fatty	8.8	7.2	7.2	2.9	26
Pork lean	6.5	3.0	6.3	16	32
Sausage	10	6.9	7.0	11	35
Turkey	2.1	5.1	2.4	3.0	13
Vegetables	1.5	0.26	0.60	1.2	3.5

Table 10. Weighted mean concentrations of PAH by food group, for all grilling methods in the simulated model. Data are given in ng/g.

Food	BaA	BaP	BbF	Chry	PAH4
Beef	4.1	3.5	2.3	4.3	14
Beef patties	5.1	5.7	2.0	3.0	16
Bread	26	19	13	13	71
Chicken with skin	4.1	1.8	3.9	3.6	13
Chicken without skin	2.7	1.0	2.0	4.0	9.8
Duck	5.8	4.8	5.1	16	32
Fish (other than salmon)	4.4	4.7	2.5	3.3	15
Salmon	1.5	0.90	1.1	3.2	6.6
Lamb	5.2	4.3	1.5	3.3	14
Other food	3.8	5.3	1.7	3.6	14
Plant based products	0.43	0.62	0.43	0.48	2.0
Pork fatty	11	8.0	8.5	5.6	33
Pork lean	6.5	3.1	6.3	16	32
Sausage	15	12	10	16	54
Turkey	2.1	5.2	2.4	3.0	13
Vegetables	1.5	0.26	0.60	1.2	3.5

The percentiles of BaP in the food groups are shown in Table 11 and illustrated as percentile plot in figure 12, appendix I. Similar tables and figures with results for BaA, BfF and Chry are shown in appendix I (section 11.1.3 simulated occurrence data).

Table 11. Simulated concentration of BaP (ng/g) given as mean and percentiles 5/10/25/50/90/95 values.

Food	Mean	P5	P10	P25	P50	P75	P90	P95
Beef	3.5	0.02	0.04	0.17	0.69	3.4	9.0	20
Beef patties	5.7	0.02	0.04	0.15	1.0	2.2	16	26
Bread	19	0.20	0.20	0.24	0.88	20	75	75
CWS - chicken with skin	1.8	0.02	0.05	0.20	0.92	2.2	3.8	5.2
CWoS – chicken without skin	1.0	0.04	0.08	0.19	0.54	1.4	2.5	3.2
Duck	4.8	0.15	0.17	2.9	4.9	8.3	8.8	9.6
Fish (other than salmon)	4.7	0.04	0.07	0.28	1.1	4.4	10	31
Salmon	0.90	0.02	0.04	0.17	0.40	1.1	1.9	4.5
Lamb	4.3	0.004	0.02	0.18	1.4	3.3	4.8	7.3
Other food	5.3	0.10	0.23	0.74	2.0	5.3	24	24
Plant based products	0.62	0.06	0.12	0.28	0.56	0.89	1.1	1.2
Pork fatty	8.0	0.01	0.03	0.17	0.86	5.3	11	43
Pork lean	3.1	0.01	0.06	0.29	0.88	2.2	3.9	23
Sausage	12	0.01	0.02	0.05	0.21	2.1	24	100
Turkey	5.2	0.02	0.05	0.13	1.0	12	14	15
Vegetables	0.26	0.01	0.02	0.05	0.11	0.23	0.60	1.1

7.1.1.1 Impact of grilling on campfire

Given the difference in mean concentrations in sausages with deterministic and probabilistic approach and based on the observation from one study by Larsson et al. (1983) which reported particularly high concentrations of PAHs in campfire grilled sausages, a sensitivity analysis was performed in which campfire grilled food from the overall data set was excluded. In the compiled dataset there are four food categories with data on campfire grilled food: sausages, beef patties, chicken with skin and bread. In particular, about 18% of the total included observations of sausages in the dataset were campfire grilled and were compiled from one study out of ten (Larsson et al., 1983). This analysis showed that grilling on campfire was associated with a substantial increase in PAHs in sausages and in bread (Tables 12 and 13). The mean simulated concentration of BaP in sausages was 1.1 ng/g without campfire grilling, and 12 ng/g with campfire grilling. Data on bread were, however, from only one study and must be interpreted with caution (Rey-Salgueiro et al., 2008).

As indicated above, VKM found that one study (Wiek and Tkacz, 2017) accidentally was left out from the compilation of studies. The study showed that campfire grilled sausages had substantially higher concentrations of PAH than sausages grilled on briquettes. This is in line with findings previously reported by Larsson et al. (1983). VKM found that inclusion of these data has only minor effect on the weighted mean PAH concentrations in sausages when assessed deterministically. VKM assumes that the impact on probabilistic occurrence data would also be small. A comparison between weighted mean with and without the data from Wiek and Tkacz (2017) is shown in Appendix I, table 40.

Table 12. Simulated distribution of BaP concentrations (ng/g) in foods with and without campfire grilling data showing mean and the percentiles 5/10/25/50/90/95.

Food	Mean	P5	P10	P25	P50	P75	P90	P95
Beef patties campfires excluded	5.3	0.02	0.04	0.14	0.94	2.1	14	24
Beef patties campfire included	5.7	0.02	0.04	0.15	0.96	2.2	16	26
Bread campfires excluded	0.65	0.20	0.20	0.20	0.25	1.5	1.5	1.5
Bread campfire included	19	0.20	0.20	0.24	0.88	20	75	75
CWS campfires excluded	1.8	0.02	0.05	0.20	0.93	2.2	3.8	5.2
CWS campfire included	1.8	0.02	0.05	0.20	0.92	2.2	3.8	5.2
Sausages campfires excluded	1.1	0.01	0.013	0.03	0.12	0.43	2.0	3.2
Sausages campfire included	12	0.01	0.02	0.05	0.21	2.1	24	100

Table 13. Simulated mean PAH concentrations (ng/g) in beef, bread, chicken with skin and sausages including and excluding data generated by grilling on campfire.

Food	BaA	BaP	BbF	Chry	PAH4
Beef patties campfires excluded	4.7	5.3	1.6	2.3	14
Beef patties campfire included	5.1	5.7	2.0	3.0	16
Bread campfires excluded	0.85	0.65	0.75	0.92	3.2
Bread campfire included	26	19	13	13	71
CWS campfires excluded	4.1	1.8	4.0	3.7	14
CWS campfire included	4.1	1.8	3.9	3.6	13
Sausages campfires excluded	1.8	1.1	1.4	2.9	7.2
Sausages campfire included	15	12	10	16	54

7.1.2 HAAs occurrence in grilled food

The occurrence of HAAs in various heat-treated food items was reviewed in 2007 (VKM 2007). The levels varied greatly, with higher concentrations in pan fried and grilled meat than in grilled fish. For PhIP and MeIQx it was reported a concentration of 480 ng/g and 7 ng/g respectively in very well-done grilled chicken although most referred studies reported lower concentrations. The content was shown to vary according to broiling- or grilling method, time, and temperature.

Since then, more data have become available on the occurrence related to cooking temperature and time, and other factors that affect the HAA content of various food items (Alaejos & Afonso, 2011, Geng et al., 2023). In the review by Alaejos & Afonso (2011) including data up to 2009 most of the publications were published prior to 2007. Here the chemical structures of 21 AIAs and 12 ACs were described. The most commonly HAAs determined in cooked food in this and later publications (Han et al., 2023; Jinap et al., 2013; Polak et al., 2020; Suleman et al., 2020; Wang et al., 2021) were PhIP, IFP, 8-MeIQx, 4,8-MeIQx, 7,8-MeIQx, IQx, IQ and MeIQ among the AIAs, and AαC, MeAαC, Glu-P-1, Glu-P-2, Harman, Norharman, Trp-P1 and Trp-P2 among the ACs. Much less or no data exist for AIAs, such as 1,5,6-TMIP, 3,5,6-TMIP, 4-CH2OH-8-MeIQx, and for ACs such as Phe-P-1, Orn-P-1, Cre-P-1, and Lys-P-1. Highest concentrations in cooked food are reported for Phip, MelQ, MelQx, DiMelQx, Harman and Norharman. As discussed in section (4.4.1) about analysis of HAA the reported occurrence data should be subjected to a thorough quality assessment, which was not possible within the time frame of the project. Therefore, VKM made a judgment that it was not possible to do an adequate assessment of exposure based on the published occurrence data.

7.2 Grill habits and consumption of grilled food

7.2.1 Grill habits in Norway

In 2006 (VKM report 2007), 53% of the respondents reported to grill more than eight times during the season, which was considered to be from May to August. It is however reasons to believe that many also grill food in April and September. In a similar survey by Matprat in 2022, 65% of the respondents reported to grill more than 12 times per season, 34% reported grilling 12-18 times per season, 26% grilled 24-48 times per season and 5% reported grilling more than 72 times per season (MatPrat, 2022). Ten percent reported that they never grilled, 21% grilled less than 12 times per season and four percent reported that they did not know how often they had grilled during the season (illustrated in figure 6). Preliminary data from the pilot to the on-going national dietary survey "Norkost 4" show similar findings about the grilling frequency among the participants as reported by MatPrat. Grilling food on campfire and campfire pans, in particular sausages and occasionally so-called "bread on a stick", is quite common in Norway. In a pilot study approximately 5% of the respondents reported that they grilled on campfire more than 13 times during a season (2023 Norkost pilot, preliminary results).

In the survey by MatPrat from 2022, 71% of the respondents reported that they had grilled sausages, 63% had grilled pork chops and 33% had grilled fish. Further, 63% reported grilling hamburgers, 40% had grilled chicken and 42% had grilled vegetables. In a similar study used in the VKM report from 2006 grilling of sausages was reported by 82% of the respondents, grilling of pork chops by 66%, grilling of steaks by 54% and 47% reported grilling of fish of the respondents (VKM, 2007). The survey in 2022 included 19 different food items and cannot be directly compared to the numbers given in 2006. However, the survey indicates that a wide range of foods are used for grilling (Table 14). Even though the majority responded that they grill sausages and pork chop it is not a measure of how frequently they consumed grilled food.

In a national representative survey commissioned by Rema 1000 (Norwegian grocery store chain) including 1001 participants from March 2023, 70% of the respondents reported that they preferred to grill meat as main course instead of fish, cheese or vegetables (YouGov for REMA1000) (https://www.rema.no/nyheter/nordmenn-elsker-aa-grille/).

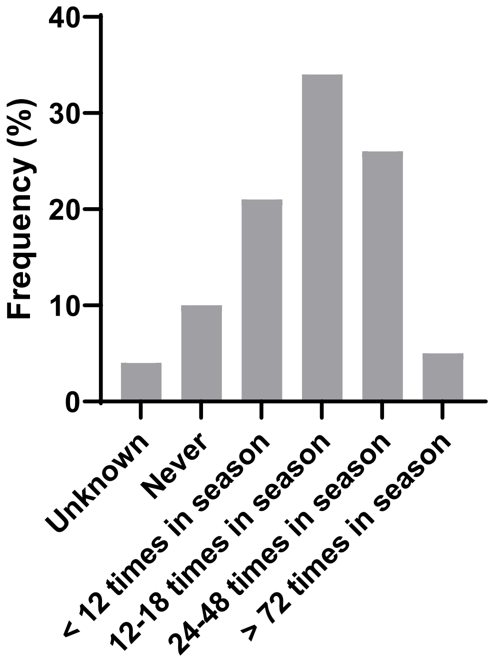

Figure 6. Distribution of grilling frequency during the prime grilling season, spring, summer and autumn (Source: Survey by Matprat, 2022).

Table 14. Food items grilled by survey respondents (Source: Survey by MatPrat, 2022, 1,008 respondents).

Food	Respondents (%)	Food	Respondents (%)
Grill sausages	71	Chicken thighs	19
Burger	63	Lamb	15
Pork chops	46	Beef fillet	13
Vegetables	42	White fish	12
Chicken fillet	40	Vegetarian products	11
Marinated meat	40	Turkey	6
Special sausages	39	Blue mussel/Shellfish	5
Beef steak	34	Reindeer	2
Pork steak	31	Other	3
Red fish	22		

7.2.2 Consumption of grilled food

The Norwegian national food consumption surveys do not include complete data on food preparation and grilling. The abovementioned percentage values of grilled food indicate which foods are grilled, not how often they are grilled. This makes it challenging to estimate consumption of grilled food. In the VKM 2007 report, the risk assessment of PAHs in grilled food was based on intake data from Rohrmann (Rohrmann et al., 2002). The estimated average dietary intake of grilled food amongst the (female) Norwegian participants in this study was 3.5 g/day. Only 4.5% (n=118) of the meals eaten were reported as grilled (oven grilled, electric or gas grill), while 2.1% of the meals were charcoal grilled (n=53). However, these data are old and probably outdated, confined to women and from a very small and not necessarily representative population. Due to the absence of consumption data it was decided to use consumption scenarios combined with the available estimated PAH occurrence data.

7.2.3 Scenarios used for consumption of grilled food in this study.

As described in the protocol and in the absence of consumption data it was decided to use consumption scenarios combined with the available PAH occurrence data. The absence of up-to-date data on the consumption of grilled food and insufficient information on types of food used for grilling, makes a dietary assessment imprecise.

Two different scenario food plates were constructed to illustrate possible consequences of different food preferences on the intake of PAHs from grilled foods. The composition of the two scenario plates was intended to represent different dietary habits. Several types of foods can be grilled, however, based on the available surveys on grill habits described above, grilling meat and various meat products are most likely the grilled food groups that the majority consume. In addition, fish and particularly salmon, is frequently grilled. It was decided to make one scenario food plate with fat rich meat including pork ribs, hamburger, sausages, and chicken with skin. The other scenario food plate contained lean meat (beef steak, pork fillet, chicken fillet) and salmon. The first scenario with fat-rich grilled meat is suspected to contain higher levels of PAHs than the second scenario with lean meat and salmon. Each of the plates is meant to represent one dinner serving of grilled food with a portion size of 200g. The two scenario plates are described in detail in section 2.3.1.1.

By using these two different plates, neither the consumption of grilled vegetables or plant-based meat substitutes nor grilled bread were included. Not many articles were found that measured PAHs in grilled vegetables or plant-based food or bread, which means that the concentration of PAH in such food is largely unknown. The few collected occurrence data showed, however, that the concentrations in grilled vegetables and plant-based product were in the lower concentration range, and will therefore probably contribute only marginally to the total PAH exposure. One paper reported high PAH concentrations in campfire grilled bread, and low concentration in campfire grilled bread wrapped in aluminium foil, indicating that smoke from the fuel may contaminate the bread with PAHs (Rey-Salgueiro et al., 2008).

7.3 Dietary exposure to PAHs

7.3.1 Probabilistic estimate of PAH content in the two scenario plates

The content of PAH4 and BaP from one dinner plate with 200 grams of grilled food from the two scenarios using the probabilistic approach are shown in Tables 15 and 16. The concentration data of PAHs included data from all types of grilling methods, including campfire grilled food, as described in section 7.1.1 and the content per plate described in Figure 7. The lower percentiles can indicate PAH content in a plate of food that is gently grilled or grilled on electrical grills where it is expected that minimal amounts of PAHs are produced. The higher percentiles may correspond to situations where the food is grilled using methods known to increase formation of PAHs in the food, such as high temperature grilling with charcoal or even campfire for a longer grill time leading to well done food.

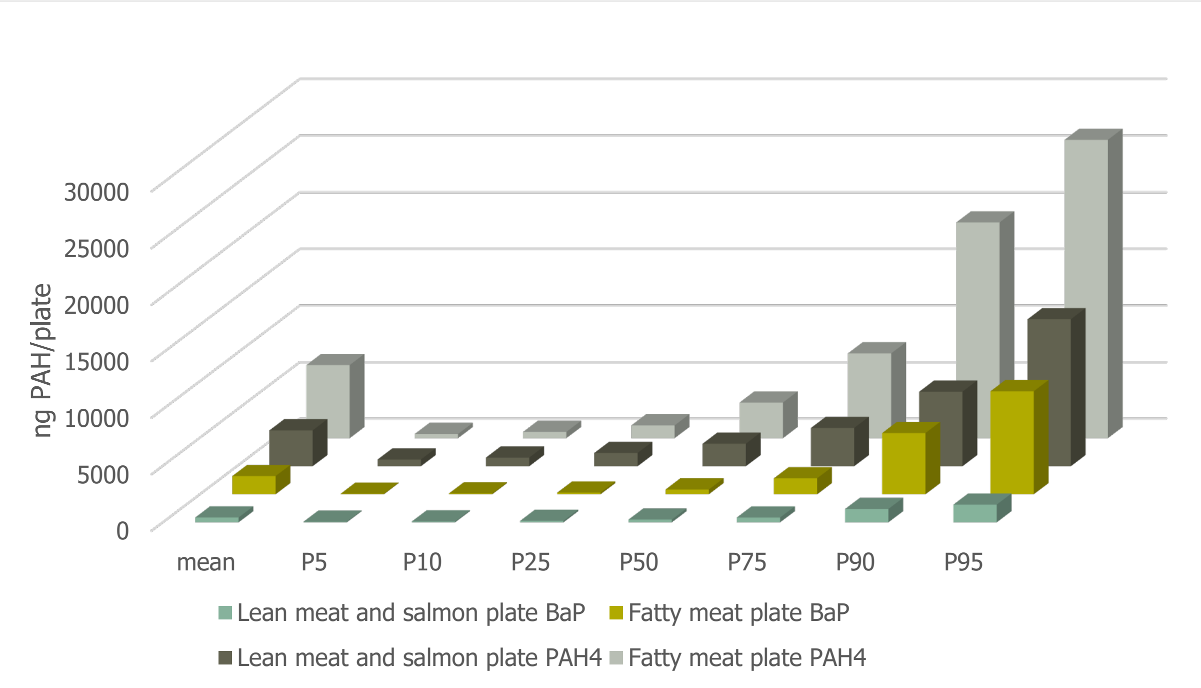

Figure 7. PAH content per plate. The x axis shows mean and percentiles of BaP and PAH4 content for the fat rich meat plate and lean meat and salmon plate (Y-axis, ng/plate).

Table 15 shows the mean BaP, mean PAH4 and percentile contents per plate, used in the scenarios. The results indicate higher content of PAH in the fatty plate than the plate with lean meat and salmon. Table 16 shows the contribution of the various grilled food on the scenario plates to the amount of BaP and PAH4 per plate. The data indicate that PAH from grilled sausages and fat rich pork, followed by beef patties contributed the most to the amount of PAH on the scenario plates.

Table 15. Simulated content of BaP and PAH4 in the two scenario plates.

BaP (ng/plate)	**mean**	**P5**	**P10**	**P25**	**P50**	**P75**	**P90**	**P95**
Fatty meat plate	1610	46	73	145	424	1412	5417	9117
95% CI	1464-1703	39-53	66-81	133-158	387-465	1288-1575	3983-6443	8177-9883
Lean meat and salmon plate	429	54	76	131	241	423	1181	1567
95% CI	402-459	49-59	70-81	123-140	227-254	398-456	1115-1270	1445-1685
PAH4 (ng/plate)	**mean**	**P5**	**P10**	**P25**	**P50**	**P75**	**P90**	**P95**
Fatty meat plate	6469	367	550	1132	3153	7502	19065	26356
95% CI	6069-6842	324-415	484-601	1050-1265	2879-3399	6910-8200	17070-21511	24752-28803
Lean meat and salmon plate	3170	597	767	1172	1992	3384	6596	13001
95% CI	3013-3331	543-636	731-812	1119-1234	1904-2117	3213-3620	5859-7296	9345-14010

Table 16. Mean simulated content of BaP and PAH4 distributed by food item on the two scenario plates.

Fat rich meat plate	**Food amount per plate (g)**	**Mean BaP (ng)**	**Mean PAH4 (ng)**
Chicken with skin	20	36	269
Beef patties	60	342	950
Pork, fatty	60	483	1989
Sausage	60	737	3249
SUM	200	1610	6468
Lean meat and salmon plate	**Food amount per plate (g)**	**Mean BaP (ng)**	**Mean PAH4 (ng)**
Beef	50	179	720
Chicken without skin	50	50	495
Salmon	50	45	334
Pork, lean	50	156	1627
SUM	200	429	3170

CWS: chicken with skin; CWoS: chicken without skin

7.3.2 Impact of grilling on campfire on the PAH content in the scenario plates

None of the foods with data from grilling on campfire (see 7.1.1) were part of the lean meat and salmon plate but were present on the fat rich plate. Excluding campfire grilled food from the fat rich plate had a substantial impact on the mean, median and higher percentiles content for both PAH4 and BaP (Tables 17, 18 and figure 8). Notably, sausages contributed substantially to the sum PAH4 when campfire was included. By excluding campfire grilled food from the fat rich plate, the amount of PAH4 on the plate was similar to the amount of PAH4 on the plate with lean meat and salmon. The levelling out between the two plates may partly be explained by the higher estimated concentration of chrysene in the pork that was categorized as lean (see section 7.1.1). The concentration of BaP in the plate with fat rich meat was still higher than in the plate with lean meat and salmon when excluding campfire grilled food from the concentration data.

Table 17. Content of PAH4 and BaP (ng/plate) in the fat-rich plate showing the effect of inclusion of campfire grilled results.

	mean	**P5**	**P10**	**P25**	**P50**	**P75**	**P90**	**P95**
PAH4, campfire excluded	3529	305	435	828	1788	4252	7839	13194
PAH4, campfire included	6469	367	550	1132	3153	7502	19065	26356
BaP, campfire excluded	904	40	64	120	277	727	1964	3854
BaP, campfire included	1610	46	73	145	424	1412	5417	9117

Table 18. Mean simulated content of BaP and PAH4 distributed by food item on the scenario plate with fat rich meat, including and excluding food grilled on campfire.

Fat rich meat plate	Plate (g)	Campfire excluded		Campfire included	
		Mean BaP (ng)	Mean PAH4 (ng)	Mean BaP (ng)	Mean PAH4 (ng)
CWS	20	36	271	36	269
Beef patties	60	317	827	342	950
Pork, fatty	60	483	1988	483	1989
Sausage	60	66	433	737	3249
SUM	200	904	3529	1610	6468

CWS: chicken with skin

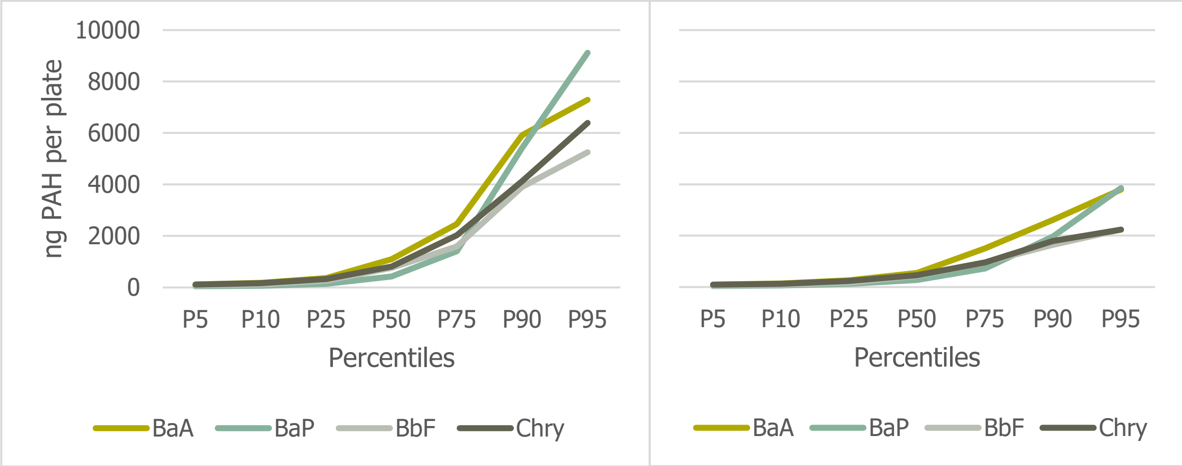

Figure 8. Distribution of BaA, BaP, BbF and Chry for the fatty meat scenario plate with campfire grilled food included (left) and without campfire grilled food (right).

The content of the individual PAHs had a quite wide range from percentile 5 to 95 as illustrated in Figure 8. The concentrations of the four PAHs are clearly higher when the campfire grilled food is included in the occurrence data, in particular above the median.

7.3.3 Exposure to PAH from the background diet

Several attempts made to estimate background dietary exposure to PAHs have shown large variations. A summary of assessments of dietary intake of PAHs is shown in Table 19.

In a Norwegian context, dietary BaP intakes has been estimated in the Norwegian Mother, Father and Child cohort study (MoBa). The women were recruited from all over Norway during 1999-2008 and included 50 651 pregnant women. The estimated mean ± SD dietary BaP exposure was 149 ± 48 ng/day (Duarte-Salles et al 2013). The intake was based on total diet consumption using a semi-quantitative food frequency questionnaire (FFQ) in combination with a database on BaP content in food prepared for this study (Duarte-Salles et al., 2010). The database compiled available mean BaP concentration data of food items in the FFQ. Milk and yoghurt, cereals, fruits, sweets and meat contributed 59% of the total BaP exposure intake. Non-alcoholic beverages, vegetables, fats and oils, snacks, fish and other foods contributed 41% of the total BaP exposure. Although this MoBa study did not include specific information on the intake of grilled food, it gives a reasonable estimate of the total exposure to BaP in a large group of women.

In a similar study from Spain, it was estimated a mean dietary exposure of 181 ng/day among pregnant non-smoking women (Duarte-Salles et al., 2010). The Joint FAO/WHO Expert Committee on Food Additives ((JECFA, 2006)) estimated a mean intake of 4 ng/kg bw per day corresponding to a daily intake of 280 ng with a body weight of 70 kg. EFSA calculated a median intake of 3.9 ng/kg bw and 19.5 ng/kg bw, corresponding to 235 ng BaP and 1,168 ng PAH4 per day respectively assuming a body weight of 60 kg (EFSA, 2008). In the risk assessment by EFSA, based on PAH occurrence data from across Europe and Norwegian consumption data, a median intake of 252 ng BaP and 1449 ng PAH4 per day (4.2 and 24.1 ng/kg b.w.) was calculated for the Norwegian population. In a market basket study from Sweden, in which PAHs were analysed in the most common consumed food items, it was estimated a mean intake of BaP and PAH4 of 50 ng and 276 ng per person per day, respectively (Abramsson-Zetterberg et al., 2014). These numbers correspond to 0.82 and 4.5 ng/kg body weight per day, given a body weight of 60 kg. In a French total dietary study from 2013 dietary intake of BaP and PAH4 was assessed to be 0.19 ng/kg and 1.48 ng/kg b.w. per day respectively (Veyrand et al., 2013). The large variation in the exposure estimates summarised in Table 19 may be due to the selection of food for the calculations, the actual concentrations in the food at the time of the survey and how samples with concentrations below the LOQ are included in the calculations. In the assessment of EFSA 2008 and JECFA 2006 a relatively high proportion of foodstuff suspected to have high PAH concentrations, such as grilled and some preserved foodstuff, was included in the occurrence data, which may pose a risk of overestimating the dietary intake of the substances.

VKM decided to use the data from the dietary BaP intake estimated in the MoBa study by Duarte-Salles et al., (2013) as dietary background exposure to PAHs. This study used PAH occurrence data collected in Duarte-Salles et al, (2010) in which food analysed before 1990, food suspected to have an origin from PAH polluted areas and extreme outliers were excluded from the dataset. In this study it was estimated a mean daily dietary intake of 149 ng BaP among pregnant Norwegian women. With a bodyweight of 70 kg this implies a daily exposure of BaP of 2.1 ng/kg bw per day. PAH from meat and fish products contributed to approximately 15% of the total exposure. Duarte-Salles et al., (2013) did not estimate daily exposure to PAH4. Background exposure to PAH4 was, therefore, estimated based on a presumed concentration ratio of 5 between sum PAH4 and BaP, as this ratio between sum PAH4 and BaP was reported as the median ratio of the mean dietary consumption of PAHs across European countries (EFSA 2008).

Table 19. Overview of estimated dietary intake of PAHs from different countries.

Study	Population	Year of food collection/analysis	Treatment of ND data	Intake per day (ng BaP)	Intake per day (ng PAH4)
(Dennis et al., 1983)	UK population	1979	Mean LB	250	1150
(de Vos et al., 1990)	Netherlands	1984-86	Mean UB	290	2540
(Butler et al., 1993)	10 US homes	1987	NR	87-195	NR
(White, 2002)	UK population	2000	Mean UB	112*	400
(Falcó et al., 2003)	Spanish adult males	2000	½ of LOD	128	1339
(Falcó et al., 2003)	Spanish adult females	2000	½ of LOD	97	NR
(Turrio-baldassarri et al., 1996)	Italy	1993-1995	Regional maximum	170	NR
(EFSA, 2002)	Austria	1989**	Median	50	275
(EFSA, 2002)	Sweden	1985**	Mean	80	NR
(Ibáñez et al., 2005)	Spanish adult females	1982-2001	NR	120	NR
(Martí-Cid et al., 2008)	Spanish male adults	2006	½ of LOD	89	NR
(Martí-Cid et al., 2008)	Spanish female adults	2006	½ of LOD	73	NR
(Martorell et al., 2010)	Catalonian males	2008	½ of LOD	76*	NR
(Martorell et al., 2010)	Catalonian females	2008	½ of LOD	64*	NR
(Duarte-Salles et al., 2010)	Spanish adult females	1990-2009	½ of LOD	181	NR
(JECFA, 2006)	13 countries	2005**	NR	280*	NR
(EFSA, 2008)	EU population	2005-2007	Mean UB	235	1168
(EFSA, 2008)	Norwegian population	2005-2007	Mean UB	252	1449
(Australia, 2006)	Australian males	2004	Mean UB	91*	NR
(Australia, 2006)	Australian females	2004	Mean UB	84*	NR
(Duarte-Salles et al., 2013)	Norway, adult females	1990-2009	½ of LOD	149	NR
(Veyrand et al., 2013)	French population	2007-2009	½ of LOD	13*	103*
(Abramsson-Zetterberg et al., 2014)	Sweden	2010	½ of LOD	49	270
(Di Bella et al., 2020)	Italian adults	2018	½ of LOD	35*	1444*
(Zapico et al., 2022)	Spanish adults	2020/2021**	NR	30	NR
(Grigoriou et al., 2022)	Greek adults	2020	½ of LOD	44*	242

Calculated from a body weight of 70 kg. Year of survey*

7.3.4 Exposure to PAH from the probabilistic scenario plates with grilled food

The exposure to PAH was calculated from a dietary intake of BaP and PAH4 in two grill scenarios with an added background exposure from other dietary sources.

To avoid overestimating PAH exposure by adding exposure from grilled food to the exposure from other meat or fish on the day of grilling, the contribution from other meat and fish was subtracted from the total diet on the day of grilling. This was done by subtracting 15% of the daily background intake of 2.1 ng/kg bw /day (0.315 ng/kg/day), which is the proportion contributed from meat and fish according to (Duarte-Salles et al., 2010). VKM noted however that this subtraction of the background contribution of PAHs from fish and meat on the days of grilling had only minor effect on the estimates. Exposures to PAH for the two scenarios were created for 1 to 100 servings per year, which may cover the frequencies of grilling by the Norwegian population. The intake of PAHs was expressed on a bw basis (ng/kg bw per day) assuming a body weight of 70 kg. The estimated exposures were used to calculate MOEs.

Table 20 shows exposure of PAH4 and BaP (ng pr kg bw) with a bw of 70 kg from consumption of one plate whereas Tables 21-26 show the estimated exposures of BaP and PAH4 from the two grill scenarios. Tables 21 and 22 show exposures with data from campfire grilled food included, and tables 25 and 26 show exposures when concentration data from campfire grilled food are excluded.

Table 20. Simulated exposure to BaP and PAH4 (ng/kg bw), from consumption of one scenario plate.*

BaP	**mean**	**P5**	**P10**	**P25**	**P50**	**P75**	**P90**	**P95**
Fatty meat plate	23	0.66	1.4	2.1	6.1	20	77	130
Lean meat and salmon plate	6.1	0.77	1.1	1.9	3.4	6.0	17	22
PAH4	**mean**	**P5**	**P10**	**P25**	**P50**	**P75**	**P90**	**P95**
Fatty meat plate	92	5.2	7.9	16	45	107	272	377
Lean meat and salmon plate	45	8.5	11	17	29	48	94	186

*assuming bw 70 kg

Table 21. BaP exposure from the scenario of fat rich meat (200 g per meal and a bw of 70 kg), with a presumed background exposure with data from campfire grilled food included, per year.

Number of grilled meals per year	**Exposure of BaP (ng/kg bw/day)**				
	Mean	**Median**	**P75**	**P90**	**P95**
0	**2.1**	**2.1**	**2.1**	**2.1**	**2.1**
1	2.2	2.1	2.2	2.3	2.5
5	2.4	2.2	2.4	3.2	3.9
10	2.7	2.3	2.6	4.2	5.7
15	3.0	2.3	2.9	5.3	7.4
20	3.3	2.4	3.2	6.3	9.2
30	4.0	2.6	3.7	8.4	13
40	4.6	2.7	4.3	11	16
50	5.2	2.9	4.8	13	20
60	5.8	3.0	5.4	15	23
70	6.5	3.2	5.9	17	27
80	7.1	3.4	6.5	19	31
90	7.7	3.5	7.0	21	34
100	8.3	3.7	7.5	23	38

Table 22. PAH4 exposure from the scenario of fat rich meat (200g per meal and a bw of 70 kg), with a presumed background exposure with data from campfire grilled food included.

Number of grilled meals per year	**Exposure of PAH4 (ng/kg bw/day)**				
	Mean	**Median**	**P75**	**P90**	**P95**
0	**10.5**	**10.5**	**10.5**	**10.5**	**10.5**
1	11	11	11	11	12
5	12	11	12	14	16
10	13	12	13	18	21
15	14	12	15	22	26
20	15	13	16	25	31
30	18	14	19	33	41
40	20	15	22	40	52
50	23	16	25	48	62
60	25	18	28	55	72
70	28	19	31	62	82
80	30	20	34	70	93
90	33	21	37	77	103
100	35	22	39	85	113

Table 23. BaP exposure from the scenario of lean meat and salmon (200g per meal and a bw of 70 kg), with a presumed background exposure.

Number of grilled meals per year	**Exposure of BaP (ng/kg bw/day)**				
	Mean	**Median**	**P75**	**P90**	**P95**
0	**2.1**	**2.1**	**2.1**	**2.1**	**2.1**
1	2.1	2.1	2.1	2.1	2.2
5	2.2	2.1	2.2	2.3	2.4
10	2.3	2.2	2.3	2.6	2.7
15	2.3	2.2	2.3	2.8	3.0
20	2.4	2.3	2.4	3.0	3.3
30	2.6	2.4	2.6	3.5	3.9
40	2.7	2.4	2.7	3.9	4.5
50	2.9	2.5	2.9	4.4	5.1
60	3.1	2.6	3.0	4.8	5.7
70	3.2	2.7	3.2	5.3	6.3
80	3.4	2.8	3.4	5.7	6.9
90	3.5	2.9	3.5	6.2	7.5
100	3.7	3.0	3.7	6.6	8.1

Table 24. PAH4 exposure from the scenario of lean meat and salmon (200g per meal and a bw of 70 kg), with a presumed background exposure.

Number of grilled meals per year	**Exposure of PAH4 (ng/kg bw/day)**				
	Mean	**Median**	**P75**	**P90**	**P95**
0	**10.5**	**10.5**	**10.5**	**10.5**	**10.5**
1	11	11	11	11	11
5	11	11	11	12	13
10	12	11	12	13	16
15	12	12	12	14	18
20	13	12	13	16	21
30	14	13	14	18	26
40	15	13	16	21	31
50	16	14	17	23	36
60	18	15	18	26	41
70	19	16	19	28	46
80	20	16	21	31	51
90	21	17	22	33	56
100	22	18	23	36	61

Table 25. BaP exposure from the scenario of fat rich meat (200g per meal and a bw of 70 kg), with a presumed background exposure with data from campfire grilled food excluded.

Number of grilled meals per year	**Exposure of BaP (ng/kg bw/day)**				
	Mean	**Median**	**P75**	**P90**	**P95**
0	**2.1**	**2.1**	**2.1**	**2.1**	**2.1**
1	2.1	2.1	2.1	2.2	2.2
5	2.3	2.1	2.2	2.5	2.8
10	2.4	2.2	2.4	2.9	3.6
15	2.6	2.2	2.5	3.2	4.3
20	2.8	2.3	2.7	3.6	5.1
30	3.1	2.4	2.9	4.4	6.6
40	3.5	2.5	3.2	5.1	8.1
50	3.8	2.6	3.5	5.9	10
60	4.2	2.7	3.8	6.7	11
70	4.5	2.8	4.0	7.4	13
80	4.9	2.9	4.3	8.2	14
90	5.2	3.0	4.6	8.9	16
100	5.6	3.1	4.9	10	17

Table 26. PAH4 exposure from the scenario of fat rich meat (200g per meal and a bw of 70 kg), with a presumed background exposure with data from campfire grilled food excluded.

Number of grilled meals per year	**Exposure of PAH4 (ng/kg bw/day)**				
	Mean	**Median**	**P75**	**P90**	**P95**
0	**10.5**	**10.5**	**10.5**	**10.5**	**10.5**
1	11	11	11	11	11
5	11	11	11	12	13
10	12	11	12	14	16
15	13	11	13	15	18
20	13	12	14	17	21
30	15	12	15	20	26
40	16	13	17	23	31
50	17	14	19	26	36
60	19	14	20	29	41
70	20	15	22	32	46
80	21	16	23	35	51
90	23	16	25	38	57
100	24	17	27	41	62

8 Risk characterization

8.1 Grilled food and health outcomes

In the current scoping review of epidemiological studies on health impact of consumption of grilled food compared to consumption of food prepared by other cooking methods, no new insights were identified. As a result, there is a notable data gap regarding systematic reviews on this topic. It is important to note, however, that the scope of this assessment did not include a systematic review of findings in new primary studies. The knowledge on grilling of food and health outcomes obtained in the 2007 report, thus, remains relevant. Although this was a narrative review based on a literature search following other principles, it included results from original data.

8.2 Risk characterizations of PAHs from grilled food

Several process contaminants in grilled food were examined (Chapter 4). However, VKM found the data on other contaminants than PAH too limited to do a meaningful assessment.

In the occurrence data on PAHs in grilled food, all papers reported BaP, and the majority reported the PAHs included in PAH4. VKM decided to characterize the risk from PAH in grilled food using the MOE approach. The $BMDL_{10}$ values derived by EFSA (see 5.1) for BaP alone (0.07 mg/kg bw per day) and for the sum PAH4 (0.34 mg/kg bw per day) were used as RPs in the risk characterization. MOE is calculated by dividing the RP by the exposure. According to EFSA (2008) an MOE above 10,000 is of low public health concern. The risk was characterized with background exposure from the rest of the diet included.The MOEs obtained by the probabilistic exposure calculations are presented below. MOEs were also calculated based on the deterministic exposure calculations using the mean PAH4 and BaP concentrations and these are presented in the Appendix 11.2 and 11.3.

VKM considers that exposure to PAH resulting in MOE below 10,000 is of public health concern.

Lean meat and salmon plate scenario

Tables 27 and 28 show the calculated MOEs for the lean meat and salmon plate scenario. The findings suggest that when consuming grilled lean meat and salmon 100 times a year with an average BaP and PAH4 concentration, the MOEs remain above 10,000. For PAH4 and BaP, the MOEs for high (95-percentile) concentrations are below 10,000 when consuming grilled lean meat and salmon approximately 50 and 70 times per year, respectively. The difference in MOEs between BaP and PAH4 is primarily due to the proportional higher concentrations of chrysene in the pork that was categorized as lean.

Table 27. MOEs of BaP exposure from the scenario of lean meat and salmon plate.

Number of grilled meals per year	MOE with a presumed background exposure				
	Mean	**Median**	**P75**	**P90**	**P95**
0	33000	33000	33000	33000	33000
1	33000	33000	33000	33000	32000
5	32000	33000	32000	30000	29000
10	31000	32000	31000	27000	26000
15	30000	31000	30000	25000	23000
20	29000	31000	29000	23000	21000
30	27000	30000	27000	20000	18000
40	26000	29000	26000	18000	15000
50	24000	28000	24000	16000	14000
60	23000	27000	23000	15000	12000
70	22000	26000	22000	13000	11000
80	21000	25000	21000	12000	10000
90	20000	24000	20000	11000	9300
100	19000	24000	19000	11000	8600

Shaded background indicates MOE<10000.

Table 28. MOEs of PAH4 exposure from the scenario of lean meat and salmon plate (200g per meal, bw of 70 kg).

Number of grilled meals per year	MOE with a presumed background exposure				
	Mean	**Median**	**P75**	**P90**	**P95**
0	32000	32000	32000	32000	32000
1	32000	32000	32000	32000	31000
5	31000	31000	31000	29000	26000
10	29000	30000	29000	26000	22000
15	28000	29000	27000	24000	19000
20	26000	28000	26000	22000	17000
30	24000	27000	24000	19000	13000
40	22000	25000	22000	16000	11000
50	21000	24000	20000	15000	9500
60	19000	23000	19000	13000	8300
70	18000	22000	17000	12000	7400
80	17000	21000	16000	11000	6700
90	16000	20000	15000	10000	6100
100	15000	19000	15000	9500	5600

Shaded background indicates MOE<10000.

Scenario with fat-rich plate of meat

Tables 29 and 30 show the calculated MOEs for the fat rich meat plate scenario for BaP and PAH4, respectively. The finding suggests that when consuming this scenario plate in average 70 times a year with an average BaP or PAH4 concentration, the MOE remains above 10,000. For high (90-and 95-percentile) concentrations of BaP the MOEs were below 10,000 when consuming grilled fat-rich meat approximately 15-25 times per year. The MOEs for PAH4 were in the similar range as those for BaP (Table 30).

Table 29. MOEs of BaP exposure from the scenario of fat rich meat plate.

Number of grilled meals per year	**MOE with a presumed background exposure**				
	Mean	**Median**	**P75**	**P90**	**P95**
0	33000	33000	33000	33000	33000
1	32000	33000	32000	30000	29000
5	29000	32000	30000	22000	18000
10	26000	31000	26000	17000	12000
15	23000	30000	24000	13000	9400
20	21000	29000	22000	11000	7600
30	18000	27000	19000	8300	5500
40	15000	26000	16000	6600	4400
50	13000	24000	15000	5500	3500
60	12000	23000	13000	4700	3000
70	11000	22000	12000	4100	2600
80	9800	21000	11000	3700	2300
90	9100	20000	10000	3300	2100
100	8400	19000	9300	3000	1900

Shaded background indicates MOE<10000.

Table 30. MOEs of PAH4 exposure from the scenario of fat rich meat plate.

Number of grilled meals per year	**MOE with a presumed background exposure**				
	Mean	**Median**	**P75**	**P90**	**P95**
0	32000	32000	32000	32000	32000
1	32000	32000	32000	30000	29000
5	29000	31000	28000	24000	22000
10	26000	29000	25000	19000	16000
15	24000	28000	23000	16000	13000
20	22000	26000	21000	13000	11000
30	19000	24000	18000	10000	8200
40	17000	22000	15000	8400	6600
50	15000	21000	14000	7100	5500
60	13000	19000	12000	6200	4700
70	12000	18000	11000	5400	4100
80	11000	17000	10000	4900	3700
90	10000	16000	9300	4400	3300
100	9600	15000	8600	4000	3000

Shaded background indicates MOE<10000.

Tables 31 and 32 show the calculated MOEs for the fat rich meat plate scenario when concentration data from campfire grilled food was excluded. When excluding the campfire grilled food, the MOE remained above 10,000 when consuming a plate of grilled fat-rich meat 100 times a year with an average BaP and PAH4 exposure. For 95P exposure to BaP and PAH4, the MOEs were below 10,000 when consuming a plate of grilled fat-rich meat approximately 30 and 50 times or more per year respectively. These figures are similar to the calculated MOEs for PAH4 in the lean meat and salmon plate scenario. This observation indicates that the reported PAH concentrations in campfire grilled food may contribute considerably to the scenario-related exposure and associated risk to PAHs.

As mentioned in 7.1.1 the available data did not allow differentiation on grilling method. PAHs generated from all types of grilling methods were, therefore, included in the data set of which approximately 65% of the data were reported from charcoal grilled food. The MOEs for the P90/P95 exposure may represent a worst-case scenario where the food in general is grilled with methods leading to high PAH levels in the food such as well-done food grilled on lump coal or briquettes at high temperatures, on lump coal with wood chips, or on campfire. The MOEs calculated from the mean, median and 75 percentile concentrations of PAHs in the grilled food may represent use of varied grill methods that in most cases do not cause substantial PAH formation and will probably apply to most grilling situations.

The two scenarios used in the assessment are based on four different food types on each plate and their contribution to PAH intake differ. Grilled fat rich pork appears to have a higher potential to accumulate PAHs than grilled chicken with skin, beef and probably in most circumstances grilled sausages (except when grilled on campfire). People have different preferences for what to grill, and changing the composition of food on the plate will change the PAH load and ultimately the potential health risk. Moreover, the present exposure estimations are mainly based on concentration data from charcoal grilled food, which implies that the assessment may overestimate the potential health risk for people that primarily grill with gas as fuel or use an electric grill.

Table 31. MOEs of BaP exposure from the from the fat rich meat plates, excluding meat grilled on campfire.

Number of grilled meals per year	**MOE with a presumed background exposure**				
	Mean	**Median**	**P75**	**P90**	**P95**
0	33000	33000	33000	33000	33000
1	33000	33000	33000	32000	31000
5	31000	33000	31000	28000	25000
10	29000	32000	29000	24000	19000
15	27000	31000	28000	22000	16000
20	25000	30000	26000	19000	14000
30	22000	29000	24000	16000	11000
40	20000	28000	22000	14000	8600
50	18000	27000	20000	12000	7300
60	17000	26000	19000	11000	6300
70	16000	25000	17000	9400	5600
80	14000	24000	16000	8600	5000
90	13000	23000	15000	7800	4500
100	13000	23000	14000	7200	4100

Table 32. MOEs of PAH4 exposure from the from the fat rich meat plates excluding meat grilled on campfire.

Number of grilled meals per year	**MOE with a presumed background exposure**				
	Mean	**Median**	**P75**	**P90**	**P95**
0	32000	32000	32000	32000	32000
1	32000	32000	32000	31000	31000
5	30000	31000	30000	28000	26000
10	29000	30000	28000	25000	22000
15	27000	30000	26000	23000	19000
20	26000	29000	25000	21000	16000
30	23000	27000	22000	17000	13000
40	21000	26000	20000	15000	11000
50	20000	25000	18000	13000	9400
60	18000	24000	17000	12000	8200
70	17000	23000	16000	11000	7300
80	16000	22000	14000	9800	6600
90	15000	21000	14000	9000	6000
100	14000	20000	13000	8300	5500

8.3 HAAs and other heat-induced process contaminants in grilled food

The lack of well characterized exposure and hazard hampers the ability to conduct a comprehensive characterization of the health risk associated with the exposure to the many HAAs from grilled food. It is noted though, that the concentration of HAAs in the published studies may be higher in grilled food, compared with other cooking methods, particularly when grilled well-done. Grilling of food may be associated with higher and less controllable surface temperature than other cooking methods. As most of the HAAs are genotoxic and carcinogenic in rodents and with some being classified as potential human carcinogens, their formation during grilling may therefore raise health concern.

Although not precisely known, it is plausible that several of the other heat-induced contaminants, e.g., acrylamide that is formed in heat-treated carbohydrate rich food, occur at higher concentrations in grilled food than in food prepared by other cooking methods due to higher and less controllable temperature in grilling.

9 Uncertainties

The uncertainty of this risk assessment is influenced by the uncertainties arising from the different stages of the risk assessment, including the selection of the hazardous contaminants, evaluation of the hazard of the contaminants, collection of occurrence data of the contaminants, and compilation of consumption data for the grilled foods. The major uncertainties and their impact on the risk are outlined below.

9.1 Human studies

The systematic literature search carried out to identify systematic reviews on studies in humans on consumption of grilled food and health outcomes in comparison with other cooking methods resulted in no eligible publications. Consequently, it was not feasible to conduct a scoping review to assess the cumulative health effects of heat-induced process-contaminants from grilled food. There may, however, from single primary studies still be available information, that could provide valuable insights into issue of the health effects of process contaminants in grilled food, but analysing these primary sources was beyond the scope of this work.

9.2 Heat-induced process contaminants

9.2.1 Identification of heat-induced contaminants in grilled food

The identification of heat-induced contaminants in grilled food was based on several sources of information: non-systematic literature search in scientific published literature and reports from international and national food safety organisations, searches in reference lists of retrieved articles and a call for data was made through the EFSA focal point network on occurrence data of process contaminants in grilled food. A low risk is presumed of missing important process contaminants in grilled food that are reported in the literature. However, high heat in combination with various substances in the food may create a range of different unknown substances in the combustion process, such as various halogenated substances. Some substances that could potentially contaminate the food from the heat sources, but are less frequently reported in the literature might, have been overlooked in the search.

9.2.2 Occurrence data and factors affecting formation of PAHs and HAAs in grilled food

Of the identified heat-induced contaminants PAHs and HAAs came out as the most prevalent ones, with most of the data on PAHs and less data on HAAs. The uncertainty in occurrence data can be divided into the following elements: (1) differences in type, design, and use of grill and fuel, (2) differences in type of food, (3) sampling and analytical uncertainty (4) missing data due to search strategy.

9.2.2.1 Grilling devices and fuel

Differences in design and use of the grilling device might be of importance for the formation of hazardous contaminants, but these are often not reflected in the available literature. The following differences in grill design have shown to have a significant impact on the measured concentration of contaminants in the grilled food: distance of the food to the heat source, devices reducing dripping of fat into the heat source, and position of the heat source under or above the food. Factors influencing the measured concentration of contaminants during grilling include the waiting time between ignition and start of grilling, selected temperature, open or closed lid, frequency of food turning, and the prevention of fat dripping. For charcoal grills and campfires, the type and quality of the fuel can also influence the concentration of the contaminants in the foods.

9.2.2.2 Food

There are two different sources of uncertainty related to the food: (1) variations in the food composition itself and (2) assumptions made by categorizing specific food items into very few general categories. Even a well-defined food item like pork fillet fitting directly into a category used in the scenarios has natural variations in texture and in the content of fat, protein, carbohydrates, and other constituents. The grilled meat surface area to mass ratio is usually not reported, which may influence the reported PAH levels. Furthermore, in the various studies, food items might have been fresh or frozen, treated with different amounts of salt, nitrite, sugar, spices, herbs, or marinated with oil, wine etc. In addition, it is anticipated that grill habits such as grill temperature, duration, and desired doneness, flipping frequency etc. may vary significantly. Only very few of these variations are reported, but all might have at least some influences on the concentration of process contaminants, and hence, introduce uncertainties into the occurrence data, however, it is not possible to quantify these uncertainties. Since there is a lack of occurrence data for many grilled food types, it was necessary to estimate concentrations for these types by assuming similar concentrations as in comparable food types. To what extent this introduces an error its magnitude is unknown.

9.2.2.3 Sampling and analysis

Uncertainties related to the chemical analysis of the selected contaminants are caused by insufficient extraction of the analytes, loss during sample clean-up, errors due to wrong identification or calibration under instrumental analysis, and instrument fluctuations. For well-established analyses like PAHs these factors are well known and regular international interlaboratory studies show that the analytical uncertainty for PAH compounds is in the range of ± 15–25%. The analysis of HAA compounds is much more demanding compared to PAHs and still in development. This explains the paucity of data on HAAs. The lack of international interlaboratory studies hampers quantification of hampers quantification of the analytical uncertainty for HAAs, but it is presumed to be far higher than ± 50%.

9.2.2.4 Search strategy and selection of published literature

The studies used to collect concentration data of PAHs in grilled food were not subjected to systematic quality assessment. This may introduce a risk of bias in the occurrence data. Critical factors in this regard include reporting of analytical performance, such as LOD/LOQ, adequate reporting of grill methods and food characteristics, proper use of statistics and documentation of all data.

9.3 Consumption of grilled food

VKM lacks the data necessary to describe the consumption of grilled food in Norway. Therefore, the uncertainties associated with the consumption of grilled food in this assessment are directly linked to the scenarios used in the risk assessment.

In the absence of consumption data on grilled food, two scenario plates were constructed using commonly grilled food items, and exposure to these plates is estimated using frequencies known to cover the frequencies of consumption of the Norwegian population. The portion size of 200 g prepared food is considered a conservative approach, given that the standard portion size for the food items used is 150 g. These scenarios are considered realistic examples but are not true consumptions.

9.4 Exposure to PAHs and other process contaminants

9.4.1 Background PAHs exposure from the general diet

The dietary background exposure of PAHs in the general Norwegian population is not known and is subject to great uncertainty. VKM decided to use the exposure data from the pregnant women in the Norwegian MoBa study by Duarte-Salles et al. (2013), which estimated a daily intake of 149 ng, or 2.1 ng BaP/kg bw per day for a person with a body weight of 70 kg. This is in the upper range level compared to other recent studies on dietary exposures to PAH (see table 19). Previous studies on dietary exposure to PAHs show large variation in exposure, from a daily exposure of 13 ng BaP from a French market basket study to 290 ng from an older Dutch study (see Table 19). The uncertainty in the background exposure can lead to both over- and underestimation of the exposures and the associated health risk. Other sources and routes of PAH exposure are air pollution and smoking, but these were not considered.

9.4.2 Probabilistic PAH exposure from the scenario plates

Probabilistic methods were used both to estimate occurrence distribution of PAHs in various food items and in the estimation of the distribution of the PAH content of the scenario-plates. The probabilistic method better reflects the width in PAH occurrence than the deterministic method, which only give an estimate of the average exposure.

When estimating exposure from the scenario plates, the occurrence levels were presumed to be uncorrelated across food types. In practice, the same consumer is likely to follow similar grilling routines and use the same grilling equipment independent of food types. If true, that would imply positively correlated occurrence of PAHs in foods on the same plate scenario and a wider distribution of exposures from the plates. As data are lacking that would allow estimation of the correlations across food types, VKM acknowledge that the chosen approach is likely to results in low percentiles being biased up and high percentiles being biased down.

The biases at the PAH4 level and the food level counterbalance each other, however, data are lacking to assess relative importance of the two potential biases.

9.5 Hazard characterization

VKM used the previous hazard characterizations of PAHs conducted by EFSA (2008), and the uncertainties in hazard characterization in EFSA 2008 is applicable in the present assessment.

9.6 Risk characterization

The general opinion is that an MOE of 10,000 or higher, taking into consideration overall uncertainties, is of low public health concern. The uncertainties in the MOE estimation lie in the undelaying data (exposure as well as the $BMDL_{10}$ reference point), which should be taken into account when interpreting an MOE.

For the MOE calculations, a body weight of 70 kg may seem low. However, this is meant to represent both women and men. A lower body weight with the same intake will lead to a higher relative exposure which will imply a lower MOE. A higher body weight with the same intake will lead to a relatively lower exposure which imply a higher MOE. Similar, a portion size of 200 g prepared food may seem large and can be considered as a conservative approach, but it is meant to represent both men and women.

Most data on occurrence of process contaminants in grilled food is on PAHs, for which also hazard characterization was available. A quantitative assessment was performed on PAHs only. Less is known about the contribution of other process-induced substances present in grilled food to potential health effects and if they amplify the effects of PAHs.

9.7 Summary of uncertainties in the assessment

The uncertainties in the consumption of grilled food (food types and frequency) and in the occurrence of PAHs and other process contaminants in grilled food are large. Available data only allowed a quantitative assessment of PAHs. Exposure scenarios were constructed, and estimated exposures are considered to cover the variation of PAH exposure from grilled food. The uncertainty in the estimated PAH exposure from the rest of the diet is also subjected to large uncertainty. This may lead to both over- and underestimation of the risk from PAHs in grilled food consumption, although the estimated intake from other dietary sources is in the upper range compared to other recent studies on dietary exposures to PAH (see table 19). Due to lack of data, the health risk associated with exposure to other heat-induced contaminants in grilled food could not be assessed. Consequently, the total risk associated with consumption of grilled food remains uncertain.

10 Conclusions and answers to the terms of reference

The overall aim of the present assessment was to identify health risks associated with consumption of grilled food and to conduct a risk assessment of exposure to process contaminants formed in grilled foods with the Norwegian population as target population. The health risks associated with grilling of food is assessed based on new knowledge since the previous VKM opinion from 2007.

TOR1: *Identify process contaminants which are formed to a greater extent by grilling than by frying and create an overview of reported amounts of these process contaminants in various types of grilled food.*

- The following substances and groups of substances associated with the grilling of food were identified: polycyclic aromatic hydrocarbons (PAHs), PAH analogues such as chlorinated-PAHs (Cl-PAHs), nitro-PAHs, polyphenols, heterocyclic aromatic amines (HAAs), 3- monochloropropane-1,2-diol (3-MCPD) and glycidyl esters, acrylamide, nitrosamines, nitrite, harmful Maillard reaction products, biogenic amines, 7-ketocholesterol, acridine derivatives, anthraquinone (ATQ), pyrazines, advanced glycation end products (AGE).

- The concentration data on grilled food items other than meat and fish, such as vegetables and bread, are scarce.

- For two groups of compounds there is substantial or plausible evidence for higher concentration in grilled food than in fried food:
    - PAHs are frequently reported in grilled food. There is substantial evidence of higher concentrations of PAHs in grilled food than in fried food. Although there is limited information on their occurrence, it is plausible that PAH-analogues occur in higher concentrations in grilled food.

    - HAAs are frequently reported in grilled food and are regularly found in higher concentrations in grilled foods than in fried food as the surface temperature may be higher when grilling.

- For the remaining heat-induced substances the information is too limited to conclude on their occurrence in grilled food in comparison with other types of heat-treatment.

- Concentration data for PAHs in grilled food (mostly meat and meat products) was collected from a total of 81 studies. Generally, the studies provided insufficient information on food item such as the body part of meat, fat content and grill method.

- The highest concentrations of PAHs (BaP and PAH4) were found in campfire grilled sausages and bread.

- Concentration data for BaP, regardless of grilling method, indicated variability ranging from levels below quantification to more than 100 ng/g in fatty pork meat and sausages. Most of the grilled food items had median concentrations below approximately 1 ng/g, but varied from 0.1 to 4.1 ng/g across all food items.

- The occurrence of single HAAs in grilled food varied greatly, from <LOD to about 50 ng/g. The concentration could, however, reach up to 240 ng/g for PhIP in very well-done grilled chicken, and with more in meat than in fish. Highest concentrations in cooked food are reported for Phip, MeIQ, MeIQx, DiMeIQx, Harman and Norharman.

TOR2: *Elucidate factors (for example grill type, grill method and food) that are important for the formation of the identified process contaminants in grilled food.*

- The type of heat source can influence the content of PAHs and HAAs differently. Main types of heat sources for grilling are electricity, gas, charcoal (lump charcoal, briquettes) and firewood. Other factors that may influence the formation of PAHs and HAAs include type of fuel, direct or indirect grilling, type of meat, distance from the heat source, grilling temperature, and grilling time.

PAHs

- Contamination of food with PAHs may occur through: (1) deposition on the food of fuel-related PAHs in smoke, (2) deposition of smoke from incomplete combustion (pyrolysis) of dripping fat onto the heating source or hot surfaces. (3) Over-heating the food resulting in burned surface during cooking.

    1. Electric heating and gas emit no or little PAHs, while lump charcoal, and briquettes and in particular firewood may contribute significantly to PAH contamination. When using charcoal, the PAH emission is higher in the initial period after lighting of the grill.

    2. Dripping fat on the heat source is a significant source of PAHs in grilled food and is related to the fat content of the food. Diverting the smoke from the food or avoiding fat dripping directly on the heat source may reduce PAH deposition on the food. Marination is shown to both reduce and increase the content of PAHs.

    3. Temperature, proximity to the heat source, frequent flipping of the food, and cooking time are important for avoiding burning the food during grilling.

HAAs

- HAAs are mainly formed in the crust or gravy by heating. Variables like (1) temperature and time, (2) presence of precursors, such as creatine, reducing sugars and free amino acids in the food and (3) method of cooking significantly affect the formation of HAAs.

1. The number of HAAs and concentrations of individual HAAs increase with rising temperature, while regular turning of the meat during cooking reduce the surface temperature and mitigate HAA formation. Amino-imidazo-azaarenes (AIAs) are formed at lower temperatures (>100-300°C) than aminocarbolines (ACs) (>300°C). Longer cooking times and doneness are related to higher amounts of HAAs.

2. Protein rich food with creatine and reducing sugars, e.g. more in lean meat than in fatty meat, fish or mixed products, increase HAA formation.

3. Marinating the food with plant extracts and phenolic compounds appears to reduce HAA formation, probably because of their activity as antioxidants and scavenging of free radicals and carbonyl compounds. Microwaving beef and chicken prior to charcoal grilling reduce the content of HAAs formed.

TOR 3: *If possible, based on available information, assess the health risks associated with the consumption of grilled food compared to fried food.*

By conducting an umbrella review of systematic reviews of epidemiological studies published since 2006 no additional knowledge on the association between consumption of grilled food and health outcomes in humans was identified. Therefore, the conclusion drawn in the previous VKM 2007 assessment remains pertinent, suggesting a possible association between intake of well-done fried or grilled meat and cancer in the colon, rectum, prostate, breast, and pancreas.

VKM estimated PAH exposure in grilled food quantitatively based on scenarios of consumption of grilled food also including a background exposure of PAH from the rest of the diet. From the exposure scenarios MOEs were calculated using RPs for BaP and PAH4 identified by EFSA (2008).

Due to lack of data, the risk from HAA exposure and other heat-induced contaminants could not be characterised.

PAHs

The available dietary information was not sufficient for allowing an estimation of the consumption of grilled food in the Norwegian population. Therefore, two grilled food consumption scenarios were used to illustrate possible consequences of different food preferences on the intake of PAHs from grilled foods. The scenarios included only meat and salmon because of lack of occurrence data in other grilled food items, such as vegetables, bread and meat imitates.

- Two different scenario plates with grilled food were constructed, each with 200 g food. One plate contained meat with high fat content (pork ribs, hamburger, sausages, and chicken) and one plate contained lean meat and fish (beef steak, pork fillet, chicken fillet and salmon). The content of BaP or PAH4 on each plate were calculated using probabilistic modelling of PAH concentration distributions of the different food items and PAH content distributions of the two scenario-plates. Exposure scenarios for the two scenario plates with included background PAH exposure were created for 1 to 100 servings per year, which covered the actual frequencies of grilling by the Norwegian population.

- Risk was characterized by the MOE approach using $BMDL_{10}$s from the EFSA risk assessment of PAHs in food from 2008 as reference points for indicators BaP and PAH4. MOEs for PAH exposure from the two scenario plates were calculated.

- For the plate with lean meat and salmon the use of mean, median and 75 percentile exposure to BaP and PAH4 up to 100 servings per year, resulted in MOEs above 10,000. At the 90 percentile and 100 servings the MOEs were approximately 10,000 for BaP and slightly below for PAH4.

- For the plate with fat reach meat the MOEs for the scenario related exposures remained above 10,000 (including background exposure) when consuming grilled fat rich meat with a mean BaP and PAH4 content up to 100 times a year. At concentrations equivalent to the 95 percentile of BaP and PAH4 the MOEs were below 10,000 when consuming grilled fat rich meat approximately 15 and 25 times a year respectively.

- The impact of campfire grilling on exposure estimates and the associated MOEs was examined in a sensitivity analysis. Data on campfire grilled food was only available for food items on the fat rich meat plate. Excluding campfire grilled food from the fat rich plate had a substantial impact on the mean, median and higher percentiles values for both PAH4 and BaP.The use of mean, median and 75 percentile exposure to BaP and PAH4 and more than 100 servings per year, resulted in MOEs above 10,000. At a 95 percentile content of BaP and PAH4 the MOEs were above 10,000 for up to 30 servings per year.

- Using PAH4 as an indicator instead of BaP gave approximately the same result as that of BaP, with slightly higher MOEs for the fat rich meat plate and slightly lower for the lean meat and salmon plate.

- VKM considers that exposure to PAH resulting in MOE below 10,000 is of public health concern.

- The database did not allow creation of exposure scenarios reflecting various grilling methods. The MOEs calculated from the mean, median and 75 percentile exposure to PAHs likely represent the use of varied grill methods and food not causing substantial PAH formation. This will probably apply to most grilling situations. The MOEs calculated for the 90- and 95-percentile exposures to PAH may represent frequent use of grilling methods known to increase formation of PAHs in the food, such as high temperature grilling with charcoal or even campfire for a longer grill time leading to well done food.

As an overall outcome for PAHs, VKM notes a public health concern for those who often (more than 15-25 times per year) consume fat rich meat that is grilled in a way that leads to higher PAH content. Higher PAH content may be seen in grilled food when fat has dripped on the heat source and/or in food grilled well-done and/or use of charcoal or particularly campfire as heat source.

HAAs and other heat-induced contaminants.

- The concentrations of HAAs seem to be higher in grilled meat than in fried meat, particularly when well done. Grilling of food is associated with higher and less controllable surface temperature than frying. As most of the HAAs are genotoxic and carcinogenic in rodents and some have been classified as possible human carcinogens, their formation during grilling may be of concern.
- Although not precisely known, it is plausible that several other heat-induced contaminants, such as of PAH-analogues, can occur in higher concentrations in grilled food than in fried food, as the mechanism of formation is presumed to be similar to that of the PAHs or due to a presumably higher and less controllable temperature in grilling.

Uncertainties

- The uncertainties in this assessment are large. Exposure to PAH was estimated from simulated occurrence data and consumption scenarios. The health risk from exposure to PAHs in consumed grilled food characterised by the MOE approach may both be over- and underestimated. Due to lack of data the health risk associated with exposure to other heat-induced contaminants in grilled food that PAH could not be assessed. Due to high and less controllable temperature during grilling, it is likely that HAAs and some other heat-induced contaminants may be present in higher concentrations in grilled food than in fried food. Therefore, the total risk associated with presence of process contaminants in grilled food is likely to be higher than that for PAH alone.

Data gaps

- Systematic reviews on health outcomes related to consumption of grilled food in comparison with other cooking methods are lacking.

- Data on consumption of grilled food, food items, frequency of grilling and grilling method applied are missing.

- Data on occurrence of PAH and other heat-induced contaminants in food prepared by different grilling methods and by other comparable cooking methods are missing.

- Toxicological data on individual HAAs for better hazard characterization considering toxicokinetic differences between rodents and humans are missing.

- Occurrence data on HAAs in grilled food analysed with controlled and validated analytical methods are missing.

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11 Appendix I

11.1 Occurrence data

11.1.1 LB, simulated values and UB concentrations of PAHs

The collected concentration data were estimated using lower bound estimates, upper bound estimates and simulated values for non-detects (i.e. concentrations reported as below LOQ). There was only minor difference between these estimates as shown for mean PAH4 concentrations in the various food types (Figure 9).

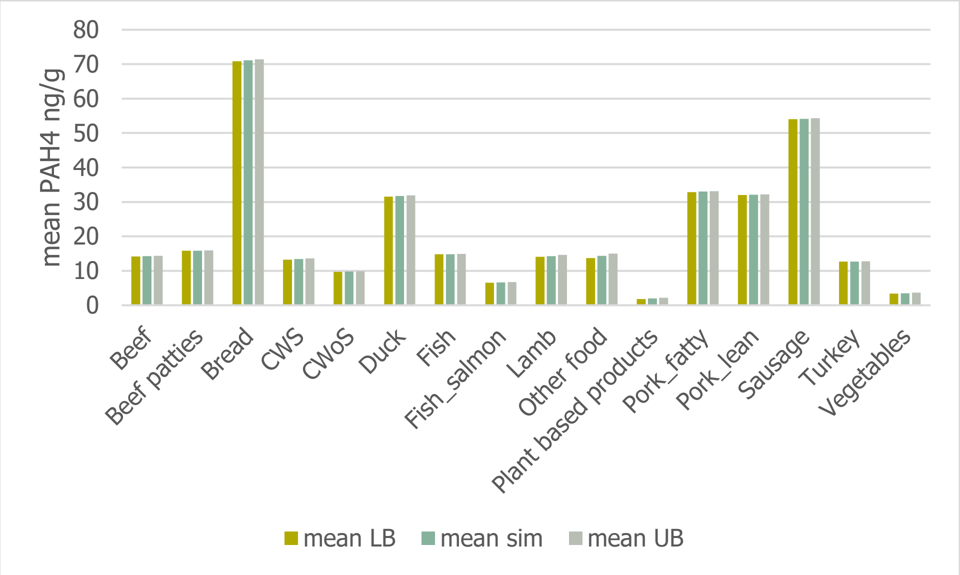

Figure 9. The mean sum PAH4 by food groups, calculated using LB, the simulated values below LOQ and with UB approaches. CWS: chicken with skin; CWoS: chicken without skin.

11.1.2 Occurrence data, deterministic approach

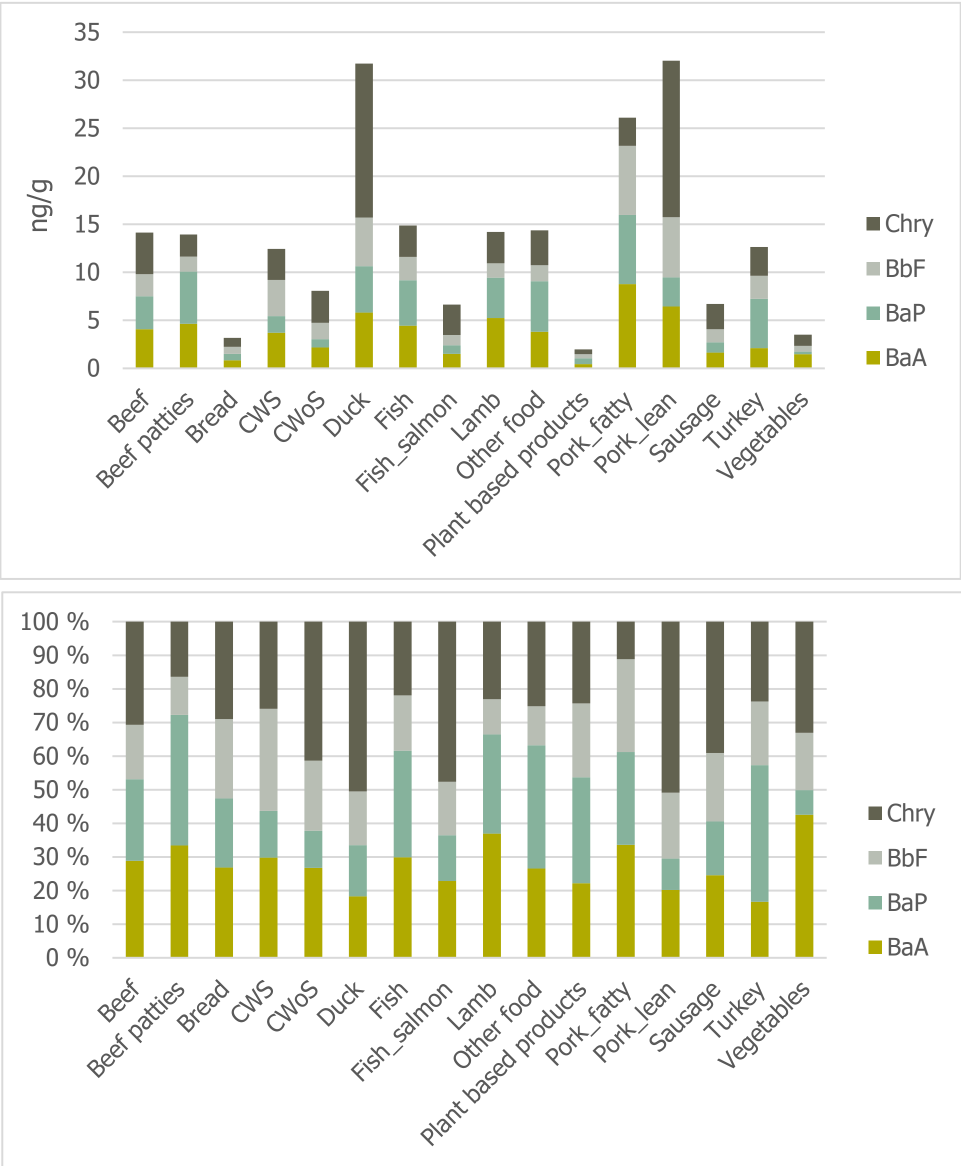

Figure 10. Mean concentrations in ng/g estimated by deterministic approach (upper panel) including food grilled on campfire and percent distribution (lower panel) for of BaA, BaP, BbF and Chry (sum PAH4) in 16 food categories. CWS: chicken with skin; CWoS: chicken without skin.

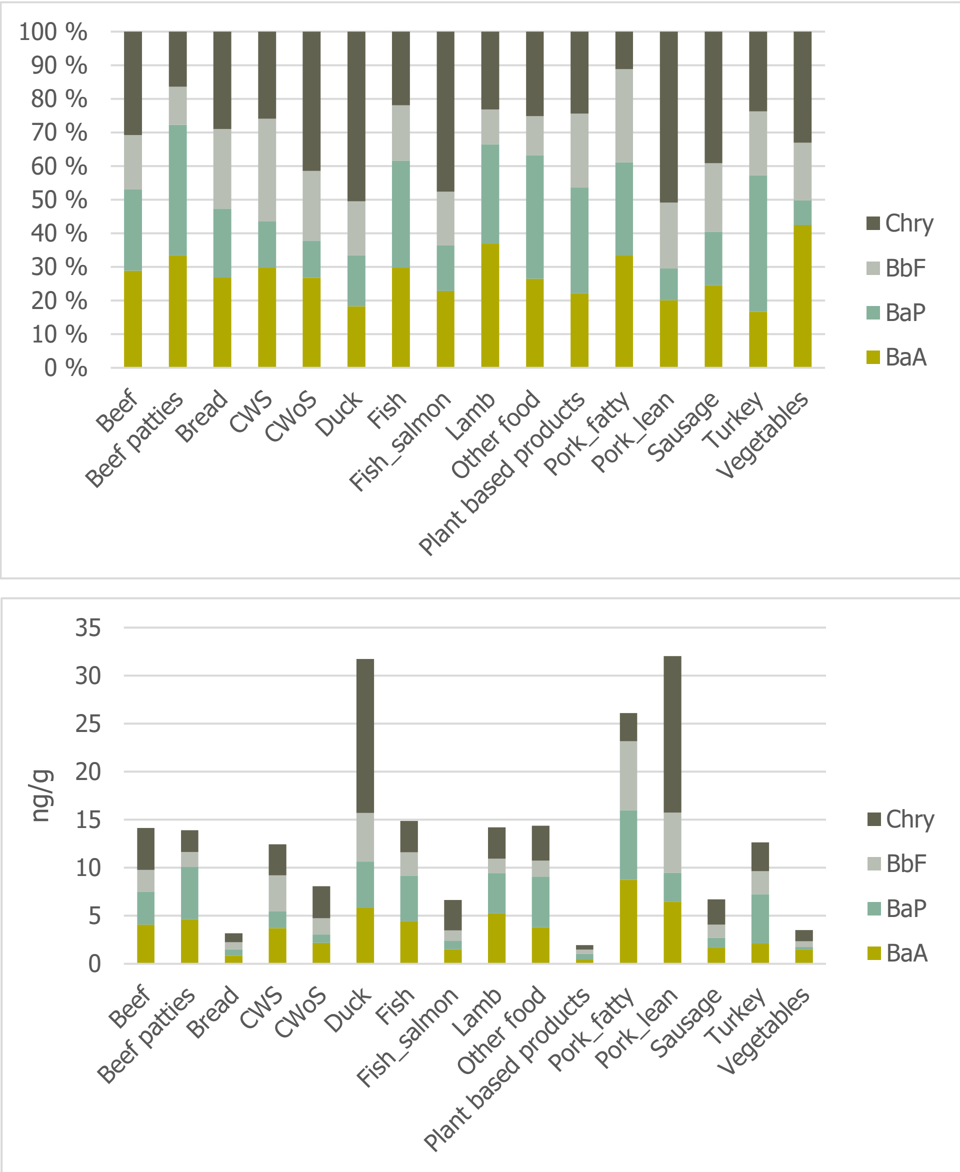

***Figure 11**. Mean concentrations in ng/g estimated by deterministic approach (upper panel) excluding campfire grilled food and percent distribution (lower panel) for of BaA, BaP, BbF and Chry (sum PAH4) in 16 food categories. CWS: chicken with skin; CWoS: chicken without skin.*

11.1.3 Simulated occurrence data

Table 33. Mean and percentiles BaA (ng/g) for the eight food groups.

Food	Mean	P5	P10	P25	P50	P75	P90	P95
Beef	4.1	0.03	0.12	0.48	1.7	4.6	9.5	18
Beef patties	5.1	0.04	0.08	0.46	0.78	4.0	17	30
Bread	26	0.02	0.04	0.40	1.3	27	100	100
CWS	4.1	0.03	0.13	0.73	2.3	6.0	12	14
CWoS	2.7	0.21	0.37	0.96	2.0	3.3	6.5	9.0
Duck	5.8	0.08	0.16	1.6	2.7	7.6	20	25
Fish	4.4	0.85	1.1	2.2	3.3	5.5	6.9	8.6
Fish salmon	1.5	0.01	0.06	0.31	0.76	1.8	4.9	6.9
Lamb	5.2	0.01	0.03	0.30	3.9	9.8	10	10
Other food	3.8	0.15	0.30	0.72	3.7	6.4	7.8	8.1
Plant based products	0.43	0.03	0.05	0.15	0.32	0.45	0.98	1.5
Pork fatty	11	0.06	0.17	0.34	1.5	13	32	46
Pork lean	6.5	0.16	0.32	1.5	2.8	4.4	9.9	49
Sausage	15	0.02	0.02	0.29	2.0	13	56	100
Turkey	2.1	1.9	2.0	2.0	2.1	2.2	2.3	2.3
Vegetables	1.5	0.02	0.04	0.09	0.80	1.8	3.8	5.4

* CWS: chicken with skin; CWoS: chicken without skin

Table 34. Mean and percentiles BbF (ng/g) for the eight food groups.

Food	Mean	P5	P10	P25	P50	P75	P90	P95
Beef	2.3	0.02	0.06	0.29	0.86	2.7	5.7	9.4
Beef patties	2.0	0.05	0.09	0.20	0.46	1.8	5.1	12
Bread	13	0.02	0.05	0.12	1.1	14	50	50
CWS	3.9	0.02	0.07	0.30	1.8	4.3	11	17
CWoS	2.0	0.09	0.14	0.54	1.3	2.5	5.2	7.6
Duck	5.1	0.17	0.18	0.30	4.1	9.7	11	12
Fish	2.5	0.07	0.15	0.62	1.8	2.5	4.6	5.1
Fish salmon	1.1	0.02	0.04	0.24	0.62	1.3	4.1	4.7
Lamb	1.5	0.01	0.03	0.06	1.0	1.7	2.4	5.1
Other food	1.7	0.09	0.18	0.45	1.3	1.8	4.5	5.3
Plant based products	0.43	0.04	0.08	0.22	0.36	0.45	0.74	1.4
Pork fatty	8.5	0.03	0.07	0.24	1.9	11	22	31
Pork lean	6.3	0.41	1.1	1.9	2.8	7.0	16	22
Sausage	10	0.02	0.02	0.16	1.1	10	37	656
Turkey	2.4	2.2	2.2	2.3	2.4	2.5	2.6	2.7
Vegetables	0.60	0.01	0.02	0.06	0.23	0.49	0.91	2.3

* CWS: chicken with skin; CWoS: chicken without skin

Table 35. Mean and percentiles Chry (ng/g) for the eight food groups.

Food	Mean	P5	P10	P25	P50	P75	P90	P95
Beef	4.3	0.02	0.07	0.19	1.0	5.4	15	19
Beef patties	3.0	0.04	0.06	0.31	0.88	2.2	5.9	17
Bread	13	0.08	0.15	0.38	1.4	14	50	50
CWS	3.6	0.02	0.09	0.59	1.9	4.6	10	14
CWoS	4.0	0.11	0.17	1.3	3.1	5.5	9.5	13
Duck	16	0.30	0.32	4.1	7.0	32	34	44
Fish	3.3	0.58	0.62	1.2	1.8	3.6	4.8	7.6
Fish salmon	3.2	0.06	0.17	0.44	1.1	3.2	8.5	20
Lamb	3.3	0.02	0.13	0.48	1.9	4.2	6.3	8.1
Other food	3.6	0.11	0.21	0.53	2.5	4.9	11	12
Plant based products	0.48	0.06	0.10	0.23	0.42	0.54	0.80	1.4
Pork fatty	5.6	0.01	0.03	0.23	0.89	5.2	21	30
Pork lean	16	0.41	0.66	1.9	4.0	11	44	142
Sausage	16	0.04	0.05	0.55	3.2	15	54	97
Turkey	3.0	2.4	2.5	2.7	3.0	3.3	3.5	3.7
Vegetables	1.2	0.02	0.04	0.13	0.50	0.64	2.5	4.8

* CWS: chicken with skin; CWoS: chicken without skin

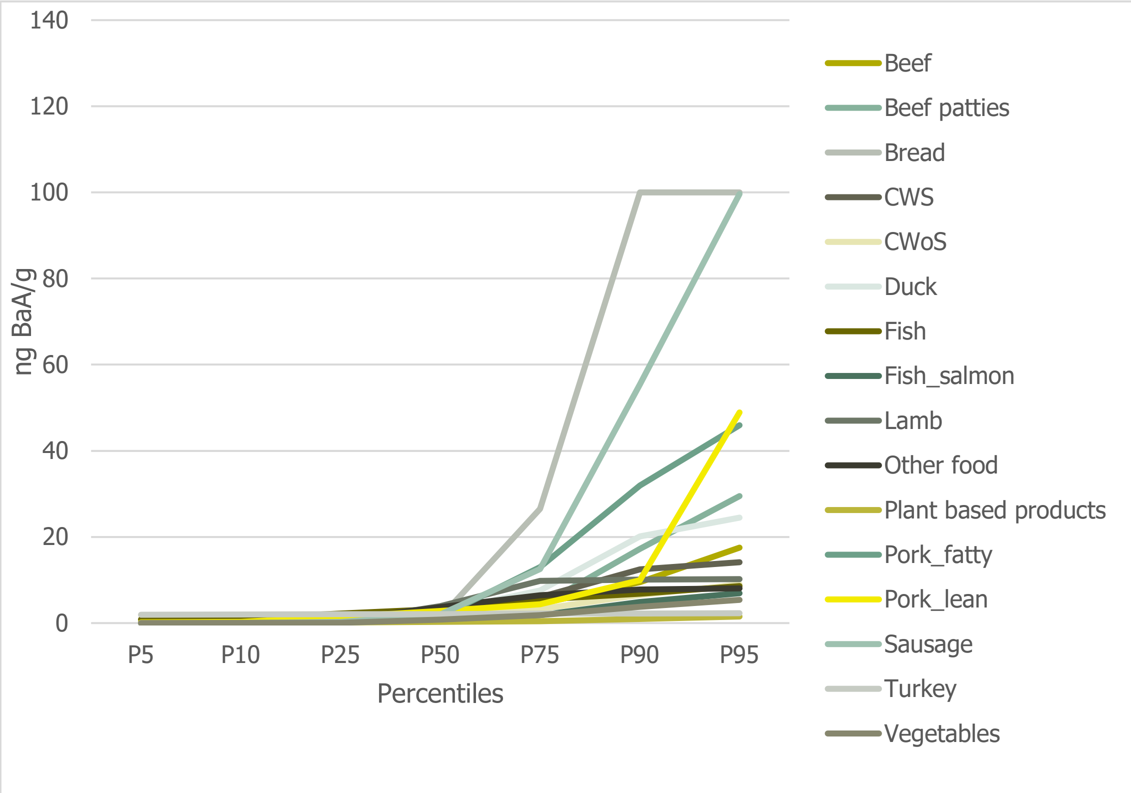

Figure 13. Percentile plot for BaA simulated values (ng/g).

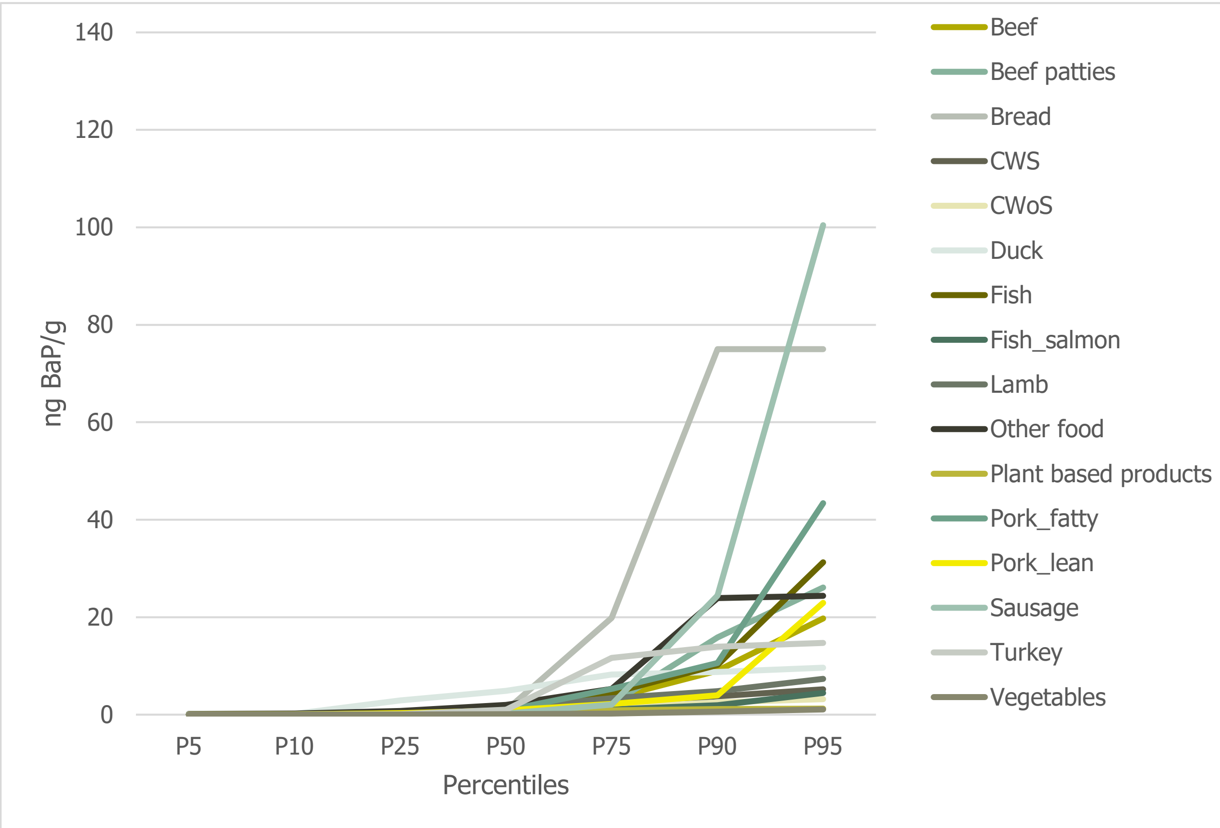

Figure 12. Percentile plot for BaP simulated values (ng/g).

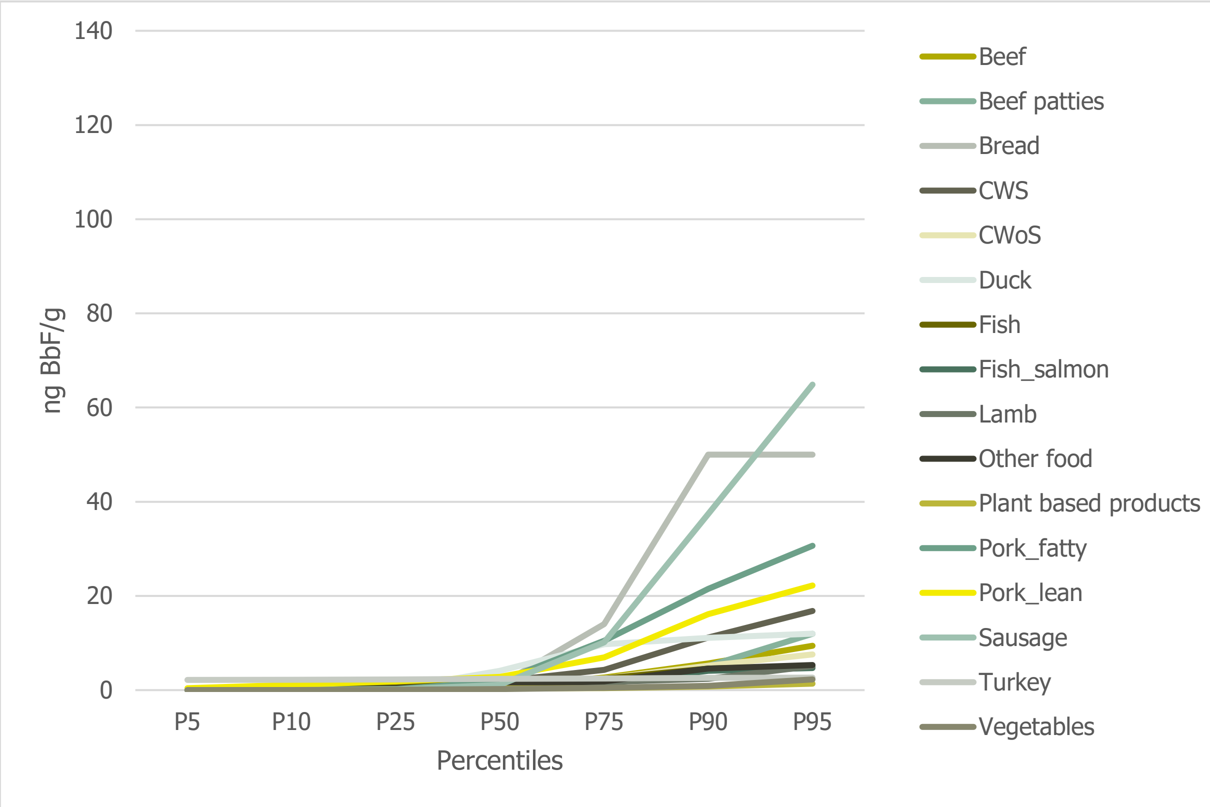

Figure 14. Percentile plot for BbF simulated values (ng/g).

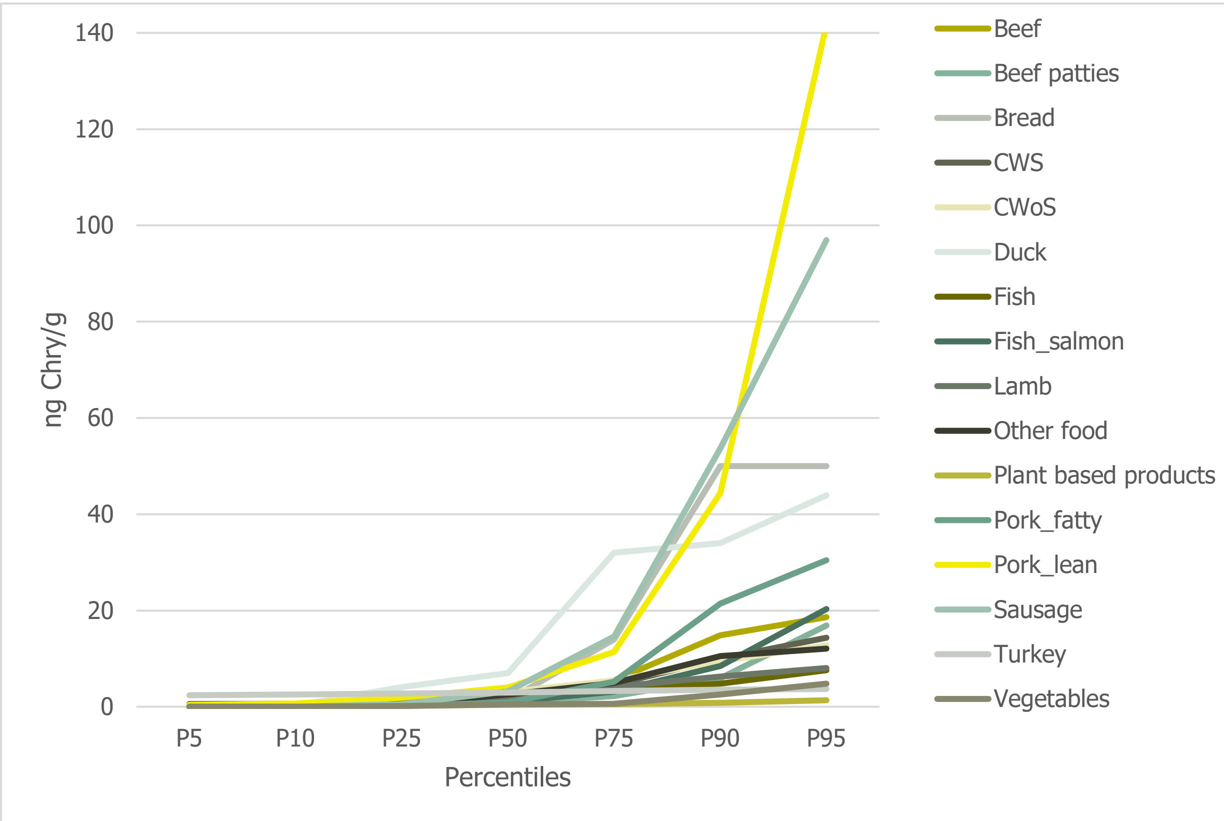

Figure 15 Percentile plot for Chry simulated values (ng/g).

11.2 PAH content from the two scenario-plates estimated by deterministic method

Mean exposure to BaP and PAH4 from a dinner plate with 200 grams of meat or fish from the two described scenarios was calculated based on the occurrence data in Tables 9 and 10 and shown again in Tables 36 and 37. The concentration data of PAHs included data from all types of grilling methods. It was not possible to make a reasonable differentiation between the various methods due to lack of information in the reported studies.

The PAH exposures from each of the two scenario-plates estimated by deterministic method were compared with the similar estimates with the probabilistic method (Table 36). The differences in the estimated exposures were most profound in the fat rich plate in which the probabilistic method estimated considerable higher mean exposures of BaP and sum PAH4 compared to the deterministic method. The difference can largely be explained by the estimated concentrations in sausages, which deviated considerable between the two methods. As shown in tables 9 and 10 in section 7.1.1 the mean concentration of BaP and PAH4 estimated in sausage by deterministic and probabilistic methods were 6.9 and 12 and 35 and 54 respectively.

Table 36. Mean estimated exposures to BaP and PAH4 from a scenario plate with 200g fat rich meat. The data are based on mean concentrations of BaP and mean sum PAH4 based on deterministic and probabilistic methodology.

Fat rich plate	1 plate (g)	Mean BaP, deter. (ng)	Mean BaP, prob. (ng)	Mean PAH4, deter. (ng)	Mean PAH4, prob. (ng)
CWS	60	34	36	246	269
Beef patties	60	350	342	958	950
Pork_fatty	60	432	483	1565	1989
Sausage	20	416	737	2099	3249
SUM	200	1232	1610	4867	6468

Table 37. Estimated concentrations of BaP and PAH4 on a plate with 200g lean meat and salmon. The data are shown as mean concentrations of BaP and mean sum PAH4 from the estimates with use of deterministic and probabilistic methodology.

Lean plate	1 plate (g)	Mean BaP, deter. (ng)	Mean BaP, prob. (ng)	Mean PAH4, deter. (ng)	Mean PAH4, prob. (ng)
Beef	50	171	179	707	720
CWoS	50	44	50	404	495
Fish_salmon	50	45	45	332	334
Pork_lean	50	151	156	1601	1627
SUM	200	411	429	3044	3170

11.2.1 Exposure to PAH from the deterministic scenario plates with grilled food

The risk characterization of PAH exposure was calculated from a dietary intake of BaP and PAH4 in two grill scenarios from the PAH concentrations estimated by the deterministic method with an added background exposure from other dietary sources as explained in 7.3.3.

Table 38. BaP and PAH4 exposure from the scenario of fat rich meat (200g per meal and a bw of 70 kg), with a presumed background exposure with data from campfire grilled food included.

Number of meals	Fat rich plate		Lean meat and salmon	
	BaP	PAH4	BaP	PAH
0	2.1	10.5	2.1	10.5
1	2.4	11	2.1	11
5	2.3	11	2.2	11
10	2.6	12	2.3	12
15	2.8	13	2.3	12
20	3.0	14	2.4	13
30	3.5	16	2.6	14
40	4.0	18	2.7	15
50	4.5	20	2.9	16
60	4.9	22	3.0	17
70	5.4	24	3.2	19
80	5.9	25	3.3	20
90	6.4	27	3.5	21
100	6.8	29	3.6	22

11.3 Risk characterizations of PAHs from scenarios by deterministic method

MOEs were calculated deterministic from the two grill scenarios from a mean PAH concentration (table 9) with a presumed background exposure of 149 ng/day, or 2.1 ng/kg/day and 10.5 ng/kg/day BaP and PAH4 respectively for a person with a body weight of 70 kg (as described in section 9.4.1). Table 39 shows the MOEs for BaP and PAH4 exposure from the two scenario plates, calculated for mean concentrations: MOEs below the critical MOE of 10,000 were not observed. The finding suggests that when consuming grilled fat rich meat or lean meat and salmon 100 times a year with an average BaP and PAH concentration, the MOE remains above 10,000.

Table 39. MOEs of deterministic mean BaP and PAH4 exposure from the scenario plates of fat rich meat and lean meat, with background exposure (200 g per meal, bw of 70 kg), consumption from one to 100 meals per year.

Number of meals	Fat rich plate		Lean meat and salmon	
	BaP	PAH4	BaP	PAH
0	33000	32000	33000	32000
1	33000	32000	33000	32000
5	30000	30000	32000	31000
10	27000	28000	31000	29000
15	25000	26000	30000	28000
20	23000	24000	29000	27000
30	20000	21000	27000	24000
40	18000	19000	26000	23000
50	16000	17000	24000	21000
60	14000	16000	23000	20000
70	13000	14000	22000	18000
80	12000	13000	21000	17000
90	11000	12000	20000	16000
100	10000	12000	19000	15000

Table 40. Weighted mean UB PAH concentrations (ng/g) in grilled sausages assessed deterministically, for all grilling methods with and without the inclusion of the data reported by Wiek and Tkacz, (2017).

Grilled sausages	BaA	BaP	BbF	Chry	PAH4
Wiek and Tkacz, (2017) excluded	10	6.9	7.0	11	35
Wiek and Tkacz, (2017) included	9.5	7.2	7.4	9.9	34