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Ranking of substances for monitoring in foods, drinks and dietary supplements - based on risk and knowledge gaps

Scientific Opinion of the Scientific Steering Committee of the Norwegian Scientific Committee for Food and Environment

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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 answer the request from the Norwegian Food Safety Authority. The project group consisted of six VKM members and a project leader from the VKM secretariat. The Scientific Steering Committee evaluated and approved the final opinion

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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.

Declaration of interest

Potential conflicts of interest have been considered and it was concluded that none of the participants had any conflict of interest. For further information, please contact VKM.

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Summary

The Norwegian Food Safety Authority (NFSA) requested the Norwegian Scientific Committee for Food and Environment (VKM) to provide an overview of substances in foods, drinks and dietary supplements that may constitute a potential health risk for humans, based on the VKM members' expert judgements. VKM was further requested to perform a ranking of these substances. Additionally, VKM should give an overview of the foods, drinks and dietary supplements most relevant for monitoring, and describe what would be the adequate sampling procedure and number of samples. Monitoring procedures were included to ensure that the monitoring performed is representative for the occurrence of the substances in foods, drinks and/or dietary supplements consumed by the Norwegian population.

The substances requested to be included were food additives and flavourings, substances used in food contact materials, environmental contaminants, process-induced substances and natural toxins. Substances not to be included were veterinary medicine residues, illegal pharmaceuticals and pesticide residues.

The overview provided by VKM included substances belonging to the following sub-groups:

- Natural toxins; with the sub-groups mycotoxins, plant toxins, marine and freshwater algae toxins
- Metals and metalloids
- Persistent organic pollutants (POPs); with the sub-groups brominated flame retardants, dechloranes, dioxins and dioxin-like polychlorinated biphenyls (DL-PCBs), non-dioxin-like polychlorinated biphenyls (NDL-PCBs), perfluorinated and polyfluorinated alkyl substances (PFAS) and siloxanes
- Substances in food contact materials; with the sub-groups bisphenols and phthalates
- Flavourings
- Additives; with the sub-groups nitrites and nitrates, phosphates, sweeteners and synthetic antioxidants
- Process-induced contaminants; with the sub-groups acrylamide, esterified 3- and 2-monochloropropane-1,2-diol (MCPD), glycidyl fatty esters (GEs), furans, heterocyclic aromatic amines (HAAs) and polycyclic aromatic hydrocarbons (PAHs)
- «Other substances»
- Trace elements

The ranking of the substances was based on inherent toxicity (hazard) and level of exposure (both occurrence and intake). In addition, vulnerable groups, adequacy of toxicity data and exposure data were considered. A simple methodology was chosen. More advanced methodology may be used in later updates of this ranking, if found useful.

Key words: flavourings, food additives, food contact materials, metals, metalloids, natural toxins, Norwegian Scientific Committee for Food and Environment, «other substances», persistent organic pollutants, process-induced contaminants, ranking, trace element, VKM.

Sammendrag på norsk

Mattilsynet har bedt Vitenskapskomiteen for mat og miljø (VKM) om å utarbeide en oversikt over stoffer i mat, drikke og kosttilskudd som kan utgjøre en potensiell helserisiko. Oversikten skal basere seg på VKM-medlemmenes ekspertvurdering. VKM skal også vurdere og rangere stoffene ut i fra potensiell helserisiko, og beskrive hvilke matvarer, drikke og/eller kosttilskudd som det er mest relevant å overvåke for hvert av de inkluderte stoffene. For å sikre at overvåkingen er representativ for forekomst av stoffene i norsk kosthold, ble VKM også bedt om å beskrive hvordan prøver bør tas og hva som er et tilstrekkelig antall prøver.

Mattilsynet ønsket at tilsetningsstoffer, aromastoffer, stoffer som brukes ved produksjon av matkontaktmaterialer, miljøgifter og andre forurensende stoffer, prosessfremkalte stoffer og naturlige gifter skal inngå i oversikten og rangeringen. Rester av plantevernmidler og rester av legemidler skulle ikke inkluderes.

De inkluderte stoffene ble delt inn i følgende grupper og undergrupper:

- Naturlige giftstoffer, med undergruppene mykotoksiner, plantetoksiner, marine toksiner og ferskvannstoksiner
- Metaller og metalloider
- Persistente organiske miljøgifter, med undergruppene brominerte flammehemmere, dekloraner, dioksiner og dioksinlignende PCB, ikke-dioksinlignende PCB, perfluorerte organiske fluorstoffer og siloksaner
- Aromastoffer
- Tilsetningsstoffer, med undergruppene nitrater og nitritter, fosfater, søtstoffer og syntetiske antioksidanter
- Prosessfremkalte stoffer, med undergruppene akrylamid, 3-monokloropropanediol (3-MCPD) og glycidyl estere, furaner, heterosykliske aminer og polysykliske aromatiske hydrokarboner
- «Andre stoffer»
- Sporstoffer

Stoffene er rangert ut i fra hvor toksiske de er, grad av eksponering i befolkningen, mulige sårbare grupper og eventuell mangel på kunnskap om toksisitet og eksponering.

Det er brukt en enkel metodikk. Mer avansert metodikk kan eventuelt bli brukt i senere oppdateringer av denne rangeringen, hvis det viser seg å være hensiktsmessig.

Abbreviations and glossary

Abbreviations

ADI	acceptable daily intake
AGD	anogenital distance
ALT	alanine transaminase
ARfD	acute reference dose
BGAS	blue-green algae food supplements
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMI	body mass index
CRF	chronic renal failure
CVD	cardiovascular disease
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
FDA	Food and Drug Administration, USA
FFQ	food frequency questionnaire
GD	gestational day
GI	gastrointestinal
GL	guidance level
GLP	good laboratory practice
HBGV	health-based guidance value
HDL	high density lipoprotein
IARC	International Agency for Research on Cancer
LB	lower bound
LCPUFAS	long-chain polyunsaturated fatty acids
LDL	low density lipoprotein
LOD	limit of detection
LOQ	limit of quantification
ML	maximum level
MoBa	the Norwegian Mother and Child Cohort Study
MOE	margin of exposure
MOS	margin of safety
MSDI	maximised survey-derived daily intake
NCRI	negligible cancer risk intake
NFSA	Norwegian Food Safety Authority
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
OECD	the Organisation for Economic Co-operation and Development
PBPK	physiologically based pharmacokinetic model
PND	postnatal day
pTDI	provisional tolerable daily intake
pTWI	provisional tolerable weekly intake
RCT	randomized controlled trial
RFP	relative potency factors

TDI	tolerable daily intake
TDS	total diet study
TTC	threshold of toxicological concern
tTDI	temporary tolerable daily intake
TWI	tolerable weekly intake
UB	upper bound
UF	uncertainty factor
UL	tolerable upper intake level
VKM	Norwegian Scientific Committee for Food and Environment
WHO	World Health Organization

Glossary

Acceptable daily intake (ADI)
An estimate of the amount of a substance in food or drinking water that can be consumed daily over a lifetime without presenting an appreciable risk to health. It is usually expressed as milligrams of the substance per kilogram of body weight.

Benchmark dose (BMD)
The minimum dose of a substance that produces a clear, low level health risk, usually in the range of a 1-10% change in a specific toxic effect, such as cancer induction.

Benchmark dose lower confidence limit (BMDL)
The lower boundary of the confidence interval on the benchmark dose. The BMDL accounts for the uncertainty in the estimate of the dose-response that is due to characteristics of the experimental design, such as sample size.

Health-based guidance value (HBGV)
Such a value indicates the amount of a chemical in food or drinking water that a person can consume on a regular basis over a lifetime without any significant risk to health (e.g. ADI, TDI, TWI etc.).

Limit of detection (LOD)
A limit of detection is the lowest concentration of a substance that can be detected using a validated analytical method but which is too small to be measured with the required certainty.

Limit of quantification (LOQ)
The limit of quantification is the lowest concentration of a substance that can be measured with the required certainty using a validated analytical method.

Margin of exposure (MOE)
The ratio of the reference point (RP) (i.a. no observed adverse effect level (NOAEL) or the

benchmark dose lower confidence limit (BMDL)) for the critical effect to the theoretical, predicted or estimated human exposure dose or concentration.

Margin of safety (MOS)
The margin between the health-based guidance value (HBGV) (reference dose) and the actual or estimated human exposure dose or concentration. Be aware that MOS sometimes is used with the the same meaning as MOE by some experts.

No observed adverse effect level (NOAEL)
The highest concentration or amount of a substance, at which no detectable adverse effects occur in experimental animals or an exposed population.

Tolerable daily intake (TDI)
An estimate of the amount of a substance in food or drinking water, which is not added deliberately (e.g. contaminants) and which can be consumed daily over a lifetime without presenting an appreciable risk to health.

Tolerable weekly intake (TWI)
An estimate of the amount of a substance in food or drinking water, which is not added deliberately (e.g. contaminants) and which can be consumed weekly over a lifetime without presenting an appreciable risk to health.

Uncertainty factors (UF)
Numerical adjustment used to extrapolate from experimentally determined (dose–response) relationships to estimate the exposure to an agent below which an adverse effect is not likely to occur. Generally, UF is initially set at 100, with interspecies variation (x10, difference between animal and humans) and intraspecies variation (x10) taken into account. UF may be supplemented if there is any uncertainty related to the study period, reliability and other features of the toxicity tests.

Undesirable substances in food (*Definition given by the Food Safety Authority for this assignment*)
Pesticide and veterinary residues, unauthorized use levels of food additives, unauthorized substances, contaminants, natural toxins, processing contaminants and substances migrating from food contact materials.

Background as provided by the Norwegian Food Safety Authority

Undesirable substances in food

Food shall not contain levels of undesirable substances or additives that can be of health concern. There is no explicit definition, and in this assignment, pesticide and veterinary residues, unauthorized use levels of food additives, unauthorized substances, contaminants, natural toxins, processing contaminants and substances migrating from food contact materials, will be referred to as undesirable substances in foods. Monitoring is an important tool to reveal potential substances of concern in foods as well as to maintain and ensure consumer safety. In order to prioritize which substances to monitor, the Norwegian Food Safety Authority (NFSA) needs a knowledge-based ranking of contaminants that may be a potential health risk for the Norwegian consumers.

Several undesirable substances are included in the EU/EEA regulations, and for many of these substances there are maximum levels (MLs) established for the different food categories. The MLs are generally based on risk assessments and other aspects such as good agricultural and production practices, as well as assessments of what is practically achievable. The MLs cannot be too low, causing most of the food to be discarded.

Monitoring

Several monitoring programs are conducted by the Norwegian Food Safety Authority (NFSA). With respect to undesirable substances, two monitoring programs («Pesticide residues» and «Veterinary residues») are conducted on livestock animals each year. Norway is committed to perform these monitoring programs according to the EEA agreement. Furthermore, large monitoring programs on undesirable substances in seafood are performed yearly («Veterinary residues in fish» and «Environmental contaminants in wild fish, marine oils and in fish and seafood from contaminated harbors and fjords»). In addition, smaller monitoring programs on other undesirable substances are conducted yearly. The prioritization of which substances to examine differ from year to year and is based upon i.e. changed dietary habits or new knowledge about specific substances. To ensure that the monitoring data can be applied in the management of safe foods, NFSA needs risk-based knowledge regarding which substances to examine, which food categories to monitor, and how many samples that should be included in the monitoring programs for each substance and food category.

Terms of reference as provided by the Norwegian Food Safety Authority

NFSA asks The Norwegian Scientific Committee for Food and Environment (VKM) to provide an overview with a risk ranking of substances in foods, drinks and dietary supplements that may pose a potential health risk for Norwegian consumers. There is no upper limit of number of substances that can be included in the overview. The assignment is divided into three parts:

Part 1

Provide an overview of substances in foods, drinks and dietary supplements that may potentially pose a health risk and include scientific reasons or arguments for each substance. Potential health risks should be assessed based on both toxicity and exposure, when this information is available. The list of substances should be based on the VKM members' expert judgements.

Substances that should be included:

- Food additives and flavourings
- Substances used in food contact materials
- Contaminants
- Process-induced substances
- Natural toxins

Substances that should be excluded (these substances are already covered by the two extensive monitoring programs that Norway is committed to according to the EEA agreement) are veterinary residues, illegal pharmaceuticals and pesticide residues.

Part 2

To assess and rank the substances on the list developed in part 1, according to potential health risk.

Part 3

For each of the substances on the list from part 1, VKM is asked to describe

- Which food, drinks and/or dietary supplements are most relevant for monitoring
- What is adequate sampling procedure and number of samples to ensure monitoring that is representative for the occurrence in foods consumed by the Norwegian population

1 Substances in foods, drinks and dietary supplements that may pose a potential health risk

A list of substances or groups of substances in foods, drinks and dietary supplements that may potentially pose a health risk was prepared based on the VKM members' expert judgements. Thus, the substances included in this list were not chosen based on a systematic approach. Since the list of substances (Table 1-1) was prepared by expert judgements and not by any systematic method, the list of included substances is not exhaustive and the list may be revised/extended later. Systematic methodology can be used in later updates of this list, if found useful. The list was prepared by members of the VKM Scientific Steering Committee, members of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics, members of the Panel on Contaminants and members of the Panel on Animal Feed.

The substances included in the list are natural toxins, metals and metalloids, persistent organic pollutants, substances used in food contact materials, food additives and flavourings, process-induced contaminants, so-called «other substances» (see Chapter 10) and trace elements. Veterinary residues, illegal pharmaceuticals and pesticide residues were not included, according to the mandate by the Norwegian Food Safety Authority. In this work, the emphasis has been on substances that are currently not being monitored.

Most substances in the present ranking are listed individually. However, for practical purpose, some substances that were risk-assessed as a group were included as a group.

A total of 79 single substances or groups of substances was included. An overview of the substances is given in Table 1-1.

Table 1-1. An overview of the included substances (79 single substances or groups of substances).

Substance group	Sub-group	Substance
Natural toxins	Mycotoxins	Aflatoxins (AFLAs)
		Alternariol (AOH) and Alternariol methyl ether (AME)
		Deoxynivalenol (DON) and modified forms
		Enniatins (ENNs)
		Ochratoxin A (OTA)
		Patulin (PAT)
		T-2 (T2) and HT-2 (HT2) toxins and modified forms
		Zearalenone (ZEN) and modified forms
	Plant toxins	Solanine and Chaconine
		Cyanogenic glucosides

Substance group	Sub-group	Substance
		Erucic acid
		Glucosinolates
		Pyrrolizidine alkaloids (PAs)
		Tropane alkaloids (TAs)
	Marine algae toxins	Azaspiracids (AZAs)
		Tetrodotoxin (TTX) and analoges
	Freshwater algae toxins	Microcystins (MCs)
Metals and metalloids		Aluminium (Al)
		Arsenic (As) – organic and inorganic
		Cadmium (Cd)
		Chromium (Cr)
		Lead (Pb)
		Methylmercury (MeHg)
		Nickel (Ni)
Persistent organic pollutants (POPs)	Brominated flame retardants	Polybrominated diphenyl ethers (PBDEs) (including Decabromodiphenyl ether (DecaBDE)), Hexabromocyclododecane (HBCDD), Hexabromobenzene (HBB), Decabromo-diphenyl ethane (DBDPE), 1,2-Bis(2,4,6-tribromophenoxy)ethane (BTBPE) and 2,4,6-Tribromophenol (TBP)
	Dechloranes	Dechlorane plus (syn-DP and anti-DP)
	Dioxins and Dioxin-like polychlorinated biphenyls (DL-PCBs)	Dioxins and DL-PCBs
	Non-dioxin-like polychlorinated biphenyls (NDL-PCBs)	PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180 and PCB6
	Perfluorinated and polyfluorinated alkyl substances (PFAS)	Perfluorooctane sulfonate (PFOS), Perfluorooctanoic acid (PFOA), Perfluorohexane sulfonic acid (PFHxS), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Perfluoroundecanoic acid (PFUnDA) and Perfluoroheptane sulfonate (PFHpS)
	Siloxanes	Octamethylcyclotetrasiloxane (D4), Decamethylcyclopentasiloxane (D5) and Dodecamethylcyclohexasiloxane (D6)
Substances in food contact materials	Bisphenols	Bisphenol A (BPA), Bisphenol S (BPS), Bisphenol F (BPF) and Bisphenol AF (BPAF)
	Phthalates	Bis(2-ethylhexyl)phthalate (DEHP), Butyl-benzyl-phthalate (BBP), Di-butylphthalate (DBP), Di-isodecyl phthalate (DIDP), Di-isononyl phthalate (DINP)
Flavourings		Caffeine
Additives	Nitrites and nitrates	Sodium and potassium salts of nitrite and nitrate
	Phosphates	Phosphoric acid-phosphates

Substance group	Sub-group	Substance
	Sweeteners	Sucralose
		Acesulfame K (E950)
	Synthetic antioxidants	Butylated hydroxyanisole (BHA, E320)
		Butylated hydroxytoluene (BHT, E321)
		Ethoxyquin (EQ)
Process-induced contaminants	Acrylamide	Acrylamide
	Esterified 3- and 2-monochloropropane-1,2-diol (MCPD) and glycidyl esters (GEs)	Glycidyl fatty esters (GEs), 3-Monochloropropanediol (3-MCPD) and its fatty esters
	Furans	Furan, 2-Methylfuran and 3-Methylfuran
	Heterocyclic aromatic amines (HAAs)	2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), HAAs in general
	Polycyclic aromatic hydrocarbons (PAHs)	Polycyclic aromatic hydrocarbons (PAHs)
«Other substances» *		L-Aspartic acid, L-Carnitine and L-Carnithine-L-tartrate, Coenzyme Q10 (CoQ10), Conjugated linoleic acids (CLAs), Creatine, Curcumin, L-Cysteine and L-Cystine, Docosahexaenoic acid (DHA), Docosapentaenoic acid (DPA), Eicosapentaenoic acid (EPA), D-Glucurono-γ-lactone, Inositol, Lycopene, L-Methionine, Piperine, Taurine, L-Tyrosine
Trace element		Iodine

*«Other substances»: substances other than vitamins or minerals that have a nutritional and/or physiological effect according to the food supplement directive 2002/46/EC. They are added mainly to food supplements, but also to energy drinks and other foods.

2 Ranking methodology

At the start of this work, the members of the project group familiarised themselves with available methods used for ranking of chemicals. The choice of methodology was discussed in the VKM Scientific Steering Committee. Because of time contraints set by the Norwegian Food Safety Authority, a simple methodology was chosen for this first attempt of making such a ranking list of chemicals based on risk and knowledge gaps. More advanced methodology can be used in later updates of this ranking, if found useful.

The ranking of the substances was based on their inherent toxicity (hazard) and level of exposure (based on both occurrence and intake). In addition, vulnerable groups, adequacy of toxicity data and of exposure data (occurrence and/or intake) were considered. The following considerations were used to rank the substances:

Either

1. If there are available health-based guidance values (HBGV), such as acceptable daily intake (ADI), tolerable daily intake (TDI) or tolerable weekly intake (TWI), including temporary or provisional and group values, the scoring is based on whether exposure per day or per week is above or below these values. When setting e.g. ADI or TDI, the no observed adverse effect level (NOAEL) or the benchmark dose lower confidence limit (BMDL) in the critical study is divided by appropriate uncertainty factors (UF). Alternatively, when available, quantitative data for toxicity and exposure is used to calculate the margin of exposure (MOE) or the margin of safety (MOS). MOE is the ratio of the NOAEL or BMDL for the critical effect to the estimated human exposure dose or concentration. MOS is the margin between the HBGV and the estimated human exposure dose or concentration. Be aware that MOS sometimes is used with the same meaning as MOE by some experts. Depending on the values for MOE or MOS, the substance will be given the score 2 for high MOE or MOS, 4 for medium MOE or MOS or 6 for low MOE or MOS. Depending on whether the exposure is well below, close to or above the ADI/TDI/TWI, the substance will be given the score 2, 4 and 6, respectively.

Or

2. The inherent toxicity (hazard) of the substance is evaluated. The scores given are 1 for low toxicity, 2 for medium toxicity or 3 for high toxicity.
3. The level of exposure to the substance is evaluated. The scores given are 1 for low exposure, 2 for medium exposure or 3 for high exposure.

And

4. Vulnerable groups may e.g. be high exposure groups in the population, for instance because of certain dietary habits, or especially vulnerable population groups, for

example due to certain genetic variants, diseases, drug use or age/life phase. The scores given are 0 for no specific vulnerable groups, 0.5 when the exposure is somewhat higher for one or more groups in the population/one or more groups in the population are somewhat more vulnerable, or 1 when the exposure is very high for one or more groups in the population/one or more groups in the population are especially vulnerable.

5. Adequacy of data on toxicity are scored 0 for sufficient toxicity data, 0.5 when some toxicity data are lacking or 1 when little toxicity data are available.
6. Adequacy of data on exposure are scored 0 for sufficient data to calculate the exposure, 0.5 when some exposure data are lacking, or 1 for little exposure data are available.

An overview of the points used to rank the substances according to potential health risk and knowledge gaps is given in Table 2-1. When quantitative data on toxicity and exposure are available, the substance is scored according to points 1, 4, 5 and 6. When either quantitative data on toxicity or exposure are unavailable, the substance is scored according to the points 2, 3, 4, 5 and 6.

The highest possible score is 9 whether based on sum of scoring in points 1, 4, 5 and 6 or based on sum of scoring in points 2, 3, 4, 5 and 6. The lowest possible score is 2.

Table 2-1. Method used for the ranking of the substances. When quantitative data on toxicity and exposure were available, points 1, 4, 5 and 6 were scored. When either quantitative data on toxicity or exposure were unavailable, points 2, 3, 4, 5 and 6 were scored. Acceptable daily intake (ADI); Benchmark dose lower confidence limit (BMDL); Health-based guidance value (HBGV); Margin of exposure (MOE); Margin of safety (MOS); No observed adverse effect level (NOAEL); Tolerable daily intake (TDI); Tolerable weekly intake (TWI).

1. Quantitative data are available for both toxicity and exposure (MOE/MOS/ADI/TDI/TWI) • If there are available HBGVs, such as ADI, TDI or TWI, including temporary or provisional and group values, the scoring is based on whether exposure per day or per week is above or below these values. • Alternatively, when available, quantitative data for toxicity and exposure is used to calculate MOE or MOS. MOE is the ratio of NOAEL or BMDL for the critical effect to the estimated human exposure dose or concentration. MOS is the margin between the HBGV and the estimated human exposure dose or concentration. Be aware that some experts use MOS with the the same meaning as MOE.	
If the exposure is above the ADI/TDI/TWI or MOE/MOS is too low*	Score = 6.0
If the exposure is close to the ADI/TDI/TWI or MOE/MOS is at the edge of acceptable value	Score = 4.0
If the exposure is well below the ADI/TDI/TWI or MOE/MOS is more than sufficiently high*	Score = 2.0
2. The intrinsic toxicity of the substance/substance group	
High toxicity	Score = 3.0

Medium toxicity	Score = 2.0
Low toxicity	Score = 1.0
3. Exposure from foods	
High exposure	Score = 3.0
Medium exposure	Score = 2.0
Low exposure	Score = 1.0
4. Vulnerable groups	
If the exposure is very high for one or more groups in the population/one or more groups in the population are especially vulnerable due to, for example, certain genetic variants, diseases, drug use or age/life phases (<1 year, puberty, pregnant/nursing, elderly)	Score = 1.0
Exposure is somewhat higher for one or more groups in the population/one or more groups in the population are somewhat more vulnerable due to, for example, specific genetic variants, diseases, drug use or age/life stages	Score = 0.5
There are no specific groups in the population with high exposure/no population groups that are very vulnerable due to, for example, specific genetic variants, diseases, drug use or age/life stages	Score = 0.0
5. Adequacy of data on toxicity	
Little data available on toxicity	Score = 1.0
Some toxicity data are lacking	Score = 0.5
There is sufficient toxicity data	Score = 0.0
6. Adequacy of data on exposure (occurrence and/or intake)	
Little data available on exposure	Score = 1.0
Some exposure data are lacking	Score = 0.5
There is sufficient exposure data	Score = 0.0

* MOE is too low/MOE is sufficiently high:

- For substances that are genotoxic and carcinogenic (substances for which no threshold of toxicity can be identified), too low MOE would in general be <10,000 based on $BMDL_{10}$ (the lower limit of a one-sided 95% confidence interval on BMDL corresponding to 10% tumor incidence over control). Other considerations of sufficiently large MOE to conclude on low risk may be done from case to case based on the data available.
- For non-genotoxic substances (substances for wich a treshold can be identified), a too low MOE would be <100 based on no observed adverse effect level (NOAEL) or BMDL. Other considerations of sufficiently large MOE may be done based on the data available.

In this ranking there are very different groups of substances included, for instance both genotoxic and non-genotoxic substances. For some substances, there are a lot of toxicity data and/or exposure data available and several risk assessments have been performed by competent insitutions, whereas very limited toxicity data and no or few risk assesments were available for other substances. The reasoning behind and the basis for the scoring as low, medium or high in the various questions will therefore be somewhat different for the various

groups of chemicals. Because of this plurality, the methodology used is more or less consistent and suitable for the various groups of substances. The tables of ranked substances should therefore be read together with the main text, where calculations are included and explanations are given for the scoring. At the end of each chapter, references to the risk assessments, i.e. from EFSA or VKM, and scientific publications used to decide on the ranking, are listed. The readers are referred to these dockuments for further details. The ranking is associated with uncertainty, and when in doubt on how to score, the medium score was chosen.

3 Ranking of natural toxins

An overview of the scoring and ranking of the included natural toxins is given in Table 3-1. A detailed description follows after the table.

Table 3-1. Summary table for scoring of natural toxins.

Subgroup	Substance	1. MOE/MOS/ ADI/TDI/TWI	2. Toxicity	3. Exposure	4. Vulnerable groups	5. Lack of toxicity data	6. Lack of exposure data	Total score	Comments
Mycotoxins	Aflatoxins (AFLAs)	6.0	-	-	0.5	0.5	0.5	7.5	• Occurrence is monitored, but better analytical methods are available • Increased occurrence due to climate change expected • Exposure exceeds level of accepted lifetime cancer risk
	Alternariol (AOH) and Alternariol methyl ether (AME)	-	2.0	2.0	0.5	1.0	0.5	6.0	• Occurence data missing • Higher exposure in children expected • Toxicity data limited. Toxicokinetic data missing

Subgroup	Substance	1. MOE/MOS/ ADI/TDI/T WI	2. Toxicity	3. Exposure	4. Vulnerabl e groups	5. Lack of toxicity data	6. Lack of exposure data	Total score	Comments
	Deoxynivalenol (DON) and modified forms	4.0	-	-	1.0	0.5	0.5	6.0	• TDI exceeded by Norwegian children • New analytical methods available • Effects of chronic low-level toxicity unclear
	Enniatins (ENNs)	-	1.0	3.0	0.5	1.0	1.0	6.5	• Updated occurrence data are lacking • New sensitive analytical methods available • Toxicity data insufficient
	Ochratoxin A (OTA)	4.0	-	-	0	0.5	1.0	5.5	• Updated occurrence data are lacking • New analytical methods available • Exposure in Norway not assessed • Human health risk from dietary exposure unclear
	Patulin (PAT)	2.0	-	-	0	0.5	1.0	3.5	• Provisional tolerable daily intake established in 1995 • Toxicokinetic data are lacking • Exposure in Norway not assessed

Subgroup	Substance	1. MOE/MOS/ ADI/TDI/TWI	2. Toxicity	3. Exposure	4. Vulnerable groups	5. Lack of toxicity data	6. Lack of exposure data	Total score	Comments
	T-2 (T2) and HT-2 (HT2) toxins and modified forms	6.0	-	-	1.0	0.5	1.0	8.5	• Exposure in high-consumers exceeds new group TDI • Occurrence data for Norwegian grain insufficient • New available analytical methods (low LOD) should be used • Toxcicity data for metabolites missing
	Zearalenone (ZEN) and modified forms	2.0	-	-	0	1.0	0.5	3.5	• Occurrence data for Norwegian grain are old • Consumption of maize increases • Toxicity data for modified forms scarce • New analytical methods available
Plant toxins	Solanine and Chaconine	-	2.0	2.0	1.0	0.5	1.0	6.5	• Little or no chronic toxicity data (no TDI) • No good data on total exposure (intake and occurrence) from potatoes and all other relevant vegetables in Norway or EU • Vulnerable groups may be pregnant women and their fetus

Subgroup	Substance	1. MOE/MOS/ ADI/TDI/TWI	2. Toxicity	3. Exposure	4. Vulnerable groups	5. Lack of toxicity data	6. Lack of exposure data	Total score	Comments
	Cyanogenic glucosides	4.0			0.5	1.0	0	6.0	
	Erucic acid	4.0	-	-	0.5	0.5	0	5.0	
	Glucosinolates		1.0	1.0	0.5	1.0	0.5	4.0	• Low toxicity, may also be beneficial
	Pyrrolizidine alkaloids (PAs)	6.0	-	-	1.0	0.5	0.5	8.0	• High consumers of tea and herbal infusions, food supplements based on plant extracts or pollen can have high chronic exposure • Acute toxicity is also possible • 17 PAs suggested monitored, no Norwegian data
	Tropane alkaloids	4.0	-	-	0.5	1.0	0.5	6.0	• Most analytical data available are below the level of quantification • High consumers (in particular children) may exceed acute ARfD
Marine algae toxins	Azaspiracids (AZAs)	4.0	-	-	1.0	0.5	1.0	6.5	
	Tetrodotoxin (TTX) and TTX analoges	4.0	-	-	1.0	0.5	1.0	6.5	

Subgroup	Substance	1. MOE/MOS/ ADI/TDI/T WI	2. Toxicity	3. Exposure	4. Vulnerabl e groups	5. Lack of toxicity data	6. Lack of exposure data	Total score	Comments
Freshwater algae toxins	Microcystins (MCs)	4.0	-	-	0.5	1.0	1.0	6.5	

3.1 Subgroup mycotoxins

Mycotoxin occurrence in Norway is dependent on the percentage of imported grain, and the amount imported varies from year to year. The occurrence of mycotoxins is expected to change in the warmer and more humid climate, and aflatoxins, ochratoxin A and fumonisins will probably increase in crops and food products in middle and Northern Europe.

3.1.1 Deoxynivalenol (DON) and modified forms

Deoxynivalenol (DON) is a mycotoxin primarily produced by *Fusarium* fungi, occurring predominantly in cereal grains. DON and modified forms are the most common mycotoxins in Norwegian-grown cereals. The modified forms include 3-acetyl-deoxynivalenol (3-Ac-DON), 15-acetyl-deoxynivalenol (15-Ac-DON) and deoxynivalenol-3-glucoside (DON-3-glu), are all produced in plants. The relative ratios of concentrations of 3-Ac-DON, 15-Ac-DON and DON-3-glu to DON were determined as 10%, 15% and 20%, respectively. Since 3-Ac-DON and 15-Ac-DON are largely deacetylated and DON-3-glucoside cleaved in the intestines, the same toxic effects as for DON can be expected. The TDI of 1 µg/kg bw per day, that was established for DON, is therefore used as a group TDI for the sum of DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside (EFSAl, 2017). The TDI was based on reduced body weight gain in mice for which a no observed adverse effect level (NOAEL) of 100 µg/kg bw per day was determined.

3.1.1.1 ***Scores***

MOE/MOS/ADI/TDI/TWI: score 4.0

The tolerable daily intake (TDI; 1µg/kg bw per day) is exceeded by up to 3.5 times in infants and small children. In these calculations, only occurrence data for DON have been considered. Accordingly, the MOE value for DON is higher than 100 in Norwegian adults, but below 30 in children. Including the modified forms would most probably lead to a further decrease of the MOE (VKM, 2013).

Toxicity (background information)

DON binds to ribosomes, leading to inhibition of protein synthesis and subsequently also RNA and DNA synthesis. This binding also induces ribotoxic stress and activates different mitogen-activated protein kinases (MAPKs). Activation of MAPKs explains several effects of DON, such as apoptosis or survival of cells, inflammatory effect and oxidative stress. The main clinical effects of exposure to DON are reduced weight gain, inflammation and reduced immune responses. DON is shown to upregulate the expression of proinflammatory genes and several other genes related to communications between the innate and the adaptive immune systems and to cell–cell signalling (Wentzel et al., 2016). DON also altered the expression of several genes involved in gastrointestinal disease, inflammatory disease and response network. Furthermore, DON affected the gastrointestinal barrier, which could be associated with intestinal inflammatory disease in humans (Cano et al., 2013). DON

increased the permeability through the gut epithelial layer both *in vivo* and *in vitro* (Akbari et al., 2014). Effects of chronic low-level DON exposure on the neurodevelopment have not been investigated so far.

DON is hydrophilic, heat stabile, easily absorbed in the gut (bioavailability 50-90%), distributed to tissues (can cross the blood-placenta and blood-brain barriers) and eliminated with intermediate velocity in most species (half-life 1-4 h) with the exception of birds.

Exposure (background information)
Human health risk of acute DON intoxication was assessed using epidemiological data of mycotoxicosis and a group-ARfD of 8 µg/kg bw per eating occasion was calculated. Estimates of acute dietary exposures were below this dose and did not raise a health concern in humans. However, the estimated mean chronic dietary exposure was above the group TDI in infants, toddlers and other children, and at high exposure also in adolescents and adults, indicating a potential health concern. The same has been shown in a study estimating DON exposure in the Norwegian population (Sundheim et al., 2017). Based on food consumption and occurrence data, the mean exposure to DON in years with low and high levels of DON in the flour, respectively, were in the range of or up to two times TDI in 1-year-old infants and 2-year-old children. In years with high mean DON concentration, the high (95-percentile) exposure exceeded the TDI by up to 3.5 times in 1-, 2- , 4- and 9-year-old children. The assessment concluded that exceeding the TDI in infants and children is of concern. The estimated dietary DON intakes in adolescent and adult populations are in the range of the TDI or below, and are not a health concern. Acute human exposure to DON is not of concern in any age group.

Vulnerable groups: score 1.0
The dietary exposure of infants and children is above the TDI, which is of concern. Infants in Norway have higher consumption of cereal-based foods than other European children.

Lack of toxicity data: score 0.5
There are relatively little data on the effects of chronic low-level exposure to DON. Studies in rodents and pigs have shown possible effects on the immune activity, gut health and neurodevelopment at DON levels below the current NOAEL.

Lack of exposure data: score 0.5
Data on chronic low-level exposure to DON, especially in infants and toddlers, are lacking.

Total score = 6.0 for deoxynivalenol (DON) and modified forms

3.1.1.2 ***References***

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3.1.2 Zearalenone (ZEN) and modified forms

Zearalenone (ZEN) is a phenolic resorcylic acid lactone mycotoxin produced by several *Fusarium* species, particularly *Fusarium graminearum*. ZEN can be modified in plants, fungi and animals by phase I and phase II metabolism. Modified forms of ZEN include its reduced phase I metabolites, i.e. α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL), α-zearalanol (α-ZAL) and β-zearalanol (β-ZAL), zearalanone (ZAN) and its phase II derivatives, such as those conjugated with glucose (zearalenone-14-glucoside (ZEN14G)), sulphate (zearalenone-14-sulphate (ZEN14S)) and glucuronic acid (ZENGlc) (EFSA, 2016).

ZEN occurs worldwide in all types of grains. Maize and wheat bran contain the highest concentrations, but grains and grain-based food such as breakfast cereals, bread and bakery wares make the largest contribution to the estimated dietary intake in Europe due to high consumption. Vegetable oils may also contribute to the overall dietary intake of ZEN (VKM, 2013). There is only limited information on the occurrence of the modified forms in grain. However, it has been reported that α-ZEL and β-ZEL occur in amounts of up to 58% and 21% of ZEN, respectively, in cereal-based foods. ZEN14Glc represented an additional 42%, while both α- and β-ZEL14Glc accounted for additional 20%. ZEN14S was less prevalent (EFSA, 2014; EFSA, 2016).

Wide interspecies differences in ZEN toxicokinetics have been documented. Prehepatic, hepatic end extrahepatic ZEN metabolism has been reported. Metabolite profiles are species-

dependent and may affect the species-sensitivity to the toxin. The main ZEN metabolites are α-ZAL, β-ZAL, with only very limited amounts of α-ZEL, β-ZEL and other reductive metabolites being produced. The reduced metabolites retain or increase the estrogenic potency of the parent compound (EFSA, 2016). After oral exposure, ZEN and its metabolites are rapidly absorbed, distributed to several organs and quickly excreted, mainly via the biliary route as glucuronides.

3.1.2.1 ***Scores***

MOE/MOS/ADI/TDI/TWI: score 2.0

Based on estrogenicity data in the most sensitive animal species, the pig, and taking into account comparisons between pigs and humans, EFSA established a TDI for ZEN of 0.25 µg/kg bw per day (EFSA, 2011). The TDI was redefined as a group TDI in 2016, including ZEN and all modified forms. EFSA also considered it appropriate to include glucuronides of ZEN and its phase I metabolites in this group TDI. To account for differences in *in vivo* estrogenic potency, each phase I metabolite was assigned a potency factor relative to ZEN to be applied to exposure estimates of the respective ZEN metabolites. It was assumed that conjugates (phase II metabolites) of ZEN and its phase I metabolites, which per se have no estrogenic activity, will be cleaved releasing ZEN and its phase I metabolites (EFSA, 2016).

Estimates of chronic dietary exposure to ZEN based on the available occurrence data are below or in the region of the TDI for all age groups and not a health concern.

Toxicity (background information)

Acute toxicity of ZEN is low (EFSA, 2011), so that an ARfD for ZEN has not been set. The main biological activity of ZEN is its estrogenicity, i.e. the ability to act like the endogenous steroidal sex hormone 17-β estradiol. ZEN binds to estrogenic receptors (ERs) and has a stronger affinity to ER-α than to ER-β. ZEN and its modified forms differ considerably in their estrogenic activity. Based on their «uterotrophic activity» assessed in rodents, ZEN and its modified forms are ranked as follows: α-ZEL > α-ZAL > ZEN = ZAN = β-ZAL > β-ZEL. ZEN can activate the pregnane X receptor (PXR) and increase the transcription of a number of genes, including several CYPs (EFSA, 2016).

A group TDI of 0.25 µg/kg bw per day expressed as ZEN equivalents was established for ZEN and its modified forms (phase I and phase II metabolites). To account for differences in estrogenic potencies *in vivo*, each modified form was assigned a potency factor relative to ZEN to be applied to exposure estimates of the respective ZEN metabolites. The relative potency factors (RPFs) to be applied for the different modified forms are 1.0 for ZENGlcs and ZEN Sulfs; 60 for α-ZEL, α-ZELGlcs and α-ZELSulfs; 0.2 for β-ZEL, β-ZELGlcs and β-ZELSulfs; 1.5 for ZAN, ZANGlcs and ZANSulfs; 4.0 for α-ZAL, α-ZALGlcs and a-ZALSulfs; 2.0 for b-ZAL, b-ZALGlcs and b-ZALSulfs; 1.0 for cis-ZEN, cis-ZENGlcs and cis-ZENSulfs; 8.0 for cis-α-ZEL, cis-α-ZELGlcs and cis-α-ZELSulfs; 1.0 for cis-β-ZEL, cis-β-ZELGlcs and cis-β-ZELSulfs. In addition, it is assumed that glucuronides of ZEN and its phase I metabolites have the same

RPFs as their aglycones because they will be cleaved during enterohepatic circulation releasing ZEN and its phase I metabolites.

Exposure (background information)
The dietary exposure to ZEN was estimated based on occurrence data in Norwegian cereal products and consumption data from national dietary surveys. The lowest and highest mean ZEN concentrations in 2008 – 2011 for sieved wheat flour, milled wheat flour, wheat bran and oat flakes were used to estimate the intake in different age groups in the Norwegian population. The estimated intakes of ZEN were below the TDI for all age groups. Exposure to ZEN as considered of no concern for all age groups (VKM, 2013).

Vulnerable groups: score 0.0
Specific vulnerable groups have not been identified. However, ZEN exposure has been associated with the development of breast cancer in adult women and late puberty in adolescent girls (EFSA, 2016).

Lack of toxicity data: score 1.0
Data on the estrogenicity of the modified forms (phase I and phase II metabolites) of ZEN is scarce. More data on the occurrence of the modified forms of ZEN in food (including food of animal origin) and feed are needed in order to characterise risks using the group TDI and the RPFs. Furthermore, more data on toxicokinetics of the modified forms of ZEN are needed, particularly information on the absorption and bioavailability of phase II metabolites of ZEN that are present in food and feed. To reduce the uncertainty associated with the establishment of the RPFs, estrogenicity of the modified ZEN, in particular of α-ZEL, comparative to ZEN, should be investigated in pigs, the most sensitive species for ZEN toxicity.

Lack of exposure data: score 0.5
The consumption of maize-based products in Norway has increased in recent years. Thus, Norwegian consumers might be exposed to maize-specific mycotoxins at higher extent than before. The monitoring of maize-based products for ZEN should be intensified. There is limited data on the occurrence of modified forms of ZEN in food and feed.

Total score = 3.5 for zearalenone (ZEN) and modified forms

3.1.2.2 *References*

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3.1.3 T-2 (T2) and HT-2 (HT2) toxins and modified forms

T-2 toxin (T2) and HT-2 toxin (HT2) are type A trichothecenes produced by various *Fusarium* species. HT2 is deacetylated T2. *In vivo*, T2 is rapidly metabolised to HT2. Modified forms of T2 and HT2 result from phase I and phase II metabolism of T2 and HT2 in fungi, plants and mammals. Relevant phase I metabolites include 19-OH-T2, neosolaniol (NEO) and 19-OH-HT2, T2-triol and T2-tetraol. Known phase II metabolites are T2-3-glucose (T2-3-Glc), T2-3-diglucose (T2-3-diGlc), T2-3-sulfate (T2-3-Sulf), T2-3-glucuronic acid (T2-3-GlcA), 3-acetyl-T2 (3-Ac-T2), 3-feruolyl-T2 (3-Fer-T2), HT2-3-glusose (HT2-3-Glc), HT2-diglucose (HT2-diGlc), HT2-glucuronic acid (HT2-GlcA) and HT2-malonylglucose (HT2-MalGlc) (EFSA, 2017). Modified forms may add 10% to the concentration of T2 and HT2 in food and feed (EFSA, 2014).

T2 and HT2 and their modified forms occur in all major wheat-, barley- and oat-producing parts of the world. The highest concentrations are found in oats, both in Norway and worldwide, but wheat is the main contributor to the daily intake of T2 and HT2 in Norway due to the high wheat consumption. Maize can contain T2 and HT2 in warmer climates (VKM, 2013). Compiled occurrence data from different European countries show the highest levels of the sum of T2 and HT2 within the food category «Grains and grain-based products» for «Grains for human consumption» and «Breakfast cereals», in particular in oat-containing commodities (EFSA, 2017).

3.1.3.1 ***Scores***

MOE/MOS/ADI/TDI/TWI: score 6.0
In 2011, a group tolerable daily intake (group TDI) of 0.1 µg/kg bw was established for the sum of T2 and HT2 based on reduced antibody response to a specific antigen seen in a subchronic study with pigs (EFSA, 2011). All exposure estimates were below the group TDI of 0.1 µg/kg bw, and consequently, EFSA concluded that there was no health concern (EFSA, 2011). An ARfD of 0.3 µg for T2 and HT2/kg bw was established based on acute emetic events in mink.

In 2017, based on new toxicity data, a $BMDL_{10}$ of 3.33 µg T2/kg bw per day was calculated. An uncertainty factor (UF) of 200 was used; an additional factor of 2 was added to the standard UF because a subchronic study was used and by noting that the toxic effect

reached no plateau at the end of the study. The new group TDI for T2 and HT2 of 0.02 (rounded from 0.017) µg/kg bw was established. Acute emetic events in mink upon exposure to both T2 and HT2 were identified as critical effects for setting an ARfD for T2 and HT2, and calculations for BMD resulted in a $BMDL_{10}$ of 2.97 µg T2 or HT2/kg bw per day. Using an UF of 10, a group ARfD of 0.3 µg T2 and HT2 per kg bw was established. An interspecies factor was not applied as it was assumed that humans are not more sensitive than mink towards this effect (EFSA, 2017). Molarity-based relative potency factors (RPF) have been assigned to the different modified forms.

The mean dietary exposure (ng/kg bw per day) in the total European population ranged from 4.4 to 63 in infants, 9.0 to 65 in toddlers, 8.5 to 62 in other children, 4.4 to 39 in adolescents, 2.5 to 26 in adults, 2.3 to 23 in the elderly and 5.7 to 21 in the very elderly. The maximum values for most population groups exceed the new group TDI of 20 ng/kg bw per day, which is of concern.

Toxicity (background information)
T2 inhibits protein, RNA and DNA synthesis. There are indications that T2 induces apoptosis and in some cell types necrosis, as well as lipid peroxidation that affects cell membrane integrity. T2 induces hematotoxicity and myelotoxicity associated with impairment of hematopoiesis in bone marrow (reduction of total leukocyte count), which is considered as the critical effect under chronic exposure (used to set the TDI). New *in vivo* acute toxicity studies showed that T2 and HT2 have anorectic effects in pigs upon short-term exposure.

Since T2 is rapidly metabolised to HT2, the toxicity of T2 might partly be attributed to HT2. No *in vivo* studies on hematotoxicity of modified forms of T2 and HT2 have been identified, but it is assumed that the phase I metabolites have a similar mode of action. The phase I metabolites of NEO, T2-triol and T2-tetraol are therefore included in the group TDI with T2 and HT2. Because phase I metabolites show different potencies in the inhibition of protein synthesis and other toxic effects, it was decided to assign molarity-based relative potency factors (RPFs) for their inclusion in the group TDI. These RPFs are 1 for T2, HT2 and 19-OH-T2; 0.3 for NEO and 19-OH-HT2; and 0.1 for T2-triol and T2-tetraol. It was further assumed that the phase II metabolites are hydrolysed to their aglycones after ingestion so they were included in the group TDI. Thus, T2-3-Glc, T2-3-diGlc, T2-3-Sulf, T2-3-GlcA, 3-Ac-T2, 3-Fer-T2, HT2-3-Glc, HT2-diGlc, HT2-GlcA and HT2-MalGlc are considered with a RPF of 1. NEO-Glc was included by using a factor 0.3 and T2-triol-Glc and T2-tetraol-Glc by applying a factor of 0.1 (EFSA, 2017).

The toxicokinetic data for T2 and HT2 are fragmentary. Bioavailability has not been quantified. Absorption is presumably rather fast. The toxins are distributed rapidly to the organs, but do not accumulate. They can pass through the placenta-barrier and the blood-brain barrier. Metabolism is rapid and complex leading to the generation of many different metabolites. T2 and its metabolites are excreted in urine and feces, mainly as glucuronides. Data on the toxicokinetics of modified forms (phase I metabolites and phase II metabolites) of T2 or HT2 are not available (EFSA, 2011; EFSA, 2017).

Exposure (background information)
Since no data were provided on modified forms of T2 and HT2, a potential presence of modified forms was not considered in this assessment. The maximum values for most population groups exceed the new group TDI of 20 ng/kg bw per day.

An assessment of exposure to T2 and HT2 in the Norwegian population concluded that the dietary intake could not be estimated because the majority of analysed grain samples were determined to be below the limit of detection (LOD). Therefore, scenarios were made to illustrate the potential intakes of sum of T-2 and HT-2 toxins, probably over-estimating them. VKM (2013) indicated that the dietary intake of the sum of T-2 and HT-2 toxins in 1- and 2-year-olds may exceed the TDI (old TDI of 0.1 µg/kg bw per day), while 4-year-olds with high exposure had an intake in the range of the TDI. According to the exposure scenarios, the exposures to the sum of T2 and HT2 toxins in 9- and 13-year-olds were below the TDI. Furthermore, both the mean and high exposures in adults were below the TDI. It was concluded that according to the exposure scenarios, the dietary intake of the sum of T2 and HT2 was potentially of concern for the youngest age groups (VKM, 2013). Modified forms were not considered.

Vulnerable groups: score 1.0
The chronic dietary exposure to the sum of T2 and HT2 was estimated to be two- to threefold higher in the young population groups («Infants», «Toddlers» and «Other children») than that estimated for the adult population groups («Adults», «Elderly» and «Very elderly»). (EFSA, 2017).

Lack of toxicity data: score 0.5
Toxicity data for T2 and HT2 phase I metabolites are missing.

Lack of exposure data: score 1.0
Occurrence data for T2 and HT2 in Norwegian grain and grain products are scarce and rather old. Improved analytical methods would allow the detection of lower concentrations. Data for modified forms are not available.

Total score = 8.5 for T-2 (T2) and HT-2 (HT2) toxins and modified forms

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3.1.4 Alternariol (AOH) and Alternariol methyl ether (AME)

Alternariol (AOH) and alternariol methyl ether (AME) are benzopyrone mycotoxins produced by *Alternaria alternata*. The fungus grows on pre- and postharvest crops (VKM, 2013). High relative humidity in summer may lead to proliferation of *Alternaria* and thus a potential contamination with Alternaria toxins. A large variety of Alternaria toxic metabolites have been described, but knowledge about their occurrence and toxicity is very limited.

Alternaria toxins occur in many types of food. They are present in cereals, vegetables (tomatoes, carrots, potatoes) and in fruits such as apples and grapes. Oil seeds like sunflower seeds, rapeseeds and olives may also be infected. Currently, there are no regulations for the presence of Alternaria toxins in food or feed. In 2016, occurrence data on four main toxins, AOH, AME, tenuazonic acid (TeA) and tentoxin (TEN) were collected. The highest mean levels of AOH were reported for some grains, in particular «Buckwheat» (lower bound (LB) = 27.9 µg/kg, upper bound (UB) = 33.1 µg/kg) and «Oats» (LB = 35.3 µg/kg, UB = 39.7 µg/ kg). AOH was also present in diverse samples of tomato-based products e.g. «Tomato puree» (LB = 4.6 µg/kg, UB = 17.1 µg/kg). The reported levels of AME were lower than those reported for AOH, with few exceptions. The highest mean levels were found in samples of tree nuts and oil seeds, in particular «Chestnuts» (LB = 16.8 µg/kg, UB = 17.5 µg/kg) and «Sesame seeds» (LB = 11.3 µg/kg, UB = 11.8 µg/kg). The highest levels of all Alternaria toxins were reported for TeA reaching mean concentrations of 351.2 µg/kg (LB = UB) in tomatoes and several tomato-based products. Dried cereals that have to be reconstituted with water contained TeA average values of 496–497 µg/kg (LB–UB) in more than 90% of the samples (EFSA, 2016).

3.1.4.1 ***Scores***

MOE/MOS/ADI/TDI/TWI (background information)
A TDI has not been set, and since a NOAEL has not been determined, the MOE cannot be calculated.

The threshold of toxicological concern (TTC) approach has been used by EFSA to assess the relative level of concern for dietary exposure of humans to these mycotoxins (EFSA, 2011). This was based on the following considerations: (1) there are few or no relevant toxicity data on Alternaria toxins, (2) the chemical structure of several of them is known, (3) dietary exposure data exist for some of them.

For the genotoxic Alternaria toxins, AOH and AME, the estimated chronic dietary exposure exceeded the relevant TTC value indicating a need for additional toxicity data. The dietary exposure estimates for non-genotoxic tentoxin and tenuazonic acid were lower than the relevant TTC value of 2.5 ng/kg bw per day, and considered unlikely to be a human health concern (EFSA, 2016).

In 2016, the highest exposure to AOH was estimated in «Toddlers», with the mean exposure between 3.8 and 71.6 ng/kg bw per day (EFSA, 2016), meaning that all toddlers exceeded the TTC. The 95-percentile exposure was between 11.4 and 270.5 ng/kg bw per day (LB–UB), exceeding the TTC with up to 100 times.

AME exposure in toddlers reached a mean exposure between 3.4 and 38.8 ng/kg bw per day (LB–UB) and a 95-percentile exposure between 10.3 and 97.3 ng/kg bw per day (LB–UB), exceeding the TTC up to 50 times.

It is, however, uncertain, if the TTC (set in 2011) is still relevant since a newer toxicity study in mice showed no genotoxicity at an oral dose as high as 2,000 mg/kg (Schuchardt et al., 2014).

Toxicity: score 2.0
AOH and AME are mutagenic *in vitro* and there is also limited evidence for carcinogenic properties.

However, there are few or no relevant toxicity data on Alternaria toxins (EFSA, 2011). AOH, AME, TeA and altertoxins (ATX) are described to induce harmful effects in animals, including fetotoxic and teratogenic effects. Culture extracts of *A. alternata* as well as individual mycotoxins such as AOH and AME are mutagenic and clastogenic in various *in vitro* systems. In addition, it has been suggested that in certain areas in China Alternaria toxins in grains might be responsible for oesophageal cancer.

Experiments performed in rodents with purified Alternaria toxins indicated that the acute toxicity is in the following order: ALT > TeA > AME and AOH. These data are not suitable for the risk assessment of Alternaria toxins since the risk for public health related to these toxins is not expected to result from acute exposures (EFSA, 2011). The TTC approach was

therefore used for the assessment of human health risk. For the genotoxic AOH and AME, it was concluded that the estimated chronic dietary exposure exceeded the relevant TTC value of 2.5 ng/kg bw per day, indicating a need for additional toxicity and occurrence data. The TTC for TeA and TEN was identified as 1,500 ng/kg bw per day.

A mice study in 2014 with repeated oral application of 2,000 mg/kg AOH showed no toxic or genotoxic effect of AOH in bone marrow and no systemic genotoxicity (Schuchardt et al., 2014).

There is little relevant information available on the absorption, distribution and excretion of Alternaria toxins in animals and humans. One rat study for AOH showed poor absorption, rapid metabolism and no tissue accumulation. *In vitro* metabolism of AOH and AME lead to the formation of 7 hydroxylated metabolites, mostly to catechol metabolites that can be conjugated with glucuronic acid and sulphate (EFSA, 2011). In 2014, an *in vivo* oral toxicokinetic study in mice was performed with 200, 1,000 and 2,000 mg/kg bw radiolabelled and unlabelled AOH (Schuchardt et al., 2014). The study revealed low systemic absorption, with about 90% of the total dose excreted via feces and up to 9% via urine. Blood levels did not exceed 0.06% of the administered dose during the first 24 h after administration. Thus, target organ toxicity would most likely be restricted to the gastrointestinal tract. Four metabolites (8-hydroxy-AOH, 4-hydroxy-AOH, 10-hydroxy-AOH and 2-hydroxy-AOH) were detected. After repeated application of the highest dose, a micronucleus assay revealed no toxic or genotoxic effect of AOH in bone marrow and the comet assay with liver tissue did not indicate systemic genotoxicity (Schuchardt et al., 2014).

Exposure: score 2.0

In a risk assessment in 2011 on the presence of Alternaria toxins in feed and food, AOH, AME, tenuazonic acid, iso-tenuazonic acid, altertoxins, tentoxin, altenuene and AAL-toxins were assessed (EFSA, 2011). A lower bound-upper bound (LB-UB) approach was used for the assessment of the occurrence data, since the data were below the LOD for many Alternaria toxins. The lower bound assigns a value of zero to left-censored results; the upper bound assigns the value of LOD or LOQ to results below the LOD and LOQ, respectively. The highest concentrations for AOH, AME, TeA and TEN were found in the food group «Legumes, nuts and oilseeds» and in particular in sunflower seeds. Mean concentrations of AOH in this food group were in the range of 22 µg/kg (LB mean) to 26 µg/kg (UB mean) with a maximum of 1,200 µg/kg. For AME the mean values were in the range 11 (LB) to 12 µg/kg (UB), with a maximum of 440 µg/kg. TeA was present in higher concentrations (LB mean = 333 µg/kg; UB mean = 349 µg/kg; maximum = 5,400 µg/kg). Mean concentrations of TEN ranged from 47 (LB mean) to 50 µg/kg (UB mean) with a maximum of 880 µg/kg.

Based on published occurrence data on about 300 feed and agricultural commodities in Europe, AOH was found in 31% of the feed and agricultural commodity samples at concentrations from 6.3 to 1,840 µg/kg (maximum found in sunflower seeds). AME was found in 6% of the samples with levels ranging from 3 to 184 µg/kg (maximum found in cereals). ALT was found in 73% of the samples with concentrations between 6.3 and 41

µg/kg (maximum found in wheat grains). TeA was present in 15% of the samples with levels varying between 500 and 4,310 µg/kg (maximum found in oats).

A limited dietary exposure assessment focusing only on adults (≥18 to <65 years old) was performed. The dietary exposure in adults was estimated only for AOH, AME, TeA and TEN. The estimated mean chronic dietary exposure in the adult population across dietary surveys, using LB and UB mean concentrations, was in the following ranges: AOH: 1.9 - 39 ng/kg bw per day; AME: 0.8 - 4.7 ng/kg bw per day; TeA: 36 - 141 ng/kg bw per day; TEN 0.01 - 7 ng/kg bw per day (the ranges represent the minimum LB to maximum UB from the different countries). The 95-percentile exposure estimates were 2 to 3 times higher than the mean dietary exposure estimates (EFSA, 2011).

In 2016, EFSA performed a dietary exposure assessment of Alternaria toxins for the European population (EFSA, 2016). The highest exposure to AOH was estimated in «Toddlers», with the mean exposure between 3.8 and 71.6 ng/kg bw per day (minimum lower bound–maximum upper bound, (LB–UB)) and the 95-percentile exposure between 11.4 and 270.5 ng/kg bw per day (LB–UB). Overall, «Fruit and fruit products» were the most important contributors to the dietary exposure to AOH. The highest exposure to AME was estimated in «Toddlers», with mean exposure between 3.4 and 38.8 ng/kg bw per day (LB–UB) and 95-percentile exposure between 10.3 and 97.3 ng/kg bw per day (LB–UB). Overall, the main contributors to the dietary exposure to AME were «Vegetable oil» and «Pome fruits» (pears).

Vulnerable groups: score 0.5
It is expected that the dietary exposure in children might be higher compared to adults by a factor of 2 to 3. Similarly, vegetarians might have higher exposure due to the higher intake of food of plant origin (EFSA, 2011; EFSA, 2016).

Lack of toxicity data: score 1.0
Toxicity data for AOH and AME (and even more for other Alternaria toxins) are very limited. *In vitro* experiments show a genotoxic potential, while *in vivo* the low absorption rate might hinder sufficient uptake and systemic toxicity. The data are, however, insufficient to draw a conclusion on genotoxicity and systemic toxicity. A NOAEL has not been determined, and a TDI has not been set.

Lack of exposure data: score 0.5
Data on the occurrence of AOH and AME in Norwegian cereals are lacking.

Total score = 6.0 for alternariol (AOH) and alternariol methyl ether (AME)

3.1.4.2 ***References***

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3.1.5 Enniatins (ENNs)

Enniatins (ENNs) are secondary fungal metabolites that are mainly produced by *Fusarium* species (VKM, 2013). ENNs are six-membered cyclic depsipeptides commonly composed of three D-2-hydroxyisovaleric acid (Hiv) residues linked alternately to three N-methyl-L-amino acid residues (N-Me-R), which are used for distinguishing between the individual enniatins. Enniatin B (ENNB), a (N-Me-Val-Hiv)3 – molecule is the most prevalent ENN. Other important ENNs are enniatin B1 (ENNB1), enniatin A (ENNA) and enniatin A1 (ENNA1). ENNs are considerably heat-resistant and have been detected in prepared products in considerable concentrations.

ENNs belong to the most commonly found contaminants in grain and grain-based products. In Mediterranean countries, wheat and sorghum contain up to 493 and 696 mg/kg ENN (Fæste et al., 2011). ENN levels in Norwegian wheat and oats were analysed in 2000-2002 (VKM, 2013). The yearly medians in wheat, barley and oats were 126-730, 153-493 and 19-65 µg/kg, respectively, with yearly maximum concentrations 1,590-7,400, 1,213-5,100 and 223-440 µg/kg, respectively. ENNs have been shown to be carried-over through the food chain. No limits for ENNs in food or feed have been set by relevant authorities.

3.1.5.1 ***Scores***

MOE/MOS/ADI/TDI/TWI (background information)
Only limited data are available for ENNs toxicity and exposure. Considering the recently defined NOAEL for ENNB in female mice (0.18 mg/kg bw per day) (Maranghi et al., 2018) and the European exposure estimates for the sum of ENNs (EFSA, 2014), i.e. a mean chronic exposure from 0.42 to 1.82 µg/kg bw/ per day and the 95-percentile exposure from 0.91 to 3.28 µg/kg bw per day, a preliminary MOE value in the range of 100 - 430 for mean ENNs exposure and 55 - 200 for the 95-percentile exposure can be calculated. A TDI has not been defined.

Toxicity: score 1.0

The cyclopeptidic ENNs form ionophores with hydrophobic groups on the outside and polar groups in the core, resembling a disc in the three-dimensional conformation. They can transport monovalent and divalent cations, either in sandwiched complexes or by creating channels in biological membranes (VKM, 2013). The primary toxic effect of ENNs is related to their ionophoric properties. ENNB with up to 100 µM did not show genotoxicity, but demonstrated cytotoxicity at low micromolar concentrations. The observed activities included specific inhibition of acyl-coenzyme A cholesterol acyltransferase, depolarization of mitochondria, inhibition of osteoclastic bone resorption and induction of apoptosis in cancer cells, as well as interactions with ATP-binding cassette transporters like P-glycoprotein (VKM, 2013). The lack of correlation between *in vitro* and *in vivo* toxicity is presumably the result of low bioavailability.

The toxicokinetic parameters of ENNB have been investigated *in vitro* for several species (Fæste et al., 2011). ENNB and ENNB1 are metabolised to at least 10 phase I metabolites by hydroxylation, carboxylation and oxidative demethylation reactions (Ivanova et al., 2017). The predicted systemic elimination was intermediate and the predicted bioavailabilities ranged from 20 to 63%. A preliminary study on ENNB1 toxicokinetics in pigs determined high bioavailability (up to 90% and rapid elimination) (Devreese et al., 2014), whereas a study on ENNB and ENNB1 in chicken showed poor absorption (5 and 11% bioavailability), considerable distribution into tissues and a high elimination rate (Fraeyman et al., 2016). The lipophilic ENNs accumulates in organs and can cross barriers, reaching the brain and placenta.

There are no reports of natural cases of mycotoxicosis in humans or animals. EFSA stated that acute exposure to ENNs, such as ENNB, does not indicate concern for human health, but a concern might be the chronic exposure (EFSA, 2014). However, recently the *in vivo* toxicity and genotoxicity of ENNB in mice have been studied (Maranghi et al., 2018). The results support a genotoxic effect in bone marrow and liver cells after acute treatment, but not after repeated exposure. Immunotoxic ENNB effects were observed in both genders, suggestive of a suppressive/inhibiting activity. The ENNB treatment affected spleen, brain and thyroid in both sexes, and thymus, kidneys, adrenals and reproductive system in female mice only, and duodenum in male mice only. Overall, for these endpoints, taking into account also the severity of the effects, female mice seem more susceptible to repeated oral exposure to ENNB. For subchronic toxixicity, the NOAEL for female mice was established at 0.18 mg/kg bw per day based on histomorphometrical effects on thymus, uterus and spleen. In male mice, the NOAEL was 1.8 mg/kg bw per day (enterocyte vacuolization in duodenum and increased reactive oxygen species and reduced glutathione brain levels). For reproductive and developmental toxicity, the maternal NOAEL was 1.8 mg/kg bw per day (decreased white pulp area and increased red/white pulp area ratio in spleen) and the developmental NOAEL for offspring was 18 mg/kg bw per day.

A TDI for ENNs has not been established.

Exposure: score 3.0
In 2013, VKM concluded that an assessment of ENNs and beauvericin in grain in Norway could not be performed due to the lack of occurrence and toxicity data. However, VKM recognised the presence of ENNs in Norwegian grains and considered that they may be of potential risk for human health (VKM, 2013).

In 2014, EFSA estimated exposure for the sum of ENN A, A1, B and B1 in the European population (EFSA, 2014). The most important contributors to the chronic dietary exposure to beauvericin and the sum of ENNs were grains and grain-based products. The mean chronic exposure to the ENNs ranged from 0.42 to 1.82 µg/kg bw per day and the 95-percentile exposure ranged from 0.91 to 3.28 µg/kg bw per day. The highest acute exposure estimates of the sum of ENNs were 4.67 µg/kg bw per day (mean) and 10.1 µg/kg bw per day (95-percentile). Toddlers were in general the age group with the highest dietary chronic and acute exposure to ENNs. EFSA concluded that acute exposure to ENNs does not indicate concern for human health. There might be a concern with respect to chronic exposure, but no firm conclusion could be drawn and a risk assessment was not possible to perform for dietary exposure to ENNs, due to the overall lack of toxicity data (EFSA, 2014). At the moment, EFSA is further collecting occurrence data for a future risk assessment (Prosperini et al., 2017).

Vulnerable groups: score 0.5
ENNs can transfer via the placenta to the fetus and into the brain. Toddlers have the highest dietary chronic and acute exposure to ENNs.

Lack of toxicity data: score 1.0
Relevant toxicity data are lacking (Properini et al., 2017). Research on toxicological effects induced by ENNB is in progress. In 2018, the *in vivo* toxicity and genotoxicity of ENNB were studied in mice (Maranghi et al., 2018).

Lack of exposure data: score 1.0
Occurrence data on ENNs in Norwegian grain and grain products are sporadic and rather old. Data are needed for the assessment of human and animal risk from dietary ENNs exposure.

Total score = 6.5 for enniatins (ENNs)

3.1.5.2 ***References***

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3.1.6 Aflatoxins (AFLAs)

Aflatoxins (AFLAs) are difuranocoumarin mycotoxins produced by two species of *Aspergillus*, *A. parasiticus* and *A. flavus*, commonly found in areas with hot and humid climates. Aflatoxin B1 (AFB1) is the most important compound with respect to prevalence and toxicity. Other important AFLA are aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2) and the hydroxylated AFB1- and AFB2-metabolites aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2).

AFLA can occur in ground nuts, tree nuts, maize, rice, figs and other dried foods, spices, crude vegetable oils and cocoa beans, as a result of fungal contamination before and after harvest. AFM1 and AFM2 are mainly found in milk. The carry-over of AFB1 from animal feed into the milk as AFM1 has been estimated to be 1-2%, but it can reach up to 6% in high-yielding dairy cows. The maximum permissible level for AFM1 in milk in the EU is 0.05 µg/kg (EU, 2001). AFLA is also transferred into eggs.

EFSA has assessed human health risk from dietary exposure to AFLA several times. In 2007, consequences of an increase of the EU maximum levels for processed almonds, hazelnuts

and pistachios from 4 µg/kg to 8 or 10 µg/kg for the sum of AFB1, B2, G1 and G2 were assessed (EFSA, 2009). It was concluded that the proposed increase would add about 1% to the estimated total dietary exposure of people from all sources and therefore on cancer risk. In 2009, EFSA evaluated an increase of the maximum level for total AFLA from 4 µg/kg to 10 µg/kg for other tree nuts, such as Brazil nuts and cashews, and concluded that public health would not be adversely affected (EFSA, 2009). It was, however, pointed out that the number of highly contaminated foods reaching the market should be reduced. In 2012, the possible emergence of AFLA in cereals in Europe due to climate change was modelled showing a risk for an increase of *A. flavus* contamination in maize, both in +2°C and +5°C scenarios, and a very low risk for wheat and none for rice (Battilani et al., 2012). Maize samples in Norway analysed for the sum of AFB1 and AFB2 before 2011 containeda mean middle bound concentration of 0.6 µg/kg (VKM, 2013). In 2013, the occurrence of the sum of AFB1, B2, G1 and G2 was determined in cereals and cereal-derived products on behalf of EFSA (EFSA, 2013). For cereals and their milling products, the maximum mean value at LB was found in samples of unspecified grain milling products (2.21 µg/kg) while the maximum mean value at UB was found in oat milling products (2.60 µg/kg). For processed cereal products the maximum mean value at the LB was found in fine bakery wares (0.45 µg/kg), while the maximum mean value at the UB was found in raw pasta (1.87 µg/kg). In 2018, a possible increase of the maximum level for total AFLA from 4 to 10 µg/kg in peanuts and processed products thereof was evaluated (EFSA, 2018). The mean concentration of AFLA in peanuts was determined as 2.65/3.56 µg/kg (lower bound (LB)/upper bound (UB)) with a maximum of 1,429 µg/kg. The mean concentration in peanut butter was 1.47/1.92 µg/kg (LB/UB) with a maximum of 407 µg/kg.

3.1.6.1 ***Scores***

MOE/MOS/ADI/TDI/TWI: score 6.0

A MOE value of 10,000 or higher was used by EFSA for the risk assessment of dietary exposure to total AFLA (EFSA, 2007; EFSA, 2009). It was based on the lowest $BMDL_{10}$ (10 % extra cancer risk) value of 870 ng/kg bw per day.

However, in 2017, a linear non-threshold model was adopted (JECFA, 2017). In 2018, EFSA assessed cancer risk for AFLA in peanuts (exposure scenarios resulting in levels of 0.04–4.28 ng/kg bw per day), estimating an additional AFLA-induced cancer risk in the range of 0.001 to 0.333 per year per 100,000 persons (EFSA, 2018). An excess lifetime cancer risk of 5-10 or less is considered to be of low risk for public health, which corresponds to a yearly excess cancer risk of 0.014 additional cancer cases per 100,000 assuming a lifetime expectancy of 70 years. The calculated AFLA-induced cancer risks exceed the low-risk value at the current maximum level (4 µg), and the risk is increased by a factor of 1.6–1.8 at the elevated level (10 µg).

Toxicity (background information)

AFLA is readily absorbed after oral exposure. AFB1 is metabolised to various metabolites, including the endo- and exo-epoxides of AFB1, the 4-hydroxy-metabolite AFM1 as well as the

glutathione-conjugated metabolite AFB1-N7-Gua, which is excreted as aflatoxin–N-acetylcysteine in urine. The liver is the major site of AFLA metabolism. AFB1-exo-8,9-epoxide is hydrolysed to 8,9-dihydrodiol, which is unstable and rearranges to a dialdehyde reacting with proteins such as albumin. Aflatoxin B1-N7-Gua also undergoes sequential metabolism and is excreted as aflatoxin–N-acetylcysteine in urine (VKM, 2013). The half-life of AFB1 in humans is long (>64 h).

AFB1 is transformed to its DNA-reactive form, AFB1-exo-8,9-epoxide, in the liver, which binds to liver proteins and inhibit their functionalities, potentially resulting in acute aflatoxicosis. Alternatively, it can bind to DNA, leading to aflatoxin-induced hepatocellular carcinoma. AFB1 is mutagenic in bacterial systems and in eukaryotes leading predominantly to a G>T mutation. The AFLA–DNA adduct is unstable and undergoes depurination, leading to its urinary excretion. AFLA also bind to proteins such as albumin (AF-alb) via the formation of aflatoxin B1-8,9-dihydrodiol. There is a high correlation between the presence of AFLA-DNA adducts in the liver, their urinary excretion and the formation of the serum albumin adduct (VKM, 2013).

There are reports of acute/sub-acute human and animal aflatoxicosis, which may lead to lethal hepatotoxicity, but the critical effect for human risk assessments is the carcinogenic effect (VKM, 2013). The International Agency for Research on Cancer (IARC) concluded that «*naturally occurring aflatoxins are carcinogenic to humans (Group 1)*» (IARC, 1993; IARC, 2012; JECFA, 1999). AFLA are assessed as a group since the toxicological profiles of the most important naturally occurring AFLA (AFB1, B2, G1, and G2) appear to be similar. The genotoxic carcinogenicity of AFM1 is approximately 10 times lower than that of AFB1, and it was concluded that *«AFM1 is possibly carcinogenic to humans (Group 2B)»* (IARC, 1993; IARC, 2012; JECFA, 2001).

A linear dose-response relationship has been demonstrated for toxic effects of AFB1 in at least two animal species, down to doses of less than 0.1 pg/kg bw per day. No TDI or similar levels for safe intake have been established for human consumption as a NOAEL cannot be determined for the carcinogenic potential of AFLA (EFSA, 2007; EFSA, 2018).

In 2007, EFSA derived a $BMDL_{10}$ on a background risk of 10.5% of 870 ng/kg bw per day from a Chinese study on mortality from liver cancer, and a $BMDL_{01}$ of 78 ng/kg bw per day on a background risk of 0.17–0.50% was derived from African studies on liver cancer. A MOE value of 10,000 or higher was used by EFSA for the risk assessment of dietary exposure to total AFLA (EFSA, 2007).

Co-exposure to hepatitis viruses, in particular hepatitis B, has a strong impact on the carcinogenic risk to AFLA. In epidemiological studies, there is an interaction with hepatitis B infection, and subjects positive for hepatitis B surface antigen (HBsAg) show at least a multiplicative risk when present together with AFLA exposure (FAO/WHO, 2017; EFSA, 2018).

In 2017, JECFA supported a linear non-threshold model in AFB1 cancer risk assessment due to thehepatotumourigenic effects of AFB1 in rats and trout at doses approaching human exposure (JECFA, 2017). Using averaging of different models, cancer potency estimates of 0.017 (mean) and 0.049 (95% UB) per 105 person years per ng/kg bw for HBsAg– individuals and 0.269 (mean) and 0.562 (95% UB) per 105 person years per ng/kg bw for HBsAg+ individuals were calculated. HBsAg+ seroprevalence ranges between 0.01% and 5.61% in EU countries (JECFA, 2017; EFSA, 2018).

Exposure (background information)
EFSA has performed several scenario calculations for the evaluation of a proposed increased of AFLA maximum levels in certain nuts (EFSA, 2007). The overall average exposure to AFLA in the European population from the consumption of almonds, hazelnuts, pistachios, other nuts, oilseeds, maize, dried fruits and spices was estimated to range from 0.35 to 1.93 ng/kg bw per day. In 2009, the exposure to almonds, hazelnuts, pistachios, other tree nuts and other food was estimated to range from 0.09 to 1.986 ng/kg bw per day (EFSA, 2009). In 2018, mean chronic exposure to total AFLA from peanut and peanut-derived products was estimated in scenarios for consumers only as ranging from 0.04–2.74 ng/kg bw per day for the current maximum level (4 µg/kg) and 0.07–4.28 ng/kg bw per day for the increased maximum level (10 µg/kg) (EFSA, 2018). The exposure to AFLA in Norwegian grain products has been considered to be of no concern (VKM, 2013).

Vulnerable groups: score 0.5
Children and vegetarians may have a higher exposure to AFLA than the mean of the population due to a higher percentage of nut consumption (EFSA, 2007). Regarding AFLA exposure from peanuts, the highest values were calculated for adolescents and other children (EFSA, 2018).

Lack of toxicity data: score 0.5
The EFSA CONTAM Panel has recommended that a full risk assessment on human dietary exposure from AFLA in food should be carried out (EFSA, 2018).

Lack of exposure data: score 0.5
Occurrence data for AFLA in Norwegian grain and food products with regard to possible changes due to climate change are needed.

Total score = 7.5 for aflatoxins (AFLAs)

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3.1.7 Ochratoxin A (OTA)

Ochratoxin A (OTA) is a storage mycotoxin produced by *Aspergillus* and *Penicillium* fungi in both tropical and temperate regions mainly under humid conditions (VKM, 2013). The OTA molecule contains a dihydrocoumarin linked to β-phenylalanine via an amide bond. OTA is heat-stable.

Contamination of food commodities with OTA, including cereals and cereal products, pulses, coffee, beer, grape juice, dry wine fruits and wine as well as cacao products, and nuts and spices, has been reported from all over the world (EFSA, 2006). Carry-over of OTA into meat, milk and eggs is negligible.

Maximum levels (MLs) are established for OTA in foodstuffs such as cereals, dried vine fruit, coffee and some spices. In 2017, the EU proposed additionally MLs for dried figs and dried apricots or all dried fruit, mixtures of spices, sunflower and pumpkin seeds, pistachios, hazelnuts or all tree nuts, liquorice placed on the market for the final consumer, herbs and herbal teas, and cocoa powder. In Norwegian grain products, OTA is considered of no concern (VKM, 2013). The yearly mean OTA concentrations measured in 2005-2009 in barley and oats ranged from 0.14 to 4.5 and 0.07 to 0.21 µg/kg, respectively, with yearly maximum concentrations of 0.8-40.0 and 0.5-2.1 µg/kg, respectively. OTA has also been detected in wheat (imported and Norwegian) in 1990-1998 with yearly means of 0.1-0.9 µg/kg. OTA might be present in higher concentrations in imported food (maize etc.).

3.1.7.1 ***Scores***

MOE/MOS/ADI/TDI/TWI: score 4.0

In 2006, EFSA derived a tolerable weekly intake (TWI) of 120 ng/kg bw per week on the basis of the lowest observed adverse effect level (LOAEL) of 8 µg/kg bw per day for early markers of renal toxicity in pigs (the most sensitive animal species), and by applying a composite uncertainty factor of 450 for the uncertainties in the extrapolation of experimental

data derived from animals to humans as well as for intra-species variability (EFSA, 2006). An update of the assessment was not required based on the newer toxicity data (EFSA, 2010).

In 2008, JECFA concluded, as EFSA before, that due to accumulation of OTA in the kidneys the establishment of a tolerable weekly intake would be more relevant than a TDI. JECFA set a provisional TWI (PTWI) of 100 ng/kg bw per day (JECFA, 2008).

In 2010, Health Canada calculated a negligible cancer risk intake (NCRI) for OTA and defined it as «*the exposure associated with a risk level of 1:100,000 and equivalent in units to a TDI*» (Kuiper-Goodman et al., 2010). The NCRI was derived from a tumorigenicity rat study, where the OTA dose associated with a 5% increase in tumour incidence above background (TD05) was 27.4 µg/kg bw. The TD05 was adjusted to 19.6 µg/kg bw with regard to the study period (5 days out of 7 days) and by applying a safety factor of 5,000 (considered equivalent to linear extrapolation to zero exposure based on a non-threshold carcinogenicity concept), resulting in a NCRI value of 3.9 ng/kg bw perday, which was rounded to 4 ng/kg bw per day (Bui-Klimke and Wu, 2015; Mitchell et al., 2017). Additionally, Health Canada developed a TDI based on a BMD10 of 1.56 µg/kg bw per day derived from the pig nephrotoxicity study (Kuiper-Goodman et al., 2010; Bui-Klimke and Wu, 2015). Applying a composite uncertainty factor of 500 considering species differences and study design resulted in a TDI of 3 ng/kg bw per day.

The available European occurrence data (15 to 60 ng/kg bw per week in adults) (EFSA, 2006) were below the TWI and PTWI. Considering the LOAEL of 8 µg/kg bw per day, and calculating theoretical daily exposure (2.1 to 8.6 ng/kg bw per day), MOE values of about 900-3700 could be determined, which were well above the factor of 450 applied by EFSA. High consumers would exceed the TDI of 3 ng/kg bw per day set by Health Canada (Kuiper-Goodman et al., 2010).

Toxicity (background information)

OTA is rapidly absorbed from the gastrointestinal tract (bioavailability about 40-60%), binds strongly to plasma proteins (the unbound fraction has been estimated to be as low as 0.02%) and can enter the enterohepatic recirculation through biliary secretion and reabsorption from the intestine and the kidney tubules (EFSA, 2006; JECFA, 2008; VKM, 2013; Mitchell et al., 2017). This causes secondary distribution of OTA in the serum and intestinal contents. After absorption, OTA is rapidly distributed by the blood, mainly to the kidneys, but lower concentrations are also found in the liver, muscle and fat. Specific transport proteins are probably involved in cellular uptake into kidneys, where it accumulates. Elimination is slow by urinary and fecal excretions, with a half-life in human blood of about 35 days after oral ingestion. OTA in plasma mainly occurs as the parent compound, but minor amounts of conjugates and hydroxylation products have been reported. All metabolites are considered to be less toxic than OTA. In ruminants, microorganisms in the rumen efficiently hydrolyse OTA to phenylalanine and ochratoxin α, prior to absorption. Ochratoxin α is considered to be of low toxicity (EFSA, 2006; VKM,

2013). In monogastric animals and humans, OTA is secreted into the milk, and thus breast milk may be a significant route of exposure for infants, when mothers are exposed to OTA.

OTA is genotoxic and causes DNA damage due to the formation of OTA-DNA adducts. OTA affects several biochemical pathways. It inhibits the enzyme phenylalanyl-tRNA^{Phe} synthetase, thereby blocking acylation of amino acids and consequently peptide elongation in protein synthesis. OTA reduces also the activity of glycolytic enzymes and increases the activity of gluconeogenic enzymes. It has been shown to increase lipid peroxidation and formation of reactive oxygen species (VKM, 2013).

OTA is a potent renal toxin in all animal species tested and there is indication for pathogenesis of distinct renal diseases in humans (EFSA, 2006; Bui-Klimke and Wu, 2015). The extent of renal injury is dose-dependent, but also associated with the duration of exposure, as OTA accumulates in renal tissue. OTA can cross the placenta and lead to fetal deformations in mice (Mitchell et al., 2017). IARC has classified OTA as a Group 2B (possible) human carcinogen (IARC, 1993).

Exposure (background information)
EFSA estimated that the OTA exposure in adult Europeans in the range from 15 to 60 ng/kg bw per week, including high consumers of foods containing OTA, which was below the TWI (EFSA, 2006). Data for infants and children were not available.

In Norway, exposure to OTA has been estimated from OTA detection in the blood of donors in 2001, when it was considerably below the TWI (VKM, 2013). In 2003, a newer study detected four times higher OTA blood concentrations. The correlation of dietary OTA levels to urinary OTA is, however, stronger than to serum OTA (Bui-Klimke and Wu, 2015).

In 2017, mean OTA exposure in USA was calculated as 0.18 ng/kg bw per day (95 percentile: 0.68 ng/kg bw per day) in infants consuming infant cereals, 0.02 (0.04) ng/kg bw per day in adult consumers of milk, 0.05 (0.12) ng/kg bw per day in adult coffee drinkers, 0.05 (0.18) ng/kg bw per day in 1-5 year-old children drinking cacao and 0.16 (0.60) ng/kg bw per day in adult consumers of pork (Mitchell et al., 2017), which are all below TWI, PTWI and the Canadian TDI.

Vulnerable groups: score 0
Infants consuming cereals or being nursed by OTA-exposed mothers may be exposed to elevated OTA concentrations.

Lack of toxicity data: score 0.5
Little data are available.

Lack of exposure data: score 1.0
The predictability of urinary-OTA for OTA-exposure should be verified. The exposure to OTA from dietary exposure in the Norwegian population has not been assessed. Newer exposure data are lacking for the European and Norwegian populations.

Total score = 5.5 for ochratoxin A (OTA)

3.1.7.2 *References*

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3.1.8 Patulin (PAT)

Patulin (PAT) is an unsaturated heterocyclic lactone (4-hydroxy-4H-furo[3,2-c]pyran-2(6H)-one) produced by a wide range of *Penicillium* and *Aspergillus* species, of which *P. expansum*, a common contaminant of damaged fruit such as apples, is the most important. PAT is water-soluble, stable to heat processing at pH <6, but gradually degraded during storage in the presence of sulphites, sulfhydryl groups and ascorbic acid. Fermentation of apple juice to produce alcoholic beverages degrades PAT (EFSA, 2002).

The occurrence of PAT as a natural contaminant of apple juice is a worldwide problem and international recommendations and regulations have been made for maximum levels permitted in consumer products. In 2002, EFSA performed an assessment on the dietary

intake of PAT based on occurrence data from 10 European countries (EFSA, 2002). Of the 4633 apple juice samples tested (including nectars and drinks), 57.4% were positive, containing mean PAT concentrations in the range of 1.4 to 70.6 µg/kg. Apple juice concentrates (1175 samples, 96.0% positives) contained mean PAT concentrations ranging from 3.2 to 162 µg/kg. Apple ciders (339 samples, 37.2% positives) contained mean PAT concentrations ranging from 0.8 to 153 µg/kg. Pear juices (100 samples, 17.0% positives) contained mean PAT concentrations ranging from 2.5 to 14.3 µg/kg. Grape juices (324 samples, 39.5% positives) contained mean PAT concentrations ranging from 4.3 to 24.0 µg/kg, and other fruit and citrus juices (174 samples, 2.9% positives) contained mean PAT concentrations ranging from 2.5 to 25 µg/kg. Apple purees (97 samples, 7.2% positives) contained mean PAT concentrations ranging from 1.6 to 10.0 µg/kg. Furthermore, tomato puree was considered as of relevance although the sample numbers were too small to calculate means. Baby food (312 samples, 13.8% positives) contained mean PAT concentrations ranging from 0.6 to 11.7 µg/kg. Occurrence data for fresh fruit (apples, pears and peaches) were sparse. The mean PAT concentration (64 samples, 23% positives) ranged from 0.2 to 1166 µg/kg including apples with peel. Previously, JECFA had estimated the mean content of PAT in apple juice (7 - 52% of samples positive) as 10 - 15 µg/kg (JECFA, 1990). In a subsequent evaluation, it was assumed that PAT levels in apple juice were generally below 50 µg/kg (JECFA, 1995).

3.1.8.1 ***Scores***

MOE/MOS/ADI/TDI/TWI: score 2.0

The current provisional maximum tolerable daily intake (pmTDI) for dietary exposure to PAT is 0.4 µg/kg bw per day (JECFA, 1995), based on a NOAEL of 43 µg/kg bw per day (safety factor 100). European exposure data from consumption of apple-based products have been estimated to 21 (mean)/57 (95-percentile) ng/kg bw per day in adults and 64 (mean)/199 (95-percentile) ng/kg bw per day in children (EFSA, 2002), which results in MOE-values of about 754 (mean)/2050 (95-percentile) in adults and 670 (mean)/2,120 (95-percentile) in children. Other exposure assessments have concluded with even lower PAT exposure with the exception of one Italian study (Baretta et al., 2000), which estimated the highest intake for adults drinking apple juice with pulp as 9.6 µg/kg bw per day, a value exceeding the PMTDI considerably (MOE = 4.5), and one Swedish study, calculating PAT exposure from apple juice in high consuming 4-year olds as 2.04 µg/kg bw per day (MOE = 21) and in high consuming adults as 0.65 µg/kg bw per day (MOE = 66) (Arnér, 2015).

Toxicity (background information)

PAT has antibiotic properties and is genotoxic, causing chromosomal damage, but shows no mutagenic potential in the Ames test. It shows an inhibitory effect on many enzymes, probably due to its affinity to SH-groups (JECFA, 1990). PAT has no reproductive or teratogenic effects, but shows embryotoxicity accompanied by maternal toxicity (JECFA, 1995). The LD50 in mice is 5 mg/kg bw. A study in rats on reproductive toxicity (0 to 1.5 mg PAT/kg bw per day) showed reduced weight, tumour development and a high lethality with the highest dose. A NOAEL was determined at 43 µg/kg bw per day (recalculated from the

previous 0.1 mg/kg bw per day (JECFA, 1990) under consideration of the dosing interval). The provisional tolerable weekly intake (pTWI, 7 µg/kg bw per week) was changed into a provisional maximum tolerable daily intake (pmTDI) of 0.4 µg/kg bw per day (JECFA, 1995), applying a safety factor of 100. The pmTDI of 0.4 µg/kg bw per day was endorsed by EFSA (2000).

PAT was evaluated by IARC in 1976 and 1986, which concluded that there was inadequate evidence for the carcinogenicity of PAT in experimental animals and that no evaluation could be made of the carcinogenicity of PAT to humans. Case reports or epidemiology studies of PAT carcinogenicity in humans were not available. PAT was included in category 3 as not classifiable as to its carcinogenicity to humans (IARC, 1976; IARC, 1986).

Some preliminary toxicokinetic characteristics of PAT were determined by a single oral dose of radiolabelled PAT (3 mg/kg bw) in rats (JECFA, 1990). Within 7 days approximately 49% of administered radioactivity was recovered from feces, and 36% from urine. Most of the excretion of label occurred within the first 24 h. PAT was distributed to erythrocytes and several organs (spleen, kidney, lung and liver). PAT metabolites were not observed, but the toxin has a strong affinity to sulfhydryl groups, forming adducts with cysteine and glutathione that are less toxic.

Exposure (background information)
EFSA estimated the dietary intake of PAT from consumption of apple-derived products and other fruit based on consumption data from several European countries (EFSA, 2002). Exposures to PAT in consumers of the relevant food products (59 - 77% of the total population) were calculated in adults as 21 (mean)/57 (95-percentile) ng/kg bw per day and in children as 64 (mean)/199 (95-percentile) ng/kg bw per day.

Previously, JECFA had estimated the dietary intake of PAT from apple juice containing 10-15 µg/l as in the range of less than 0.03 to 0.26 (mean) and less than 1.9 to 3.9 µg/day (95-percentile) for different age groups in the population, including children (JECFA, 1990). In 1995, JECFA estimated a maximum intake of PAT in children as 0.2 µg/kg bw per day in children, and 0.1 µg/kg bw per day in adults.

In an Italian study, exposure of infants from apple-containing baby food was estimated to be 40.9 ng/kg bw per day (Beretta et al., 2000). The highest intake for adults drinking apple juice with pulp was estimated as 9.6 µg/kg bw per day. The French Food Safety Agency (ANSES) performed a risk assessment on PAT in 2006 (ANSES, 2006). Exposure to PAT from apple-based products in the general population was estimated to 18 (mean)/57 (95-percentile) ng/kg bw per day in adults and 30 (mean)/106 (95-percentile) ng/kg bw per day in children. For adult vegetarians, exposure was estimated in the range of 34 to 50 (mean) and 90 to 120 (95-percentile) ng/kg bw per day, depending on the type of vegetarian diet. A Spanish study estimated PAT exposure from the consumption of apple juice in the adult population as low as 0.42 ng/kg bw per day (González-Osnaya et al., 2007). In Sweden, exposure to PAT from apple juice was estimated for average and high consumers to be 0.009-2.04 µg/kg bw per day and 0.003-0.65 µg/kg bw perday among 4-year olds and

adults, respectively (Arnér, 2015). In a Serbian study, PAT intake in infants from apple juice was in the range of 20 to 45 ng/kg bw per day, and from apple puree in the range of 7.2 to 41 ng/kg bw per day, while the intake from juice in small children was estimated as in the range of 26 to 56 ng/kg bw per day (Torović et al., 2017). These results were comparable to other PAT intake estimates in infants and children in different European studies reported between 2007 and 2014.

Vulnerable groups: score 0
PAT exposure in infants and young children is generally higher than in adults, but in most studies estimated as below the pmTDI. Exposure in vegetarians is higher than in the general population, but below the pmTDI.

Lack of toxicity data: score 0.5
PAT toxicity data are considerably old and insufficient to determine immunotoxicity or human carcinogenicity. The toxicokinetics parameters and biotransformation pathways are not known.

Lack of exposure data: score 1.0
Exposure data for the Norwegian populations are lacking.

Total score = 3.5 for patulin (PAT)

3.1.8.2 *References*

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3.2 Subgroup plant toxins

3.2.1 Pyrrolizidine alkaloids (PAs)

Pyrrolizidine alkaloids (PAs) are a large group of natural toxins synthesised as secondary metabolites by different plant species. Several PAs are known to be highly toxic to humans and animals as a result of their presence in food. PAs occurs in e.g. tea and herbal infusions, honey and food supplements (plant extracts and pollen-based supplemets) (EFSA, 2017).

3.2.1.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 6.0
Many PAs are genotoxic and carcinogenic.

A $BMDL_{10}$ of 237 µg/kg bw per day, calculated for increased incidence of liver hemangiosarcoma in female rats after riddelliine exposure, is the reference point for chronic risk assessment of the sum of 1,2-unsaturated PAs, assuming equal potency (EFSA, 2017).

Based on exposure assessments in EU countries there was a wide range in MOE values for mean exposure, ranging from >10,000,000 to about 4,900 (min LB–max UB across dietary surveys and age classes). At 95-percentile exposure, the median LB to UB MOE values ranged between 16,200 and 4,200.

Vulnerable groups: score 1.0
People with high consumption of tea and herbal infusions can have high chronic exposure. In addition, the consumption of herbal food supplements based on PA-producing plants could reach acute/short-term exposure levels in the range of doses associated with severe acute/short-term effects in humans (1-3 mg/kg bw per day). The EFSA CONTAM Panel (2017) concluded that exposure levels less than 100 times lower than the dose range of 1–3 mg PA/kg bw per day may be associated with the risk of acute/short-term effects.

Lack of toxicity data: score 0.5
The EFSA CONTAM Panel (2017) recommends to obtain toxicological data, in particular data on toxicokinetics, metabolic activation and carcinogenic potency, on the PAs most commonly found in food.

Lack of exposure data: score 0.5
The EFSA CONTAM Panel (2017) proposed a list of 17 PAs to be monitored in relevant food and feed. These are intermedine/lycopsamine, intermedine-N-oxide/lycopsamine-Noxide, senecionine/senecivernine, senecionine-N-oxide/senecivernine-N-oxide, seneciphylline, seneciphylline-N-oxide, retrorsine, retrorsine-N-oxide, echimidine, echimidine-N-oxide, lasiocarpine, lasiocarpine-N-oxide and senkirkine.

Total score = 8.0 for pyrrolizidine alkaloids (PAs)

3.2.1.2 *References*

- EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen HK, Alexander J, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, Nebbia CS, Oswald IP, Petersen A, Rose M, Roudot A-C, Schwerdtle T, Vleminckx C, Vollmer G, Wallace H, Ruiz Gomes JA and Binaglia M (2017). Statement on the risks for human health related to the presence of pyrrolizidine alkaloids in honey, tea, herbalinfusions and food supplements. EFSA Journal 15(7):4908. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2017.4908.

3.2.2 Solanine and chaconine

The glycoalkaloids α-solanine (CAS no. 20562-02-1) and α-chaconine (CAS no. 20562-03-2) are produced in potatoes (*Solanum tuberosum*), which belong to the nightshade family (*solanaceae*; in Norwegian «søtvierfamilien»). α-Solanine is also found in eggplants, apples, bell peppers, cherries, sugar beets, chili, tomatoes and tobacco. The only difference between α-solanine and α-chaconine is the sugars in the trisaccharide position of the molecule, i.e., glucose with two rhamnoses for α-solanine, and a glucose, galactose and a rhamnose for α-chaconine (Dolan et al., 2010). These two substances are evaluated together.

Depending on variety and storage conditions, concentrations of α-chaconine and α-solanine in potato tubers vary between 0.0005–0.64 mg/g potato (0.5–635 ppm) and 0.005–25.1

mg/g potato (5–125,100 ppm), respectively. Although glycoalkaloids are found throughout the potato tuber, the greatest concentrations are in the sprouts, peels and sun-greened areas. The Food and Drug Administration (FDA) in USA considers the maximum acceptable glycoalkaloid content to be 20–25 mg/100 g fresh potato weight (or 200–250 ppm). Under current FDA regulations, 20 milligrams solanine per 100 grams (a small potato) can render it unfit to eat (Dolan et al., 2010).

Synthesis of α-chaconine and α-solanine is stimulated by light, mechanical injury, aging and potato beetle infestation. Exposure of potatoes to light in the field or marketplace can lead to glycoalkaloid concentrations that are unsafe for human consumption. Concentrations of solanine in green or blighted potatoes have been shown to increase by seven-fold (Dolan et al., 2010).

There is presently no EU legislation for glycoalkaloids. A maximum concentration of 200 mg/kg for food items is in use in many EU countries.

EFSA is performing a risk assessment of glycolalkaloids, which is expected to be published in January 2020 («Request for a scientific opinion on the risks for animal and human health related to the presence of glycolalkaloids in feed and food, in particular in potatoes and potato-derived products», EFSA-Q-2016-00811).

3.2.2.1 ***Scores***

MOE/MOS/ADI/TDI/TWI: score 6.0
JECFA (2007) considered that, despite the long history of consumption of plants containing glycoalkaloids, the available epidemiological and experimental data from human and laboratory animal studies did not permit the determination of a safe level of intake. There is no TDI-value available. Children may be more sensitive than adults.

In 2018, the Federal Institute for Risk Assessment (BfR) in Germany established a NOAEL of 0.5 mg/kg bw per day based on the available toxicological data (the main document is in German, only summary in English). To avoid an exceedance of the NOAEL, the glycoalkaloid content in table potatoes should be no higher than 100 mg per kg potatoes.

Potato consumption is investigated in the following national surveys/studies: The Norwegian Mother and Child Cohort Study (MoBa) (pregnant women), Norkost 3 (adults), Ungkost 3 (9 and 13 years) and Småbarnskost 2 (2 years). In MoBa, the mean intake of potatoes and various potato products for pregnant women in Norway during the first half of their pregnancy was 51 g/day (data from MoBa, personal communication with Anne Lise Brantsæter, Norwegian Institute of Public Health). In Norkost 3, the mean (SD) potato intake was 83 (80) g per day for men, and 50 (57) g per day for women. In Ungkost 3, the mean (SD) potato intake, in g/day, for 13 year old boys was 35 (41), for 13 year old girls was 31 (37), for 9 year old boys was 30 (33), and for 9 year old girls was 29 (34). In Småbarnkost 2, the mean potato intake was 29 g/day for 2 year old boys and 27 g/day for 2 year old girls.

To estimate consumption per kg bw, the following body weights were used: 70 kg for adults, 50.3 kg for adolescents (13 years), 32.9 kg for children (9 years), 13.3 kg for 2 year old boys and 12.4 kg for 2 year old girls.

MOE was calculated using the NOAEL established by BfR (2018), and the exposure to solanine and chaconine was estimated using concentrations of solanine and chaconine in potato, as reported by Dolan et al. (2010) (high and low level), and intake of potatoes from different consumption studies/surveys (Norkost 3, Ungkost 3 and Småbarnkost 2) (Table 3.2.2.1-1). α-Solanine and α-chaconine are not mutagenic or only weakly mutagenic *in vitro*, are not genotoxic *in vivo*, and are therefore not considered to be mutagenic or genotoxic. Therefore, a MOE value based on NOAEL above 100 is acceptable. However, since the data are not very good (little or no chronic toxicity data probably used by BfR to establish the NOAEL), an additional factor of 3 should be added. Therefore, MOE should be at least 300 in this case.

Table 3.2.2.1-1. MOE values for different population groups. Values in bold are acceptable, i.e. ≥300. Levels (range) in potato from Dolan et al. (2010).

		Potato intake			
		Solanine level in potato tubers		**Chaconine level in potato tubers**	
Population group	**Study/survey used**	**0.005 mg/g potato**	**25.1 mg/g potato**	**0.0005 mg/g potato**	**0.64 mg/g potato**
Women	MoBa and Norkost 3	125	0.030	**1,250**	1.1
Men	Norkost 3	83	0.020	**833**	0.7
Boys, 13 years	Ungkost 3	167	0.030	**1,666**	1.1
Girls, 13 years	Ungkost 3	167	0.030	**1,666**	1.3
Boys, 9 years	Ungkost 3	100	0.020	**1,000**	0.9
Girls, 9 years	Ungkost 3	125	0.020	**1,250**	0.9
Boys, 2 years	Småbarnskost 2	50	0.009	**500**	0.4
Girls, 2 years	Småbarnskost 2	50	0.009	**500**	0.4

In conclusion, for consumption of potatoes with low levels of chaconine, MOE values are acceptable for all age groups. For potatos with high levels of chaconine, and low and high levels of solanine, MOE values are not acceptable.

In addition to exposure from potatoes and potato products, people are also exposed for these substances from several other vegetables not included in the calculations above.

Toxicity: score 2.0

The following description of toxicity is based on Dolan et al. (2010), Munne and Verta (2013) and JECFA (2007).

The symptoms of acute toxicity to α-solanine and α-chaconine are due to their ability to act as inhibitors of acetylcholinesterase and disruptors of cell membranes. For α-chaconine, the intraperitoneal (i.p.) LD50 is 19.2 to 27.5 mg/kg bw for mice and 84 mg/kg bw for rats. For α-solanine, the oral LD50 dose is 590 mg/kg bw for rats, the intraperiotoneal LD50 dose is 30 to 42 mg/kg bw for mice, 67 to 75 mg/kg bw for rats and less than 40 mg/kg bw for monkeys. Glycoalkaloid doses of 1 to 3 or 5 mg/kg bw (depending on the reference) have been reported to be acutely toxic to humans, and doses of 3 to 6 mg/kg bw have resulted in death. Symptoms of glycoalkaloid toxicity in humans include drowsiness, itchiness in the neck region, increased sensitivity (hyperesthesia), laboured breathing and gastrointestinal symptoms (abdominal pain, nausea, vomiting and diarrhea). Many alkaloids cause acute toxicity by mimicking or blocking the action of nerve transmitters. In more severe cases, neurological symptoms may be observed including drowsiness and apathy, confusion, weakness and vision disturbances, followed by unconsciousness and in some cases death. Onset of symptoms has ranged from minutes to 2 days after ingestion of toxic potatoes, but will generally occur 8 to 12 hours after ingestion, with longer incubation periods generally associated with the more severe cases. Other factors may be present in potatoes and modulate the toxicity of the steroidal glycoalkaloids.

α-Solanine and α-chaconine are not mutagenic or only weakly mutagenic *in vitro*, are not genotoxic *in vivo*, but are embryotoxic and teratogenic to experimental animals. Teratogenic effects in mammals include central nervous system abnormalities (e.g. exencephaly, cranial bleb, encephalocele and anophthalmia), mild hydronephrosis, hydroureter and irregular or fused ribs. Although one human case study reported a correlation between the severity of potato late-blight and the incidence of spina bifida, no other studies in humans have found a correlation between the consumption of potatoes and birth defects. No chronic exposure data were found. There is no evidence that α-solanine and α-chaconine are carcinogenic in animals or humans.

Acute, short-term and subchronic animal toxicity studies identified similar effects from administration of α-chaconine, α-solanine, or plants or extracts containing the glycoalkaloids. These substances often give moderate acute toxicity, mostly gastrointestinal symptoms, but can also give serious effects such as neurological symptoms and teratogenic effects at least in animals, and even death. Therefore, they are given a medium score for toxicity.

Exposure: score 2.0

The concentrations of α-chaconine and α-solanine in potato tubers reported by Dolan et al. (2010) are used for the exposure estimation. It was reported that, depending on variety and storage conditions, concentrations of α-chaconine and α-solanine in potato tubers vary between 0.0005–0.64 mg/g potato and 0.005–25.1 mg/g potato, respectively. In addition, consumption data from MoBa (pregnant women), and consumption data from the national

food consumption surveys Norkost 3 (adults), Ungkost 3 (9 and 13 years) and Småbarnskost 2 (2 years), are used.

In MoBa, the mean intake of potatoes and various potato products for pregnant women in Norway during the first half of their pregnancy was 51 g/day (data from MoBa, personal communication with Anne Lise Brantsæter, Norwegian Institute of Public Health). In Norkost 3 (Totland et al., 2012), the mean (SD) potato intake was 83 (80) g per day for men, and 50 (57) g per day for women. In Ungkost 3 (Hansen et al., 2015), the mean (SD) potato intake, in g/day, for 13 year old boys was 35 (41), for 13 year old girls was 31 (37), for 9 year old boys was 30 (33), and for 9 year old girls was 29 (34). In Småbarnkost 2 (Kristiansen et al., 2009), the mean potato intake was 29 g/day for 2 year old boys and 27 g/day for 2 year old girls. An overview of the estimated exposure to solanine and chaconine from potatos is given in Table 3.2.2.1-2.

Table 3.2.2.1-2. Estimated exposure (in mg/kg bw per day) to α-solanine and α-chaconine from potatoes. Body weights of 70 kg for adults, 50.3 kg for adolescents (13 years), 32.9 kg for children (9 years), 13.3 kg for 2 year old boys and 12.4 kg for 2 year old girls, were used. Levels (range) in potato from Dolan et al. (2010).

		Potato intake			
		α-Solanine level in potato tubers		**α-Chaconine level in potato tubers**	
Population group	**Study/survey used**	**0.005 mg/g potato**	**25.1 mg/g potato**	**0.0005 mg/g potato**	**0.64 mg/g potato**
Women	MoBa and Norkost 3	0.004	18.3	0.0004	0.47
Men	Norkost 3	0.006	29.8	0.0006	0.76
Boys, 13 years	Ungkost 3	0.003	17.5	0.0003	0.45
Girls, 13 years	Ungkost 3	0.003	15.5	0.0003	0.39
Boys, 9 years	Ungkost 3	0.005	22.9	0.0005	0.58
Girls, 9 years	Ungkost 3	0.004	22.1	0.0004	0.56
Boys, 2 years	Småbarnskost 2	0.01	54.8	0.001	1.4
Girls, 2 years	Småbarnskost 2	0.01	54.5	0.001	1.4

In all age groups and both genders, the exposure is below 100 mg/kg bw per day from potatoes and is therefore considered low. However, potatoes are a staple food in Norway, with daily consumption by many people. In addition, people are exposed also for these substances from several other vegetables not included in the calculations above. They are therefore given a medium score for exposure in all age and gender groups.

Vulnerable groups: score 1.0

Pregnant women and their fetus may be vulnerable groups since teratogenic effects are reported in animals. Children may be more sensitive than adults (JECFA, 2007).

Lack of toxicity data: score 0.5
Although the mechanism for acute toxicity is known, there are little or no data on chronic toxicity of these glycoalkaloids, and therefore no TDI has yet been established.

Lack of exposure data: score 1.0
There are no good data on total exposure (intake and occurrence) of these two glycoalkaloids from potatoes and all the other vegetables containing these substances (eggplant, apples, bell peppers, cherries, sugar beets, chili, tomatoes and tobacco) for the Norwegian or European populations.

For both solanine and chaconine, questions 2 and 3 were used instead of question 1 since MOE was calculated using the NOAEL established by BfR (not EFSA), based on little or no chronic toxicity data, and the exposure to solanine and chaconine was estimated using their concentrations in potato from a single publication from USA.

Total score = 6.5 for solanine and chaconine

3.2.2.2 ***References***

- BfR (The Federal Institute for Risk Assessment) (2018). Table potatoes should contain low levels of glycooalkaloids (solanine). BfR Opinion No 010/2018. URL: https://www.bfr.bund.de/cm/349/table-potatoes-should-contain-low-levels-of-glycoalkaloids-solanine.pdf (Main document in German, summary in English).
- Dolan LC, Matulka RA, Burdock GA (2010). Naturally occurring food toxins. Toxins 2(9):2289–2332. URL: doi: 10.3390/toxins2092289, PMID: 22069686.
- Hansen LB, Myhre JB, Johansen AMW, Paulsen MM, Andersen LF (2015). UNGKOST 3. Landsomfattende kostholdsundersøkelse blant elever i 4. -og 8. klasse i Norge, 2015. URL: https://www.fhi.no/globalassets/dokumenterfiler/rapporter/2016/ungkost-rapport-24.06.16.pdf (In Norwegian).
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- Kristiansen AL, Andersen LF, Lande B (2009). Småbarnskost – 2 år. Landsomfattende kostholdsundersøkelse blant 2 år gamle barn. The Norwegian Directorate of Health, IS-1731. URL: https://helsedirektoratet.no/Lists/Publikasjoner/Attachments/702/Smabarnskost-2007-landsomfattende-kostholdsundersokelse-blant-2-ar-gamle-barn-IS-1731.pdf (In Norwegian).
- Munne P, Verta M (2013). Selection of main contaminant expsoure pathways, Foodweb project. The Baltic environment, food and health: from habits to awareness. Finnish Environment Institute. URL: http://foodweb.ut.ee/s2/111_94_92_Selection_of_the_main_contaminant_exposure_pathway.pdf.

- Totland TH, Melnæs BK, Lundberg-Hallén N, Helland-Kigen KM, Lund-Blix NA, Myhre JB, Johansen AMW, Løken EB, Andersen LF (2012). Norkost 3. En landsomfattende kostholdsundersøkelse blant menn og kvinner i Norge i alderen 18-70 år, 2010-11. The Norwegian Directorate of Health, IS-2000. URL: https://helsedirektoratet.no/Lists/Publikasjoner/Attachments/301/Norkost-3-en-landsomfattende-kostholdsundersokelse-blant-menn-og-kvinner-i-norge-i-alderen-18-70-ar-2010-11-IS-2000.pdf (In Norwegian).

3.2.3 Tropane alkaloids (TAs)

Tropane alkaloids (TAs) are toxic secondary metabolites occurring in plants from several plant families including *Brassicaceae*, *Solanaceae* (e.g. mandrake, henbane, deadly nightshade, Jimson weed) and *Erythroxylaceae* (including cocoa). The TAs are responsible for the toxic effects of some of these plants and occur in all parts of the plant. More than 200 TAs have been described and particularly plants from the Solanaceae family have a large variety of TAs. The main TAs in plants are (-)-hyoscyamine and (-)-scopolamine. Atropine is the racemic mixture of (-)-hyoscyamine and (+)-hyoscyamine.

(-)-hyoscyamine and (-)-scopolamine are readily absorbed from the gastrointestinal tract, quickly and extensively distributed into tissues, and excreted predominantly in the urine. Known metabolic pathways in humans are demethylation and phase II conjugation of atropine, (-)-hyoscyamine and (-)-scopolamine. (-)-Hyoscyamine and (-)-scopolamine are antagonists of the muscarinic acetylcholine receptors primarily present in the autonomic effector sites innervated by parasympathetic (cholinergic postganglionic) nerves but also in the central nervous system (CNS). The effects of hyoscyamine and scopolamine occur rapidly after administration and includes pupillary dilation and neurobehavioural effects. In humans, the predominant peripheral antimuscarinic effects are decreased production of secretions from the salivary, bronchial, and sweat glands, dilation of the pupils (mydriasis) and loss of the eyes ability to focus, change in heart rate, inhibition of micturition, reduction in gastrointestinal tone and inhibition of gastric acid secretion (EFSA, 2013).

Most of the analytical results (95%) in the EFSA database were below the LOD or below the LOQ. Highest levels were, according to EFSA (2018), found in tea and herbal infusions, cereal bars and spices.

3.2.3.1 ***Scores***

MOE/MOS/ADI/TDI/TWI: score 4.0
EFSA established a group ArfD for the sum of (-)-hyoscyamine and (-)-scopolamine of 16 ng/kg bw (EFSA, 2013). Later, EFSA also estimated the acute human exposure to TAs when more data were available (EFSA, 2018). The exposure exceeded the group ArfD for the upper bound mean (UB) in toddlers and other children. The high exposure (95-percentile) exceeded the TDI for toddlers and other children for both LB and UB estimations.

The toxicity of other TAs remains largely unknown. Data on the occurrence were made available by EFSA (Mulder et al., 2016).

Vulnerable groups: score 0.5
Based on the EFSA estimation of intake, children have a higher intake than adults (EFSA, 2018).

Lack of toxicity data: score 1.0
The toxicity data are mainly for two of the more than 200 described alkaloids. The acute toxicity is of main concern. Little is known about long-term effects.

Lack of exposure data: score 0.5
There are no Norwegian data available. The occurrence data on TAs in EU are updated (Mulder et al., 2016). TAs occur mainly in imported food plants.

Total score = 6.0 for tropane alkaloids (TAs)

3.2.3.2 *References*

- EFSA (European Food Safety Authority), Arcella D and Altieri A (2018). Scientific report on human acute exposure assessment to tropane alkaloids. EFSA Journal 2018;16(2):5160. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5160 (under review).
- EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2013. Scientific Opinion on Tropane alkaloids in food and feed. EFSA Journal 2013;11(10):3386, 113 pp. doi:10.2903/j.efsa.2013.3386. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2013.3386.
- Mulder PPJ, De Nijs M, Castellari M, Hortos M, MacDonald S, Crews C, Hajslova J Stranska M (2016). Occurrence of tropane alkaloids in food. EFSA supporting publication 2016:EN-1140. doi:10.2903/sp.efsa.2016.EN-1140 https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2016.EN-1140.

3.2.4 Erucic acid

Erucic acid is a monounsaturated omega-9 fatty acid, which is present in the oil-rich seeds of the *Brassicaceae* family of plants, particularly rapeseed and mustard. It mainly enters the food chain when rapeseed oil is used in industrial food processing and home cooking in some countries (EFSA, 2016). Please note that Norwegian occurrence data in fish and fish oils were not included in the EFSA opinion on erucic acid in feed and food (2016).

3.2.4.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 4.0
Exposure >TDI for some groups of the European population, but only at 95-percentile UB

exposures. However, updated exposure assessment is needed because fish is not included and Norwegian data show high levels in wild and farmed fatty fish.

Vulnerable groups: score 0.5
Exposure >TDI for infants and other children.

Lack of toxicity data: score 0.5
There is a lack of studies with pure erucic acid. The TDI might be too conservative.

Lack of exposure data: score 0.0
Sufficient Norwegian data are available for fish and fish oil, in addition to European data for other foods.

Total score = 5.0 for erucic acid

3.2.4.2 *References*

- EFSA Panel on Contaminants in the Food Chain (CONTAM), Knutsen HK, Alexander J, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, Dinovi M, Edler L, Grasl-Kraupp B, Hogstrand C, Hoogenboom L (Ron), Nebbia CS, Oswald I, Petersen A, Rose M, Roudot A-C, Schwerdtle T, Vollmer G, Wallace H, Cottrill B, Dogliotti E, Laakso J, Metzler M, Velasco L, Baert K, Ruiz JAG, Varga E, Dorr B, Sousa R and Vleminckx C (2016). Scientific Opinion on erucic acid in feed and food. EFSA Journal 14(11): 4593, 173 pp. doi:10.2903/j.efsa.2016.4593 https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2016.4593
- Sissener et al. (2018). Erucid acid (22:1n-9) in fish feed, farmed, and wild fish and seafood products. Nutrients 10: 1443.

3.2.5 Cyanogenic glucosides

Foods such as apricot kernels, almonds, linseeds, bamboo and cassava contain cyanogenic glycosides. There may be great variation in content between plant varieties, e.g. sweet and bitter cassava.These substances contain chemically bound cyanide that can be released when the plant cells are damaged by for example grinding or chewing, as the cyanogenic glucosides are brought in contact with their degrading enzymes. The amount that is released is dependent on the food source and processing/preparation. Cyanide is acutely toxic by binding to haemoproteins causing perturbation of oxygen transport.

3.2.5.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 4.0

In 2016, EFSA CONTAM Panel established an ARfD of 20 µg/kg bw for cyanide (CN) from apricot kernels, and in 2019 this was extended to be applicable for all dietary sources of CN (EFSA, 2019). EFSA also conducted an exposure assessment showing that the mean intake

did not exceed the ARfD for any age groups. At the 95-percentile the ARfD was in some surveys exceeded up to 2.5 fold for children and adolescents. It was considred that it was unlikely that the exposure to CN from cyanogenic glucosides in food consumed in European surveys would lead to any adverse effects given the conservatism in the exposure assessment and derivation of the ARfD.

Vulnerable groups: score 0.5
Children and adolescents.

Lack of toxicity data: score 1.0
Lack of bioavailability and chronic toxicity data.

Lack of exposure data: score 0.0
Lack of exposure data.

Total score = 5.5 for cyanogenic glucosides

3.2.5.2 *References*

- EFSA Panel on Contaminants in the Food Chain (CONTAM) (2019). Evaluation of the health risks related to the presence of cyanogenic glycosides in foods other than raw apricot kernelsdoi: 10.2903/j.efsa.2019.5662 https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/j.efsa.2019.5662.
- EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain) (2016). Scientific opinion on the acute health risks related to the presence of cyanogenic glycosides in raw apricot kernels and products derived from raw apricot kernels. EFSA Journal 2016;14(4):4424, 47 pp. doi:10.2903/j.efsa.2016.4424.

3.2.6 Glucosinolates

The food plants belonging to the family *Brassicaceae* or *Cruciferae* include many vegetables, which contain a large number of glucosinolates. Components of the diet are e.g. cauliflower, cabbages, broccoli and Brussels sprouts. Their seeds are used for production of edible oils such as rape seed oil. Press cakes containing glucosinolates are used for feed. Glucosinolates are responsible for the flavour of brassica derived products such as mustard and horsraddish. Their degradation products, i.a. isothiocyaniates and oxazolidinethiones are relased upon the action of myrosinases and have been assigned a wealth of health benefical effects such as anti-genotoxic effects, anti-tumourigenic effects, induction of phase II detoxication enzymes, as well as adverse effects, e.g. genotoxic effects, inhibition of ABC-transporters. They may exhibit liver and kidney toxicity, and inhibit transport of iodine into the thyroid gland and together with iodine deficiency induce goiter («cabbage goiter»).

3.2.6.1 ***Scores***

Toxicity: score 1.0
Glusinolates may exhibit liver and kidney toxicity, and inhibit transport of iodine into the thyroid gland and induce goiter. No health based guidance values for glucosinolates have been established. Their toxicity is considered to be low

Exposure: score 1.0
Exposure to glucosinolates is related to intake of brassica vegetables. There are some reviews of human exposure to glucosinolates. The exposure is considered to generally be within safe limits.

Vulnerable groups: score 0.5
Iodine deficient groups are vulnerable for inhibitors of iodine transport. In particular pregnant women and pherhaps also lactating women as transport of iodine to the fetus and breast milk might be compromised.

Lack of toxicity data: score 1.0
There is a general lack of toxicity data.

Lack of exposure data: score 0.5

Total score = 4.0 for glucosinolates

3.2.6.2 ***References***

- Latté KP, Appel K-E, Lampen A (2011). Health benefits and possible risks of broccoli – An overview. Food and Chemical Toxicology 49: 3287–3309.

3.3 Subgroup marine algae toxins

3.3.1 Azaspiracids (AZAs)

Azaspiracids (AZAs) have been associated with food poisoning since the first incident in 1995, when a food poisoning episode in The Netherlands was attributed to Irish mussels (Mytilus edulis) harvested at Killary Harbor. Symptoms were stomach cramps, vomiting, severe diarrhea and general nausea. Since then, AZAs are regularly reported to be present in shellfish along the coast of Norway, and shellfish are therefore included in the Norwegian Food Safety Authority's surveillance of algal toxins in blue mussels. Crabs are not uncluded in this surveillance.

The mechanism or mechanisms whereby AZAs exert their toxic effects are still unknown (Munday, 2014). The toxicological information on AZAs is inadequate. No LD50s of AZA are available either by oral administration or by injection.

EFSA has established an ARfD based on one incident of human poisoning involving AZAs due to lack of other data. A lowest observed adverse effect level (LOAEL) resulting in AZA poisoning was estimated at 113 µg AZA1 equivalents per person (1.9 µg AZA1 equivalents/kg body weight for a 60 kg adult). Uncertainty factors were required to extrapolate from the LOAEL to a no observed adverse effect level (NOAEL), and for variability within the human population. The CONTAM Panel in EFSA decided that the usual factor of 10 for human variability was not required because the reported incident was expected to have occurred in sensitive, rather than average, individuals (EFSA, 2008). However, an additional factor of three was applied because the available data related to a small number of individuals from a single incident. Consequently, the CONTAM Panel established an ARfD of 0.2 µg AZA1 equivalents/kg bw.

3.3.1.1 *Scores*

Quantitative data for intake and toxicity: score 4.0

ARfD = 0.2 µg/kg bw of AZA-1 equivalents (EFSA, 2008). Two unpublished pilot studies from 2013 and 2014 from the west coast of Norway showed the brown meat from crabs to contain levels up to and also above the ARfD. This was found although there was no warning of AZA-contamination of the shellfish in the same area.

Vulnerable groups: score 1.0
People eating brown crab meat regularly. There is a difference whether only the white meat or also the brown meat is consumed, since the highest concentration occurs is in the brown meat. If brown meat is avoided, we may lower the scoring to 0.5 or possibly also to 0, because almost all of the AZAs are found in the brown meat and only trace levels in the white meat.

Lack of toxicity data: score 0.5
Data are needed to characterize the mode of action.

Lack of exposure data: score 1.0
Information on occurrence in Norwegian crabs is limited. There is no correlation between AZAs found in shellfish and AZAs found in crabs.

Total score = 6.5 for azaspiracids (AZAs)

3.3.1.2 *References*

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3.3.2 Tetrodotoxin (TTX) and TTX analoges

Tetrodotoxin (TTX) is traditionally associated with seafood from tropical regions, but recently TTX was detected in bivalve mollusks in more temperate European waters, i.e. the UK (Turner et al., 2015) and the Netherlands (Gerssen et al., 2018). One poisoning episode has been reported from eating part of a trumpet shellfish (*Charonia sauliae*) in Spain (Fernández-Ortega et al., 2010).

TTX is a sodium channel blocker and can cause serious poisoning and even death after ingestion (Munday, 2014). TTX is a hydrophilic heat-stable toxin, assumed produced by bacteria, and so far 25 naturally occurring analogues of TTX have been detected and many of these have also been shown to have toxicity potential.

In 2017, EFSA performed a risk assessment on TTX in shellfish (Knutsen et al., 2017). An ARfD for TTX of 0.25 µg/kg bw was derived, based on effects in mice. This implied that the TTX concentration in a large portion of 400 g shellfish, consumed by a 70 kg person, should not exceed 44 µg TTX/kg shellfish.

According to the Dutch study, 6 of their samples (3 samples in 2015, 2 samples in 2016 and only one in 2017) taken in the sanitary survey program exceeded the limit of 44 µg/kg of TTX (Gerssen et al., 2018). Furthermore, within the sanitary survey samples only oysters exceeded this limit. According to the British study, TTX concentrations ranged from approximately LOQ (3 µg/kg TTX in shellfish tissue) to a maximum of 120 µg/kg (Turner et al., 2015). TTX analogues were quantified at lower levels, typically 10–15% of the total TTX content. The maximum summed concentration quantified of all TTX analogues was 137 µg/kg TTXs in one oyster sample.

3.3.2.1 ***Score***

Quantitative data for intake and toxicity: score 4.0
ARfD is 0.25 µg/kg bw (EFSA, 2017). Levels reported from shellfish in UK and the Netherlands are above the ARfD, and may indicate a risk of exposure also in Norway.

Vulnerable groups: score 1.0
People eating shellfish.

Lack of toxicity data: score 0.5
Further information on the acute oral toxicity of TTX and its analogues is needed. Chronic effects should also be investigated (EFSA, 2017).

Lack of exposure data: score 1.0
Data on presence in Norwegian seafood is lacking. TTX was found in 14 out of 29 samples of blue mussels (*Mytilus Edulis*) and oysters (*Crassostrea gigas*) in the UK (Turner et al., 2015) and oyster and mussels in the Netherlands (Gerssen et al., 2018) recently. Poisoning has been reported in Spain from eating part of a trumpet shellfish (*Charonia sauliae*) from the Atlantic (Fernández-Ortega et al., 2010)

Total score = 6.5 for tetrodotoxin (TTX) and TTX analoges

3.3.2.2 ***References***

- Fernández-Ortega, J.F., Santos, J.M.M.-d.l., Herrera-Gutiérrez, M.E., Fernández-Sánchez, V., Loureo, P.R., Rancaño, A.A., Téllez-Andrade, A., 2010. Seafood intoxication by tetrodotoxin: First case in europe. The Journal of Emergency Medicine 39, 612–617.
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3.4 Subgroup freshwater algae toxins

3.4.1 Microcystins (MCs)

Microcystins (MCs) are cyclic heptapeptides produced by various cyanobacteria such as Microcystis, Planktothrix, Anabaena and Nostoc. MC-LR is one of the most commonly investigated analogues, allthough more than 250 variants are reported. MCs are also the most widespread of the cyanobacterial toxins (Buratti et al., 2017). MCs are hepatotoxic, hydrophilic and heat stable. Several human poisoning episodes are described, among them an episode in Caruaru in Brazil where 130 patients received dialysis containing approximately 19.5 µg/L MCs (MC-YR, MC-LR and MC–AR) in the water and developed acute neurotoxicity

and subacute hepatotoxicity, whereupon 76 of the patients died (Carmichael et al., 2001). In 2014, the city of Toledo, Ohio, was without drinking water for three days due to MCs in the water (Buratti et al., 2017).

Among the several routes by which humans may be exposed to cyanotoxins, the oral route is the most important, occurring by consumption of contaminated drinking water or food. Human exposure from food can be due to consumption of fish, crops, food supplements based on algae, or items of animal origins, following the use of contaminated water for irrigation or in farming activities (Testai et al., 2016). Literature suggests that cyanotoxins can be accumulated in food at concentrations higher than provisional limits set for MC-LR in drinking water. In particular, several investigations on contaminated blue-green algae food supplements (BGAS) have shown levels of contamination exceeding the proposed provisional guidance value. Assumptions on the variable daily consumption of these products have evidenced a risk for chronic consumers (Testai et al., 2016).

To protect consumers from the adverse effects of cyanobacterial peptide toxins, WHO proposed a provisional upper limit in drinking water of 1 µg/L for MC-LR and a TDI of 0.04 µg/kg bw (WHO, 2011). The Oregon Health Division (USA) set a provisional regulatory standard of 1 µg/g MC-LR equivalents per dry weight product in supplements of bluegreen algae (Gilroy et al., 2000). However, this standard has no legal status outside Oregon, although used for orientation in other countries.

3.4.1.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 4.0
The TDI is 0.04 µg/kg bw per day (Testai et al., 2016; WHO, 2011). Exposure is unknown in Norway, however, it is a recurring problem around the great lakes and in Florida in USA, in Serbia and China.

Vulnerable groups: score 0.5
People taking algal supplements may be exposed.

Lack of toxicity data: score 1.0
The data available are mainly data for MC-LR and a few other analogues, whereas it is limited for the other 250 analogues. Long-term exposure studies (2-years) are lacking.

Lack of exposure data: score 1.0
Information on presence in Norwegian drinking water and algal supplements are scarce.

Total score = 6.5 for microcystins (MCs)

3.4.1.2 *References*

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4 Ranking of metals and metalloids

An overview of the scoring and ranking of the included metals and metalloids is given in Table 4-1. A detailed description follows after the table.

Table 4-1. Summary table for scoring of metals and metalloids.

Substance	1. MOE/MOS /ADI/TDI/ TWI	2. Toxicity	3. Exposure	4. Vulnerable groups	5. Lack of toxicity data	6. Lack of exposure data	Total score	Comments
Aluminium (Al)	4.0	-	-	0.5	0.0	0.0	4.5	
Inorganic arsenic (As)	6.0	-	-	0.5	0.0	0.0	6.5	Chemical speciation of arsenic in food should be performed Note that very little data are available on toxicity and exposure for arsenolipids/arsenosugars. Thus, the scores are uncertain
Organic arsenic (As)	-	1.0	1.0	0.0	1.0	1.0	4.0	
Cadmium (Cd)	6.0	-	-	0.5	0.0	0.0	6.5	
Chromium (Cr)	2.0	-	-	0.0	0.0	1.0	3.0	CrVI most toxic, CrIII less toxic
Lead (Pb)	6.0	-	-	1.0	0.0	0.5	7.5	
Methylmercury (MeHg)	6.0	-	-	1.0	0.0	0.0	7.0	
Nickel (Ni)	2.0	-	-	1.0	0.0	0.0	3.0	Nickel allergic persons may exceed threshold

4.1 Aluminium (Al)

Aluminium is the most abundant metallic element in the earth's crust. Aluminium sulphates and sodium aluminium phosphates are registered food additivies in baking powder and anti-caking agents. Aluminium may be present in food both as a result of its use as food additive and as a contaminant leaching out of packaging and cookware material to acidic food. Oral bioavailability is low, 0.1-0.4%. Neurodevelopmental toxicity following pre- and postnatal exposure has been observed in experimental animals.

4.1.1 Scores

MOE/MOS/ADI/TDI/TWI: score 4.0
EFSA (2008) established a TWI of 1 mg/kg bw. The mean dietary exposure in Norway varied from 0.22 to 0.89 mg/kg bw per week and was comparable to exposure in other European countries (VKM, 2013). High consumers of food with aluminium, the 95-percentile, had an estimated exposure of 0.5-1.9 mg/kg bw per week and exceded the TWI, but their exposure was below the provisional TWI (pTWI) established by JECFA of 2 mg/kg bw (VKM, 2013; WHO, 2011). Exposure to aluminium from cosmetics products may occur.

Vulnerable groups: score 0.5
High consumers, 1 to 2 year old children.

Lack of toxicity data: score 0.0
Sufficient data are available.

Lack of exposure data: score 0.0
Sufficient data are available.

Total score = 4.5 for aluminium (Al)

4.1.2 References

- Alexander J and Oskarsson A (2019). Toxic Metals. In: Chemical hazards in foods of animal origin. Food safety assurance and veterinary public health. Editors: Smulders FJM, Rietjens IMCM and Rose M. Wageningen Academic Publishers, 2019. Pp. 157-180.
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4.2 Inorganic and organic arsenic (As)

Arsenic is a metalloid that occurs in many different chemical forms in the environment and in food. Fish and seafood are the main contributor to the dietary exposure to total arsenic, and a high consumption of fish and seafood leads to a high dietary exposure to total arsenic. Arsenic from seafood is mainly as organic arsenic, whereas less than one and up to a few percent may occur as inorganic arsenic. Inorganic arsenic forms are trivalent arsenite (AsIII) and pentavalent arsenate (AsV). Organic arsenic in seafood changes in composition in the food web. Arsenosugars are dominating in algae and shellfish, whereas arsenobetaine becomes more prevalent higher up in the food web. In fin fish and in cod, arsenobetain is the dominating species. In more recent years arsenic bound to lipids, i.a. fatty acids, phospholipids etc, have been characterised. Arsenolipids have been found in the lipid phase in several seafoods including algae and cod liver. Methylation of arsenic takes place both in environmental organsims and in humans who forms monomethyl- and dimethyl arsenic. Methylation takes place in complicated stepwise reduction – oxidative methylation process. Generally, the trivalent species are the most toxic with monomethyl arsenic as the most reactive. In humans, inorganic arsenic is methylated and excreted as dimethyl arsenic and to a less extent monomethyl arsenic. Arsenosugars and lipids split off dimethylarsenic upon metabolism. Arsenic, mainly as inorganic arsenic, may also occur in cereals, particularly in rice grown in fields irrigated with water high in arsenic. Dimethyl arsenic may also be present in rice from 10-40%. In other parts of the world arsenic in drinking water is a huge health problem. Inorganic arsenic is well known as an acute poison and as a public health issue related to presence in drinking water and food causing skin problems, cancer and cardiovascular diseases. Dimethyl arsenic causes cancer in rats and mice. Regarding organic arsenic compounds including arsenolipids and arsenosugars there is little information on both their occurance and toxicity. Arsenobetain is excreted unchanged and has been considered to have low toxicity.

4.2.1 Scores

Arsenic is a metalloid that occurs in many different chemical forms in the environment and in food. Fish and seafood are the main contributors to the dietary exposure to total arsenic, and a high consumption of fish and seafood leads to a high dietary exposure to total arsenic. Exposure to arsenic via seafood is mainly to organic arsenic.

MOE/MOS/ADI/TDI/TWI: score 6.0 for inorganic arsenic
Inorganic arsenic is carcinogenic. The reference points for its carcinogenic effect have been established by EFSA (2009) and JECFA (2011): EFSA $BMDL_{01}$ 0.3-8 µg/kg bw per day, JECFA $BMDL_{05}$ 3 µg/kg bw per day.

Dietary exposure to inorganic arsenic in the Norwegian population was estimated by EFSA (2014). The Norwegian exposure levels were the highest among the European populations. A high exposure to total arsenic for Norwegian adults was also estimated in the Norwegian Fish and Game study (Birgisdottir et al., 2013). There was little variation in the estimated dietary exposures to inorganic arsenic for the European populations (EFSA, 2014). In the European populations, the main contributors to dietary exposure of inorganic arsenic were the food groups «grain-based processed products rice and non rice-based», «milk and dairy products» and «drinking water» (EFSA, 2014). There is no information regarding specific dietary patterns of Norwegian sub-populations possibly leading to a higher exposure to inorganic arsenic.

The dietary exposure to inorganic arsenic is within the range of the $BMDL_{01}$ established by EFSA (2009).

Arsenolipids and arsenosugars occur in seafood, particularly those low in the food web, such as algae and shellfish. There is little information on both their occurance and toxicity. These compounds may split off dimethyl arsenic. This compound is carcinogenic in rats and mice. No assessments of these compounds have been conducted by EFSA or WHO.

Toxicity: score 1.0 for organic arsenic
The toxicity is not well characterised.

Exposure: score 1.0 for organic arsenic
Little information on exposure is available.

Vulnerable groups: score 0.5 for inorganic arsenic; 0.0 for organic arsenic
High consumers of rice (inorganic arsenic).

Lack of toxicity data: score 0.0 for inorganic arsenic; score 1.0 for organic arsenic
There is lack of toxicity data for organic arsenic compounds in particular those from seafood, e.g. arsenic bound to sugars and lipids.

Lack of exposure data: score 0.0 for inorganic arsenic; score 1.0 for organic arsenic

Total score = 6.5 for inorganic arsenic and 4.0 for organic arsenic

4.2.2 References

- Alexander J and Oskarsson A (2019). Toxic Metals. In: Chemical hazards in foods of animal origin. Food safety assurance and veterinary public health. Editors: Smulders

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4.3 Cadmium (Cd)

Cadmium occurs naturally together with zinc and lead in minerals and can vary considerably among soil types, and is high in soils from alum shale. Antropogenic soures to soil are phosphate fertilisers and deposition from the atmeosphere and sewage sludge. The use of cadmium is restricted to avoid further environmental contamination. Cadmium is taken up in plants from the soil. The uptake is dependent of i.a. plant species and cultivar, soil and pH. Cereal and vegetable products are the main sources among non-smokers, whereas tobacco smoke is the mainsource in smokers. About 5% of cadmium is taken up in the intestinal tract and it accumulates in the kidney and liver with a half life ranging from 20 to 40 years. In practise it accumulate life long into old age. Cadmium is primarily toxic to the kidney, and can also cause bone demineralisation. At very high doses it may cause chronic nephropathy and severe osteomalacia.

4.3.1 Scores

MOE/MOS/ADI/TDI/TWI: score 6.0
Cadmium is primarily toxic to the kidney, and can also cause bone demineralisation. EFSA (2009) established a TWI for cadmium of 2.5 µg/kg bw. The exposure in the European population is in the range of the TWI. The 95-percentile, 3.66 µg/kg bw per week, exceed the TWI (EFSA, 2009).

Vulnerable groups: score 0.5
Individuals with empty iron-stores have an enhanced intestinal absorption of cadmium.

Lack of toxicity data: score 0.0
Sufficient data are available.

Lack of exposure data: score 0.0
Sufficient data are available.

Total score = 6.5 for cadmium (Cd)

4.3.2 References

- Alexander J and Oskarsson A (2019). Toxic Metals. In: Chemical hazards in foods of animal origin. Food safety assurance and veterinary public health. Editors: Smulders FJM, Rietjens IMCM and Rose M. Wageningen Academic Publishers, 2019. Pp. 157-180.
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4.4 Chromium (Cr)

Chromium occurs in two main form, CrIII and CrVI. In nature chromium mainly occurs in the trivalent state. This is also the cae with biological material where CrVI is rapidly reduced to CrIII. Dietary chromium is mainly in the form of CrIII. CrIII has been suggested to play a role in glucose metabolism. Exposure to CrVI can take place via drinking water. The latter is highly toxic and carcinogenic. CrVI compounds are easily transported across biological membranes in the airways and gastrointestinal tract, whereas the transport of CrIII is much slower. Upon reduction of CrVI to CrIII reactive chromium intermediates may form andbind to macromolecules such as proteins and DNA and cause enzyme inhibition, allergenicity and DNA damage. CrVI is highly toxic and carcinogenic in particular upon inhalation of aerosols and may cause lung cancer. Chromium compounds may also induce skin contact allergy**.**

4.4.1 Scores

MOE/MOS/ADI/TDI/TWI: score 2.0
Chromium occurs in two main form, CrIII and CrVI. The latter is highly toxic and carcinogenic. A TDI of 0.3 mg/kg bw per day for CrIII was established by EFSA (EFSA, 2014). The exposures in European populations were well below the TDI. Exposure to CrVI can take place via drinking water. BMDLs derived by EFSA for diffuse epithelial hyperplasia of duodenum in female mice ($BMDL_{10}$) and for haematotoxicity in rats ($BMDL_{05}$) and calculated MOE values indicated no public health concern.

Vulnerable groups: score 0.0
No vulnerable groups have been identified.

Lack of toxicity data: score 0.0
Sufficient data are available.

Lack of exposure data: score 1.0
Little information on exposure is available.

Total score = 3.0 for chromium (Cr)

4.4.2 References

- EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain) (2014). Scientific Opinion on the risks to public health related to the presence of chromium in food and drinking water. EFSA Journal 2014;12(3):3595, 261 pp. doi:10.2903/j.efsa.2014.3595.

4.5 Lead (Pb)

Lead is in soil both from natural geological sources and from antropogenic activity. The main use is in lead batteries, but also in ammunition, crystal glass, and in cable sheathing and solders. Exposure has been reduced after lead in petrol and paint and other products were regulated or banned. There are many food sources of lead in the diet, the major contributing were beverages, including fruit and vegetable juices, vegetables, starchy roots and tubers and legumes, nuts and oil seeds, in addition to grain and products thereoff. Only on average 8% is absorbed in the intestine, the absorption being higher in children. Exposure to lead is associated with a number of adverse effects. EFSA (2010) identified developmental neurotoxicity in young children and cardiovascular effects and nephrotoxicity in adults as the critical effects for the risk assessment.

4.5.1 Scores

MOE/MOS/ADI/TDI/TWI: score 6.0
EFSA (2010) identified developmental neurotoxicity in young children and cardiovascular

effects and nephrotoxicity in adults as the critical effects for the risk assessment. For developmental neurotoxicity, a $BMDL_{01}$ was 0.5 µg/kg bw. For effects on prevalence of chronic kidney disease the $BMDL_{10}$ was 0.63 µg/kg bw, and for effects on systolic blood pressure the $BMDL_{01}$ was 1.50 µg/kg bw. Exposure assessment in European population showed almost no margins to the BMDLs, in particular for cognitive effects.

Vulnerable groups: score 1.0
Fetus and children. High consumers of game shot with lead ammunition.

Lack of toxicity data: score 0.0
Sufficient data are available.

Lack of exposure data: score 0.5
Data on small game shot with lead ammunition is needed.

Total score = 7.5 for lead (Pb)

4.5.2 References

- Alexander J and Oskarsson A (2019). Toxic Metals. In: Chemical hazards in foods of animal origin. Food safety assurance and veterinary public health. Editors: Smulders FJM, Rietjens IMCM and Rose M. Wageningen Academic Publishers, 2019. Pp. 157-180.
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4.6 Methylmercury (MeHg)

Environmental sources of mercury are both natural and antropogenic. Mercury undergoes a complex transformation and cycles in the atmosphere. Mercury occurs in three forms, elemental/ metallic mercury, inorganic mercury (Hg^{22+}, $Hg^{2+}$and methylmercury (MeHg, the most prevalent of the organic forms). Methylmercury is bioaccumulated and biomagnified in the marine food web. Mercury in food occurs mostly as MeHg and less as inorganic mercury (iHg). Fish and other seafood are the main sources of mercury in the diet. Predatory fish species can contain high levels of mercury. Total mercury is measured in food. In seafood 80-100% is MeHg. iHg is nephrotoxic, and EFSA established a TWI of 4 µg/kg bw (EFSA, 2012). MeHg passes membranes and physiological barriers such as the placenta and the blood brain barrier and is neurotoxic with the prenatal and postnatal stage being the most

vulnerable stages. EFSA established a TWI for MeHg of 1.3 µg/kg bw for neurodevelopmental effects (EFSA, 2012).

4.6.1 Scores

MOE/MOS/ADI/TDI/TWI: score 6.0
Mercury in food occurs mostly as methylmercury (MeHg) and less as inorganic mercury (iHg). iHg is nephrotoxic, and EFSA established a TWI of 4 µg/kg bw (EFSA, 2012). MeHg is neurotoxic with the prenatal and postnatal stage being the most vulnerable stage. EFSA established a TWI of 1.3 µg/kg bw for neurodevelopmental effects (EFSA, 2012). Total mercury is measured in food. In seafood 80-100% is MeHg. The 95-percentile estimated exposure is in the range of the TWI. High consumers of fish with high levels of mercury may exceed the TWI for MeHg, whereas iHg is not of concern. Mercury exposure from fish in Norway was evaluated by VKM in 2019 and different scenarios were developed. VKM concluded that *«Eating fish with a low mercury concentration will not lead to an exposure exceeding the TWI, even at a high weekly intake of fish (1000 g). Eating only fish with a high mercury concentration leads to an exposure exceeding the TWI when consuming more than one portion of fish per week (150 g). The mean weekly intake of fish in pregnant women (217 g) therefore leads to an exposure exceeding the TWI if only fish with a high mercury concentration is consumed. When eating three weekly portions of fish consisting of only fish with an assumed high concentration of mercury, the fish can contain up to 0.28 mg/kg ww before the TWI is reached».*

Vulnerable groups: score 1.0
Pregnant women. There is dietary advice for women in childbearing age.

Lack of toxicity data: score 0.0
Sufficient data are available.

Lack of exposure data: score 0.0
Sufficient data are available.

Total score = 7.0 for methylmercury (MeHg)

4.6.2 References

- Alexander J and Oskarsson A (2019). Toxic Metals. In: Chemical hazards in foods of animal origin. Food safety assurance and veterinary public health. Editors: Smulders FJM, Rietjens IMCM and Rose M. Wageningen Academic Publishers, 2019. Pp. 157-180.
- EFSA Panel on Contaminants in the Food Chain (CONTAM) (2012). Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012;10(12):2985. doi:10.2903/j.efsa.2012.2985.
- VKM, Heidi Amlund, Kirsten Eline Rakkestad, Anders Ruus, Jostein Starrfelt, Jonny Beyer, Anne Lise Brantsæter, Sara Bremer, Gunnar Sundstøl Eriksen, Espen

Mariussen, Ingunn Anita Samdal, Cathrine Thomsen and Helle Katrine Knutsen (2019). Scenario calculations of mercury exposure from fish and overview of species with high mercury concentrations. Opinion of the Panel on Contaminants of the Norwegian Scientific Committee for Food and Environment. VKM report 2019:3, ISBN: 978-82-8259-319-9, ISSN: 2535-4019. Norwegian Scientific Committee for Food and Environment (VKM), Oslo, Norway.

4.7 Nickel (Ni)

Nickel in food may originate from kitchen utensils and certain plants accumulating nickel from the soil, e.g. cocoa and soy beans. Whereas nickel by inhalation may cause cancer, oral nickel appear not to be carcinogenic. It may in experimental animals cause toxic effects on kidneys, lung, spleen and other myeloid tissues and reproductive toxicity. Systemic nickel following oral intake may aggravate nickel contact allergic dermatitis in sensitized individuals.

4.7.1 Scores

MOE/MOS/ADI/TDI/TWI: score 2.0
Nickel in food may originate from kitchen utensils and certain plants that accumulate nickel, e.g. cocoa. EFSA (2015) derived a $BMDL_{10}$ of 0.28 mg/kg bw for reproductive toxicity. The estimated exposure of the European population is between 80 and 150 µg/person per day and of no concern.

Vulnerable groups: score 1.0
A $BMDL_{10}$ of 1.1 µg/kg bw was derived for aggravation of nickel-induced dermatitis in nickel allergic individuals, which may affect up to 15% of women. Intake of nickel could be a problem for this group.

Lack of toxicity data: score 0.0
Sufficient data are available.

Lack of exposure data: score 0.0
Sufficient data are available.

Total score = 3.0 for nickel (Ni)

4.7.2 References

- Alexander J and Oskarsson A (2019). Toxic Metals. In: Chemical hazards in foods of animal origin. Food safety assurance and veterinary public health. Editors: Smulders FJM, Rietjens IMCM and Rose M. Wageningen Academic Publishers, 2019. Pp. 157-180.
- EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain) (2015). Scientific Opinion on the risks to public health related to the presence of nickel in

food and drinking water. EFSA Journal 2015;13(2):4002, 202 pp. doi:10.2903/j.efsa.2015.4002.

5 Ranking of persistent organic pollutants (POPs)

An overview of the scoring and ranking of the included persistent organic pollutants (POPs) is given in Table 5-1. A detailed description follows after the table.

Table 5-1. Summary table for scoring of persistent organic pollutants (POPs).

Subgroup	Substance	1. MOE/MOS/ADI/TDI/TWI	2. Toxicity	3. Exposure	4. Vulnerable groups	5. Lack of toxicity data	6. Lack of exposure data	Total score	Comments
Brominated flame retardants	Polybrominated diphenyl ethers (PBDEs) (including decabromodiphenyl ether (DecaBDE))	2.0	-	-	0.5	0.5	0.5	3.5	Ongoing risk assessment by EFSA
	Hexabromocyclododecane (HBCDD)	2.0	-	-	0.0	0.5	0.5	3.0	
	Hexabromobenzene (HBB)	-	1.0	1.0	0.0	1.0	1.0	4.0	
	Decabromo-diphenyl ethane (DBDPE)	-	1.0	1.0	0.0	1.0	1.0	4.0	
	1,2-Bis(2,4,6-tribromophenoxy)ethane (BTBPE)	-	1.0	1.0	0.0	1.0	1.0	4.0	
	2,4,6-Tribromophenol (TBP)	2.0	-	-	0.0	1.0	1.0	4.0	
Dechloranes	Dechlorane plus (syn-DP and anti-DP)	-	1.0	2.0	0.0	1.0	1.0	5.0	

Subgroup	Substance	1. MOE/MOS/ADI/TDI/TWI	2. Toxicity	3. Exposure	4. Vulnerable groups	5. Lack of toxicity data	6. Lack of exposure data	Total score	Comments
Dioxins and Dioxin-like polychlorinated biphenyls (DL-PCBs)	Dioxins and DL-PCBs	6.0	-	-	1.0	0.5	0.5	8.0	Occurrence data in composite fish meals (fish cakes, fish fingers etc.) is in particular lacking
Non-dioxin-like polychlorinated biphenyls (NDL-PCBs)	NDL-PCBs	-	2.0	2.0	1.0	0.5	0	5.5	Occurrence data in composite fish meals (fish cakes, fish fingers etc.) is in particular lacking
Perfluorinated and polyfluorinated	Perfluorooctane sulfonate (PFOS), Perfluorooctanoic acid (PFOA)	6.0	-	-	0.5	0.5	1.0	8.0	

Subgroup	Substance	1. MOE/MOS/ADI/TDI/TWI	2. Toxicity	3. Exposure	4. Vulnerable groups	5. Lack of toxicity data	6. Lack of exposure data	Total score	Comments
alkyl substances (PFAS)	Perfluorohexane sulfonic acid (PFHxS), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Perfluoroundecanoic acid (PFUnDA) and Perfluoroheptane sulfonate (PFHpS)	-	2.0	2.0	0.5	1.0	1.0	6.5	
Siloxanes	Octamethylcyclotetra-siloxane (D4)	2.0	-	-	0.5	0.5	0.5	3.5	
	Decamethylcyclopenta-siloxane (D5)	2.0	-	-	0.5	0.5	0.5	3.5	
	Dodecamethylcyclohexa-siloxane (D6)	2.0	-	-	0.5	1.0	0.5	4.0	

5.1 Subgroup brominated flame retardants

5.1.1 Polybrominated diphenyl ethers (PBDEs), including decabromodiphenyl ether (DecaBDE)

5.1.1.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 2.0
The EFSA CONTAM Panel (2011a) received data on 19 PBDE congeners in 3971 food samples. A toxicity survey was performed and neurodevelopment was identified as the most critical endpoint. Eight congeners were considered, BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183 and BDE-209, but sufficient toxicity data were only available for BDE-47, BDE-99, BDE-153 and BDE-209. The EFSA CONTAM Panel derived BMDLs for the PBDE congeners BDE-47, BDE-99, BDE-153 and BDE-209. However, due to uncertainties in the database, EFSA did not use the BMDLs to establish HBGVs. Instead a MOE for health risk was calculated.

The panel calculated a MOE value by comparing the minimum lower bound and maximum upper bound dietary intake for the different PBDE congeners with the estimated human intake associated with the body burden at the $BMDL_{10}$. The $BMDL_{10}$ was derived from effects on neurodevelopment in mice as the critical endpoint. For average upper bound consumers, the MOE values for BDE-47, BDE-99, and BDE-153 were 90, 6.5 and 23, respectively. MOE for BDE-209 was approximately 97,000 for 1 to 3 year old children, which was the group with the highest maximum intake. The panel argued that the calculated MOE values were sufficient to cover interspecies differences in sensitivity for the effects observed and concluded that a MOE value larger than 2.5 indicated no health concerns.

Vulnerable groups: score 0.5
EFSA identified high exposed children (1 to 3 years) as a potential vulnerable group (EFSA, 2011a). For young children with an average and high consumption, the maximum upper bound dietary intake resulted in MOE values of 1.4 and 0.7, respectively. The estimation was based on analysis of one sample in the category «Food for infants and children», which had a high concentration of BDE-99. It was, therefore, speculated if the calculated MOE was an overestimation.

Lack of toxicity data: score 0.5
The EFSA panel did not find the available toxicity data sufficient to establish a HBGV.

Lack of exposure data: score 0.5
Updated information on occurrence in Norwegian food is lacking. The use of PBDEs are, however, phased out and levels are decreasing.

Total score = 3.5 for polybrominated diphenyl ethers (PBDEs), including decabromodiphenyl ether (DecaBDE)

5.1.2 Hexabromocyclododecane (HBCDD)

5.1.2.1 ***Scores***

MOE/MOS/ADI/TDI/TWI: score 2.0
EFSA received data in 1914 food samples, and all studies were performed on technical HBCDD (EFSA, 2011b). Risk assessment of individual stereoisomers was not possible. A toxicity survey was performed and neurodevelopmental effects on behavior was identified as a critical endpoint. The EFSA panel derived a $BMDL_{10}$ of 0.79 mg/kg bw. However, due to uncertainties in the database, EFSA did not use the BMD to establish a HBGV, but instead calculated a MOE value by comparing the minimum lower bound and maximum upper bound dietary intake of HBCDD with the $BMDL_{10}$. EFSA argued that a MOE value larger than 8 implied no health concern. A factor of 2.5 was considered sufficient to cover inter-species differences for the observed effects. Due to uncertainties in the elimination half-life in humans, it was concluded that the MOE also should cover individual differences in elimination kinetics with a factor of 3.2. For children of the age of 3 to 10 years with an average or high consumption, the maximum upper bound dietary intake resulted in MOE values of 1,600 and 700, respectively. For adult consumers, the MOE value was higher. It was concluded that the current dietary exposure to HBCDD does not raise a health concern (EFSA, 2011b).

Vulnerable groups: score 0.0
There were no particular vulnerable groups.

Lack of toxicity data: score 0.5
Not sufficient data available to set a HBGV.

Lack of exposure data: score 0.5
Updated information on occurrence in Norwegian food is lacking. Levels are decreasing.

Total score = 3.0 for hexabromocyclododecane (HBCDD)

5.1.3 Hexabromobenzene (HBB)

5.1.3.1 ***Scores***

Toxicity: 1.0
EFSA (2012) reviewed so-called emerging and novel brominated flame retardants. HBB was identified as a substance with high potential for bioaccumulation. This assumption was based on the chemical properties of the compound, not on experimental data. The toxicity of HBB has not been extensively studied, but oral exposure of rats indicated a relatively low toxicity. Chronic doses (15-375 mg/kg bw/day) have shown an increase in porphyrines in rat urine. Pregnant rats administered 200 mg/kg/day from GD5 to GD15 showed no teratogenic effects on the pups. A single intraperitoneal dose of 10,000 mg/kg bw is considered as lethal dose..

Exposure: score 1.0
Most studies in food show levels <LOQ (Cequier et al., 2015; EFSA, 2012).

Vulnerable groups: score 0.0
No indication of susceptible groups.

Lack of toxicity data: score 1.0
The toxicity is not well characterized. The studies available report low toxicity (EFSA, 2012).

Lack of exposure data: score 1.0
Very little information on exposure is available. HBB is listed by EFSA as a concern, due to the high bioaccumulation factor.

Total score: 4.0

5.1.4 Decabromo-diphenyl ethane (DBDPE)

5.1.4.1 *Scores*

Toxicity: score 1.0
EFSA (2012a) reviewed so-called emerging and novel brominated flame retardants. DBDPE was predicted as a substance with high potential persistence, but with a less bioaccumlation potential. The toxicity of DBDPE has not been extensively studied, but oral exposure of rats indicated low toxicity. Oral administration of 100 mg/kg bw per day in rats for 90 days revealed few signs of toxicity. A significant decrease in triiodothyronine (T3) levels was observed. No evidence of maternal toxicity, developmental toxicity or teratogenicity was observed in rats or rabbits treated with up to 1250 mg/kg bw per day during gestation.

Exposure: score 1.0
Reviewed by EFSA (2012a), most studies in food show levels <LOQ.

Vulnerable groups: score 0
There were no particular vulnerable groups.

Lack of toxicity data: score 1.0
The toxicity has not been well characterized.

Lack of exposure data: score 1.0
Little information on exposure is available. The levels are likely to increase.

Total score = 4.0 for hexabromobenzene (HBB)

5.1.5 1,2-Bis(2,4,6-tribromophenoxy)ethane (BTBPE)

5.1.5.1 ***Scores***

Toxicity: score 1.0
The EFSA CONTAM Panel (2012a) reviewed so-called emerging and novel brominated flame retardants. BTBPE was identified as a substance with high potential for bioaccumulation. This assumption was based on the chemical properties of the compound, not on experimental data. Rat studies showed that BTBPE is poorly absorbed in the organism. Oral exposure of rats indicated a low toxicity, and no effect was observed on rats orally exposed to 35 mg/kg bw per day through the diet for 14 days. Acute lethal dose for rat and dogs is >10 g/kg bw.

Exposure: score 1.0
Listed by EFSA as a concern due to high bioaccumulation factor (EFSA, 2012a).

Vulnerable groups: score 0.0
There were no particular vulnerable groups.

Lack of toxicity data: score 1.0
The toxicity is not well characterized.

Lack of exposure data: score 1.0
Little information on exposure is available.

Total score = 4.0 for 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE)

5.1.6 2,4,6-Tribromophenol (TBP)

5.1.6.1 ***Scores***

MOE/MOS/ADI/TDI/TWI: score 2.0
EFSA (2012b) reviewed brominated phenols and their derivates other than tetrabromobisphenol A. 2,4,6-tribromophenol (TBP) was the dominating substance. A toxicity survey was performed and main targets were identified as liver and kidney. In a repeated oral exposure study on rats, both male and pregnant female rats were fed up to 1000 mg/kg bw per day for 45-48 days. A NOAEL of 100 mg/kg bw was estimated for both sexes. A worst case exposure of 40 ng/kg bw per day for high consumers of marine food was estimated, which indicated a MOE value of six orders of magnitude if a NOAEL of 100 mg/kg bw was considered. It was concluded that current dietary exposure to TBP does not raise a health concern.

Vulnerable groups: score 0.0
No indication of susceptible groups.

Lack of toxicity data: score 1.0
The toxicity is not well characterized.

Lack of exposure data: score 1.0
Very little information on exposure is available. A report from the Norwegian Environment Agency stated that there is no registration of use volumes in EU, which may indicate less use of the substance in Europe (Norwegian Environment Agency, 2016).

Total score = 4.0 for 2,4,6-tribromophenol (TBP)

5.1.7 References

- Cequier E, Marcé RM, Becher G, Thomsen C (2015). Comparing human exposure to emerging and legacy flame retardants from the indoor environment and diet with concentrations measured in serum. Environ Int. 74:54-9. doi: 10.1016/j.envint.2014.10.
- EFSA Panel on Contaminants in the Food Chain (CONTAM) (2012a). Scientific Opinion on Emerging and Novel Brominated Flame Retardants (BFRs) in Food. EFSA Journal 2012;10(10):2908 [133 pp.] doi:10.2903/j.efsa.2012.2908.
- EFSA Panel on Contaminants in the Food Chain (CONTAM) (2012b). Scientific Opinion on Brominated Flame Retardants (BFRs) in Food: Brominated Phenols and their Derivatives. EFSA Journal 2012;10(4):2634. [42 pp.] doi:10.2903/j.efsa.2012.2634.
- EFSA Panel on Contaminants in the Food Chain (CONTAM) (2011a). Scientific Opinion on Polybrominated Diphenyl Ethers (PBDEs) in Food. EFSA Journal 2011;9(5):2156. [274 pp.] doi:10.2903/j.efsa.2011.2156.
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- Norwegian Environment Agency (2016). Substance evaluation conclusion as required by reach article 48 and evaluation report for 2,4,6-tribromophenol. EC No 204-278-6. https://echa.europa.eu/documents/10162/6e2fea36-f7d1-4e90-874f-c02ff853f6c6.

5.2 Subgroup dechloranes

5.2.1 Dechlorane plus (syn-DP and anti-DP)

5.2.1.1 *Scores*

Toxicity: score 1.0
ECHA (2017) concluded that dechlorane plus does not meet the classification criteria for mutagenicity, toxicity to reproduction or specific target organ toxicity. The data were considered to be conclusive but not sufficient for classification for these endpoints. Carcinogenicity data are lacking (and are not required at the registration tonnage). There is some evidence for potential liver impairment in mice (Wu et al., 2012), but the significance of these findings was unclear.

Exposure: score 2.0
Exposure data are lacking, but dechloranes have been measured in human samples with high detection frequency and at levels similar to the more well known PBDEs (Cequier et al., 2015). Dechloranes have also been found at all levels of terrestrial and marine food chains (The Norwegian Environment Agency et al., 2017; Norwegian Institute for Air Research et al., 2018, Norwegian Institute for Air Research et al., 2017).

Vulnerable groups: score 0.0
Lack of data.

Lack of toxicity data: score 1.0
The toxicity is not well characterized.

Lack of exposure data: score 1.0
Little information on exposure is available.

Total score = 5.0 for dechlorane plus (syn-DP and anti-DP)

5.2.1.2 *References*

- Cequier E, Marcé RM, Becher G, Thomsen C (2015). Comparing human exposure to emerging and legacy flame retardants from the indoor environment and diet with concentrations measured in serum. Environ Int. 74:54-9. doi: 10.1016/j.envint.2014.10.
- ECHA (2017). Appendix 1 Detailed summaries of data cited in the main report (*Annex XV report https://echa.europa.eu/documents/10162/6ba01c40-009a-8388-1556-d8caa50d2b4f*) https://echa.europa.eu/documents/10162/13638/ec_-_dechlorane_plus_annex_xv_svhc_appendix_en.pdf/86c6520a-cdc8-86bf-cc86-57beef04bc6f.

- The Norwegian Environment Agency, Ruus A, Bæk K, Petersen K, Allan I, Beylich B, Schlabach M, Warner N, Borgå K, Helberg M (2018). Environmental Contaminants in an Urban Fjord 2017. ISBN: 978-82-577-7103-4. https://www.miljodirektoratet.no/globalassets/publikasjoner/m1131/m1131.pdf.
- Norwegian Institute for Air Research, Heimstad ES, Nygård T, Herzke D, Bohlin-Nizzetto P (2018). Environmental pollutants in the terrestrial and urban environment 2017. https://www.miljodirektoratet.no/globalassets/publikasjoner/m1076/m1076.pdf
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- Wu B, Liu S, Guo X, Zhang Y, Zhang X, Li M, Cheng S (2012). Responses of mouse liver to dechlorane plus exposure by integrative transcriptomic and metabonomic studies. Environ Sci Technol 2;46(19):10758-64. doi: 10.1021/es301804t.

5.3 Subgroup Dioxins and Dioxin-like PCBs (DL-PCBs)

5.3.1 Scores

MOE/MOS/ADI/TDI/TWI: score 6.0
Exposure >TWI (2 pg TE/kg bw per week) set by EFSA (2018).

Vulnerable groups: score 1.0
All groups have exposure >TWI, young women and children are sensitive groups.

Lack of toxicity data: score 0.5
The toxicity is well characterised. However, data on relative potency of individual DL-compounds are needed, in particular for PCB-126.

Lack of exposure data: score 0.5
Information on levels in composite food (e.g. fish gratin, fish cakes) and to some extent in land-based food (butter, cheese, eggs) from Norway is missing. This is particularly important for food where the degree of self-sufficiency is high.

Total score = 8.0 for dioxins and dioxin-like PCBs (DL-PCBs)

5.3.1.1 *References*

- EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen HK, Alexander J, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L,Grasl-Kraupp B, Hogstrand C, Nebbia CS, Oswald IP, Petersen A, Rose M, Roudot A-C, Schwerdtle T,Vleminckx C, Vollmer G, Wallace H, Fürst P, Håkansson H, Halldorsson T, Lundebye A-K, Pohjanvirta R,Rylander L, Smith A,

van Loveren H, Waalkens-Berendsen I, Zeilmaker M, Binaglia M, Gomez Ruiz JA,Horvath Z, Christoph E, Ciccolallo L, Ramos Bordajandi L, Steinkellner H and Hoogenboom LR (2018). Scientific Opinion on the risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food. EFSA Journal 2018;16(11):5333, 331 pp.https://doi.org/10.2903/j.efsa.2018.5333.

5.4 Subgroup Non-dioxin-like PCBs (NDL-PCBs)

5.4.1 Scores

Toxicity: 2.0
For PCBs, the literature on toxicity, toxicological effects, tolerance limits and nutritional risk (including MOE, BMD, TDI and TWI calculations) is strongly dominated by DL-PCB.

NDL-PCB congeners are usually considered of low toxicity. Toxicity assessment of NDL-PCB congeners in natural PCB mixtures is difficult because more toxic DL-PCB congeners often occur in the mixture at low concentrations which can be difficult to measure chemically but which can nevertheless produce toxic effects in test organisms.

Exposure: score 2.0
Since NDL-PCBs are hardly degradable and highly fat soluble, they are enriched in the food chain and can be measured at particularly high concentrations in certain types of seafood with particularly high fat content (e.g. cod liver). The concentration of NDL-PCB is normally significantly higher than the DL-PCB. They can therefore be more easily quantified with low uncertainty and are therefore measured as indicator PCBs.

Vulnerable groups: score 1.0
This point is very similar to dioxins/DL-PCBs. Potentially sensitive groups for NDL-PCBs are young women, nursing babies and people with a high consumption of fatty fish and fish products, seagull eggs and brown crab meat.

Lack of toxicity data: score 0.5
The toxic mechanisms of action of NDL-PCB have not yet been fully elucidated. Interacting effects between different PCB compounds are also likely and challenging to calculate. Toxic contributions from more toxic PCB congeners (and other types of substances, e.g. polychlorinated dibenzofurans) which may be present at low (>LOQ) levels may complicate effect and risk assessments. This is especially true when effect testing is performed on complex mixtures. Further complications may arise since it may be difficult to distinguish the effects of hydrocarbones and metabolites of PCBs, especially for endocrine disrupting effects.

Lack of exposure data: score 0.0
There is a substantial amount of data available of chemical levels of NDL-PCBs in various types of biological and non-biological samples. In recent years, PCB6 has increasingly been

used as a grouping consisting of the six most common NDL-PCBs. These are also referred to as the six indicator PCBs and are easier to measure analytically than the DL-PCB because they normally occur in so much higher concentrations.

Total score = 5.5 for non-dioxin-like PCBs (NDL-PCBs)

5.4.2 References

- EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen HK, Alexander J, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L,Grasl-Kraupp B, Hogstrand C, Nebbia CS, Oswald IP, Petersen A, Rose M, Roudot A-C, Schwerdtle T,Vleminckx C, Vollmer G, Wallace H, Fürst P, Håkansson H, Halldorsson T, Lundebye A-K, Pohjanvirta R,Rylander L, Smith A, van Loveren H, Waalkens-Berendsen I, Zeilmaker M, Binaglia M, Gomez Ruiz JA,Horvath Z, Christoph E, Ciccolallo L, Ramos Bordajandi L, Steinkellner H and Hoogenboom LR (2018). Scientific Opinion on the risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food. EFSA Journal 2018;16(11):5333, 331 pp.https://doi.org/10.2903/j.efsa.2018.5333.
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- WHO-IPCS (2003). Polychlorinated biphenyls: human health aspects, Concise International Chemical Assessment Document. The International Programme on Chemical Safety (IPCS), Agency for Toxic Substances and Disease Registry, Geneva.

5.5 Subgroup perfluorinated and polyfluorinated alkyl substances (PFAS)

Per- and polyfluoroalkyl substances (PFAS) are a group of man-made chemicals that includes perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), and many other chemicals. PFAS have been manufactured and used in a variety of industries around the globe. PFOA and PFOS have been the most extensively produced. Both chemicals are very persistent in the environment and in the human body.

5.5.1 Perfluorooctane sulfonate (PFOS) and Perfluorooctanoic acid (PFOA)

5.5.1.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 6.0
Average exposure >pTWI in several dietary surveys (EFSA, 2018).

Vulnerable groups: score 0.5
Higher exposure in high consumers of fish.

Lack of toxicity data: score 0.5
Lack of data on mode of action.

Lack of exposure data: score 1.0
Data on levels in drinking water is lacking, and there is a need for more data in food with lower LOQ.

Total score = 8.0 for perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA)

5.5.1.2 *References*

- EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen HK, Alexander J, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, Nebbia CS, Oswald IP, Petersen A, Rose M, Roudot A-C, Vleminckx C, Vollmer G, Wallace H, Bodin L, Cravedi J-P, Halldorsson TI, Haug LS, Johansson N, van Loveren H, Gergelova P, Mackay K, Levorato S, van Manen M and Schwerdtle T (2018). Scientific Opinion on the risk to human health related to the presence of perfluorooctanesulfonic acid and perfluorooctanoic acid in food. EFSA Journal 2018;16(12):5194, 284 pp.https://doi.org/10.2903/j.efsa.2018.5194.

5.5.2 Perfluorohexane sulfonic acid (PFHxS), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Perfluoroundecanoic acid (PFUnDA) and Perfluoroheptane sulfonate (PFHpS)

5.5.2.1 *Scores*

Toxicity: score 2.0
Lack of data, the score is based on similarity with PFOS/PFOA.

Exposure: score 2.0
Lack of data. Measured levels in humans suggest widespread exposure at somewhat lower levels than PFOS and PFOA.

Vulnerable groups: score 0.5
Higher exposure in breast fed infants and high consumers of fish expected.

Lack of toxicity data: score 1.0

Lack of exposure data: score 1.0

Total score = 6.5 for perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA) and perfluoroheptane sulfonate (PFHpS)

5.5.2.2 *References*

- EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen HK, Alexander J, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, Nebbia CS, Oswald IP, Petersen A, Rose M, Roudot A-C, Vleminckx C, Vollmer G, Wallace H, Bodin L, Cravedi J-P, Halldorsson TI, Haug LS, Johansson N, van Loveren H, Gergelova P, Mackay K, Levorato S, van Manen M and Schwerdtle T (2018). Scientific Opinion on the risk to human health related to the presence of perfluorooctanesulfonic acid and perfluorooctanoic acid in food. EFSA Journal 2018;16(12):5194, 284 pp.https://doi.org/10.2903/j.efsa.2018.5194.

5.6 Subgroup siloxanes

5.6.1 Octamethylcyclotetrasiloxane (D4)

5.6.1.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 2.0
MOS values are higher than 60,000 for most groups (teens and adults) exposed to D4 (Gentry et al., 2017).

Vulnerable groups: score 0.5
Women: Decreased reproductive capability observed in female rats. The relevance for human reproductive risk assessment is questionable (Franzen et al., 2017).

Infants: A Swedish study reported that 11 of 39 human breast milk samples contained one or more of the cyclic siloxanes (D4, D5 and D6). The maximum concentrations of D4, D5 and D6 were 10 µg/l, 4.5 µg/l and 4.8 µg/l, respectively (IVL, 2005).

Children: The results from a Monte Carlo analysis indicated that oral intakes in children are <10 times greater than intakes estimated for adults. MOS values were estimated for oral intake only for teens and adults, as the physiologically based pharmacokinetic (PBPK) model cannot conduct simulations for infants. However, the large MOS values calculated for teens and adults would suggest that even for children, the MOS values resulting from ingestion of food containing D4 should be greater than 1,000,000 (Gentry et al., 2017).

Lack of toxicity data: score 0.5
The information on human toxicity is limited.

Following oral administration, 12 – 52% of D4 is absorbed in rats (Danish Ministry of the Environment, 2014).

Acute toxicity: A single dose study in human healthy volunteers did not show any immunotoxic or pro-inflammatory effects after inhalation of D4 (10 ppm, 1 hour) (Danish

Ministry of the Environment, 2014). Animal studies have reported a low potential for acute toxicity following dermal, oral or inhalation exposure to D4 (Franzen et al., 2017).

Chronic toxicity: Liver: Rats exposed to D4 or D5 (oral or inhalation) have shown reversible hepatomegaly (both hyperplasia and hypertrophy). Several studies have reported an induction of hepatic cytochrome P450 enzymes in rats exposed to D4 or D5, which is similar to the enzyme induction observed after exposure to phenobarbital. Thus, D4 and D5 are considered to be enzyme inducers in rat liver (Franzen et al., 2017; Danish Ministry of the Environment, 2014). Importantly, a similar hepatic effect has not been observed in guinea pigs after exposure to D4, indicating species-specific effects (Danish Ministry of the Environment, 2014). Lung: Rat studies have demonstrated effects in the lung including interstitial inflammation, increased lung weight, alveolar macrophage accumulation/aggregation and alveolar histiocytosis after repeated D4 exposure (Danish Ministry of the Environment, 2014).

Reproductive effects: One- and two-generation inhalation studies have reported effects in female rats at concentrations of 500 ppm and greater: decreases in the number of corpora lutea, with an associated decrease in number of uterine implantation sites, total number of pups born and the mean live litter size. Based on this, the reproductive NOAEC (no observed adverse effect concentration) for D4 was determined to be 300 ppm (Franzen et al., 2017). The decrease in female rat reproductive capability after inhalation of D4 is consistent with impaired ovulation due to a suppression or shift in the luteinizing hormone (LH) surge (Franzen et al., 2017). This effect might be due to inhibition of preovulatory prolactin (Quinn et al., 2007a). D4 may act as a dopamine agonist, and thereby reduce the release of prolactin (Dekant et al., 2017). Whereas prolactin is required for normal ovulation in rats, it does not appear to play a role in human ovulation (Porcile et al., 1990; Yasui et al., 1990). Therefore, the impairment of fertility in female rats exposed to D4 is of questionable relevance for human reproductive risk assessment. Another contributing factor may be that D4 acts as a weak estrogen or anti-estrogen (Danish Ministry of the Environment, 2014).

Genotoxicity and mutagenicity: D4 is not considered to be genotoxic or mutagenic (Franzen et al., 2017). Endometrial adenomas have been observed in female rats exposed to D4 or D5. The neoplastic effects observed after D4 exposure have been attributed to a hormonal dysregulation resulting from interaction of D4 with the dopamine D2-receptor. Data from rat studies suggest that D4 can act as a dopamine D2-receptor agonist causing a reduction in prolactin. A reduction of prolactin in the rat causes luteolysis and new ovarian follicle stimulation resulting in estrogen dominance, which causes persistent endometrial stimulation leading to uterine tumours. Prolactin is not luteotropic in non-human primates and humans (Danish Ministry of the Environment, 2014).

Developmental effects: No developmental effects of D4 were observed in rats or rabbits following inhalation exposure (700 ppm from gestation day 6 through 15 in rats and 500 ppm from gestation day 6 through 18 in rabbits) or after oral exposure of rabbits (1,000 mg/kg bw per day from gestation day 7 through 19) (Franzen et al., 2017).

Immunotoxicity: No immunotoxic or pro-inflammatory effects have been observed after oral exposure to D4 in human volunteers (Franzen et al., 2017; SCCP, 2005).

D4 is classified as hazardous, with the human health risk phrase R62, Repr. Cat. 3 (reproductive toxicity), in the Hazardous Substances Information System (HSIS) (Safe Work Australia). D4, D5 and D6 meet the criteria for very persistent (vP) and very bioaccumulative (vB) chemicals (REACH/ECHA). ECHA's Member State Committee has agreed that D4, D5 and D6 are REACH substances of very high concern (SVHCs), based on persistent, bioaccumulative and toxic (PBT) properties.

Lack of exposure data: score 0.5
There is only limited information on D4 concentrations in food (e.g. fish, other seafood and mammals) (Norwegian Environment Agency, 2013; Danish Ministry of the Environment, 2014). Benthic feeding fish (perch, whitefish, burbot) have been reported to have lower cyclic siloxane concentrations than pelagic fish at comparable trophic levels (Norwegian Environment Agency, 2013).

Certain food products are processed using antifoam containing D4 (SCCP, 2005).

Gentry et al. (2017) have performed a PBPK analysis for the general public considering both inhalation of indoor and outdoor air in the home environment, exposure to D4 in environmental media (e.g. ingestion of water, soil, air, fish and other foods) and ingestion of anti-gas medication etc. Exposure to environmental media was also considered for fishermen where the consumption of fish was assumed to be the main source of protein. The mean reported oral intake of D4 determined from the Monte Carlo analysis ranged from 0.005 mg/kg bw per day for males and females in the general public ages 60 and older to 0.007 mg/kg bw per day for male and female subsistence fishermen 12 to 19 years of age. The 90th percentile of oral intake to D4 was approximately 0.009 mg/kg bw per day for males in the general public or subsistence fisherman 20 to 59 years of age (Gentry et al., 2017).

The exposure estimates associated with the use of models and the choice of variables related to the use of consumer products are uncertain (quantity and frequency of use, absorbed fraction and environmental parameters).

Total score = 3.5 for octamethylcyclotetrasiloxane (D4)

5.6.2 Decamethylcyclopentasiloxane (D5)

5.6.2.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 2.0
The MOS values determined for the mean oral consumption for men, women and teenagers in both the general public and a population of fishermen were all above 15,000,000 (Franzen et al., 2016).

The lowest MOS value was 880 and was associated with the use of hand and body lotion in women. MOS values reported for the use of antiperspirant/deodorant roll-on products and aerosols were 2,300–2,500 in women (Franzen et al., 2016).

Vulnerable groups: score 0.5
Infants: A Swedish study reported that 11 of 39 human breast milk samples contained one or more of the cyclic siloxanes (D4, D5 and D6). The maximum concentrations of D4, D5 and D6 were 10 µg/L, 4.5 µg/L and 4.8 µg/L, respectively (IVL, 2005).

Lack of toxicity data: score 0.5
The information on human toxicity is limited.

Liver toxicity: Rats exposed to D4 or D5 (oral or inhalation) showed reversible hepatomegaly as a result of hepatic hyperplasia and hepatic hypertrophy. Several studies have reported an induction of hepatic cytochrome P450 enzymes in rats exposed to D4 or D5, which is similar to the enzyme induction observed after exposure to phenobarbital. Thus, D4 and D5 are considered to be enzyme inducers in rat liver (Franzen et al., 2017; Danish Ministry of the Environment, 2014).

Lung toxicity: Rat studies have demonstrated interstitial inflammation, increased lung weight, alveolar macrophage accumulation/aggregation, and multifocal alveolitis after repeated D5 exposure (Danish Ministry of the Environment, 2014).

Genotoxicity and mutagenicity: D5 is not considered to be genotoxic. Long-term exposure in female rats (24 months, 160 ppm) has been associated with an increase in uterine adenocarcinomas. This tumorogenic effect may be species-specific with no risk or relevance to human health (Franzen et al., 2016).

Reproductive effects: No reproductive toxicity was observed in the available studies on D5.

D4, D5 and D6 meet the criteria for very persistent (vP) and very bioaccumulative (vB) chemicals (REACH/ECHA). D5 and D6 can be considered PBT because of D4 impurities. ECHA's Member State Committee has agreed that D4, D5 and D6 are all REACH substances of very high concern (SVHCs), based on persistent, bioaccumulative and toxic (PBT) properties.

Lack of exposure data: score 0.5
The highest contributors to D5 exposure in adults have been suggested to be consumer products like body lotion, hair spray, foundation, after shave etc. (Franzen et al., 2016).

There is only limited information on D5 concentrations in food (e.g. fish, other seafood and mammals) (Norwegian Enviroment Agency, 2013; Danish Ministry of the Environment, 2014). The intake from consumption of D5 from food, water and soil combined is estimated to be 0.005–0.0076 mg/kg bw per day for men/women and teenagers. These intakes also

include D5 from antifoam used in processing of food, and the consumption of D5 from the use of lipstick (Franzen et al., 2016).

The estimates of human exposure associated with the use of models and the choice of variables related to the use of consumer products, are uncertain (quantity and frequency of use, absorbed fraction and environmental parameters).

Total score = 3.5 for decamethylcyclopentasiloxane (D5)

5.6.3 Dodecamethylcyclohexasiloxane (D6)

5.6.3.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 2.0
A MOE value of approximately 40,000 has been reported for the general population in Canada (Health Canada, 2008).

Vulnerable groups: score 0.5
Infants: A Swedish study reported that 11 of 39 human breast milk samples contained one or more of the cyclic siloxanes (D4, D5 and D6). The maximum concentrations of D4, D5 and D6 were 10 µg/l, 4.5 µg/l and 4.8 µg/l, respectively (IVL, 2005).

Lack of toxicity data: score 1.0
Following oral administration, approximately 12% of D6 is absorbed in rats (Danish Ministry of the Environment, 2014).

D6 has a low acute toxicity (after oral intake). However, data regarding acute inhalation toxicity, irritation, sensitisation, repeated dose toxicity, toxicity to reproduction, mutagenicity, genotoxicity or carcinogenicity are limited (Danish Ministry of the Environment, 2014).

A 4-week rat study reported increased liver weight, periportal lipidosis and thyroid follicular cell hypertrophy after oral D6 exposure (basis for the critical effect level). The critical effect level for repeated-dose toxicity of D6 has been considered to be oral intake of 100 mg/kg bw per day via (Health Canada, 2008).

D4, D5 and D6 meet the criteria for very persistent (vP) and very bioaccumulative (vB) chemicals (REACH/ECHA). D5 and D6 can be considered PBT because of D4 impurities. ECHA's Member State Committee has agreed that D4, D5 and D6 are REACH substances of very high concern (SVHCs), based on persistent, bioaccumulative and toxic (PBT) properties.

Lack of exposure data: score 0.5
There is some information on D6 levels in the environment (air, waste water, sediments, fish and mammals). However, there is only limited information on the exposure in humans (Danish Ministry of the Environment, 2014).

The estimates of intake from environmental media and diet range from 28.7 µg/kg bw per day for adults aged 60 years and older to 87 µg/kg bw per day for children aged 6 months to 4 years. The most significant contribution to daily intake from environmental media is inhalation of indoor air, based on a study of 400 homes in Sweden (Kaj et al., 2005; Health Canada, 2008).

The estimates of human exposure associated with the use of models and the choice of variables related to the use of consumer products, are uncertain (quantity and frequency of use, absorbed fraction and environmental parameters).

Total score = 4.0 for dodecamethylcyclohexasiloxane (D6)

5.6.4 References

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6 Ranking of substances in food contact materials

An overview of the included scoring and ranking of substances in food contact materials is given in Table 6-1. A detailed description follows after the table.

Table 6-1. Summary table for scoring of substances in food contact materials.

Subgroup	Substance	1. MOE/MOS/ADI/TDI/TWI	2. Toxicity	3. Exposure	4. Vulnerable groups	5. Lack of toxicity data	6. Lack of exposure data	Total score	Comments
Bisphenols	Bisphenol A (BPA)	2.0	-	-	0.0	0.5	0.5	3.0	EFSA ongoing work: hazard asessment of BPA
	Bisphenol S (BPS), bispenol F (BPF) and bisphenol AF (BPAF)	-	2.0	2.0	0.5	1.0	1.0	6.5	Lack of data on both toxicity and exposure
Phthalates	Bis(2-ethylhexyl)phthalate (DEHP)	2.0	-	-	0.5	0.5	0.5	3.5	In addition to the reproductive effects, immunotoxic, neurotoxic and metabolic effects needs evalution
	Butyl-benzyl-phthalate (BBP)	2.0	-	-	0.5	0.5	0.5	3.5	
	Di-butylphthalate (DBP)	2.0	-	-	0.5	0.5	0.5	3.5	
	Di-isodecyl phthalate (DIDP)	2.0	-	-	0.5	0.5	0.5	3.5	
	Di-isononyl phthalate (DINP)	2.0	-	-	0.5	0.5	0.5	3.5	

6.1 Subgroup bisphenols

6.1.1 Bisphenol A (BPA)

A temporary tolerable daily intake (pTDI) for external oral exposure to bisphenol A (BPA) in humans of 4 µg/kg bw was establiseh by the EFSA CEF Panel (2015). The pTDI was based on mean relative kidney weight in mice. EFSA also estimated the BPA exposure (EFSA CEF Panel, 2015). For external exposure, diet was shown to be the main BPA source in all population groups. The estimated BPA dietary intake was highest in infants and toddlers (up to 0.875 µg/kg bw per day). Women of childbearing age had dietary exposures comparable to men of the same age (up to 0.388 µg/kg bw per day). EFSA concluded that there was no health concern for any age group from dietary exposure and low health concern from aggregated exposure. The uncertainty around dietary intake estimates was relatively low.

6.1.1.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 2.0
Exposure calculated by EFSA (EFSA, 2015) was below the temporary TDI for BPA for all population groups.

Vulnerable groups: score 0.0
Potential vulnerable groups include pregnant women, infants and children. For these groups, the estimated exposure was below the tTDI.

Lack of toxicity data: score 0.5
Data are needed to establish a permanent TDI.

Lack of exposure data: score 0.5
Sufficient data are available to estimate the exposure from foods.

Total score = 3.0 for bisphenol A (BPA)

6.1.2 Bisphenol S (BPS), Bispenol F (BPF) and Bisphenol AF (BPAF)

6.1.2.1 *Scores*

Toxicity: score 2.0
There is not sufficient toxicological data available to assess the toxicity (ANSES, 2013).

Exposure: score 2.0
Biomonitoring data shows an increasing exposure.

Vulnerable groups: score 0.5
The score 0.5 was given due to lack of data.

Lack of toxicity data: score 1.0
No threshold for toxicity has been established for bisphenol S, F or AF (ANSES, 2013).

Lack of data to estimate exposure from foods: score 1.0
To estimate the exposure to bisphenol S, F and AF, data on their occurrence in foods are needed.

Total score = 6.5 for bisphenol S (BPS), bispenol F (BPF) and bisphenol AF (BPAF)

6.1.3 References

- EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids) (2015). Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: Executive summary. EFSA Journal 2015;13(1):3978, 23 pp. doi:10.2903/j.efsa.2015.3978.
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6.2 Subgroup phthalates

Background information for the ranking
The phthalates di-butylphthalate (DBP), butyl-benzyl-phthalate (BBP), bis(2-ethylhexyl)phthalate (DEHP), di-isodecyl phthalate (DINP) and di-isononyl phthalate (DIDP) are listed and authorised in the positive list in Annex 614 I (Table 1) of Regulation (EC) No 10/20117 on plastic materials and articles intended to come into contact with food.

Hazard
The former EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) re-evaluated DBP, BBP, DEHP, DIDP and DINP for use in the manufacture of plastic food contact materials (FCM), and as a result it issued five separate opinions in 2005 (EFSA, 2005a; b; c; d; e). In addition, the AFC Panel published a statement regarding the possibility of allocating a group TDIfor those five phthalates (EFSA, 2005f), after having reviewed these phthalates individually. The available evidence supported that DBP and DEHP exerted pivotal effects on germ cell development/depletion, BBP on epididymal spermatozoa concentration and DINP and DIDP on the liver. While the three phthalates DBP, DEHP and BBP seemed to act on the same target organ (the testis), the profile of their effects at the hormonal and cellular level was not identical and their individual modes of action (MoA) had yet to be demonstrated. The AFC Panel then concluded in 2005 that a group TDI could not be allocated to these five phthalates in consideration of their different pivotal effects.

The European Chemicals Agency (ECHA) published an opinion (and a background document) on an Annex XV dossier proposing restrictions on DBP, di-isobutylphthalate (DIBP), DEHP and BBP (ECHA, 2017 a; b). Most of the information ECHA used were not available for EFSA in 2005. Therefore, EFSA was requested to update its 2005 opinions on DBP, BBP and DEHP in the context of FCM.

In 2018, the EFSA CEP Panel started to work on this new risk assessment of the five phthalates regulated for use in plastic FCM, according the terms of reference as provided in the updated mandate:

«In accordance with Article 12(3) of Regulation (EC) No 1935/20045, the European Commission asks EFSA to update its 2005 opinions on the safety assessment of di-butylphthalate (DBP, FCM No 157), butyl-benzyl-phthalate (BBP, FCM No 159), and Bis(2-ethylhexyl)phthalate (DEHP, FCM No 283), which have been authorised for use as plasticisers and technical support agents in plastic Food Contact Materials (FCM).»

A draft of this EFSA opinion is at present available for public consultation (EFSA, 2019). In this opinion, EFSA concluded that for all the five phthalates, the critical effects and the individual TDI values were fully in line with what EFSA established in 2005. With regards to the grouping of phthalates, the CEP Panel considered the anti-androgenic effect, i.e. reduction of the fetal testosterone production in rats, as a common mode of action and critical step for reproductive toxicity. On this basis, the CEP Panel included DBP, BBP, DEHP and DINP into the same group TDI. Although the Panel considered liver effects to be the most sensitive endpoint for DINP, it also noted its anti-androgenic capability. To account for the different potencies towards these endpoints an additional assessment factor of 3.3 was used in the group TDI.

DIDP was not included in the group TDI as its reproductive effects (i.e. decreased survival rate in F2) are not considered to be associated with anti-androgenicity. Therefore, DIDP maintained its individual TDI for liver effects of 0.15 mg/kg bw per day.

The group TDI was calculated by means of relative potency factors with DEHP taken as the index compound as it has the most robust toxicological dataset. The relative potency factors were calculated from the ratio of the TDI for DEHP to the HBGVs for the three other phthalates. The group TDI was established to be 0.05 mg/kg bw per day, expressed as DEHP equivalents. For further details, please see this draft opinion.

Exposure estimation in the EFSA 2019 draft assessment of phthalates
Occurrence data on phthalates in food were obtained from the literature referenced in the ECHA RAC opinion (2017a) on DBP, BBP and DEHP and complemented with additional literature search on DINP and DIDP and on specific foods not covered in the literature from ECHA RAC. Occurrence data available in the EFSA Chemical Occurrence database was not suitable for exposure assessment because of severe limitations, e.g. high LOQs and LODs and high percentage of left-censored data.

Estimates of dietary exposure (ranges of the min-max estimates for all ages, all surveys and all countries) were obtained by combining occurrence data with the consumption data from the EFSA Comprehensive Database and were as follows:

- DBP mean of (0.042 - 0.769) and 95-percentile of (0.099 - 1.503), µg/kg bw per day
- BBP mean of (0.009 - 0.207) and 95-percentile of (0.021 - 0.442), µg/kg bw per day
- DEHP mean of (0.446 - 3.459) and 95-percentile of (0.902 - 6.148), µg/kg bw per day
- DINP mean of (0.232 - 4.270) and 95-percentile of (0.446 - 7.071), µg/kg bw per day
- DIDP mean of (0.001 – 0.057) and 95-percentile of (0.008 – 0.095), µg/kg bw per day)

These estimates were in reasonably good agreement with those reported in total diet studies (TDS) for the UK, Ireland and France.

Exposure estimation for the adult Norwegian population
A study estimated phthalate exposures for the Norwegian adult population from a market survey of 37 food items and beverages including 1-3 brand names per food product (Sakhi et al., 2014). The selection of food items and beverages was based on two criteria: (i) basic food items that are commonly consumed in a typical Norwegian diet, and (ii) foods and beverages that are likely to contain these chemicals.
The estimated exposures were as follows (data from 2010-2011, median, middle-bound values, i.e. values below the LOQ are replaced by LOQ/2):
DBP: 30 ng/kg bw per day
BBP: 18 ng/kg bw per day
DEHP: 384 ng/kg bw per day
DINP: 402 ng/kg bw per day
DIDP: 33 ng/kg bw per day

6.2.1 Bis(2-ethylhexyl)phthalate (DEHP) (CAS no. 117-81-7)

6.2.1.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 2.0

EFSA (2005c) established a TDI for DEHP of 0.05 mg/kg bw per day, which was re-confirmed in the draft of EFSA (2019). The exposure to DEHP was found to be 0.446 - 3.459 and 95-percentile of 0.902 - 6.148 µg/kg bw per day (EFSA, 2019), below the TDI (50 µg/kg bw per day). The estimated exposure for adults in Norway (384 ng/kg bw per day) is also well below the TDI value for DEHP (Sakhi et al., 2014).

Vulnerable groups: score 0.5
Overall, the CEP Panel did not identify any study reviewed by ECHA (2017a; b) which could give rise to a LOAEL or NOAEL lower than those previously identified by EFSA (2005). The CEP Panel concurred with the choice of both EFSA (2005b) and ECHA (2017a) on the critical effect on the testis in F1-animals in a three-generation reproductive toxicity study in rats,

reported by Wolfe and Layton (2003), from which a NOAEL of 4.8 mg DEHP/kg bw per day was identified.

Since DEHP has adverse reproductive effects, which are transferred onto future generations, the fetus is vulnerable to the effects of this substance.

Lack of toxicity data: score 0.5
In addition to the reproductive toxicity of DEHP, there is literature on immunotoxic, neurotoxic and metabolic effects that was not taken into consideration by ECHA (2017) and EFSA (2019), as well as new data on reproductive effects after 2017 not evaluated by EFSA (2019). However, there are reports claiming that these other effects may occur at lower doses than the doses observed having reproductive toxicity. Therefore, this could lead to an underestimation of the risk based on the reproductive toxicity.

Lack of exposure data: score 0.5
The exposure data used by EFSA (2019) had limitations.
Intake: The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) provides a compilation of existing national information on food consumption at individual level. These are the most complete and detailed food consumption data currently available in the EU. However, because of the differences in the methods used for data collection, direct country-to-country comparisons can be misleading.
Occurrence: Considering the i) limited number of samples per food category; ii) the predominance of left-censored data for the large majority of food categories and phthalates; iii) the relatively high LOQs, and iv) the limited availability of information on packaging material, the CEP Panel decided to perform an alternative exposure assessment based on occurrence data on phthalates from the literature.

Total score = 3.5 for bis(2-ethylhexyl)phthalate (DEHP)

6.2.2 Butyl-benzyl-phthalate (BBP) (CAS no. 85-68-7)

6.2.2.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 2.0

EFSA (2005b) established a TDI for BBP of 0.5 mg/kg bw per day, which was re-confirmed in the draft of EFSA (2019). The exposure to BBP was found to be 0.009 - 0.207 and 95-percentile of 0.021 - 0.442 µg/kg bw per day (EFSA, 2019), below the TDI (500 µg/kg bw per day). The estimated exposure for adults in Norway (18 ng/kg bw per day) is also well below the TDI value for BBP (Sakhi et al., 2014).

Vulnerable groups: score 0.5
Overall, the CEP Panel did not identify any study reviewed by ECHA (2017a; b) which could give rise to a LOAEL or NOAEL lower than those previously identified by EFSA (2005b). The CEP Panel (2019) concurred with the choice of both EFSA (2005b) and ECHA (2017a) on the

critical effect, reported by Tyl *et al.* (2004), of reduced anogenital distance (AGD) in F1- and F2- males at birth in the 250 mg BBP/kg bw per day group, from which a NOAEL of 50 mg BBP/kg bw per day was identified.

Since BBP has adverse reproductive effects, which are transferred onto future generations, the fetus is vulnerable to the effects of this substance.

Lack of toxicity data: score 0.5
In addition to the reproductive toxicity of BBP, there is literature on immunotoxic, neurotoxic and metabolic effects that was not taken into consideration by ECHA (2017) and EFSA (2019), as well as new data on reproductive effects after 2017 not evaluated by EFSA (2019). However, there are reports claiming that these other effects may occur at lower doses than the doses observed having reproductive toxicity. Therefore, this could lead to an underestimation of the risk based on the reproductive toxicity.

Lack of expsoure data: score 0.5
The exposure data used by EFSA (2019) had limitations.
Intake: The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) provides a compilation of existing national information on food consumption at individual level. These are the most complete and detailed food consumption data currently available in the EU. However, because of the differences in the methods used for data collection, direct country-to-country comparisons can be misleading.
Occurrence: Considering the i) limited number of samples per food category; ii) the predominance of left-censored data for the large majority of food categories and phthalates; iii) the relatively high LOQs, and iv) the limited availability of information on packaging material, the CEP Panel decided to perform an alternative assessment of exposure based on occurrence data on phthalates from the literature.

Total score = 3.5 for butyl-benzyl-phthalate (BBP)

6.2.3 Di-butylphthalate (DBP) (CAS no.84-74-2)

6.2.3.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 2.0

EFSA (2005a) established a TDI for DBP of 0.01 mg/kg bw per day, which was re-confirmed in the draft of EFSA (2019). The exposure to DBP was found to be mean of 0.042 - 0.769 and 95-percentile of 0.099 - 1.503 µg/kg bw per day, below the TDI (10 µg/kg bw per day). The estimated exposure for adults in Norway (30 ng/kg bw per day) is also well below the TDI value for DBP (Sakhi et al., 2014).

Vulnerable groups: score 0.5

Overall, the CEP Panel did not identify any study reviewed by ECHA (2017a; b) which could give rise to a LOAEL or NOAEL for DBP lower than those previously identified by EFSA (2005a). The CEP Panel concurred with the choice of both EFSA (2005a) and ECHA (2017a) on the critical effect reported by Lee et al. (2004) of reduced spermatocyte development and effects on the mammary gland in a developmental toxicity study in rats, which occurred at a LOAEL of 2 mg DBP/kg bw per day. After dietary exposure on gestation day (GD) 15 to postnatal day (PND) 21, the effects were reduced spermatocyte development on PND21 and mammary gland changes in adult males in all treated groups.

Since DBP has adverse effects manifested after birth from gestational and postnatal exposure, the fetus is vulnerable to the effects of this substance.

Lack of toxicity data: score 0.5
In addition to the reproductive and developmental effects of DBP, there is literature on immunotoxic, neurotoxic and metabolic effects that was not taken into consideration by ECHA (2017) and EFSA (2019), as well as new data on reproductive effects after 2017 not evaluated by EFSA (2019). However, there are reports claiming that these other effects may occur at lower doses than the doses that have reproductive and developmental toxicity. Therefore, this could lead to an underestimation of the risk based on the reproductive and developmental toxicity.

Lack of exposure data: score 0.5
The exposure data used by EFSA (2019) had limitations.
Intake: The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) provides a compilation of existing national information on food consumption at individual level. These are the most complete and detailed food consumption data currently available in the EU. However, because of the differences in the methods used for data collection, direct comparisons among countries can be misleading.
Occurrence: Considering the i) limited number of samples per food category; ii) the predominance of left-censored data for the large majority of food categories and phthalates; iii) the relatively high LOQs, and iv) the limited availability of information on packaging material, the CEP Panel decided to gather occurrence data on phthalates also from the literature to perform an alternative assessment of exposure.

Total score = 3.5 for di-butylphthalate (DBP)

6.2.4 Di-isodecyl phthalate (DIDP) (CAS no. 68515-49-1 and 26761-40-0)

6.2.4.1 ***Scores***

MOE/MOS/ADI/TDI/TWI: score 2.0

EFSA (2005e) established a TDI for DIDP of 0.15 mg/kg bw per day, which was re-confirmed in the draft of EFSA (2019). The exposure to DIDP was found to be 0.001 – 0.057 and 95-percentile of 0.008 – 0.095 µg/kg bw per day (EFSA, 2019), below the TDI (150 µg/kg bw per day). The estimated exposure for adults in Norway (33 ng/kg bw per day) is also well below the TDI value for DIDP (Sakhi et al., 2014).

Vulnerable groups: score 0.5
In the EFSA opinion on DIDP (EFSA, 2005e), the AFC Panel based its risk assessment on the effects on liver in dogs with a NOAEL of 15 mg/kg bw per day (Hazleton, 1968) and on a NOAEL of 33 mg DIDP/kg bw per day for decreased survival in the F2-offspring in a two-generation reproductive toxicity study in rats (Exxon, 1997, 2000 published by Hushka et al., 2001). The Panel applied an uncertainty factor of 100 to derive a TDI of 0.15 mg DIDP/kg bw per day.

Overall, the CEP Panel concurred with the NOAEL of 33 mg DIDP/kg bw per day for reproductive effects in rats (based on pup mortality), which was also identified by EFSA in 2005 and ECHA in 2013, and agreed that DIDP did not exhibit anti-androgenic activity.

Since DIDP affects the mortality of pups, newborn children may be vulnerable to the effects of this substance.

Lack of toxicity data: score 0.5
In addition to the effects on the liver, reproduction and development of DIDP, there may be literature on immunotoxic, neurotoxic and metabolic effects that was not taken into consideration by EFSA (2019). These other effects may occur at lower doses than the doses observed having effects on the liver, reproduction and development. Therefore, this could lead to an underestimation of the risk based on the effects on the liver, reproduction and development.

Lack of exposure data: score 0.5
The exposure data used by EFSA (2019) had limitations.
Intake: The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) provides a compilation of existing national information on food consumption at individual level. These are the most complete and detailed food consumption data currently available in the EU. However, because of the differences in the methods used for data collection, direct comparisons among countries can be misleading.
Occurrence: Considering the i) limited number of samples per food category; ii) the predominance of left-censored data for the large majority of food categories and phthalates; iii) the relatively high LOQs, and iv) the limited availability of information on packaging material, the CEP Panel decided to gather occurrence data on phthalates also from the literature to perform an alternative assessment of exposure.

Total score = 3.5 for di-isodecyl phthalate (DIDP)

6.2.5 Di-isononyl phthalate (DINP) (CAS no. 68515-48-0 and 28553-12-0)

6.2.5.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 2.0

EFSA (2005d) established a TDI for DINP of 0.15 mg/kg bw per day, which was re-confirmed in the draft of EFSA (2019). The exposure to DINP was found to be 0.232 - 4.270 and 95-percentile of 0.446 - 7.071 µg/kg bw per day (EFSA, 2019), below the TDI (150 µg/kg bw per day). The estimated exposure for adults in Norway (402 ng/kg bw per day) is also well below the TDI value for DINP (Sakhi et al., 2014).

Vulnerable groups: score 0.5

In the EFSA opinion on DINP (EFSA, 2005d), the AFC Panel based its risk assessment on the effects on the liver, reproduction and development. The Panel considered that the pivotal effect was the effect on the liver (increased incidence of spongiosis hepatis), increased levels of liver enzymes and increased absolute and relative liver and kidney weights from the study in Fisher 344 rats by Exxon 1855 (1986; also cited as Lington, 1997). The AFC Panel (EFSA, 2005d) identified a NOAEL of 15 mg DINP/kg bw per day for non-peroxisomal proliferation-related chronic hepatic and renal effects in rats, and applied an uncertainty factor of 100 to derive a TDI of 0.15 mg DINP/kg bw per day.

Overall, regarding reproductive and developmental effects of DINP the CEP Panel concurred with the NOAEL identified in the ECHA opinion (ECHA, 2013) of 50 mg DINP/kg bw per day based on the decreased fetal testosterone production and histopathological changes (multinucleated gonocytes (MNGs)) in the rat fetus after exposure on GD12 to GD19 reported in the study of Clewell et al. (2013a). The additional studies mentioned by ECHA support this NOAEL for reprotoxic effects.

Since DINP has adverse effects on the fetus, the fetus is vulnerable to the effects of this substance.

The CEP Panel noted that two CAS numbers exist for DINP, i.e. CAS No. 68515-48-0 for 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, and CAS No. 28553-12-0 for 1,2-benzenedicarboxylic acid, 1,2-diisononyl ester. Considering that the first formulation is a «cruder» version of DINP, including also decyl fractions, the question arises whether both formulations have equivalent toxicological profiles. Consequently, the Panel reviewed a paper from Hannas et al. (2011), who demonstrated that both formulations induced a virtually identical dose-dependent reduction of fetal testicular testosterone production. The authors reported that «curve fit results comparing these two DINP formulations are statistically indistinguishable». Based on the equivalent potency of both formulations for the induction of the described effect, the Panel concludes that no differentiation of the two DINP formulations is needed in the assessment of the reproductive toxicity.

Lack of toxicity data: score 0.5
In addition to the effects on the liver, reproduction and development of DINP, there may be literature on immunotoxic-, neurotoxic and metabolic effects that was not taken into consideration by ECHA (2018) and EFSA (2019). These other effects may occur at lower doses than the doses observed having effects on the liver, reproduction and development. Therefore, this could lead to an underestimation of the risk based on the effects on the liver, reproduction and development.

Lack of exposure data: score 0.5
The exposure data used by EFSA (2019) had limitations.
Intake: The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) provides a compilation of existing national information on food consumption at individual level. These are the most complete and detailed food consumption data currently available in the EU. However, because of the differences in the methods used for data collection, direct comparisons among countries can be misleading.
Occurrence: Considering the i) limited number of samples per food category; ii) the predominance of left-censored data for the large majority of food categories and phthalates; iii) the relatively high lLOQs, and iv) the limited availability of information on packaging material, the CEP Panel decided to gather occurrence data on phthalates also from the literature to perform an alternative assessment of exposure.

Total score = 3.5 for di-isononyl phthalate (DINP)

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7 Ranking of flavourings

An overview of the scoring and ranking of the flavouring is given in Table 7-1. A detailed description follows after the table.

Table 7-1. Summary table for scoring of flavourings.

Subgroup	Substance	1. MOE/MOS/ADI/TDI/TWI	2. Toxicity	3. Exposure	4. Vulnerable groups	5. Lack of toxicity data	6. Lack of exposure data	Total score	Comments
Flavourings	Caffeine	4.0	-	-	0.5	1.0	1.0	6.5	Lack of occurrence data, and lack of toxicity data on chronic exposure

7.1.1 Caffeine

EFSA established the following intake levels of caffeine for different population groups unlikely to cause adverse effects (EFSA, 2015):

For the general adult population (not including pregnant women):

- Single intake of caffeine up to 200 mg (about 3 mg/kg bw for a 70-kg adult)
- Intakes up to 400 mg per day (about 5.7 mg/kg bw per day for a 70-kg adult) consumed throughout the day
- Caffeine intake of about 1.4 mg/kg bw may increase sleep latency and reduce sleep duration in adults

For children and adolescents:

- A daily intake of 3 mg/kg bw per day does not give rise to safety concerns
- Intakes of about 1.4 mg/kg bw may increase sleep latency and reduce sleep duration

For pregnant women and the fetus:

- Intake of 200 mg per day (about 3 mg/kg bw for a 70-kg adult) consumed throughout the day

For lactating women and the breastfed infant:

- Single intakes of caffeine up to 200 mg (about 3 mg/kg bw) and habitual caffeine consumption at doses of 200 mg per day

There are several dietary caffeine sources, including e.g. caffeine-containing soft drinks, coffee drinks, cocoa-containing products and food supplements. In a risk assessment by VKM (2015), it was concluded that a dose of 300 mg caffeine from food supplements may represent a risk of general adverse health effects and sleep disturbances in children (10 years and above), adolescents (14 to <18 years), pregnant women and fetus and lactating women and the breastfed infant. Consumed as a single dose, 300 mg of caffeine from food supplement may represent a risk of general adverse health effects and sleep disturbance in adults (≥18 years).

In a risk assessment by VKM (2019), including only the age groups 9-18 years, the following conclusions were reached for caffeine intake from energy drinks:

- In the age group 8-12 years, high chronic intake of energy drinks may represent a risk for sleep disturbance for children if all consumed energy drinks contain either 40 or 55 mg caffeine/100 ml.
- In the age group 13-15 years, high chronic intake of energy drinks may represent a risk for sleep disturbance for adolescents if all consumed energy drinks contain

either 32, 40 or 55 mg caffeine/100 ml, and a risk for general adverse health effects for energy drinks containing 40 or 55 mg caffeine/100 ml.
- In the age group 16-18 years, high chronic intake of energy drinks may represent a risk for sleep disturbance for adolescents if all consumed energy drinks contain either 32, 40 or 55 mg caffeine/100 ml.
- The highest acute intake estimates of energy drinks, if all consumed energy drinks contain either 15, 32, 40 or 55 mg caffeine/100 ml and above, may all represent a risk for sleep disturbance and general adverse health effects in all age groups.

For caffeine exposure from food and beverages (not including energy drinks):

- Among consumers and non-consumers of energy drinks aged 16-18 years and consumers aged 13-15 years, who had a high exposure of caffeine from other beverages than energy drinks, this exposure may represent a risk for sleep disturbance.
- For consumers of energy drinks aged 10-12 years who have a high intake of caffeine from other beverages than energy drinks, this exposure may represent a risk for sleep disturbance and general adverse health effects.

7.1.1.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 4.0
The intake was above the reference point for toxicity for high intake of caffeine-containing beverages for several age groups, and for intake of 300 mg caffeine from food supplements.

Vulnerable groups: score 0.5
Groups in the population that may be more susceptible to the adverse effects of energy drinks and caffeine include individuals with predispositions to certain heart conditions. The reference point of 3 mg per kg body weight per day may not necessarily protect individuals in susceptible groups.

Lack of toxicity data: score 1.0
Studies on chronic exposure to caffeine and studies including doses that represent high acute intake are needed.

Lack of exposure data: score 1.0
There was a lack of occurrence data for caffeine in foods and beverages.

Total score = 6.5 for caffeine

7.1.1.2 *References*

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8 Ranking of additives

An overview of the scoring and ranking of the included additives is given in Table 8-1. A detailed description follows after the table.

Table 8-1. Summary table for scoring of additives and flavourings.

Subgroup	Substance	1. MOE/MOS/ADI/TDI/TWI	2. Toxicity	3. Exposure	4. Vulnerable groups	5. Lack of toxicity data	6. Lack of exposure data	Total score	Comments
Nitrites and nitrates	Sodium and potassium salts of nitrite and nitrate	4.0	-	-	0.5	0.5	0.5	5.5	Naturally occurring nitrate not included in exposure (EFSA, 2017) Ongoing risk assessment of nitrates/nitrites in feed by EFSA
Phosphates	Phosphoric acid-phosphates	6.0	-	-	1.0	0.5	0.0	7.5	Naturally occurring phosphates included in exposure (EFSA, 2019)
Sweeteners	Acesulfame K (950)	-	1.0	2.0	0.0	0.5	1.0	4.5	Persistent in the environment
	Sucralose (E955)	2.0	-	-	0.0	0.0	1.0	3.0	Persistent in the environment
Synthetic antioxidants	Butylated hydroxyanisole (BHA) (E320)	2.0	-	-	0.5	0.5	1.0	4.0	

Subgroup	Substance	1. MOE/MOS/ADI/TDI/TWI	2. Toxicity	3. Exposure	4. Vulnerable groups	5. Lack of toxicity data	6. Lack of exposure data	Total score	Comments
	Butylated hydroxytoluene (BHT) (E321)	4.0	-	-	0.0	0.0	1.0	5.0	Extensively used. Several sources and exposure routes
	Ethoxyquin (EQ)	-	2.0	2.0	0.5	1.0	1.0	6.5	Lack of occurrence data, lack of toxicity data on transformation products, lack of intake data

8.1 Subgroup nitrites and nitrates

8.1.1 Sodium and potassium salts of nitrite and nitrate

Sodium and potassium salts of nitrite and nitrate are commonly used as food additives in e.g. meat to prevent bacterial growth and to achieve desirable reddish colours. Nitrate is found naturally in some foods, e.g. spinach, and may also enter the food chain from contamination of water. EFSA evaluated nitrite and nitrate in two separate risk assessments in 2017.

8.1.1.1 *Scores*

MOE/MOS/ADI/TDI/TWI: 4.0

Nitrites, and nitrate that is converted to nitrite in the body, may be transformed into nitrosamines, many of which are carcinogenic. Neither nitrate nor nitrite were considered genotoxic by EFSA (2017a; b), however, there was some evidence for a positive association between dietary nitrite and gastric cancer, and also for both nitrite and nitrate from processed meat and colorectal cancer. There was insufficient evidence for a positive association between nitrite alone in processed meat and other types of cancer. Nevertheless, EFSA considered the formation of methemoglobinaemia as the most relevant endpoint for assessing the toxicity of both nitrite and nitrate converted to nitrite in the body. Methemoglobinaemia prevents normal oxygen delivery to the tissues and may cause tissue hypoxia, in addition to other changes in hematological parameters. Using a BMD approach, EFSA derived an ADI of 0.07 mg nitrite ion/kg bw per day, and retained the existing ADI for nitrate of 3.7 mg nitrate ion/kg bw per day (EFSA, 2017a; b).

Using several conservative scenarios, the exposure to nitrite as a food additive was calculated not to go beyond the ADI, although a slight exceedance was calculated for children at the highest percentile. However, if all sources of dietary nitrite were included (food additives, naturally occurring nitrite and contamination), the ADI would be exceeded for infants, toddlers and children at the mean intake and for all age groups at the highest exposure.

If considering the amount of nitrite from meat converted to a nitrosamine (N-nitroso-dimethylamine (NDMA)) in the body with an intake at the level of the ADI, the MOE was calculated to be 42,000. Thus, the formation of nitrosamines from nitrite added at approved levels to meat products were not considered a concern for human health. Overall, EFSA concluded that nitrosamines formed in the body from nitrite at approved levels in meat was not of concern for human health. However, when calculating the risk from exposure to N-nitroso compounds (NDMA and N-nitrosodiethylamine (NDEA)) already present in meat products, MOE at mean exposure was <10,000 in toddlers, children and adolescents, and <10,000 in all age groups at high level exposure.

Vulnerable groups: 0.5
Toddlers, children and adolescents exceeded the ADI in some exposure scenarios and had a MOE value less than 10,000 if all sources of nitrosamines were considered.

Lack of toxicity data: 0.5
EFSA recommended several studies to follow up knowledge gaps uncovered in their risk assessments.

Lack of exposure data: 0.5
EFSA recommended further studies on the levels of nitrosamines formed in meat products and large-scale epidemiological studies on nitrite, nitrate and nitrosamine intake and their association with certain cancer types in order to reduce the uncertainties adressed in their risk assessments.

Total score = 5.5 for sodium and potassium salts of nitrite and nitrate

8.1.2 References

- EFSA Panel on Food Additives and Nutrient Sources added to Food (2017a). Scientific Opinion on the re-evaluation of potassium nitrite (E 249) and sodium nitrite (E 250) as food additives. EFSA Journal 15(6):4786.
- EFSA Panel on Food Additives and Nutrient Sources added to Food (2017b). Scientific Opinion on the re-evaluation of sodium nitrate (E 251) and potassium nitrate (E 252) as food additives. EFSA Journal 15(6):4787.

8.2 Subgroup phosphates

8.2.1 Phosphoric acid-phosphates

Phosphorus (P) is an essential nutrient vital for life and is naturally present in all foods. Phosphoric acid-phosphates (di-,tri- and polyphosphates) are food additives that also may be found naturally occurring in foodstuff. Analytical methods are not able to differentiate between naturally occurring and added forms of P in food. Since P is an essential nutrient, the term «Tolerable Upper Intake level» (UL) is also used to denote acceptable intake levels.

8.2.1.1 *Scores*

MOE/MOS/ADI/TDI/TWI: 6.0
In a recent risk assessment, EFSA (2019) considered phosphates to be of low acute oral toxicity and of no concern with respect to genotoxicity, carcinogenicity, reproductive toxicity or developmental toxicity. From numerous studies with different animal models and human studies, the only significant adverse effect of phosphates was calcification of the kidney and tubular nephropathy.

In a chronic rat study with sodium triphosphate and kidney damage as adverse outcome, the NOAEL was reported to be 76 mg P/kg bw per day (EFSA, 2019 and references therein). Adding a background dietary P level of 91 mg/kg bw per day to this NOAEL gives a total value of 167 mg P/kg bw per day. Using a safety factor of 4 to account for interspecies and interindividual differences, the ADI value was derived to be ~40 mg/kg bw per day (EFSA, 2019). Based on analytical data of the total P content of foods, EFSA (2019) calculated an exposure that exceeded the proposed ADI for infants, toddlers and children at the mean level of intake and at the 95-percentile the ADI was exceeded also for adolescents. Similarly, the mean dietary intakes of P from for toddlers (2 year) and children (4 and 9 years) in Norway (VKM, 2017) showed that the ADI was exceeded at the mean intake, while for adolescents (13 years) and adults (≥18 years) the ADI was exceeded at the 95-percentile.

Table 8.2.1-1. Phosphorus intake and exposure in the Norwegian population for ages 2, 4, 9, 13 and 18 (VKM 2017).

	Phosphorus from diet alone, mean (mg/day)	Phosphorus from diet alone, P95 (mg/day)	Average weight (kg) girl/boy	Exposure (mg P/kg bw per day) at mean P intake	Exposure (mg P/kg bw per day) at P95 intake
Adults (n=1787)	1725	2855	60 (girls) 71 (boys)	29 (girls) 24 (boys)	47 (girls) 40 (boys)
13 years (n=687)	1361	2257	47	29	48
9 years (n=636)	1304	1996	31	42	64
4 years (n=399)	1120	1662	17	66	98
2 years (n=1674)	1102	1787	12.5	88	143

Weights are derived from Norwegian weight development charts (http://www.vekststudien.no/en/).

The mean P intakes in the Norwegian population (VKM, 2017) did not exceed the provisional tolerable upper intake levels of 3,000 mg/day showing a discrepancy between the recently derived ADI and the suggested provisional UL for total intake of P at 3,000 mg P/day, (VKM 2017).

Vulnerable groups: 1.0
EFSA (2019) noted that the recently derived ADI should not apply to people with moderate to severe reduction in renal function. As much as 10% of the population might have chronic kidney disease with reduced renal function and may not tolerate the amount of P set at the level of ADI.

Lack of toxicity data: 0.5
Although EFSA (2019) noted that there were numerous toxicology studies available on P,

they also commented that most of them were quite old and not performed according to current standards.

Lack of exposure data: 0.0
Food consumption and concentration data for P are readily available at both European and national levels.

Total score = 7.5 for phosphoric acid-phosphates

8.2.2 References

- EFSA Panel on Food Additives and Flavourings (2019). Scientific Opinion on the re-evaluation of phosphoric acid–phosphates – di-, tri- and polyphosphates (E 338–341, E 343, E 450–452) as food additives and the safety of proposed extension of use. EFSA Journal 2019;17 (6):5674.
- VKM (2017). Assessment of dietary intake of phosphorus in relation to tolerable upper intake level. Opinion of the Panel on Nutrition, Dietetic Products, Novel Food and Allergy of the Norwegian Scientific Committee for Food Safety. VKM Report 2017: 18, ISBN: 978-82-8259-275-8, Oslo, Norway.
- IOM (1997) Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride, in: N. A. Press (Ed.), Institute of Medicine, Washington D.C.

8.3 Subgroup sweeteners

8.3.1 Acesulfame K (E950)

Acesulfame K is permitted in a wide range of food products and beverages

8.3.1.1 *Scores*

MOE/MOS/ADI/TDI/TWI
An ADI of 9 mg/kg bw day was established by the SCF (2000), based on the NOAEL in a 2-year dog study. The exposure from beverages is below the ADI. However, since we have no intake estimates from foods and beverages, points 2 and 3 are addressed.

Toxicity: score 1.0

Exposure: score 2.0
Exposure to acesulfame K from soft drinks was estimated by VKM (2014). This exposure was well below the ADI for all included age groups.

Vulnerable groups: score 0.0
No vulnerable groups were identified.

Lack of toxicity data: 0.5
Some data on toxicity are lacking.

Lack of exposure data: score 1.0
Occurrence data are needed to estimate the exposure from foods and beverages.

Total score = 4.5 for acesulfame K

8.3.1.2 *References*

- SCF (Scientific Committee on Food) 2000. Opinion Re-evaluation of acesulfame K with reference to the previous SCF opinion of 1991. Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out52_en.pdf.
- VKM (2014). Risk assessments of aspartame, acesulfame K, sucralose and benzoic acid from soft drinks, "saft", nectar and flavoured water. Opinion of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of the Norwegian Scientific Committee for Food Safety. VKM Report 2014: 26. https://vkm.no/download/18.a665c1015c865cc85bb7ae2/1501776952080/8055794778.pdf.

8.3.2 Sucralose (E995)

Sucralose is permitted in a wide range of food products and beverages.

8.3.2.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 2.0
The ADI is 15 mg/kg bw per day (SCF, 2000). In a review of the safety of sucralose (Magnuson et al., 2017), it was concluded that the estimated intakes of sucralose remain well below ADI values, even using conservative approaches, such as the maximum use levels. This is supported by the VKM risk assessment of sucralose (2014), concluding that the estimated exposure to sucralose from soft drinks was well below the ADI for all age groups.

Vulnerable groups: score 0.0
No specific vulnerable groups identified.

Lack of toxicity data: score 0.0
Sufficiently data are available to identify and characterise the toxicity.

Lack of exposure data: score 1.0
There is a lack of data on concentrations of sucralose in foods.

Total score = 3.0 for sucralose

8.3.2.2 *References*

- Magnuson BA, Roberts A, Nestmann ER (2017). Critical review of the current literature on the safety of sucralose. Food Chem Tox 106:324-355. doi: 10.1016/j.fct.2017.05.047.
- SCF (Scientific Committee on Food) 2000. Opinion of the Scientific Committee on Food on sucralose. Available online: http://ec.europa.eu/food/fs/sc/scf/out68_en.pdf.
- VKM (2014). Risk assessments of aspartame, acesulfame K, sucralose and benzoic acid from soft drinks, "saft", nectar and flavoured water. Opinion of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of the Norwegian Scientific Committee for Food Safety. VKM Report 2014: 26. https://vkm.no/download/18.a665c1015c865cc85bb7ae2/1501776952080/8055794778.pdf.

8.4 Subgroup synthetic antioxidants

8.4.1 Butylated hydroxyanisole (BHA) (E320)

Butylated hydroxyanisole (BHA) is a synthetic antioxidant authorised as both a food and feed additive in the EU and most recently evaluated as a food additive by EFSA in 2011. It is used as an antioxidant in fats and oils and in many processed foods such as soups, sauces, breakfast cereals and fine bakery wares.

8.4.1.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 2.0

A previous ADI of 0.5 mg/kg bw was revised by EFSA in 2011. The previous ADI was based on the occurrence of tumors in the forestomach of rodents. However, the latter evaluation concluded that rodent forestomach tumors were not due to genotoxicity, but rather through a thresholded mechanism of action subsequent to pro-oxidant effects and formation of reactive oxygen species, and that this manifestation was not of relevance to man. Consequently, the ADI was set at 1.0 mg/kg bw. This was based on a NOAEL of 100 mg/kg bw per day for growth retardation, increased mortality and behavioural effects in rat pups.

An exposure scenario was calculated based on a stepwise approach with both crude and refined estimates. A crude estimate gave a theoretical maximum daily exposure to BHA of 1.25 mg/kg bw per day for adults and children. A refined, yet conservative, estimate gave a theoretical maximum daily exposure of 0.7 mg/kg bw per day for children and 0.14 mg/kg bw per day for adults showing that the exposure was below the ADI. Using the refined estimate, the MOE values would be ~140 for children and ~700 for adults, making the MOE values sufficiently high.

Vulnerable groups: score 0.5
Reproduction and developmental studies have been performed in rats, mice, rabbits, pigs and monkeys. Although results differed among the species, growth retardation, increased mortality and behavioural effects were observed in rodent pups. Considering the theoretical maximum daily exposure of 0.7 mg/kg bw per day for children, it could be argued that children is a vulnerable group with a somewhat higher exposure in the population.

Lack of toxicity data: score 0.5
Some knowledge gaps were pointed out by EFSA (2011), e.g. no two-generation reproduction toxicity studies were available.

Lack of exposure data: score 1.0
The above-mentioned exposure scenarios were based on an assumption that foodstuff contained the maximum permitted concentrations of BHA, i.e. a conservative estimate. Given the lack of concentration data in actual foodstuffs, little data is available on exposure.

Total score = 4.0 for butylated hydroxyanisole (BHA)

8.4.1.2 *References*

- EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS); Scientific Opinion on the re- evaluation of butylated hydroxyanisole–BHA (E 320) as a food additive. EFSA Journal 2011;9(10):2392. [49 pp.] doi:10.2903/j.efsa.2011.2392.

8.4.2 Butylated hydroxytoluene (BHT) (E321)

An ADI for butylated hydroxytoluene (BHT) of 0.25 mg/kg bw per day was established by EFSA (2012). The ADI was based on a NOAEL of 25 mg/kg bw per day derived from two 2-generation studies in rats based on effects on litter size, sex ratio and pup body weight gain during the lactation period, using an uncertainty factor of 100.

BHT is authorised as an additive in food and feed.

EFSA estimated the exposure of children and adults to BHT, and concluded that it is unlikely that the ADI is exceeded at the mean. At the 95-percentile, the ADI may be exceeded for children in some European countries, whereas it is not exceeded for adults.

The exposure assessment was conservative, using a worst-case scenario of combined exposure to BHT from the food categories where use as a food additive is authorised.

8.4.2.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 4.0
Intake is mostly below the ADI (however, in a worst-case scenario, the 95-percentile is above for children in Finland and The Netherlands).

Vulnerable groups: score 0.0

Lack of toxicity data: score 0.0
Sufficiently data were available to identify and characterise the toxicity.

Lack of exposure data: score 1.0
There is a lack of data on concentrations of BHT in foods and beverages.

Total score = 5.0 for butylated hydroxytoluene (BHT)

8.4.2.2 *References*

- EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) (2012). Scientific Opinion on the reevaluation of Butylated hydroxytoluene BHT (E 321) as a food additive. EFSA Journal 2012;10(3):2588. [43 pp.] doi:10.2903/j.efsa.2012.2588.

8.4.3 Ethoxyquin (EQ)

The synthetic antioxidant ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (EQ)) has been permitted for use in animal feed for pets, livestock and in particular farmed fish to protect lipids and fat-soluble vitamins against oxidation. It has been widely used in global transport of fish meal, partly due to the requirement of the International Maritime Organisation (IMO) that fish meal be stabilized by either EQ or butylated hydroxytoluene (BHT) to prevent spontaneous combustion during overseas transport and storage (IMO, 2014). In the EU, EQ was previously permitted for use in feed, but not in food. Although EQ is not permitted as a food additive, the presence of EQ in feed results in transfer of both the mother compound EQ and its many transformation products (Merel et al., 2019) into the edible part of the farmed animal. In a risk assessment published in 2015, EFSA stated that there was not sufficient data to conclude on the safety of EQ, leading to a suspension of its authorization (EC 2017/962) within the EU. Nonetheless, a transition period until March 2020 allows feed produced from certain materials containing EQ to be placed on the market.

Although the mother compound EQ can be measured with relative ease, there are many transformation products of EQ, e.g. an ethoxyquin dimer (Merel et al., 2019), that is present in farmed animals that have received EQ-containing feed. Thus, other compounds than just EQ itself may be present in farmed produce and may pose a risk to the consumer. Indeed, EFSA stated that EQ itself was not genotoxic, carcinogenic and does not cause developmental toxicity. However, EQ-related transformation products, such as ethoxyquin quinone imine, have shown structural alerts for mutagenicity, carcinogenicity and DNA binding (EFSA 2015).

8.4.3.1 *Scores*

MOE/MOS/ADI/TDI/TWI
The Joint Food and Agricultural Organization and World Health Organization Meeting on

Pesticide Residues (JMPR, 1998) proposed an ADI of 0.005 mg/kg bw per day for EQ and some of its metabolites based on a toxicity study in dogs. However, EFSA (2015) decided that there was not sufficient data to propose an ADI for EQ. Moreover, given the lack of data on consumer exposure (EFSA, 2015), no calculation of MOE is possible.

Toxicity: score 2.0
Although EQ itself may have low intrinsic toxicity, the lack of data on toxicity of the various transformation products of EQ makes it difficult to evaluate a toxicity score for EQ. However, the lack of data and subsequent uncertainty warrants a conservative approach.

Exposure: score 2.0
Due to lack of concentration data in most food items except fish, no estimates of consumer exposure to EQ could be made by EFSA (2015). However, Lundebye et al. (2010) showed that if the EQ dimer (EQDM) was included in the exposure calculation, the combined intake of EQ and EQDM from a 300 g portion of Atlantic salmon to a 60 kg person could approach the ADI established by the JMPR for EQ.

Vulnerable groups: score 0.5
No vulnerable groups were identified by EFSA (2015). However, the lack of data and subsequent uncertainty warrants a conservative approach.

Lack of toxicity data: score 1.0
Although toxicity data on EQ and to some degree EQDM is available, there is a lack of data on the many transformation products of EQ.

Lack of exposure data: score 1.0
Due to lack of concentration data in most food items except fish, no estimates of consumer exposure to EQ and its transformation products could be made by EFSA (2015).

Total score = 6.5 for ethoxyquin (EQ)

8.4.3.2 *References*

- Commission Implementing Regulation (EU) 2017/962 of 7 June 2017 suspending the authorisation of ethoxyquin as a feed additive for all animal species and categories. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32017R0962 (Accessed 13th of August 2019).
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015. Scientific opinion on the safety and efficacy of ethoxyquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline) for all animal species. EFSA Journal 2015;13(11):4272.
- IMO (International Maritime Organisation), 2014. International Maritime Dangerous Goods Code. IMO Publishing, London, United Kingdom.
- JMPR, 1998. Ethoxyquin. JMPR Evaluations. http://www.inchem.org/documents/jmpr/.

- Merel, S., Regueiro, J., Berntssen, M. H. G., Hannisdal, R., Ørnsrud, R., and Negreira, N. (2019) Identification of ethoxyquin and its transformation products in salmon after controlled dietary exposure via fish feed, Food Chemistry 289.
- Lundebye, A.-K., Hove, H., Måge, A., Bohne, V.J.B., Hamre, K., 2010. Levels of synthetic antioxidants (ethoxyquin, butylated hydroxytoluene and butylated hydroxyanisole) in fish feed and commercially farmed fish. Food Addit. Contam. Part A 27 (12).

9 Ranking of process-induced contaminants

An overview of the scoring and ranking of the included process-induced contaminants is given in Table 9-1. A detailed description follows after the table.

Table 9-1. Summary table for scoring of process-induced contaminants.

Subgroup	Substance	1. MOE/MOS/ADI/TDI/TWI	2. Toxicity	3. Exposure	4. Vulnerable groups	5. Lack of toxicity data	6. Lack of exposure data	Total score	Comments
Acrylamide	Acrylamide	6.0	-	-	1.0	0.5	0.5	8.0	
Esterified 3- and 2-monochloropropane-1,2-diol (MCPD) and glycidyl esters (GEs)	3-Monochloropropane-1,2-diol (3-MCPD) and its fatty acid esters	4.0	-	-	0.5	0.5	0.5	5.5	
	Glycidyl fatty acid esters (GEs)	6.0	-	-	0.5	1.0	0.5	8.0	
Furans	Furan, 2-Methylfuran and 3-Methylfuran	6.0	-	-	0.5	1.0	1.0	8.5	Lack of occurrence data
Heterocyclic amines (HAAs)	2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)	-	3.0	2.0	0.5	1.0	0.5	7.0	Lack of toxicity data on other endpoints than mutagenicity, genotoxicity and carcinogenicity. Lack of data on intake in Norway,
	HAAs in general	-	3.0	2.0	0.5	1.0	0.5	7.0	

									especially considering preparation methods and doneness
Polycyclic aromatic hydrocarbons (PAH)		4.0	-	-	1.0	0.5	0.5	6.0	

9.1 Acrylamide

Acrylamide is a low molecular weight, water-soluble organic chemical formed in carbohydrate-rich foods from naturally present carbohydrates (reducing sugars) and amino acids (asparagine) during cooking or other heat processing. It is in addition a widely used industrial chemical and is also formed in tobacco smoke.

9.1.1 Scores

MOE/MOS/ADI/TDI/TWI: score 6.0

Acrylamide is classified by IARC as a Group 2A probable human carcinogen. The MOE values for neoplastic effects reported for European adolescents and adults ranged from 189-425 for mean exposure and from 85-213 for 95-percentile exposure (EFSA, 2015). EFSA concluded that the MOE values across all age groups were substantially lower than 10,000, indicating a health concern. Likewise, the estimated acrylamide exposures in Norwegian adolescents and adults were within the exposure range for the corresponding European age groups. Similar results were also found for children. VKM reached the same conclusion as EFSA, which is that the MOE values across all age groups were lower than 10,000 and therefore indicating a health concern (EFSA, 2015).

For non-neoplastic effects of dietary acrylamide exposure, the MOE values across all age groups indicate no health concern for average or 95-percentile exposure neither in EU nor in Norway. However, in the 1-year-old Norwegian toddlers the MOE value of the 95-percentile exposure was close to 125.

Vulnerable groups: score 1.0

Children had the highest exposure, but all groups had MOE values <10,000.

Lack of toxicity data: score 0.5

The toxicity is well characterised, the genotoxicity of acrylamide, as well as of its reactive metabolite epoxide glycidamide, has been studied extensively. However, there is a lack of data on developmental outcomes.

Lack of exposure data: score 0.5

There is no information on acrylamide concentrations in home-cooked meals. The information on acrylamide concentrations in a new type of crisp bread and biscuits is scarce and need updating. The EU commission has set recommendations for maximum permitted levels of acrylamide in several food products (EU, 2017).

Total score = 8.0 for acrylamide

9.1.2 References

- EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2015. Scientific Opinion on acrylamide in food. EFSA Journal 2015;13(6):4104, 321 pp. doi:10.2903/j.efsa.2015.410.
- EU (2017). COMMISSION REGULATION (EU) 2017/2158 of 20 November 2017 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32017R2158.
- VKM (2015). Risk assessment of dietary exposure to acrylamide in the Norwegian population. Opinion of the Panel on Contaminants, ISBN: 978-82-8259-187-4, Oslo, Norway. https://vkm.no/download/18.2994e95b15cc5450716151db/1498142208319/40af783860.pdf.

9.2 Esterified 3- and 2-monochloropropane-1,2-diol (MCPD) and glycidyl esters (GEs)

3-MCPD is a kidney toxicant and at somewhat higher concentration decrease sperm motility. EFSA has established a TDI of 2 µg/kg bw per day for 3-MCPD and its fatty acid esters (EFSA, 2018).

9.2.1 Monochloropropane-1,2-diol (3-MCPD) and its fatty acid esters

9.2.1.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 4.0
In European surveys, the TDI is not exceeded in the adult population. A slight exceedance of the TDI was observed in the high consumers of the younger age groups and in particular in the scenarios on infants receiving formula only.

Vulnerable groups: score 0.5
Infants consuming formula only may exceed the TDI.

Lack of toxicity data: score 0.5
Lack of data on developmental and neurodevelopmental effects and chronic studies on male reproductive toxicity and fertility.

Lack of exposure data: score 0.5
Lack of occurrence data, exposure likely underestimated. There are maximum limits (MLs) set by the EU-commission for 3-MCPD in hydrolysed vegetable protein and soy sauce (EU, 2018).

Total score = 5.5 for monochloropropane-1,2-diol (3-MCPD) and its fatty acid esters

9.2.1.2 ***References***

- EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen HK, Alexander J, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L, Grasl-Kraupp B, Hoogenboom LR, Nebbia CS, Oswald IP, Petersen A, Rose M, Roudot A-C, Schwerdtle T, Vleminckx C, Vollmer G, Wallace H, Lampen A, Morris I, Piersma A, Schrenk D, Binaglia M, Levorato S and Hogstrand C (2018). Scientific Opinion on the update of the risk assessment on 3-monochloropropane diol and its fatty acid esters. EFSA Journal 2018;16(1):5083, 48 pp. https://doi.org/10.2903/j.efsa.2018.5083.
- EU (2018). COMMISSION REGULATION (EU) 2018/290 of 26 February 2018 amending Regulation (EC) No 1881/2006 as regards maximum levels of glycidyl fatty acid esters in vegetable oils and fats, infant formula, follow-on formula and foods for special medical purposes intended for infants and young children. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018R0290.

9.2.2 Glycidyl fatty acid esters (GEs)

Glycidyl esters (GEs) are formed during high temperature processing of fats and oils (200°C) and is converted to glycidol following ingestion. Glycidol is genotoxic and carcinogenic.

9.2.2.1 ***Scores***

MOE/MOS/ADI/TDI/TWI: score 6.0
The MOE values for mean exposure were 11,300-10,200 across age groups and surveys, and 4,900-51,000 at 95-percentile exposure. An exposure scenario for infants receiving formula only resulted in MOE values of 5,500 (mean) and 2,100 (95-percentile). MOE values of 25,000 or higher were considered of low health concern.

Vulnerable groups: score 0.5
Infants consuming formula only, and children consuming marine oil supplements.

Lack of toxicity data: score 1.0
More extensive testing of the dose-response for carcinogenesis from chronic lifetime oral administration of glycidol and its esters in rats would reduce uncertainty in the risk assessment.

Lack of exposure data: score 0.5
Data on GEs in refined fish oil is lacking (Norway-specific concern). Impact on exposure unknown.

Total score = 8.0 for glycidyl fatty acid esters (GEs)

9.2.2.2 *References*

- EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen HK, Alexander J, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L, Grasl-Kraupp B, Hoogenboom LR, Nebbia CS, Oswald IP, Petersen A, Rose M, Roudot A-C, Schwerdtle T, Vleminckx C, Vollmer G, Wallace H, Lampen A, Morris I, Piersma A, Schrenk D, Binaglia M, Levorato S and Hogstrand C (2018). Scientific Opinion on the update of the risk assessment on 3-monochloropropane diol and its fatty acid esters. EFSA Journal 2018;16(1):5083, 48 pp. https://doi.org/10.2903/j.efsa.2018.5083.
- EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain) (2016). Scientific opinion on the risks for human health related to the presence of 3- and 2-monochloropropanediol(MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food. EFSA Journal 2016;14(5):4426. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2016.4426.
- EU (2018). COMMISSION REGULATION (EU) 2018/290 of 26 February 2018 amending Regulation (EC) No 1881/2006 as regards maximum levels of glycidyl fatty acid esters in vegetable oils and fats, infant formula, follow-on formula and foods for special medical purposes intended for infants and young children. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018R0290.

9.3 Subgroup furans

Furan has been found in a number of foods such as coffee, canned and jarred foods, including baby food containing meat and various vegetables.

9.3.1 Scores

MOE/MOS/ADI/TDI/TWI: 6.0

In 2012, VKM concluded that the exposure to furan in all age groups, particularly among infants and children, is of health concern. EFSA came to similar conclusion in 2017 (EFSA, 2017).

Furan is hepatotoxic in rats and mice. Cholangiofibrosis in rats and hepatocellular adenomas/carcinomas in mice are the most prominent effects. The reactive furan metabolite cis-but-2-ene-1,4-dialdehyde (BDA) binds covalently to amino acids, proteins and DNA.

The evidence of chromosomal damage *in vivo* is limited, and the mechanism is poorly understood. There is evidence for indirect mechanisms involved in carcinogenesis, including oxidative stress, gene expression alterations, epigenetic changes, inflammation and increased cell proliferation.

The most exposed group is infants, mainly through consumption of ready-to-eat jarred or canned foods. Exposure in other population groups is mainly from consumption of grain-based foods and coffee, depending on age and consumer habits.

Vulnerable groups: 0.5
The most exposed group is infants.

Lack of toxicity data: 1.0
Due to lack of toxicity data, a TDI has not been established.

Lack of exposure data: 1.0
Occurrence data are needed.

Total score = 8.5 for furans

9.3.2 References

- EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen HK, Alexander J, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, Nebbia CS, Oswald IP, Petersen A, Rose M, Roudot A-C, Schwerdtle T, Vleminckx C, Vollmer G, Chipman K, De Meulenaer B, Dinovi M, Mennes W, Schlatter J, Schrenk D, Baert K, Dujardin B and Wallace H (2017). Scientific opinion on the risks for public health related to the presence of furan and methylfurans in food. EFSA Journal 2017;15(10):5005,142 pp.https://doi.org/10.2903/j.efsa.2017.5005.
- VKM (2012). Risk assessment of furan exposure in the Norwegian population. Opinion of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics and the Panel on Contaminants of the Norwegian Scientific Committee for Food Safety. https://vkm.no/download/18.175083d415c86c573b5d8007/1500742472221/7b023a9623.pdf.

9.4 Subgroup heterocyclic aromatic amines (HAAs)

HAAs are a family of heat-induced food toxicants that was discovered about 30 years ago by Professor Sugimura. Currently, about 25 HAAs have been identified in cooked meat, fish, and poultry products as well as in cigarette smoke and diesel exhaust. HAAs can be divided into two distinct families: aminoimidazoazaarenes, and carbolines or pyrolytic HAAs. Aminoimidazoazaarenes are formed by Maillard reaction (a chemical reaction between amino acids, creatine/creatinine and sugars), whereas carbolines and pyrolytic HAAs are formed at elevated temperatures. The main source of human exposure to HAAs is via cooked proteinaceous foods, however, the levels of HAAs are highly dependent on the type of meat, cooking time and cooking temperature, and generally increase with the level of «doneness». The cooking method also influences HAA formation; it has been shown that high-temperature methods (pan-frying, grilling and barbecuing) cause the highest HAA concentrations, especially for 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) (IARC, 2015).

Among the HAA, 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethyl-imidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), PhIP, 3-amino-1,4-dimethyl-5*H*- pyrido[4,3-*b*]indole (Trp-P-1), 3-amino-1-meth- yl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2), 2-amino9*H*-pyrido[2,3-*b*]indole (AαC), 2-amino-3- methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC) and 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx) have been found in cooked red meat and processed meat (IARC, 2015). With the exception of 4,8-DiMeIQx, which was never evaluated, these HAAs have been evaluated by the IARC Monographs as having sufficient evidence of carcinogenicity in experimental animals (IARC, 1983; 1986; 1993; 2015).

9.4.1 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) (CAS no. 105650-23-5)

9.4.1.1 *Scores*

MOE/MOS/ADI/TDI/TWI: score 6.0

Nationwide food consumption surveys do not present questions on food preparation methods, and consumption of barbequed food in the general population is unknown. VKM (2007) performed worst-case estimates of exposure to one HAA (PhIP). These show estimated daily intake of 27 ng PhIP/kg bw from barbequed and fried food, of which 8 ng/kg bw came from barbequed meat. This exposure estimate is associated with a high uncertainty (VKM, 2007).

Based on the worst-case exposure calculations of an intake of 30 grilled meals per year, a high PhIP content in the grilled food and results from animal experiments, an intake of 8 ng/kg bw per day from grilled food alone was estimated, resulting in a MOE value of 250,000. When including the contribution of PhIP from fried foods, the calculated MOE value is approximately 75,000 for PhIP. The calculation is associated with high uncertainty, but the size of the MOE indicated that lower exposure than those found in the worst-case calculations is desirable. This support findings in epidemiological studies (VKM, 2007). The VKM Panels are of the opinion that based on the uncertainties related to these calculations, MOE should be approximately 100,000 for PhIP in order to give sufficient protection. Potential mixture effects of other HAA present simultaneous with PhIP were not taken into consideration (VKM, 2007). Studies indicate that PhIP may be more potent in humans than in rats (VKM, 2007).

Toxicity: score 3.0

After metabolic activation with S-9 mix, HAAs can be assigned to the group of the most strongly mutagenic compounds. PhIP is mutagenic in Ames test, binds to DNA and forms adducts (dG-C8-PhIP). It is the most genotoxic HAA in mammalian cells *in vitro.* PhIP induces cancer in many organs in experimental animals (colon, small intestine, appendix, breast, prostate and lymphomas), depending on species, strain and gender.

Based on animal experiments, IARC has classified PhIP as a probable carcinogen (class2B) (IARC, 1993), reported in Gibis (2016).

Exposure: score 2.0

The HAAs occurring most often in meat are PhIP, MeIQx, 4,8-DiMeIQx, IQ, MeIQ and AαC (Gibis, 2016). PhIP is the HAA which is present in highest concentrations when meat or fish are fried at normal temperature, up to 480 ng/g (in grilled chicken). Dietary intake of the three most abundant HAAs was considered by IARC (2015). Crude correlation coefficients of PhIP intake, assessed using food frequency questionnaires (FFQ) and food diaries, were 0.22 (95% CI, 0.07-0.36) for PhIP intake.

The information about daily HAA intake, including of PhIP, can vary substantially among epidemiological studies. Alongside different eating habits of people, the type of preparation and the frequency of meat consumption also play an important role (Gibis, 2016).

Vulnerable groups: score 0.5
Persons with a very high intake of meat, especially read meat (fried, grilled or barbequed), and who eat the meat well done, will have a high exposure to PhIP, and are therefore vulnerable to the adverse effects of PhIP. In addition, persons with high activity of the metabolic enzymes, both phase I (CYP1A1, CYP1A2 and CYP1B1) and phase II (NAT, SULT, UGT) enzymes, that affect the metabolism of PhIP in the direction of bioactivation rather than detoxification or increased excretion, will be extra vulnerable to the effects of PhIP.

Lack of toxicity data: score 1.0
There are a lot of data available about the mutagenicity, genotoxicity and carcinogenicity of PhIP. However, other endpoints have not been studied equally thoroughly.

Lack of exposure data: score 0.5
The information about daily PhIP intake can be very different in epidemiological studies. Alongside different eating habits of people, the type of preparation and the frequency of meat consumption also play an important role (Gibis, 2016). There is a lack of good data on intake of PhIP in all age groups in Norway, especially taking into consideration the preparation methods and doneness.

Since the estimation of MOE done for PhIP by VKM (2007) is quite uncertain, Q2+Q3 are used instead of Q1.

Total score = 7.0 for 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)

9.4.2 HAAs in general

9.4.2.1 ***Scores***

Toxicity: score 3.0

After metabolic activation with S9 mix, HAAs can be assigned to the group of the most strongly mutagenic compounds. Trp-P-1, Trp-P-2, Glu-P-1, and Glu-P-2 are, after metabolic activation, strong mutagens for the *Salmonella* Typhimurium TA98, TA100 and TA1538 strains used in the Ames test. After the addition of S9 mix, which is necessary for metabolic activation, the IQ compounds MeIQ, IQ, 4,8-DiMeIQx, MeIQx, Glu-P-1 and Trp-P-1 acted as significantly stronger mutagens than AαC, MeAαC and PhIP. The relative mutagenic potentials of MeIQ, MeIQx, and PhIP vary with the test assay. In *Salmonella* Typhimurium strain TA98, the relative potencies were MeIQ > MeIQx > PhIP; however, they were PhIP ≥ MeIQ > MeIQx in a Chinese hamster ovary system or IQ > MeIQ > Trp-P-1 ≥ MeIQx >> PhIP in human-derived hepatoma (HepG2) cells (Gibis, 2016).

The relative mutagenicity is therefore difficult to assess and the effects of intake of certain amounts of HAAs are difficult to predict (Gibis, 2016).

HAA are strong mutagens in Ames test, causing sister chromatide exchanges and chromosomal aberrations in mammalian cells, and all the 10 substances studied so far induce cancer in several organs in rodents, i.a. in the intestines, liver and breast. IQ is also a potent liver carcinogen in monkeys (VKM, 2007).

Based on animalexperiments, IARC has classified IQ as a possible carcinogen (class 2A) and 8 other HAAs (MeIQ, MeIQx, PhIP, AαAC, MeAαAC, Trp-P-1, Trp-P-2 and Glu-P-2) as probable carcinogens (class 2B) (IARC, 1993), reported in Gibis (2016).

Exposure: score 2.0
As they are genotoxic, even small amounts are mutagenic and carcinogenic. Intake will vary a lot depending on intake of meat and the preparation methods.

The HAAs occurring most often in meat are PhIP, MeIQx, 4,8-DiMeIQx, IQ, MeIQ and AαC (Gibis, 2016).

The estimated average daily HAA intake based on intake of PhIP, MeIQx, DiMeIQx, IQ and AαC in USA was determined to be 26 ng/kg bw per day, and was roughly estimated to be 420 ng/day per person in another study in USA. In New Zealand, a total HAA intake of 164 ng/day per person was calculated in one study and around 1000 ng/day per person in another study. The mean daily HAA intake was 103 ng/day per person in a German study. From various meat and fish dishes from restaurants, HAA intake in Switzerland was calculated to be up to 400 ng/day per person. The HAA intake in Spain at 606 ng/day per person is clearly higher in comparison, which can be explained by the preparation of meat, such as grilling, barbecuing and panfrying, and the highest frequency of meat consumption in Europe. Using a questionnaire, a mean HAA intake of 160 ng/day per person was found in a study in Sweden. All these studies were referenced in Gibis (2016).

Dietary intake of the three most abundant HAAs was considered: MeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*] quinoxaline (4,8-DiMeIQx) and PhIP. Crude correlation coefficients of

HAA intake, assessed using the FFQ and food diaries, were 0.43 (95% confidence interval, CI, 0.30-0.55) for MeIQx intake and 0.22 (95% CI, 0.07-0.36) for PhIP intake (IARC, 2015).

The information about daily HAA intake can be very different in epidemiological studies. Alongside different eating habits of people, the type of preparation and the frequency of meat consumption also play an important role (Gibis, 2016).

Vulnerable groups: score 0.5
Persons with a very high intake of meat, especially read meat (fried, grilled or barbequed), and who eat the meat well done, will have a high exposure to HAAs, and are therefore vulnerable to the adverse effects of HAAs. In addition, persons with high activity of the metabolic enzymes, both phase I (CYP1A1, CYP1A2 and CYP1B1) and phase II (NAT, SULT, UGT), that affect the metabolism of HAAs in the direction of bioactivation rather than detoxification or increased excretion, will be extra vulnerable to the effects of these substances.

Lack of toxicity data: score 1.0
There are a lot of data available about the mutagenicity, genotoxicity and carcinogenicity of HAAs. However, other endpoints have not been studied equally thoroughly.

Lack of exposure data: score 0.5
The information about daily HAA intake can be very different in epidemiological studies. Alongside different eating habits of people, the type of preparation and the frequency of meat consumption also play an important role (Gibis, 2016). There are a lack of good data on intake of HAAs in all age groups in Norway, especially taking into consideration the preparation methods and doneness.

Total score = 7.0 for HAAs in general

9.4.3 References

- Gibis M (2016). Heterocyclic aromatic amines in cooked meat products: causes, formation, occurrence, and risk assessment. Comprehensive Reviews in Food Science and Food Safety, 15, 269-302. URL: https://onlinelibrary.wiley.com/doi/epdf/10.1111/1541-4337.12186.
- IARC (International Agency for Research on Cancer) (2015). Red meat and processed meat. IARC Monograph, Volume 114, ISBN 978-92-832-0180-9, ISSN 1017-1606, Lyon, France. URL: https://monographs.iarc.fr/wp-content/uploads/2018/06/mono114.pdf.
- VKM (2007). Vurdering av helserisiko ved konsum av grillet mat. Uttalelse fra Faggruppen for forurensninger, naturlige toksiner og medisinrester i matkjeden og Faggruppen for tilsetningsstoffer, aroma, matemballasje og kosmetikk. 06/505-9-endelig. URL: https://vkm.no/download/18.d44969415d027c43cf13d2b/1501076859247/dccfd1091c.pdf (In Norwegian).

9.5 Subgroup polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are a large group of chemicals consisting of two or more fused aromatic rings. The main sources are incomplete combustion of organic materials and industrial processes, and several hundred PAHs have been described. Humans may be exposed to PAHs via several exposure routes, but for the non-smoking general population food is the main route of exposure. EFSA evaluated PAHs in food in 2008.

PAHs are regarded as carcinogenic and genotoxic, but there are differences in potency and bioavailability. EFSA also concluded that a toxic equivalency factor (TEF) approach was not scientifically justified due to differences in mode of action, lack of rat carcinogenicity studies for several of the relevant compounds and evidence of poor predictivity of the carcinogenic effects of mixtures based on the proposed TEF values (EFSA, 2008).

9.5.1 Scores

MOE/MOS/ADI/TDI/TWI: score 4.0
Genotoxic compounds, MOE values were >10,000 for mean consumers. High level consumers had MOE values ranging from 9,600 to 10,800. These MOE values indicates low risk (EFSA, 2008).

Vulnerable groups: score 1.0
Increased exposure for people consuming food types with higher PAH concentrations such as mussels from contaminated waters (VKM, 2011), grilled meat, food prepared using fire etc.

Lack of toxicity data: score 0.5
PAHs comprise many compounds for which variable toxicity data are available. The exposure to PAHs is largely as mixtures, and the carcinogenic effects of the mixtures have not been predicted by current models of TEF factors.

Lack of exposure data: score 0.5
Limited data on consumption of food prepared on fire, grilled food, mussels from contaminated areas etc.

Total score = 6.0 for polycyclic aromatic hydrocarbons (PAHs)

9.5.2 References

- EFSA (2008). Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on Polycyclic Aromatic Hydrocarbons in Food. The EFSA Journal (2008) 724, 1-114.
- VKM (2011). Forhold mellom BaP og PAH4 i skjell og konsekvenser for gjeldende kostholdsråd i Norge. Uttalelse fra Faggruppen for forurensninger, naturlige toksiner og medisinrester. https://vkm.no/download/18.59777ce315d3abb2351c0c82/1501508647500/f630899d2e.pdf.

- VKM 2007. Vurdering av helserisiko ved konsum av grillet mat. Uttalelse fra Faggruppen for forurensninger, naturlige toksiner og medisinrester i matkjeden og Faggruppen for tilsetningsstoffer, aroma, matemballasje og kosmetikk. https://vkm.no/download/18.d44969415d027c43cf13d2b/1501076859247/dccfd1091c.pdf.

10 Ranking of «other substances»

An overview of the scoring and ranking of the included «other substances» is given in Table 10-1. A detailed description follows after the table.

Table 10-1. Summary table for the scoring of «other substances».

Substance	1. MOE/ADI/ TDI/TWI	2. Toxicity	3. Exposure	4. Vulnerable groups	5. Lack of toxicity data	6. Lack of exposure data	Total score	Comments
L-Aspartic acid	-	2.0	1.0	0.5	0.5	0.5	4.5	Lack of Norwegian exposure data. Lack of long-term studies in children, adolescents or adults, and pregnant/lactating women
L-Carnitine and L-Carnithine-L-tartrate	2.0	-	-	1.0	0.5	0.5	4.0	Lack of Norwegian exposure data. Some lack of toxicity data. Persons with kidney diease and high plasma levels of trimethylamine (TMA) and trimethylamine-N-oxide (TMAO) are vulnerable
Coenzyme Q10 (CoQ10)	-	1.0	1.0	0.0	0.5	0.5	3.0	Lack of Norwegian exposure data. Lack of toxicity data for children, adolescents, pregnant and lactating women
Conjugated linoleic acids (CLAs)	2.0	-	-	1.0	0.5	0.5	4.0	Lack of good exposure data. Lack of studies in children, adolescents or pregnant/lactating women or elderly. May cause reduced milk production and reduced content of milk fat. Obese men with the metabolic syndrome may be vulnerable

Substance	1. MOE/ADI/ TDI/TWI	2. Toxicity	3. Exposure	4. Vulnerable groups	5. Lack of toxicity data	6. Lack of exposure data	Total score	Comments
Creatine	2.0	-	-	0.5	0.5	0.5	3.5	Lack of Norwegian exposure data. Lack of long-term studies in children, adolescents or adults, and pregnant/lactating women. Persons with impaired renal function may be vulnerable
Curcumin	4.0	-	-	1.0	0.5	0.5	6.0	Exposure may exceed ADI. Lack of Norwegian exposure data. Little or lack of toxicity data for children, adolescents, pregnant and lactating women. Patients undergoing chemotherapy, with gallstones, liver disease and hepatitis C are vulnerable
L-Cysteine and L-Cystine	-	1.0	1.0	0.5	0.5	0.5	3.5	Lack of Norwegian exposure data. Lack of studies in children, adolescents or pregnant/lactating women. May enhance effects of nitroglycerin and isosorbide, used for angina pectoris. May form kidney stones in persons with hereditary cystinuria
Docosahexaenoic acid (DHA)	4.0	-	-	0.0	1.0	0.5	5.5	Lack of Norwegian exposure data. Lack of randomised studies in children, adolescents or pregnant women

Substance	1. MOE/ADI/ TDI/TWI	2. Toxicity	3. Exposure	4. Vulnerable groups	5. Lack of toxicity data	6. Lack of exposure data	Total score	Comments
Docosapentaenoic acid (DPA)	-	1.0	1.0	0.0	0.5	0.5	3.0	Lack of Norwegian exposure data. Lack of randomised studies in children, adolescents or pregnant/lactating women
D-Glucurono-γ-lactone	-	1.0	1.0	0.0	0.5	0.5	3.0	Lack of Norwegian exposure data. Lack of toxicity data for many endpoints, and for all age groups
Eicosapentaenoic acid (EPA)	2.0			0.0	0.5	0.5	3.0	Lack of Norwegian exposure data. Lack of randomised studies in children, adolescents or pregnant women
Inositol	-	1.0	1.0	0.5	0.5	0.5	3.5	Lack of Norwegian exposure data. Lack of toxicity data for many endpoints and population/age groups. Patients with diabetes and kidney disorders such as chronic renal failure are vulnerable
Lycopene	4.0	-	-	1.0	0.5	0.5	6.0	Exposure may approach ADI. Studies on preterm labour, low birth weight etc. are needed. Lack of Norwegian exposure data

Substance	1. MOE/ADI/ TDI/TWI	2. Toxicity	3. Exposure	4. Vulnerable groups	5. Lack of toxicity data	6. Lack of exposure data	Total score	Comments
L-Methionine	-	1.0	1.0	0.5	1.0	0.5	4.0	Lack of Norwegian exposure data. Dose-response studies are needed, both animal and human. Patients with the deficiency of cystathionine b-synthase (homozygote form) are vulnerable
Piperine	-	2.0	1.0	0.5	0.5	0.5	4.5	Lack of Norwegian exposure data. Lack of chronic toxicity data and studies in children, adolescents, pregnant or lactating women. Interaction with drugs (e.g. cancer treatment and chemotherapy)
Taurine	-	1.0	1.0	0.0	0.5	0.5	3.0	Lack of Norwegian exposure data. Lack of chronic and carcinogenicity data
L-Tyrosine		1.0	2.0	0.0	1.0	0.5	4.5	Lack of Norwegian exposure data. Lack of studies in children, adolescents or pregnant/lactating women

«Other substances» are defined as substances with nutritional and/or physiological effect, and which are not vitamins or minerals (EU, 2002). They are active substances used in food supplements and energy drinks. «Other substances» are largely unregulated at the EU level, and therefore national regulations are needed. VKM was requested by the NFSA during 2015-2017 to perform risk assessments of in total 44 «other substances» (VKM, 2017). These risk assessments were based on methodology established by VKM for evaluation of these substances, for which sufficient toxicity data are often lacking. The methodology used is described in a separate document (VKM, 2015). The recommended doses of the substances in products as sold on the Norwegian market and given by the industry (producers and/or importers) were evaluated for safety, using information of adverse health effects from previous risk assessments from EFSA and similar institutions, and scientific publications found by new literature searches. In these assessments, VKM should only assess specific doses and concentrations (one or several) of these substances used in food supplements and energy drinks, thus, these assessments are not regular risk assessments of all doses, or aimed at deciding tolerable upper levels. VKM should only assess potential negative health effects, not beneficial effects, and should evaluate the substances as single substances, not as mixtures. Further, it should not be taken into consideration whether the substances could be found in other sources such as foods, drinks or cosmetics, or were formed endogenously in the body. A number of such «other substances» were found to be of potential health risk for one or several groups of consumers (among adult men and women, adolescents and children down to the age of 10 years (food supplements) or 3 years (energy drinks)) in one or several of the evaluated doses.

In this ranking of chemicals, «other substances» are included for those substances where especially little relevant toxicity data were available from humans and/or experimental animals, and/or the risk assessments showed a potential health risk for one or several age groups in the Norwegian population at the evaluated doses.

General references

- EU (2002). DIRECTIVE 2002/46/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32002L0046&from=EN.
- VKM (2017). Risikovurderinger av «andre stoffer» i kosttilskudd og energidrikker. https://vkm.no/risikovurderinger/allevurderinger/risikovurderingeravandrestofferikosttilskuddogenergidrikker.4.645b840415d03a2fe8f256aa.html (In Norwegian).
- VKM (2015). General principles for the risk assessments of "other substances" in food supplements and energy drinks Report of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics and the Panel on Nutrition, Dietetic Products, Novel Food and Allergy of the Norwegian Scientific Committee for Food Safety. https://vkm.no/download/18.645b840415d03a2fe8f25c37/1499326301370/a75fd54bf8.pdf.

10.1 L-Aspartic acid (CAS no. 56-84-8)

10.1.1 Scores

MOE/MOS/ADI/TDI/TWI (background information)

Using a safe level of 7 mg/kg bw per day set by VKM and a mean intake from food and supplements of 93 mg/kg bw per day, the exposure exceeds the safe level by a factor 13.

Toxicity: score 2.0
Literature searches including both human and animal studies have been conducted, in addition to reviewing previous reports (IOM, 2005; VKM, 2011). According to IOM (2005), all human and animal studies on the effects of aspartic acid were of short duration and there was a lack of dose-response data. IOM (2005) therefore concluded that there are not sufficient scientific data to establish an UL for aspartic acid. IOM (2005) noted that dietary supplement doses of up to 8 g/day (approximately 120 mg/kg bw per day) had not resulted in any documented adverse effects, however, no reference was provided for this statement.

Based on the systematic literature searches, VKM did not identify any long-term studies in healthy individuals that could be used for this risk assessment. In rats, a 90-day subchronic toxicity study by Tada et al. (2008) reported a NOAEL of 697 mg/kg bw per day in males and 715 mg/kg bw per day in females. A LOAEL was identified at 1,400 mg/kg bw day with toxic effects on the kidneys (regenerative renal tubules dilation accompanied by inflammatory cell infiltration) and acinar cell hypertrophy of salivary glands.

In summary, the following information is considered in the current assessment:

Short-term human studies found no adverse health effect when L-aspartic acid was given in acute doses ranging from 1 to 10 g for time periods between one single dose and four weeks. These studies were however not designed to assess toxicity of L-aspartic acid.

Administration of large quantities of L-aspartic acid to newborn mice has produced a variety of neurotoxic effects, the most marked of which was neuronal necrosis. Neurotoxic effects of dicarboxylic amino acids in animal species other than newborn rodents are highly controversial, and the available data indicate little relevance for humans.

A 90-day subchronic toxicity study in rats, with a NOAEL of 697 mg/kg bw per day in males and 715 mg/kg bw per day in females found no neurotoxicity, however, toxic effects on kidneys and possibly salivary glands were observed at 1,400 mg/kg bw per day (LOAEL).

For the risk characterisation, the NOAEL of 697 mg/kg bw per day derived from the abovementioned subchronic toxicity study in rats is used for comparison with the estimated exposures from food supplements. Using an UF = 100, a safe level is 7 mg/kg bw per day.

Exposure: score 1.0

Based on the NHANES III (1988-1994), the overall mean intake of L-aspartic acid from food and food supplements in the United States was 6.5 g/day (IOM, 2005), which is 93 mg/kg bw per day for a 70 kg person. Men 31 through 50 years of age had the highest intake at the 99th percentile of 15.4 g/day.

Vulnerable groups: score 0.5
Neonatal rodents are sensitive to the consumption of supplemental dicarboxylic amino acids since they lack the ability to metabolise the dicarboxylic amino acids (Stegink, 1976). The newborn rodent is particularly susceptible to brain lesions, and other dietary substances such as salt and sucrose have also produced brain lesions (Stegink, 1976). Administration of large quantities of glutamate and L-aspartic acid to newborn mice produces a variety of neurotoxic effects, the most marked of which is neuronal necrosis. This finding has, however, not been reproduced in neonatal nonhuman primates by a number of other scientists when giving either glutamate or aspartame at high dosages (EFSA, 2013). However, due to lack of long term studies on L-aspartic acid intake and possible negative health effects in humans, IOM (2005) concluded that aspartic acid dietary supplements are not advisable for infants and pregnant women. Neurotoxic effects of dicarboxylic amino acids in animal species other than newborn rodents are highly controversial, and the available data indicate little relevance to humans. In the present literature review, no studies with L-aspartic acid in children were found. There are no data indicating that children and adolescents are more vulnerable than adults for L-aspartic acid.

Lack of toxicity data: score 0.5
No long-term studies on L-aspartic acid in healthy children, adolescents or adult humans were found.

There are few toxicological studies in animals where L-aspartic acid is provided as a single supplement and with an appropriate study design to investigate possible long-term adverse effects.

Lack of exposure data: score 0.5
There are no data available concerning dietary intake of L-aspartic acid in Norway.

Total score = 4.5 for L-aspartic acid

10.1.2 References

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10.2 L-Carnithine (CAS no. 541-15-1) and L-Carnithine-L-tartrate (CAS no. 36687-82-8)

10.2.1 Scores

MOE/MOS/ADI/TDI/TWI: score 2.0

29 mg/kg bw per day of L-carnitine was considered as a safe level and exposure from food was 2.9 mg/kg bw per day. Therefore, exposure was below the safe level. However, there are also contributions from endogenous synthesis and potentially from use of cosmetics and supplements.

Toxicity (background information)

EFSA (2003) established a human tolerance level of L-carnitine-L-tartrate up to 3 g/day (43 mg/kg bw per day), with respect to gastrointestinal symptoms, hematology and clinical chemistry, including markers of liver and kidney function. This is equivalent to 2 g/day (29 mg/kg bw per day) L-carnitine in healthy adults. A safety factor for interindividual variation was not included in the established value. Further, this value was based on few studies of which all but one were unavailable to VKM. The EFSA Opinion on L-carnitine-L-tartrate (EFSA, 2003) referred to five human tolerance studies of L-carnitine and L-carnitine-L-tartrate. Only one of these studies (Rubin et al., 2001) was described in some detail and was available to VKM. However, the study size was small (n=10) and the duration was short (3 weeks). An ADI based on animal studies was identified for tartaric acid of 0-30 mg/kg bw per day. These values (29 mg/kg bw per day L-carnitine, 43 mg/kg bw per day L-carnitine-L-tartrate and 30 mg/kg bw per day tartaric acid) are regarded as safe levels.

The available data indicated that L-carnitine-L-tartrate was not mutagenic.

Exposure (background information)

L-carnitine: Mean intake from food (not feed-supplemented animals) is 100-300 mg/day (Feller and Rudman, 1988, in EFSA, 2003). This will give 1.4-2.9 mg/kg bw per day for a 70 kg person. The highest intake is for high meat consumption. A newer range for human dietary intake has been provided by Rebouche (2004): <0.2 to 2.4 mg/kg bw per day (14–168 mg/day for a 70 kg adult). L-carnitine is endogenously synthesised from the amino acids lysine and methionine.

Although L-carnitine and L-carnitine-L-tartrate are used as supplements in animal food, EFSA concluded that typical supplementation of feed would not substantially increase human

exposure to carnitine from food of animal origin (EFSA, 2012). Further, EFSA (2012) concluded that as the absorption rate declines with increasing L-carnitine intake, the endogenous carnitine pool may not significantly increase.

L-carnitine (equivalents) and L-carnitine-L-tartrate are listed as ingredients in various cosmetic products, such as hair conditioners (CosIng, 2015). Adolescents and adults are likely to be exposed.

Tartaric acid: L-tartaric acid occurs naturally in fruits and wine (120-180 mg/100 ml) and L-tartaric acid and its salts are approved as food additives (typically used in baking powder, biscuits and jam) (EFSA, 2003).
Neonates, infants and young children can be exposed to L-carnitine and L-carnitine-L-tartrate through foods for particular nutritional uses. Examples of such foods are infant formulae milk (for neonates and infants), follow-on formulae milk (infants), cereal-based food and other baby foods (for infants and young children (toddlers)) (EFSA, 2003).

Vulnerable groups: score 1.0
Adverse effects of L-carnitine (-L-tartrate) are occasionally observed in vulnerable groups such as in patients with kidney disease and persons with high plasma values of trimethylamine (TMA) and trimethylamine-N-oxide (TMAO). High plasma L-carnitine levels in subjects with concurrently high TMAO levels have been associated with cardiovascular disease and adverse cardiac events in patients undergoing cardiac evaluation. Adverse effects are suspected in patients with inborn errors of metabolism. Further, interactions with certain types of drugs have been reported.

Lack of toxicity data: score 0.5
There were few human studies on adverse health effects related to L-carnitine and L-carnitine-L-tartrate, of which three were randomized controlled trials (RCTs). However, they were specifically designed to investigate the positive effects (such as in patients with deficiencies) and not negative effects of L-carnitine and L-carnitine-L- tartrate. Adverse effects may not always be recorded and if they are, they may not be properly diagnosed. Both benefit studies and the few studies on negative health effects related to L-carnitine and L-carnitine-L-tartrate in adults have high heterogeneity both in design and participant characteristics. The few studies that included children and adolescents were of relatively short duration, and have accordingly inherent uncertainty in extrapolating to long-term supplementation in these age groups. No tolerance level is set for L-carnitine or L-carnitine-L-tartrate specifically for children or adolescents. No studies are found on effects of these substances in lactating or pregnant women. There is lack of acute, sub-chronic and chronic toxicity studies of L-carnitine and L-carnitine-L-tartrate in animals.

Lack of exposure data: score 0.5
There are no data available on exposure to L-carnitine and L-carnitine-L-tartrate from Norway.

Total score = 4.0 for L-carnithine and L-carnithine-L-tartrate

10.2.2 References

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10.3 Coenzyme Q10 (CoQ10) (CAS no. 303-98-0)

10.3.1 Scores

MOE/MOS/ADI/TDI/TWI (background information)
12.9 mg/kg bw per day of CoQ10 was considered as a safe level by VKM and exposure from food was 0.04 – 0.09 mg/kg bw per day. Therefore, exposure was below the safe level. However, there is also potential contribution from use of cosmetics and supplements.

Toxicity: score 1.0
With regard to mutagenicity and genotoxicity, CoQ10 (in the form of Bio-Quinone) caused no significant changes in mutagenicity and micronucleus formation, $CoQ10H_2$ (the Kaneka QH brand) was evaluated as negative in the bacterial reverse mutation, chromosomal aberration and rat bone marrow micronucleus tests, organically synthesized CoQ10 was considered to possess no mutagenicity and CoQ10 had no genotoxic activities (Fu et al., 2009; Hidaka et al., 2008; Ikeda et al., 2005; Kitano et al., 2007; Yamaguchi et al., 2009).

The human studies on healthy subjects indicated that CoQ10 was well tolerated at doses up to 900 mg per day for 4 weeks. The forms of CoQ10 tested included CoQ10 (the oxidized form), PureSorb-Q™40 (a water soluble type of CoQ10), $CoQ10H_2$ (the reduced form) and

Kaneka Q10TM (over 98% CoQ10). No significant difference in the frequency of adverse effects as compared to placebo was reported (Hosoe et al., 2007; Ikematsu et al., 2006; Nukui et al., 2007). Hathcock and Shao (2006) performed a risk assessment of CoQ10. Using the «observed safe level» or «highest observed intake», Hathcock and Shao (2006) reported that the evidence of safety was strong at intakes of CoQ10 to up 1200 mg/day (together with vitamin E, derived from a clinical trial with a substantial cohort of 80 persons with Parkinson disease and fairly long duration of 16 months and a shorter and smaller clinical trial of 10 subjects with Huntington's disease of 6 months duration) (Hathcock and Shao, 2006; WHO, 2005).

With regard to subchronic toxicity studies, Kitano et al. (2008) reported that conservative NOAEL estimates for $CoQ10H_2$ in Sprague-Dawley strain SPF [Crj:CD(SD)IGS] rats were 600 mg/kg bw per day for males and 200 mg/kg bw per day for females after 13 weeks, based on effects on the liver, and that the NOAEL for $CoQ10H_2$ in male and female beagle dogs (HRA Beagle) was estimated to be more than 600 mg/kg bw per day. Zhipeng et al. (2007) reported that CoQ10 doses up to 3,000 mg/kg per day were well tolerated by Sprague-Dawley rats, and Honda et al. (2007) reported that the NOAEL of CoQ10 for male and female Sprague-Dawley [Crl:CD(SD)] rats was considered to be 1,200 mg/kg bw per day.

With regard to long-term toxicity studies, the lack of adverse effects, including on the liver, of CoQ10 doses up to 1,200 mg/kg per day in Crl:CD(SD)BR VAF/Plus rats for 52 weeks (Williams et al., 1999) and doses up to 1,800 mg/kg per day in beagle dogs (Hazelton Research Animal strain) for 39 weeks (Yerramilli-Rao et al., 2012) indicated the safety of CoQ10. In the chronic toxicity study in rats by Williams et al. (1999), a NOAEL of 1200 mg/kg bw per day was determined.

The values used for comparison with the estimated exposure in the risk characterization are 900 mg/day (corresponding to 12.9 mg/kg bw per day in a 70 kg adult) based on human studies (4 weeks) and the NOAEL of 1,200 mg/kg bw per day based on a chronic toxicity study in rats (52 weeks).

Exposure: score 1.0
Meat and fish are the richest natural food sources of CoQ10. The richest vegetable sources are the oils, and concentrations were found ranging from 100 to 280 mg/kg in soybean, maize and olive oil. Nuts and cereals also contain CoQ10 but in lower quantities (Pravst et al., 2010). CoQ10 obtained from the diet ranges between 3 and 6 mg/day (0.04 – 0.09 mg/kg bw per day for a 70 kg person) in developed countries (AESAN, 2012). CoQ10 is used in several cosmetic products, i.e. in various anti-aging skin creams allegedly due to its antioxidant activity (CosIng, 2015).

Vulnerable groups: score 0.0
There are no known groups vulnerable for the effects of CoQ10.

Lack of toxicity data: score 0.5

There are quite a lot of animal studies on CoQ10, and also some human studies. However, no studies on adverse health effects of CoQ10 in children, adolescents, pregnant women or lactating women were identified.

Lack of exposure data: score 0.5
There are no data available on exposure to CoQ10 from Norway.

Total score = 3.0 for coenzyme Q10 (CoQ10)

10.3.2 References

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10.4 Conjugated linoleic acids (CLAs) (CAS no. 2540-56-9)

10.4.1 Scores

The major natural CLA is cis-9,trans-11-octadecadienoic acid (c9,t11-CLA) which comprises over 90% of the CLAs in ruminant fats. The other major natural CLA isomer is trans-10,cis-12-octadecadienoic acid.

MOE/MOS/ADI/TDI/TWI: score 2.0

Using a safe level of 50 mg/kg bw per day from EFSA (2012) and a dietary intake of 2.4 mg/kg bw per day, the expsoure is below the safe level by a factor of 21.

Toxicity (background information)

Most of the cited studies from the literature searches have tested supplemental CLAs in doses of about 3.5 g/day, ranging from 0.7 to 6 g/day. Many of these have been of short duration, and the intervention periods have been from days and weeks up to 2 years. The study groups have mostly been adults with overweight and obesity.

Only one study in children has been identified. Racine et al. (2010) tested 3 g/day CLA supplement (1:1 mixture of the isomers c9,t11 and t10,c12) on change in body fat and BMI among overweight/obese children in a RCT lasting 7 months. Data of blood chemistry did not reveal any significant differences between the two study groups in the levels of low density

lipoprotein (LDL), liver enzymes, or insulin, or glucose whereas high density lipoprotein (HDL) decreased significantly in the CLA-group only.

Notably, this RCT was conducted on children who were either overweight or obese, and the age-range included was limited (6-10 years). Hence, this RCT alone cannot form the basis for any conclusion about healthy children in general by VKM in the present report.

FHI (2003) concluded that intake of CLA supplements (mainly the t10,c12 isomer) may (i) adversely affect insulin resistance among obese men with the metabolic syndrome, (ii) that use of CLAs by pregnant women may reduce birth weight and birth length of their off-springs, and (iii) that use of CLAs by lactating women may reduce their milk production and the fat content of their milk. These conclusions were supported in the SNT evaluation from 2004 (SNT, 2004).

The EFSA opinions from 2010 concluded that a dose up to 3.0 g per day for up to six months of CLA supplementation was apparently safe for use in adults (EFSA, 2010 a; b). This will be 43 mg/kg bw per day for a 70 kg person. In 2012, EFSA updated the 2010 opinion and additionally included an evaluation of the safety of consuming the CLA-rich supplement Tonalin® TG 80 at a dose of 4.5 g corresponding to 3.5 g per day of CLAs (EFSA, 2012). The EFSA (2012) statement concluded that it was safe to use this supplement for up to six months. In the EFSA (2012) statement there is no information about safety of consumption of CLAs alone; the statement concerns the consumption of CLAs as part of the two products Clarinol® and Tonalin® TG 80. The EFSA (2012) statement concluded that the safety of 3.75 g Clarinol® (corresponding to approximately 3 g CLA) and 4.5 g Tonalin® TG 80 (corresponding to approximately 3.5 g CLA) had been established for these daily doses for up to six months. Additional data reviewed in this VKM report have not invalidated this conclusion.

Concern regarding insulin resistance in obese men with metabolic syndrome was stated in reports from ANSES (2011a; 2011b) that raised concerns about indications of an unfavourable effect on biomarkers of lipid and carbohydrate metabolism as well as on antioxidant status; increased markers of oxidative stress after consumption of supplemental CLAs.

There are few animal studies that are directly relevant for this risk assessment, according to previous risk assessments (SNT, 2004), (EFSA, 2010 a; b) partly because of a phletora of feeding regimens/CLA compositions, and partly because of a wide variety in species and strains. Reviews of animal studies give some support to the findings in humans of an increase in liver hypertrophy, biomarkers of oxidative stress and infavourable lipid and carbohydrate changes upon feeding with CLAs.

Many studies with adequate design (RCTs) concern CLAs and effect on body weight, but few included safety and/or risk factors as their primary aims. Many of these studies do, however, give an overview of adverse effects, though not always detailed. In most of the RCTs there were no significant differences in adverse effects between the placebo and CLA groups.

A number of biomarkers have also been studied, using them as proxies for lipid and carbohydrate metabolism as well as of oxidative stress. The results are conflicting in that some report unwanted changes while others report no changes in the levels of these biomarkers between subjects receiving CLAs and controls receiving placebo.

Based on these previous risk assessments (AFFSA, 2005a; b; ANSES, 2011b; EFSA, 2010 a; b; FHI, 2003; SNT, 2004) the present risk assessment has not found firm support for increased blood lipid levels upon CLA supplementation to healthy individuals. Most of the studies focusing on CLA supplementation and blood lipids were of short duration, and consequently the impact of such supplementation on future cardiovascular risk is uncertain since clinically relevant atherosclerotic lesions take years to develop. Notably the EFSA (2010 a) and EFSA (2010 b) opinions put a maximal duration of safe use of CLA supplementation to six months. Moreover, the changes in blood lipids, e.g. HDL cholesterol, were small, and a dose-response effect has not been demonstrated.

As value for comparison in the risk characterisation of CLAs, VKM will use 3.5 g/day mainly based on the EFSA statement from 2012. In an adult weighing 70 kg, 3.5 g/day of CLAs corresponds to 50 mg/kg bw per day.

Exposure (background information)
The daily dietary intake in Norway of CLAs range between 20 and 170 mg (MoBa 2008, version 4). This will give 0.3 – 2.4 mg/kg bw per day for a 70 kg person. Dairy products account for about 80-90% of total intake of CLAs. Intakes of CLAs in children and adolescents are not known. CLA concentrations in milk and dairy products vary considerably, by a factor of up to 10 in studies in which large numbers of samples were analysed. Because CLA concentrations are dependent on feed composition and use of supplements, seasonal fluctuations in CLA concentrations are seen. On average, CLA concentrations in milk and dairy products range from 0.2 to 1.6 g/100 g fat.

Vulnerable groups: score 1.0
CLA supplementation to lactating mothers may cause reduced milk production and reduced content of milk fat according to data from the cross-over-study on CLA supplementation to lactating women performed by Masters et al. (2002). Use of CLAs by pregnant women may reduce birth weight and birth length of their off-springs according to Elias and Innis (2001) and cited in (FHI, 2003).

In some of the RCTs, the study populations have included overweight and/or obese, classified according to their BMI values. Most of these studies did not report any differences in adverse effects between the CLA-supplemented and the control groups. However, previous reports have cautioned about the use of CLAs among obese men with the metabolic syndrome due to an increase in markers of insulin resistance and inflammation/cardiovascular disease (Risérus et al., 2002b).

In the RCT by Racine et al. (2010) on the effect of CLA supplementation to overweight/obese children, no significant differences in adverse effects or biomarkers were

detected between the CLA-supplemented and the control groups, with the exception that HDL decreased significantly more in the CLA group.

Lack of toxicity data: score 0.5
There is lack of short- and long-term human studies of CLAs with adverse health effects as the primary outcome, that are of sufficiently good quality. The studies on adverse health effects related to CLAs in adults are heterogeneous both in design and results. There are few studies on adverse health effects related to CLAs in children and adolescents as well as in vulnerable groups such as pregnant and lactating women and the elderly.

More data on the specific metabolic effects of the various isomers present in the CLA supplements are needed. There is a need for more in-depth studies on the possible adverse effects following intake of the individual CLA isomers. Identification of more biomarkers with a direct link to CLA metabolism is also called for, and mode of actions need more elucidation.

Lack of exposure data: score 0.5
Good data for content of CLAs in foods are lacking, as well as data for intake of CLAs from foods in various population groups.

Total score = 4.0 for conjugated linoleic acids (CLAs)

10.4.2 References

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10.5 Creatine (CAS no. 6020-87-7)

10.5.1 Scores

MOE/MOS/ADI/TDI/TWI: score 2.0
Using a safe level of 43 mg/kg bw per day based on SCF (2000), VKM (2010) and AESAN (2012) and a total exposure from diet and endogen production of 28.6 mg/kg bw per day, the exposure is below the safe level.

Toxicity (background information)
SCF (2000) concluded that intake of creatine in doses not exceeding 3 g per day is unlikely to pose any risk. It was not explicitly stated how the conclusion on 3 g was reached. Furthermore, it was stated that high loading doses should be avoided. EFSA (2004) based its data mainly on SCF (2000) and concluded likewise.

VKM (2010) supported EFSA (2004) that supplementation with creatine up to 3 g per day was unlikely to pose any risks. It was stated that long-term studies with doses up to 5-10 g per day in adult athletes had shown no harmful effects.

The tested doses in studies reported by AESAN (2012) varied from about 1.0 to 30.0 g per day and usually for periods shorter than one month, and AESAN (2012) concluded that a maximum amount of 3.0 g per day of creatine monohydrate was acceptable from a safety point of view for use as a food supplement. Similar to VKM (2010), AESAN (2012) reported that long-term studies with doses up to 5-10 g per day in adult athletes had shown no adverse effects.

According to the VKM opinion from 2010, gastrointestinal and cardiac symptoms (unspecified) have been reported, but these adverse effects had not been verified in well-controlled studies.

Data from the literature searches are heterogeneous in terms of study subjects (e.g. athletes or healthy persons, i.e. study populations that may differ widely in skeletal muscle mass and endurance capacity, aspects that are likely to influence creatine metabolism), supplemental

dose of creatine, and duration of the studies. Most of the studies (including the RCTs) conclude that doses up to 3 g per day for shorter periods (1-4 weeks) are safe. The studies based on long term exposure (i.e. 1-5 years) and/or with daily creatine intake >3 g (range 5-21 g) often (i) involved few and highly trained individuals of whom some took high daily loading doses of creatine (range 2-25 g) for a short period (usually <1 week), and (ii) were designed to test clinical benefit without emphasis on adverse effects. Firm clinical endpoints, i.e. information about possible organ dysfunctions, are lacking. Overall therefore, the documentation for absence of adverse health effects of doses above 3 g per day of creatine in food supplements in the general population is limited and these doses may therefore represent a risk of adverse health effects in adults.

Due to the important role of the kidneys in creatine metabolism and clearance from the blood, the kidneys have been of particular focus in many studies. However, renal function has mostly been inadequately assessed since blood biomarkers, such as creatinine, usually have been measured. Studies with more relevant endpoints like renal perfusion, glomerular filtration rate, hormonal outputs and histology have often not been identified. Therefore, based on available data from the previous risk assessments and the literature searches in the current report, VKM has not been able to find conclusive documentation that the doses tested of creatine supplementation adversely affect renal function.

Whether creatine use in high doses will promote the formation of compounds with potential mutagenic/carcinogen effects has not been clarified, but there is currently no available evidence to support the clinical relevance of this notion. Importantly, both EFSA (2004) and AESAN (2012) quoted murine studies showing no mutagenic effects or signs of renal dysfunction at doses of 50 to 2,000 mg creatine/kg bw per day for use up to one month.

The highest dose tested in the animal experiments was a maintenance dose of 2 g/kg bw per day, and this was not associated with adverse outcomes when used for 8 weeks. This study and the results reported from other animal studies are in line with those obtained in the human studies and gave no cause of additional concern about the use of creatine. However, the animal studies mostly focused on renal function whereas other possible adverse effects were largely omitted from the analyses. Also, few doses were tested and the studies were not performed according to OECD guidelines or other approved standards. Moreover, as detailed in the description of the animal research assessed in the present report, several limitations were noted for the individual studies. Therefore, VKM has not used the results from the animal studies in the risk characterisation of the specified doses of creatine. Consequently, the data from these animal studies did not change VKM's conclusion that doses above 3 g per day may represent a risk of adverse effects in humans.

As a value for comparison in the risk characterisation of creatine, VKM will use 3.0 g per day corresponding to 43 mg/kg bw per day in a 70 kg adult. This value is based primarily on the SCF (2000) and supported by VKM (2010) and AESAN (2012), as well as the articles identified in the literature searches and stems from studies of healthy humans, and is supported by animal studies. VKM considers the evidence of absence of adverse effects from

studies providing creatine doses higher than 3 g per day to be insufficient, as these studies were characterised by low sample sizes, short duration, markedly heterogeneous study populations and poor reporting of possible adverse effects.

Exposure (background information)
Creatine can also be obtained through the diet, mainly from meat and fish. The average daily intake from the diet is about 1 g creatine, and the endogenous production also amounts to about 1 g per day (SCF, 2000), thus in total 2 g per day, which is 28.6 mg/kg bw per day for a 70 kg person.

Vulnerable groups: score 0.5
Previous risk assessments caution about the use of creatine supplements by patients suffering from impaired renal function.

Lack of toxicity data: score 0.5
There is no relevant, specific information in the four previous risk assessments (AESAN, 2012; EFSA, 2004; SCF, 2000; VKM, 2010) or the literature search relating to fetuses, children, pregnant/lactating women and the elderly. There is no information about risk related to use of creatine supplements among healthy children/adolescents aged 10-17 years.

There is lack of both short- and long-term studies in humans of creatine with adverse health effects as the primary outcome that are of sufficient quality. Usually intake of creatine supplements is limited to a few weeks or days, often related to participation in exercise activities. However, there is a lack of information about the safety in a longer-term perspective.

Identification of more biomarkers with a direct link to creatine metabolism is also called for. In order to determine possible mechanisms for adverse effects, well-designed animal studies may yield important information.

Lack of exposure data: score 0.5
There are no data available concerning dietary intake of creatine in Norway.

Total score = 3.5 for creatine

10.5.2 References

- AESAN (2012). The Spanish Agency for Food Safety and Nutrition (AESAN) - 1, The Spanish Agency for Food Safety and Nutrition (AESAN), Spain.
- EFSA (2004). Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to Creatine monohydrate for use in foods for particular nutritional uses. The EFSA Journal 36:1-6.

- SCF (2000). Opinion of the Scientific Committee on Food on safety aspects of creatine supplementation, Scientific Committee on Food, EU, Brussels, Belgium.
- VKM (2010). Assessment of creatine in sports products, Opinion of the Panel on on Nutrition, Dietetic Products, Novel Food and Allergy of the Norwegian Scientific Committee for Food Safety, Norwegian Scientific Committee for Food Safety, Oslo, Norway.

10.6 Curcumin (CAS no. 458-37-7)

10.6.1 Scores

MOE/MOS/ADI/TDI/TWI: score 4.0
The ADI for curcumin is 3 mg/kg bw per day and the exposure from food as food additive and spice is reported to be 2.3 and 1.6-7.6 mg/kg bw per day. Therefore, the expsoure may exceed the ADI. There is also potential contribution from use of cosmetics.

Toxicity (background information)
Aside from gastrointestinal symptoms, few adverse events have been reported in human studies after curcumin intake in the range of 2.9–51.4 mg/kg bw per day: one case of photosensitivity (when taken together with the antidepressant fluoxetine), one case of elevated level of serum alkaline phosphatase and three cases of elevated levels of lactate dehydrogenase. Several of the human studies referred to are RCTs, with varying degrees of randomisation and patients under medical treatment as control groups instead of healthy control subjects. Some cases of contact dermatitis and contact urticaria after topical exposure to curcumin have been described.

Curcumin did not induce gene mutations in several strains, with or without metabolic activation, in Ames test. However, one *in vitro* study found that curcumin induced recombination in *Bacillus subtilis.* Curcumin induced chromosomal aberrations, micronuclei and DNA strand breaks in several studies. Thus, curcumin apparently has a genotoxic potential *in vitro*. VKM notes that several studies had limitations, such as questionable solubility of curcumin in aqueous solutions and unknown pre-exposure degradation due to photochemical instability of curcumin.

Several negative *in vivo* micronuclei and chromosomal aberration studies of curcumin have been published. However, these studies had several limitations, such as lack of information on purity of curcumin, questionable solubility of curcumin in aqueous solutions, unknown pre-exposure degradation due to photochemical instability, a single dose used and/or lack of confirmation of cytotoxicity in the bone marrow. VKM is therefore of the opinion that the available *in vivo* studies are insufficient to completely eliminate the possibility that curcumin may be genotoxic.

Curcumin is not carcinogenic based on animal studies (NTP, 1993).

There were also some animal studies on curcumin available, including chronic toxicity, carcinogenicity, and reproductive and developmental toxicity. An ADI of 0-3 mg/kg bw per day was allocated by JECFA (2004), based on a NOAEL for reduction in body weight in F2 animals in a multigenerational reproductive toxicity study in rats by (Ganiger et al., 2007). Based on the same study, EFSA supported the ADI of 3 mg/kg bw per day set by JECFA (EFSA, 2010).

Serious adverse effects of intake of curcumin in the range of 2.9-51.4 mg/kg bw per day were not observed in the human studies published after EFSA (2010). Therefore, in the present risk assessment, the value used for comparison with the estimated exposure in the risk characterisation is the ADI of 3 mg/kg bw per day.

Exposure (background information)
EFSA (2010) stated that the intake of curcumin from the normal diet amounts to less than 7% of the ADI. Maximum curcumin intake from food as food additive and spice combined has been reported to be 2.3 and 1.6-7.6 mg/kg bw per day for adults (>18 years) and children (1-10 years for food additive; 5-12 years for spices), respectively (EFSA, 2010). Curcumin is used in cosmetics as an antioxidant and colourant (CosIng, 2015).

Vulnerable groups: score 1.0
Curcumin may interact with chemotherapeutics. Potential vulnerable groups for curcumin exposure are patients under chemotherapy for breast cancer, patients with gallstones and obstructed bile passages as well as liver diseases and hepatitis C infections. There are indications that turmeric and curcumin can be transferred through lactation (EMEA, 2010).

Lack of toxicity data: score 0.5
There were few studies on negative health effects related to curcumin in children and adolescents. No studies were found on effects of curcumin in lactating women and no relevant studies were found on pregnant women. Human RCT studies on adverse effects after chronic oral exposure to curcumin in healthy subjects are lacking.

Lack of exposure data: score 0.5

There are no data available on exposure to curcumin from Norway.

Total score = 6.0 for curcumin

10.6.2 References

- CosIng (2015). Cosmetic ingredient database – CosIng, European Commission http://ec.europa.eu/growth/tools-databases/cosing/.
- EFSA (2010). Scientific Opinion on the reevaluation of curcumin (E 100) as a food additive. EFSA Journal 8.
- EMEA (2010). Assessment Report On Curcuma Longa L. Rhizoma, European Medicines Agency (EMEA).

http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_-_HMPC_assessment_report/2010/02/WC500070700.pdf.
- Ganiger S., Malleshappa H.N., Krishnappa H., Rajashekhar G., Ramakrishna Rao V., Sullivan F. (2007) A two generation reproductive toxicity study with curcumin, turmeric yellow, in Wistar rats. Food Chem Toxicol 45:64-9. DOI: 10.1016/j.fct.2006.07.016.
- JECFA (2004). Evaluation Of Certain Food Additives And Contaminants. Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives, World Health Organization (WHO). http://apps.who.int/iris/bitstream/10665/42849/1/WHO_TRS_922.pdf.
- NTP (1993). Toxicology and Carcinogenesis Studies of Turmeric Oleoresin (CAS No. 8024-37-1) (Major Component 79%-85% Curcumin, CAS No. 458-37-7) in F344/N Rats and B6C3F1 Mice (Feed Studies), National Toxicology Program (NTP). http://ntp.niehs.nih.gov/results/pubs/longterm/reports/longterm/tr400499/abstracts/tr427/index.html.

10.7 L-Cysteine (CAS no. 52-90-4) and L-Cystine (CAS no. 56-89-3)

10.7.1 Scores

Cysteine may occur in proteins either as L-cysteine itself or as L-cystine. In addition, L-cysteine and L-cystine are available in food supplements. L-Cystine passes through the gastrointestinal (GI) tract and is immediately reduced to two L-cysteine molecules upon cell entry. L-cystine is converted to L-cysteine through cystine reductase, which requires NADH as cofactor. N-acetylcysteine (or N-acetyl-L-cysteine, NAC), which is readily converted to cysteine, is also included in the risk assessment by VKM (2015).

MOE/MOS/ADI/TDI/TWI (background information)
Using a safe level of 13 mg/kg bw per day set by VKM and a mean daily intake from all sources of 14.6 mg/kg bw per day, the exposure is approximately similar to the safe level.

Toxicity: score 1.0
There are several RCTs that have measured the efficacy of NAC at relatively high doses for up to one year. The study groups have been various patient groups ranging from children, adolescents, adults and elderly, but also some healthy subjects. In the RCTs, there were no differences in severe adverse events between the placebo and NAC groups. The following adverse effects were investigated: dizziness, fatigue, energy level, gastrointestinal discomfort, allergic reactions and muscle pain among others. In most of the studies, the results for adverse effects were based on self-reporting systems or clinical examination. A few studies also included analyses of biomarkers from blood or urine samples.

The majority of the studies have been conducted in adults. The included studies demonstrated that it is well documented that the dose 1,200 mg, and in some studies even up to 2,400 mg NAC per day, do not cause adverse effects. These doses of NAC correspond to 900 and 1,800 mg of cysteine and cystine. This is equivalent to 13 and 26 mg cysteine or cystine per kg bw in an adult per day (70 kg as default weight). In the large, recent study by Zheng et al. (2014), where 1,200 mg NAC (i.e. 900 mg cysteine) or placebo was given daily for a year to 1,000 people, NAC was not associated with increased risk of severe adverse events. These results correspond with those of the other RCTs using NAC.

Studies with doses of 500 mg NAC have been conducted in children (corresponding to 375 mg cysteine or cystine). The few studies that included children and adolescents were of relatively short duration.

Animal studies have shown that high doses can result in fatty liver and hypercholesterolemia and that it can be neurotoxic in young rodents. There are, to our knowledge, no reports from studies in humans that confirm these findings. On the contrary, we were able to identify one study that demonstrated that NAC in increasing doses increased the levels of HDL while not affecting the concentration of other lipoproteins and lipids.

Animal studies included in previous reports with high doses of cysteine over six generations in rats found a NOAEL of 175 mg/kg bw per day at the highest dose.

As value for comparison used in the risk characterisation of cysteine and cystine, VKM used 900 mg/day corresponding to 13 mg/kg bw per day. This was based on doses used in many studies in various population groups.

Exposure: score 1.0
Based on distribution data from the 1988–1994 National Health and Nutrition Examination Survey (NHANES III), the common mean daily intake for all life stage and gender groups of L-cysteine is 1.0 g per day in USA, which is 14.6 mg/kg bw per day for a 70 kg person. Men 51 through 70 years of age had the highest intakes at the 99th percentile of 2.2 g per day (IOM, 2005).

Vulnerable groups: score 0.5
NAC may enhance the effect of nitroglycerin and isosorbide, two medications commonly used to treat angina pectoris. This combination may also raise the risk of side effects, such as severe headaches and may lead to abnormally low blood pressure.

In the hereditary disease cystinuria, kidney stones are formed from circulating cystine. People with this disease should consult their physician before they take supplemental cysteine or cystine.

Lack of toxicity data: score 0.5
There is a lack of studies of adverse effects as primary outcomes of cysteine and cystine in humans. The studies which have reported negative health effects related to NAC in adults

have high heterogeneity both in design, target population and results. There are few studies on negative health effects related to NAC or L-cysteine/L-cystine in children and adolescents. In the included literature, no information was available about pregnant or nursing women.

Lack of exposure data: score 0.5
There are no data on intake of L-cysteine/L-cystine from Norway.

Total score = 3.5 for L-cysteine and L-cystine

10.7.2 Referencecs

- IOM (2005). Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. Institute of Medicine of the National Academies. https://www.nap.edu/read/10490.
- Zheng J.P., Wen F.Q., Bai C.X., Wan H.Y., Kang J., Chen P., Yao W.Z., Ma L.J., Li X., Raiteri L., Sardina M., Gao Y., Wang B.S., Zhong N.S. (2014) Twice daily N-acetylcysteine 600 mg for exacerbations of chronic obstructive pulmonary disease (PANTHEON): A randomised, double-blind placebo-controlled trial. The Lancet Respiratory Medicine 2:187-194. DOI: http://dx.doi.org/10.1016/S2213-2600%2813%2970286-8.

10.8 Docosahexaenoic acid (DHA) (CAS no. 6217-54-5)

10.8.1 Scores

MOE/MOS/ADI/TDI/TWI: score 4.0
Assuming a safe level of 14 mg/kg bw per day from EFSA (2012) and the total exposure from food and supplements of 14.7 mg/kg bw per day, the exposure is approximately similar to the safe level.

Toxicity (background information)
Only few studies with DHA supplements performed after 2011 have addressed possible adverse effects of supplementation (included safety concerns). Most of the included studies have investigated dosages that are below or at the dosage considered as safe by EFSA. EFSA concluded in 2012 that up to 1 g per day of DHA does not raise safety concern for the general population.

None of the included studies from our literature searches published from 2011 onwards had investigated bleeding complications. The included studies had investigated lipid peroxidation, immune function and glucose and lipid homeostasis. None of the studies included reported adverse effects related to these endpoints.

Although there are several human intervention trials with supplementation of DHA alone, studies addressing possible adverse effects of DHA supplements for healthy adults and the general population are missing. In 2012, EFSA assessed the impact of DHA supplementation

on bleeding time, platelet function, glucose homeostasis, LDL-cholesterol and lipid peroxidation. For DHA, it was concluded that supplemental intakes of DHA up to about 4 g per day are not considered to cause adverse effects; it was not associated with an increased risk of clinical complications (e.g. spontaneous bleeding). Regarding possible increase in LDL-cholesterol it was concluded that supplemental intakes of 2 to 4 g DHA per day are not adverse in relation to cardiovascular disease (CVD) risk. A supplemental intake of up to about 4 g DHA per day for six weeks did not induce lipid peroxidation as assessed by F2-isoprostanes. Moreover, doses up to about 5 g DHA per day for up to 16 weeks did not induce changes in lipid peroxidation. Their final conclusion was that supplemental intakes of up to 1 g per day of DHA do not raise safety concerns for the general population. No information was provided regarding how they reached their conclusion of up to 1 g DHA per day.

In this risk assessment, seven studies with both patients and healthy adults were included. The dosages of DHA ranged from 1.0 to 3.6 g DHA per day and the duration from five weeks to four years. Six out of seven studies used dosages from 1 to 2 g DHA per day. The last study included up to 3.6 g DHA per day for four years and the age spanned from 7 to 31 years, but there were few participants, n=33 in the treatment groups. The main endpoints in all studies included lipid peroxidation, inflammation, cognitive performance, blood pressure and/or biomarkers of cardiovascular diseases. No serious adverse events were found related to the main endpoints. In general, adverse events were described as gastrointestinal discomfort and were not related to dose.

In this report, one safety study of supplemental DHA on vulnerable groups, such as pregnant women, children and adolescents, was identified. Animal studies on DHA have not been included in this report as previous risk assessments have found no serious adverse events with doses of DHA up to 5 g per day and combined doses of EPA and DHA up to 6.9 g per day (VKM, 2011; EFSA, 2012).

In summary, due to a limited number of studies with supplemental doses above 1 g DHA per day, the risk associated with supplemental DHA above 1 g DHA per day could not be assessed. However, a daily dose of DHA that moderately exceed 1 g from food supplements is not considered to lead to adverse health effects in the general population (including children ≥10 years and adolescents). This will be 14 mg/kg bw per day for a 70 kg person.

Exposure (background information)

Information about intakes of DHA from the diet is scarce, but calculations performed in MoBA indicated a mean total intake (SD) from food and supplements of DHA 430 (380) mg per day among pregnant women (2002 to 2008). This will give a mean exposure from food of 6.1 mg/kg bw per day for a 70 kg person.

Mean intake of EPA, DPA and DHA from fish oil/cod liver oil in adults participating in a nationally representative dietary survey was 735 mg per day (VKM, 2014). Concentrations of the n-3 long-chain polyunsaturated fatty acid (LCPUFAS) in cod liver oil may vary, and a recommended dose of 5 ml may contain 600 mg DHA, which will be 8.6 mg/kg bw per day

for a 70 kg person. The total exposure from food and supplements may be 14.7 mg/kg bw per day.

Vulnerable groups: score 0.0
The risk assessment is based on previous risk assessments of DHA containing no information on vulnerable groups.

Lack of toxicity data: score 1.0
None of the included randomised supplementation studies were undertaken in children, adolescents or pregnant women. In summary, no value for comparison with the expsoure can be established for DPA due to lack of data.

Lack of exposure data: score 0.5
Use of DHA as single fatty acid in a supplement is relatively new and the actual intake and usage is not known in the general Norwegian population.

Total score = 5.5 for docosahexaenoic acid (DHA)

10.8.2 References

- EFSA (2012). Scientific Opinion on the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). EFSA Journal 10:2815.
- VKM (2011). Evaluation of negative and positive health effects of n-3 fatty acids as constituents of food supplements and fortified foods Opinion of the Steering Committee of the Norwegian Scientific Committee for Food Safety, Norwegian Scientific Committee for Food Safety, Oslo, Norway.

10.9 Docosapentaenoic acid (DPA) (CAS no. 24880-45-3)

10.9.1 Scores

Toxicity (background information)
Only few studies with DPA supplements performed after 2011 have addressed possible adverse effects of supplementation (included safety concerns). Most of the included studies have investigated dosages that are below or at the dosage considered as safe by EFSA. EFSA did not conclude for DPA because data were not sufficient for evaluation.

None of the included studies from the literature searches published from 2011 onwards had investigated bleeding complications. The included studies had investigated lipid peroxidation, immune function and glucose and lipid homeostasis. None of the studies included reported adverse effects related tothese endpoints.

Information about effects of DPA is scarce, but one study in 10 healthy normal weight women given 2 g of supplemental DPA served for breakfast and followed for 5 hours post-prandially indicated a different incorporation of DPA compared with EPA into various cell membranes. Furthermore, 2 g per day of DPA inhibited incorporation of other fatty acids into chylomicrons (Linderborg et al., 2013). In a study in rats, the different incorporation of DPA into the various body compartments was confirmed (Fard et al., 2014). However, the importance and relevance of these findings still have to be elucidated. In summary, no value for comparison with the expsoure could be established for DPA due to lack of data.

Exposure: score 1.0
Information about intakes of DPA from the diet is scarce, but calculations performed in MoBa indicated a mean total intake (SD) from food and supplements of DPA 43 (30) mg per day among pregnant women (2002 to 2008). This gave an intake of 0.6 mg/kg bw per day for a 70 kg person.

Mean intake of EPA, DPA and DHA from fish oil/cod liver oil in adults participating in a nationally representative dietary survey was 735 mg per day (VKM, 2014). Concentrations of the n-3 LCPUFAS in cod liver oil may vary, and a recommended dose of 5 ml may contain 60 mg DPA, which is 0.9 mg/kg bw per day for a 70 kg person. In total, the exposure from food and supplements may be 1.5 mg/kg bw per day.

Vulnerable groups: score 0.0
The risk assessment is based on previous risk assessments of DPA containing no information on vulnerable groups.

Lack of toxicity data: score 0.5
None of the included randomised supplementation studies were undertaken in children, adolescents or pregnant women.

Lack of exposure data: score 0.5
Use of DPA as single fatty acid in a supplement is relatively new and the actual intake and usage is not known in the general Norwegian population.

Total score = 3.0 for docosapentaenoic acid (DPA)

10.9.2 References

- EFSA (2012). Scientific Opinion on the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). EFSA Journal 10:2815.
- Fard S.G., Linderborg K.M., Turchini G.M., Sinclair A.J. (2014) Comparison of the bioavailability of docosapentaenoic acid (DPA, 22:5n-3) and eicosapentaenoic acid (EPA, 20:5n-3) in the rat. Prostaglandins Leukotrienes and Essential Fatty Acids 90:23-26. DOI: 10.1016/j.plefa.2013.10.001.
- Linderborg K.M., Kaur G., Miller E., Meikle P.J., Larsen A.E., Weir J.M., Nuora A., Barlow C.K., Kallio H.P., Cameron-Smith D., Sinclair A.J. (2013) Postprandial

metabolism of docosapentaenoic acid (DPA, 22:5n-3) and eicosapentaenoic acid (EPA, 20:5n-3) in humans. Prostaglandins Leukot Essent Fatty Acids 88:313-9. DOI: 10.1016/j.plefa.2013.01.010.

10.10 D-Glucurono-γ-lactone (CAS no. 32449-92-6)

10.10.1 Scores

MOE/MOS/ADI/TDI/TWI (background information)
Using an assumed safe level of 10 mg/kg bw per day set by VKM and an exposure up to 0.029 mg/kg bw per day from natural sources, the exposure is well below the safe level. However, additional exposure may come from cosmetics.

Toxicity: score 1.0
D-glucurono-γ-lactone is a human metabolite formed from glucose, and there were no structural alerts for mutagenicity or carcinogenicity (EFSA, 2009). In a study on the antimutagenic activity of lactones in *E. coli*, D-glucurono-γ-lactone was reported not to be mutagenic. Animal studies on the genotoxic or carcinogenic potential of D-glucurono-γ-lactone were not available in the included literature.

There were no studies on toxicity in humans for D-glucurono-γ-lactone alone in the included literature. There were no indications of genotoxicity, neurotoxicity, chronic toxicity, carcinogenicity, reproductive or developmental toxicity of D-glucurono-γ-lactone from animal studies.

EFSA (2009) defined a NOAEL of 1,000 mg/kg bw per day for daily oral administration of D-glucurono-γ-lactone to rats, which was the highest dose tested. The NOAEL was based on a 13-week rat study of daily oral administration of D-glucurono-γ-lactone performed under good laboratory practice (GLP). Using an UF = 100, an assumed safe level would be 10 mg/kg bw per day.

Exposure: score 1.0
D-glucurono-γ-lactone and its hydrolysis product glucuronic acid occur naturally in several dietary sources. The estimated exposure to D-glucurono-γ-lactone from naturally occurring sources in the diet was 1-2 mg per day (SCF, 2003). This will give 0.014-0.029 mg/kg bw per day. In the EU, D-glucurono-γ-lactone can be used in cosmetic products (CosIng, 2015).

Vulnerable groups: score 0.0
There was no information concerning specific groups vulnerable for D-glucurono-γ-lactone in the literature reviewed in the present risk assessment.

Lack of toxicity data: score 0.5
There is lack of an ARfD or other data on acute toxicity for D-glucurono-γ-lactone. Human

studies on D-glucurono-γ-lactone are lacking for all age groups. Adequate studies on chronic toxicity, carcinogenicity, reproduction, development or genotoxicity are lacking.

Lack of exposure data: score 0.5
There are no data available on exposure to D-glucurono-γ-lactone in the general Norwegian population.

Total score = 3.0 for D-glucurono-γ-lactone

10.10.2 References

- CosIng (2015). Cosmetic ingredient database – CosIng, European Commission http://ec.europa.eu/growth/tools-databases/cosing/.
- EFSA (2009). The use of taurine and D-glucurono-γ-lactone as constituents of the so-called "energy" drinks. Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food, EFSA Journal, European Food Safety Authority, http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/ ans_ej935_Taurine%20and%20D-glucuronolactone_op_en%2C3.pdf. pp. 1-31.

10.11 Eicosapentaenoic acid (EPA) (CAS no. 10417-94-4)

10.11.1 Scores

MOE/MOS/ADI/TDI/TWI: score 2.0
Using an estimated safe level of 25.7 mg/kg bw per day from EFSA (2012) and an exposure level from food and supplements of 10.4 mg/kg bw per day, the exposure is below the safe level by a factor of 2.5.

Toxicity (background information)
Only few studies with EPA supplements performed after 2011 have addressed possible adverse effects of supplementation (included safety concerns). Most of the included studies have investigated dosages that are below or at the dosage considered as safe by EFSA.

EFSA concluded in 2012 that up to 1.8 g per day of supplemental EPA does not raise safety concerns for adults. The safety concerns related to n-3 LCPUFAS combined or as single substances in previous reports are related to bleeding complications, immune function, peroxidation and impaired glucose or lipid homeostasis.

None of the included studies from our literature searches published from 2011 onwards have investigated bleeding complications. The included studies have investigated lipid peroxidation, immune function and glucose and lipid homeostasis. None of the studies included reported adverse effects related to these endpoints.

Four randomised controlled trials and three other human studies were included. Three of the RCTs were conducted in patients with hypertriglyceridemia. Dosages used were in the range from 1.8 to 3.8 g per day of EPA for 12 weeks. The endpoints included immune function, blood pressure and heart rate. Diarrhea, nausea, nasopharyngitis and arthralgia were the most common adverse events and no serious adverse events were reported in any of the four randomised controlled studies. Furthermore, adverse events reported were not related to dosage.

Two of the included randomised studies investigated EPA at doses above 1.8 g per day (1.9-3.8 g per day) as a single fatty acid. Supplemental intakes of EPA at doses up to about 3.8 g per day for 12 weeks did not change glucose homeostasis and similar numbers of nasopharyngitis as a measure of immune function were seen in treatment group and placebo (Ballantyne, 2010; Bays, 2011).

In 2012, EFSA did not draw conclusions concerning the safety of EPA for children or adolescents. VKM identified only one recent cross-sectional study in children (Damsgaard et al., 2014), in which the concentration of EPA in blood in 8 to 11 years old children correlated positively with blood pressure in boys. However, since no new studies with EPA supplementation had been identified in children or adolescents, no provisional safe level of use for children or adolescents could be set.

Animal studies on EPA were not included in this report as it was considered that EPA is thoroughly investigated in humans.

In summary, it is well documented that 1.8 g supplemental EPA per day is unlikely to cause adverse health effects in adults. This will give an estimated safe level of 25.7 mg/kg bw per day for a 70 kg person. In two studies, doses up to 3.8 g per day were given for 12 weeks without reported adverse effects. However, these two studies were of short duration, i.e. 12 weeks and studies of longer duration are necessary for an assessment of higher intakes of EPA.

Exposure (background information)
Information about intakes of EPA from the diet is scarce, but calculations performed in MoBa indicated a mean total intake (SD) from food and supplements of EPA around 330 (340) mg/day among pregnant women (2002 to 2008). This will give a mean intake of 4.7 mg/kg bw per day for a 70 kg person.

Mean intake of EPA, DPA and DHA from fish oil/cod liver oil in adults participating in a nationally representative dietary survey was 735 mg/day (VKM, 2014). Concentrations of the n-3 LCPUFAS in cod liver oil may vary, and a recommended dose of 5 ml may contain 400 mg EPA. This may give an intake of 5.7 mg/kg bw per day for a 70 kg person. The exposure from food and supplement may be 10.4 mg/kg bw per day.

Vulnerable groups: score 0.0

The risk assessment is based on previous risk assessments of EPA containing no information on vulnerable groups.

Lack of toxicity data: score 0.5
None of the included randomised supplementation studies were undertaken in children, adolescents or pregnant women.

Lack of exposure data: score 0.5
Use of EPA as single fatty acid in a supplement is relatively new and the actual intake and usage is not known in the general Norwegian population.

Total score = 3.0 for eicosapentaenoic acid (EPA)

10.11.2 References

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10.12 Inositol (myo-inositol, CAS no. 87-89-8,)

10.12.1 Scores

MOE/MOS/ADI/TDI/TWI (background information)
A safe level was estimated to be 2.6 mg/kg bw per day by VKM. By adding 57 mg/kg bw per day (the endogenous production in a 70 kg adult) and 7-14 mg/kg bw per day (the total dietary intake of inositol in a 70 kg adult), a total exposure of up to 71 mg/kg bw per day may be estimated. Then, the exposure may exceed the safe level by a factor of 27.

Toxicity: score 1.0
With regard to genotoxicity and mutagenicity, the properties of inositol have not been thoroughly investigated.

A review of 12 controlled clinical trials in a total of 250 adults given oral doses of 4 to 30 g inositol/person per day (equal to 57 and 429 mg/kg bw per day for a 70 kg person) over 1 to 12 months found that the most frequently reported and dose-related adverse effects were related to gastrointestinal symptoms, such as flatulence, loose stools and diarrhoea (Carlomagno and Unfer, 2011).

A NOAEL of 18 g per day (257 mg/kg bw per day for a 70 kg person) of *myo*-inositol was established in a clinical study of smokers (40-74 years) with bronchial dysplasia (Lam et al., 2006). Using an UF = 100, a safe level could be estimated to be 2.6 mg/kg bw per day.

No conventional toxicological studies were available, but the results of studies in rodent models of chronic diseases (including diabetes and cancer) suggested that the toxicity of inositol is low over an oral dose range of 450–9,000 mg/kg bw per day, as concluded by EFSA (2014). Only one study showed adverse effects (at 1800 mg/kg bw per day), including thickening of basement membranes of capillaries of the retina and glomeruli. However, a NOAEL could not be identified in these studies (EFSA, 2014).

For the present risk assessment, the human studies available were not of sufficient quality to be used alone in the risk characterisation. With regard to the animal model studies, no conventional toxicological studies were available. Results of studies in rodent models of chronic diseases (including diabetes and cancer) suggested that the toxicity of inositol is low over an oral dose range of 450–9,000 mg/kg bw per day.

The values used for comparison with the estimated exposure in the risk characterization were 57 mg/kg bw per day (the endogenous production in a 70 kg adult), 7-14 mg/kg bw per day (the total dietary intake of inositol in a 70 kg adult), and the NOAEL of 18 g per day (257 mg/kg bw per day for a 70 kg person).

Exposure: score 1.0
Inositol is ingested via the daily diet, either as *myo*-inositol or in a phosphorylated form (e.g. phytic acid or other phytates) (EFSA, 2014). It is also used as a humectant ingredient in cosmetic products for skin and hair care, including hair conditioners, creams and body lotions (CosIng, 2015; EWG, 2015). The total dietary intake of inositol in adults is estimated to range from 500 to 1,000 mg per day (7 to 14 mg/kg bw per day for a 70 kg person) (Rotstein et al., 2013).

Vulnerable groups: score 0.5
The metabolism of inositol in the human body is altered by various clinical conditions, including diabetes and kidney disorders such as chronic renal failure (CRF). High levels of circulating inositol might have toxic effects on nerve tissue and may aggravate polyneuropathy in people with CRF (VKM, 2005).

Lack of toxicity data: score 0.5
There was very little data available on toxicity of *myo*-inositol from human or animal studies. No studies on negative health effects related to inositol in infants, children, adolescents and in lactating or pregnant women were identified in the literature search. There was lack of an ARfD or other data on acute toxicity for inositol.

Lack of exposure data: score 0.5
There are no data available on exposure to inositolin the general Norwegian population.

Total score = 3.5 for inositol (myo-inositol)

10.12.2 References

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10.13 Lycopene (CAS no. 502-65-8)

10.13.1 Scores

MOE/MOS/ADI/TDI/TWI: score 4.0
ADI for lycopene is 0.5 mg/kg bw per day (EFSA, 2008) and exposure may be up to be 0.6 mg/kg bw per day, i.a. approximately at the ADI.

Toxicity (background information)
Several previous risk assessments have summarized safety studies of lycopene. An ADI of 0.5 mg/kg bw per day was established by EFSA in 2008. The ADI was derived from the NOAEL of 50 mg/kg bw per day from a 52-week toxicity study in rats, based on a partly

reversible increased level of the liver enzyme alanine transaminase (ALT), however, a dose level where the effect on the enzyme was considered not toxicologically significant. An ADI is set to cover the general population, including children.

ADI was established for lycopene from all sources (lycopene from tomatoes, synthetic lycopene and lycopene from the fungus *B. trispora*). For an adult of 70 kg bw, this value corresponds to an intake of 35 mg per day.

In 2009, JECFA concluded that, based on lycopene's low toxicity, there was no need to establish a numerical ADI. Thus, a group ADI «not specified» for lycopene from all sources (tomatoes, synthetic lycopene and lycopene from *B. trispora*) was established (JECFA, 2009).

EFSA concluded that the divergence of the scientific opinions, EFSA (2008) and JECFA (2009), was not based on data that were not available to EFSA during its evaluation of lycopene, but rather to diverging interpretation of the results in the study from which the EFSA ADI was established (Smith et al. 2005; unpublished).

There are case reports of yellow-orange skin discoloration and/or gastrointestinal discomfort after prolonged high intakes of lycopene-rich food and supplements, those effects being reversible upon cessation of lycopene ingestion (JECFA, 2006). In addition, one study indicated that lycopene increased the incidence of preterm labour and low birth weight babies. However, due to weaknesses in the reporting, VKM could not use the results from this study in the risk characterisation.

In an animal study by Jian et al. (2008), the subacute oral toxicity of lycopene produced by recombinant *Escherichia coli* was tested. Daily doses of 0, 200, 500 and 2000 mg/kg bw were administered by gavage to 10 rats/sex/group for 28 days. Sterile water was used as control. No statistically significant, dose-related effects on body weight gain, clinical signs or ophthalmoscopic parameters were observed in any treatment group. Likewise, no treatment-related or dose-related toxic effect was found in hematology, clinical chemistry, urinalysis, blood coagulation, organ weights, gross observation or histopathology. A NOAEL of 2000 mg/kg bw per day was derived for lycopene produced by recombinant *E. coli*.

Exposure (background information)

Lycopene belongs to the carotenoid group that is responsible for the red colour in many fruits and vegetables. The major sources of natural lycopene in the human diet are tomatoes and tomato-based products. Fruits like pink grapefruit, water melon, rosehip, papaya and guava are also sources of lycopene (Nguyen and Schwartz, 1999).

According to dietary surveys, regular intakes of lycopene from natural dietary sources in different populations were estimated to be on average between 0.5 and 5 mg per day, with high intakes up to about 8 mg per day (EFSA, 2008). This will give 0.007, 0.07 and 0.11 mg/kg bw per day, respectively, for a 70 kg person. High consumption of fruits and vegetables, especially tomato products, may result in occasional intakes of 20 mg lycopene per day or more (EFSA, 2008). EFSA noted that total daily exposure to lycopene from *B.*

trispora as a food colour potentially could range from 2 to 6 mg on the average and go up to 11 to 23 mg at the high level. Thus, EFSA did not exclude an occasionally combined high exposure from both natural dietary sources and food colours up to 43 mg of lycopene per day (EFSA, 2008). This will be 0.6 mg/kg bw per day for a 70 kg person, i.e. above ADI.

Lycopene is authorized as a food additive and registered as E160d. In EU, lycopene can be used in cosmetic products, as an antioxidant and a cosmetic colourant (CosIng, 2015).

Vulnerable groups: score 1.0
The results from one study indicated that lycopene increased the incidence of preterm labour and low birth weight babies (Banerjee et al., 2009).

Lack of toxicity data: score 0.5
More studies on lycopene and effects on preterm labour, low birth weight and other related endpoints are needed, as one study reported that an oral intake of lycopene increased the incidence of preterm labour and low birth weight babies (Banerjee et al., 2009).

Lack of exposure data: score 0.5
There are no data available on exposure to lycopene in the general Norwegian population.

Total score = 6.0 for lycopene

10.13.2 References

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10.14 L-Methionine (CAS no. 63-68-3)

10.14.1 Scores

MOE/MOS/ADI/TDI/TWI (background information)
VKM concluded that 3 mg/kg L-methionine per day may be regarded as a safe level, and the exposure from food and supplements was 25.7 mg/kg bw per day. Thus, the exposure exceeded this assumed safe level by a factor of approximately 10.

Toxicity: score 1.0
In 2013, VKM summarised the risk assessment of L-methionine as follows:

«In 2005, Institute of Medicine, US (IOM) concluded that it was insufficient data to establish a tolerable upper intake level (UL) for methionine. One relevant new animal and four human studies with methionine were identified after 2002. Two of the new studies in humans reported on methionine-loading tests. One study in infants showed serious adverse health effects in infants given a protein hydrolysate with L-methionine equivalent to 8800 mg/L.

There are indications that intake of methionine during the so called acute methionine-loading test is associated with adverse health effects such as dizziness, nausea, sleepiness and decreased or increased blood pressure. In the loading test, 100 mg methionine per kg bw is given after a 12-hour fast. This intake (100 mg/kg bw) of L-methionine may be regarded as the lowest observed adverse effect level (LOAEL).

Although IOM has concluded that no UL could be established for methionine it has been reported that use of methionine as a single amino acid may have adverse health effects. An intake at 100 mg/kg body weight of L-methionine may be regarded as a LOAEL. With a conservative approach and the use of an uncertainty factor of 10 for between people variations and a factor of 3 for the uncertainty of LOAEL, a tentative guidance level (GL) of 100/30 ~3 mg of L-methionine per kg bw can be suggested. In a 70 kg man this is equivalent to an intake of 210 mg per daily dosage».

No studies from this literature search fulfilled the inclusion criteria or were considered relevant for the purpose of risk assessment of L-methionine by VKM in 2016. No new evidence had thus been identified which could alter the conclusion in the VKM (2013) opinion. VKM maintains the guidance level from 2013 at 210 mg methionine per day.

Exposure: score 1.0
According to VKM (2013): "*High levels of methionine follows in egg, fish, dairy products, nuts and sesame seeds. Methionine is also found in meat, cereal grains and some other plant seeds. Most fruits and vegetables including legumes are poor methionine sources. Average methionine intake in all age groups from foods and supplements is 1.8 g per day*

(IOM, 2005). This gave an exposure of 25.7 mg/kg bw per day for a 70 kg person. According to the Norwegian Food Safety Authority, there are supplements available on the Norwegian market that contain up to 500 mg methionine per recommended daily dosage".

Vulnerable groups: score 0.5
VKM (2013): The number of patients with the deficiency of cystathionine b-synthase (homozygote form), may be 1:100,000 in Europe (Mudd et al., 1985; Mudd et al., 1995). Cystathionine b-synthase plays a pivotal role in mammalian sulfur metabolism and in the conversion of methionine to cysteine via homocysteine. This transsulfuration pathway is the only pathway capable of removing sulfur-containing amino acids under conditions of abundant intake (Finkelstein, 1998). Children with the deficiency of cystathionine b-synthase are usually identified by health personnel in their childhood. Patient groups with hyperhomocysteinemia should be advised against use of methionine supplementation, because of possible increased risk of coronary vascular disease. VKM (2016): No specific vulnerable groups were identified in the reviewed literature.

Lack of toxicity data: score 1.0
VKM (2013): To be able to set an UL, dose-response studies in animals and humans are imperative. It is of great concern that products containing single amino acids with metabolic relevance are allowed on the marked without thorough knowledge of potential toxicity. Although some studies were found where the function of the amino acids was studied, mostly in patient groups, few reported on adverse health effects, and none of these were long-term studies. More dose-response studies are needed, including both animal and human studies focusing on possible negative health effects from supplementation with methionine. Long-term studies are also necessary to re-evaluate the tentative GLs.

While even high intake of amino acids from dietary proteins seems to be of no physiological concern, the use of single amino acids added to food or as supplements might cause imbalances in the amino acid pool in the body. Very little is known about a possible effect on protein synthesis..

In this risk assessment of the amino acid methionine many questions were still left unanswered because of scanty scientific literature.

Lack of exposure data: score 0.5
There are no data available on exposure to methionine in the general Norwegian population.

Total score = 4.0 for L-methionine

10.14.2 References

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10.15 Piperine (CAS no. 94-62-2)

10.15.1 Scores

MOE/MOS/ADI/TDI/TWI (background information)
Using an assumed safe level of 0.05 mg/kg bw per day dentified by VKM and an exposure up to 0.09 µg/kg bw per day, the exposure is well below the safe level. However, additional exposure may come from cosmetics.

Toxicity: score 2.0
Available data from *in vivo* and *in vitro* studies indicated that piperine had no genotoxic potential.

Several adverse health effects were identified in animal studies, including enhanced plasma cholesterol, hepatic dysfunction and histopathological changes, immunomodulatory effects and reproductive toxicity. Two dietary toxicity studies carried out in chicks (Da Silva Cardoso et al., 2009) and mice (Dogra et al., 2004), revealed hepatotoxic and immunomodulatory changes, respectively. Both reports suggested a NOAEL of 1.12 mg/kg bw per day, the lowest dose (other doses tested were 2.25 and 4.50 mg/kg bw per day). The reported dose-response effects in these two studies were not always consistently statistically significant, conclusive or were partly contrasting. For that reason, the suggested NOAELs are not used in the risk characterisation of piperine by VKM.

The range of doses reported to cause interactions with drugs and phytochemicals when studied *in vivo*, 5 to 20 mg/kg bw per day in humans and 10 to 50 mg/kg bw per day in animals (Chinta et al., 2015; Srinivasan, 2007; Srinivasan, 2013), exceeded estimated daily intake levels of piperine. Potential interactions of orally co-administered piperine were reported and comprise (a) inhibitory activity on drug metabolising enzyme systems and P-gp for various drugs, and simultaneously, enhanced bioavailability of drugs, and (b) modulation of gene and protein expression of CYP enzymes and P-gp efflux transporters. Provided that the ingestion of piperine via pepper (food flavouring) or intake of dietary supplements containing *P. nigrum* or *P. longum* does not exceed common dietary levels, the risk of adverse piperine-drug and piperine-phytochemical interactions is minimal.

A NOAEL of 5 mg/kg bw per day was identified in 2015 by EFSA based on the dose-dependent increase in plasma cholesterol levels in males at the mid and high dose (15 and 50 mg/kg bw per day) in a 90-day toxicity study in rats. The study was performed according

to OECD Guideline (TG 408) (Bauter, 2013). Using an UF = 100, an assumed safe level could be 0.05 mg/kg bw per day.

Exposure: score 1.0
Dried, ground black pepper (*Piper nigrum*) and its variants is one of the most common spices in European/Western cuisine, and thus, a major source of piperine exposure through the diet. Other potential sources of piperine include the spice Grains of Paradise (*Aframomum melegueta*) from West Africa, and consumption of piperine (pepper)-flavoured beverages and spirits.

Based on the maximised survey-derived daily intake (MSDI) approach, the estimated exposure to piperine from natural sources when consuming black pepper as flavouring ingredient, is 6.2 µg per day and 0.07 µg per day in EU and USA, respectively (EFSA, 2015). Piperine is also used in cosmetics as a perfuming agent (CosIng, 2016). This will mean an exposure to piperine from natural sources of 0.09 and 0.001 µg/kg bw per day for a 70 kg person in EU and USA, respectively.

Vulnerable groups: score 0.5
Potential adverse effects might occur due to undesired food-drug interactions caused by the uptake of black pepper or piperine-containing food. Caution should be taken regarding dietary piperine consumption during drug administration in patients (e.g. cancer treatment and chemotherapy), particularly those who favour daily pepper spice or utilise certain pepper remedies (Wang et al., 2013). Excess intake of >10 mg doses of piperine due to high consumption of pepper or intake of dietary supplements containing *P. nigrum* or *P. longum* above common dietary levels, might lead to clinically significant interactions with several drugs (Gurley et al., 2012).

Lack of toxicity data: score 0.5
There is a lack of human studies that have investigated the effect of varying and high doses of piperine for longer periods. There is lack of chronic toxicity studies of piperine in animals. No studies on adverse health effects of piperine in children, adolescents, pregnant women or lactating women were identified.

Lack of exposure data: score 0.5
There are no data available on exposure to piperine in the general Norwegian popylation.

Total score = 4.5 for piperine

10.15.2 References

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10.16 Taurine (CAS no. 107-35-7)

10.16.1 Scores

MOE/MOS/ADI/TDI/TWI (background information)

VKM concluded that a safe level based on human studies appeared to be 21 mg/kg bw per day and the exposure from the diet was up to 0.7 mg/kg bw per day. Thus, the exposure was well below the safe level. The same conclusion would be reached if using the NOAEL from the animal experiment (given a safe level of 10 mg/kg bw per day with UF = 100). However, there may be additional exposure from cosmetics.

Toxicity: score 1.0

Based on the studies by Sirdah et al. (2002), Brons et al. (2004) and Spohr et al. (2005) (20 to 50 participants, from 8 weeks to 5 months of treatment), there are indications that an intake of 1,000-1,500 mg taurine per day (corresponding to 14.3-21.4 mg/kg bw per day in a 70 kg adult) does not cause adverse health effects. Therefore, VKM considered that it was unlikely that an intake of taurine up to approximately 21 mg/kg bw per day causes adverse

health effects. The human studies available were not of sufficient quality (due to low number of participants, non-healthy populations and short duration) to be used alone in the risk characterisation.

A NOAEL of 1,000 mg/kg bw per day for pathological changes was identified by EFSA (2009), based on a 13-week neurotoxicity study in rats. Since the NOAEL set by EFSA was based on the highest dose tested, there is a possibility that the actual NOAEL is higher than 1,000 mg/kg bw per day. Therefore, VKM applied the MOE approach combined with comparisons with the intake of approximately 21 mg/kg bw per day, which was considered unlikely to cause adverse health effects based on human studies, in the risk characterisation.

The values used for comparison with the estimated exposure in the risk characterization were 21 mg/kg bw per day (from human studies) and the NOAEL of 1000 mg/kg bw per day (rat study).

Exposure: score 1.0
Taurine occurs naturally in food (EFSA, 2009). The mean daily intake of taurine from the diet has been estimated to vary between 40 and 400 mg per day (Hayes and Trautwein, 1994). This will be 0.6-5.7 mg/kg bw per day for a 70 kg person. In EU, taurine can be used in cosmetic products, and there are no restrictions with regard to either product type or use concentrations. Taurine is a buffering agent with the purpose to assure the stability of cosmetic products (CosIng, 2015).

Vulnerable groups: score 0.0
There was no information concerning specific groups vulnerable for taurine in the literature reviewed in the present risk assessment.

Lack of toxicity data: score 0.5
There is lack of an ARfD or other data on acute toxicity of taurine. Human studies on adverse effects after long-term oral exposure to taurine are lacking. Animal studies on chronic toxicity and carcinogenicity of taurine are lacking.

Lack of exposure data: score 0.5
There are no data available on exposure to taurine in the general Norwegian population

Total score = 3.0 for taurine

10.16.2 References

- Brons C., Spohr C., Storgaard H., Dyerberg J., Vaag A. (2004) Effect of taurine treatment on insulin secretion and action, and on serum lipid levels in overweight men with a genetic predisposition for type II diabetes. Eur J Clin Nutr 58:1239-1247.
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10.17 L-Tyrosine (CAS no. 60-18-4)

10.17.1 Scores

MOE/MOS/ADI/TDI/TWI (background information)
Using a safe level of 6 mg/kg bw per day set by VKM and a mean intake 40 mg/kg bw per day, the exposure exceeds the safe level by a factor 7.

Toxicity: score 1.0
In previous risk assessments of L-tyrosine, no tolerable upper intake level was established for humans. AESAN (2012) concluded that a maximum daily amount of 1,900 mg for the sum of L-tyrosine and L-phenylalanine was acceptable from the safety point of view for use as food supplements. However, it was pointed out that increases in the intake of L-phenylalanine (diets enriched with 3-7% L-phenylalanine) implied an increase in the circulating levels of L-tyrosine and that the toxic effects of L-phenylalanine were linked to those of L-tyrosine (Benevenga and Steele, 1984; Harper et al., 1970).

For L-tyrosine, no new human studies reporting on adverse effects (or the absence of such effects) in healthy individuals were retrieved, and long-term studies in humans were still missing.

Specific information about potential negative health effects and the associated doses could only be derived from the information retrieved in one animal study (Shibui et al., 2016). A LOAEL and a NOAEL of 2,000 and 600 mg/kg bw per day, respectively, for L-tyrosine were identified in a 90-day toxicological study in rats. At 2,000 mg/kg bw per day, significant increases were found in weights of livers and kidneys in addition to increased plasma lipids and hypertrophy of centrilobular hepatocytes in both sexes.

VKM used the NOAEL at 600 mg/kg bw per day as a value for comparison in the risk characterisation of the specified doses of L-tyrosine. Using an UF = 100, the safe level was 6 mg/kg bw per day.

Exposure: score 2.0
Based on distribution data from the 1988–1994 NHANES III, the mean daily intake for all life stage and gender groups of tyrosine from food and supplements is 2.8 g per day, which will be 40 mg/kg bw per day for a 70 kg person. Men 31 through 50 years of age had the highest intakes at the 99th percentile of 6.4 g per day (IOM, 2005).

Vulnerable groups: score 0.0
Mental disorders: No direct scientific evidence that the intake levels of tyrosine affect mental function negatively has been retrieved.

Lack of toxicity data: score 1.0
Lack of human toxicity studies on adverse effects as primary outcome of L-tyrosine supplementation, with the possibility to establish a dose-response relationship: The large majority of intervention studies are designed to detect health-protective and health-promoting effects of L-tyrosine. There is a need for human studies that are well-designed (randomised, blinded, placebo-controlled, multicenter), with L-tyrosine given as a single supplement as the intervention with graded doses, of sufficient sample size, designed to study long-term effects – i.e. sufficient duration of intervention and sufficient duration of follow-up, performed in healthy subjects representative of the general population.

Fetuses, pregnant and lactating women: It is not known whether moderate supplementation with L-tyrosine has any effect on the human fetus, or whether tolerance is different in pregnant and lactating women.

Lack of data in children and adolescents: A systematic literature search in children and adolescents with no restriction concerning publication year retrieved no relevant studies, revealing a severe lack of data about potential adverse health effects of L-tyrosine in children and adolescents.

With only one study, there is a general lack of toxicological studies in rodents that are performed according to OECD Guidelines or similar, with L-tyrosine given as a single supplement as the intervention, with graded, sufficiently high doses, and designed to study long-term effects – i.e. sufficient duration of intervention.

Lack of exposure data: score 0.5
There are no data available concerning dietary intake of L-tyrosine in the general Norwegian population.

Total score = 4.5 for L-tyrosine

10.17.2 References

- AESAN (2012). Report of the Spanish Agency for Food Safety and Nutrition (AESAN) on the condition for use of certain substances other than vitamins,

minerals and plants in food supplements - 1, The Spanish Agency for Food Safety and Nutrition (AESAN), Spain.
- Benevenga N.J., Steele R.D. (1984). Adverse effects of excessive consumption of amino acids. Annu Rev Nutr 4:157-81. DOI: 10.1146/annurev.nu.04.070184.001105.
- Harper A.E., Benevenga N.J., Wohlhueter R.M. (1970). Effects of ingestion of disproportionate amounts of amino acids. Physiol Rev 50:428-558.
- IOM (2005). Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. Institute of Medicine of the National Academies. https://www.nap.edu/read/10490.
- Shibui Y., Manabe Y., Kodama T., Gonsho A. (2016). 13-week repeated dose toxicity study of l-tyrosine in rats by daily oral administration. Food Chem Toxicol 87:55-64. DOI: 10.1016/j.fct.2015.11.017.

11 Ranking of trace elements

An overview of the scoring and ranking of the included trace element is given in Table 11-1. A detailed description follows after the table.

Table 11-1. Summary table for scoring of trace elements.

Substance	1. MOE/MOS/ADI/TDI/TWI	2. Toxicity	3. Exposure	4. Vulnerable groups	5. Lack of toxicity data	6. Lack of exposure data	Total score	Comments
Iodine	-	3.0	2.0	1.0	0.5	0.5	7.0	

11.1 Iodine

The potential effect of sporadic high intakes of iodine is not known. No MOE or TWI values exist, only an upper level of 600 µg iodine per day (SCF, 2002; NNR, 2014). Large groups of the population have inadequate iodine intake (Henjum et al., 2019) and individuals with inadequate iodine intake are more sensitive to sporadic high intakes than individuals with adequate iodine intakes. In Norway, there is currently a huge interest in production and consumption of macroalgea (e.g. kelp and seaweed) and products made from these.

11.1.1 Scores

Toxicity: score 3.0
The substance has low toxicity in healthy iodine-replete individuals, but abrupt increased intakes in individuals with inadequate iodine intake often result in a temporary thyroid "shut down" or "thyroid stunning". This is particularly harmful to fetal development in the first half of pregnancy (Moleti et al., 2011).

Exposure: score 2.0
The exposure due to sporadic ingestion of seaweed may result in high exposure, but the toxicity of occasional high intakes (i.e. >UL) is largely unknown.

Vulnerable groups: score 1.0
Pregnant women and their fetuses, as well as elderly individuals with nodular goitre, patients with heart disease.

Lack of toxicity data: score 0.5
The toxicity of sporadic high intake in vulnerable individuals is largely unknown.

Lack of exposure data: score 0.5
The exposure through ingestion of seaweed, particularly dried kelp is very difficult to characterize due to large variation between as well as within species of macro algea (EU, 2018).

Total score = 7.0 for iodine

11.1.2 References

- EU (2018). COMMISSION RECOMMENDATION (EU) 2018/464 of 19 March 2018 on the monitoring of metals and iodine in seaweed, halophytes and products based on seaweed. https://eur-lex.europa.eu/legal-content/GA/TXT/?uri=CELEX:32018H0464.
- Henjum S., Abel M.H., Meltzer H.M., Dahl L., Alexander J., Torheim L.E., Brantsaeter A.L. (2019). Er inntaket av jod i befolkningen tilstrekkelig? Tidsskr Nor Laegeforen 139(2), doi: 10.4045/tidsskr.18.0319.
- Moleti M., Di Bella B., Giorgianni G., Mancuse A., De Vivo A., Alibrandi A., Trimarchi F., Vermiglio F. (2011). Maternal thyroid function in different conditions of iodine

nutrition in pregnant women exposed to mild-moderate iodine deficiency: an observational study. Ckin Endocrinol 74(6).
- NNR (2014). Nordic Nutrition Recommendations 2012 Integrating nutrition and physical activity. 5th edition, https://norden.diva-portal.org/smash/get/diva2:704251/FULLTEXT01.pdf.
- SCF (2002). Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Iodine. Scientific Committee on Food, EU. https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out146_en.pdf.

12 Best sampling practice, and foods, drinks and/or food supplements for monitoring

12.1 Best sampling practice

The ultimate goal of any sampling of foods, drink or food supplements is to obtain a representative sample that can be analysed for the compound of interest, and where the analytical result can be used to make valid inferences about the larger population of food items that is being investigated. A representative sample must therefore closely match the characteristics of the population from which it was derived. Given the possible differences in ingredients used to produce a food item, different manufacturing processes, harvesting conditions etc. it is imperative that a sound sampling strategy is employed to ensure representative values in food. It is beyond the scope of the current ranking to provide a comprehensive strategy for sampling. However, several intitatives have been undertaken in order to provide guidelines for the development of sampling strategies (FAO 2003; Esbensen et al., 2015; Ramsey et al., 2019).

12.1.1 General comments

- When possible, it would be very useful if the sampling could be used to estimate exposure of the Norwegian population as well as for monitoring.
- Methods used for validation of analytical methods should follow international standards for validation.
- When existing, multi-methods, i.e. methods that can be used to measure several chemical compounds simultaneously in a certain food, should be used in order to be able to evaluate mixture effects of chemicals.
- It is known that for some groups of substances, such as mycotoxins and PFAS, newer analytical methods exist that are not yet in use for monitoring. When possible, these newer, more sensitive methods, should be used.
- For some substances, such as dioxins and PFAS, substantial knowledge is available about their levels in raw foods. New occurrence data should preferably be collected for ready to eat foods, whereas for substances for which less data on occurrence are available, data on levels in raw foods are still needed.
- For some substances, for instance furan and acrylamide, there is a need for data on how regular consumers prepare these foods in their homes (i.e. cooking methods, temperature etc.), in order to get a more complete and correct picture of the exposure to such substances.

- For substances in plants, sampling for analyses should be taken from the part(s) of the plant containing the highest level of the substances, if known.
- There is currently an increasing trend, seen both in restaurants and in books for home cooking, to use plants in foods that have traditionally not been used in foods, and for which potential toxicity is not well studied.

12.1.2 Factors to consider before sampling

Some general factors that should be considered are:

- What is adequate sampling and number of samples to ensure a monitoring that is representative for the occurrence of a given substance in foods consumed by the Norwegian population?
- For persistant substances that may acculamulate in the environment time-trends are needed.
- Are there expected changes in exposure due to changes in use (e.g. substitution, change in diatary habits)?
- Should the samples be taken from foods that are imported, from foods that are produced in Norway, or both?
- Is the time of the year for sampling important for the result?
- Should the sample be taken from a distinct part of the animal/plant?

12.1.3 Regulations and guidance documents for best sampling practice

Several EU regulations and guidance documents addresses best sampling practices to ensure adequate sampling procedure and number of samples:

- Commission Regulation (EC) 401/2006; for the control of levels of mycotoxins
- Commission Regulation (EC) No 333/2007; for the control of levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs
- Commission Regulation (EU) 2017/644 of 5 April 2017; for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs,
- Regulation (EU) No 589/2014; methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs
- Commission Regulation (EC) 1882/2006, for the control of levels of nitrates
- Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food
- Guidance Document on Measurement Uncertainty for Laboratories performing PCDD/F and PCB Analysis using Isotope Dilution Mass Spectrometry which has been elaborated by the European Reference Laboratories in the field of contaminants in feed and food.
- Guidance document on identification of mycotoxins in food and feed
- Guidance document for competent authorities for the control of compliance with EU legislation on aflatoxins

- Guidance document for the implementation of Commission Regulation (EU) 519/2014 provides guidance for sampling of lots, in particular for the sampling of large lots and silos for the control of mycotoxins for the implementation of the provisions provided for in Regulation (EC) 401/2006 as amended by Commission Regulation (EU) 519/2014.
- Mycotoxin sampling plans for food products from different agencies:
    - EU (2014). COMMISSION REGULATION (EU) No 519/2014 of 16 May 2014 amending Regulation (EC) No 401/2006 as regards methods of sampling of large lots, spices and food supplements, performance criteria for T-2, HT-2 toxin and citrinin and screening methods of analysis. Official Journal of the European Union L147/29.
    - IARC (2003). Sampling and sample preparation methods for determining concentrations of mycotoxins in foods and feeds. IARC_SP158_Chapter 3. http://publications.iarc.fr/_publications/media/download/1373/b43c4bc7b32727c9788ece5e75c7dd8392b3e3eb.pdf
    - Food Standards Agency UK (2016). Mycotoxins sampling guidance. https://www.food.gov.uk/sites/default/files/media/document/mycotoxins-sampling-guidance.pdf
    - FAO (2014). FAO mycotoxin sampling tool. http://www.fstools.org/mycotoxins/Documents/UserGuide.pdf
    - List of CEN, EN & ISO methods & general requirements for mycotoxin analysis in food and feed. https://www.wur.nl/upload_mm/b/9/0/0c2700c3-7849-470f-bfbe-da7581fc16da_04.%20Commercially%20Available%20Services%20Mycotoxins_2018%20CEN-EN-ISO%20methods%20%28food%29.pdf
    - USDA / GIPSA / FGIS (1995) Grain Inspection Handbook - Book I: Grain Sampling. Policies and procedures for sampling grain in accordance with the regulations under the United States Grain Standards Act. www.usda.gov/gipsa
    - USDA / GIPSA / FGIS (1995) Mechanical Sampling Systems Handbook. Policies and procedures regarding the equipment requirements, installation, authorization, examination and testing of mechanical sampling systems. www.usda.gov/gipsa
    - JRC (2011). Mycotoxins Factsheet. 4th edition. JRC Technial Notes. https://ec.europa.eu/jrc/sites/jrcsh/files/Factsheet%20Mycotoxins_2.pdf

    Articles:

    - Whitaker, T., Slate, A., Doko, B., Maestroni, B., & Cannavan, A. (Eds.). (2010). Sampling procedures to detect mycotoxins in agricultural commodities. Springer. DOI: 10.1007/978-90-481-9634-0
    - WILLIAM, J. (1980). Protocols for Surveys, Sampling, Post-Collection Handling, and Analysis of Grain Samples Involved in Mycotoxin Problems. J. ASSOC. OFF. ANAL. CHEM. 63:95-102.

Extensive summary, published by a commercial laboratory:

- Romer Labs® Guide to Mycotoxins. Vol. 2: Sampling and Sample Preparation for Mycotoxin Analysis. http://www.foodriskmanagement.com/wp-content/uploads/2013/03/Sampling-and-Sample-Preparation-for-Mycotoxin-Analisis1.pdf

The list above is by no means exhaustive or comprehensive.

12.2 Foods, drinks and/or food supplements for monitoring

For each of the substances included in Table 1-1, an overview of foods, drinks and/or dietary supplements relevant for monitoring is given in Tables 12.2-1 to 12.2-9. This overview was prepared based on the VKM members' expert judgements. Due to time constraints, no literature searches were performed.

Table 12.2-1. Natural toxins: foods, drinks and/or food supplements for monitoring.

Substance	Total score	Sources	Specific comments
Aflatoxins (AFLAs)	7.5	Imported foods, especially peanuts, tree nuts, dried fruits, spices, Norwegian maize	- The LOQ should be lower - Multimethods should be used to analyse foods for several mycotoxins to be able to address mixed exposure - Methods detecting modified forms should also be used - The methods should be validated according to international guidance for validation - The need for monitoring may vary according to the climate - Samples representing foods eaten i Norway should be included - Samples representying feed used in Norway should be included
Alternariol (AOH) and Alternariol methyl ether (AME)	6.0	Cereal grains and products thereof, especially Norwegian grains, imported foods such as tomato-based products, sesame seeds and oil seeds	
Deoxynivalenol (DON) and modified forms	6.0	Cereal grains and products thereof	
Enniatins (ENNs)	6.5	Cereal grains and products thereof, especially Norwegian grains	
Ochratoxin A (OTA)	5.5	Imported foods, especially coffee, spices, dried fruits, herbal teas, tree nuts, seeds and maize	
Patulin (PAT)	3.5	Norwegian and imported fresh fruits, fruit juices (especially apple juice), baby food	
T-2 (T2) and HT-2 (HT2) toxins and modified forms	8.5	Cereal grains and products thereof, especially Norwegian wheat and oats	
Zearalenone (ZEN) and modified forms	3.5	Cereal grains and products thereof, especially maize and wheat bran, vegetable oils	

Substance	Total score	Sources	Specific comments
Pyrrolizidine alkaloids (PAs)	8.0	Tea, honey and cereal-based foods	- Samples should include herbal teas, infusions and food supplements
Solanine and Chaconine	6.5	Mostly in potato and potato-derived products, but also in some other vegetables	
Cyanogenic glucosides	5.5	Almond, linseed, apricot kernels, marzipan, persipan, cassava and bamboo shoots	
Erucic acid	5.0	Fish and other seafood	
Tropane alkaloids (TAs)	6.0	Cereals and cereal-derived products in particular, gluten-free products, food supplements and herbal teas, legumes, beans (lupins) and oilseeds and derived products	- The LOQ should not be higher than of 10 µg/kg for hyoscyamine/atropine and scopolamine and preferably below 5 µg/kg according to Commission Recommendation (EU) 2015/976
Azaspiracids (AZAs)	6.5	Shellfish	- The brown crab meat may contain high concentrations. To our knowledge, no samples are taken in Norway
Tetrodotoxin (TTX) and TTX analoges	6.5	Shellfish	- There is a lack of Norwegian data
Microcystins (MCs)	6.5	Drinking water, algal supplements	

Table 12.2-2. Metals and metalloids: foods, drinks and/or food supplements for monitoring.

Substance	Total score	Sources	Specific comments
Aluminium (Al)	4.5	Drinking water and agricultural products, cereal products produced with baking powder	
Inorganic arsenic (As)	6.5	Grain-based processed products such as rice and wheat bread, seafood, algal products, milk and dairy products, and drinking water	- The proportion of inorganic arsenic in seafoods needs to be evaluated
Organic arsenic (As)	2.0	Seafood	- Data on organic arsenic species are needed
Cadmium (Cd)	6.5	Cereals and cereal products, vegetables, nuts and pulses, starchy roots or potatoes, meat and meat products, products of liver and kidney	- Whole fish analyses of fish like nalyses of fish like sardines and anchovies
Chromium (Cr)	3.0	Drinking water	
Lead (Pb)	7.5	Game meat (large and small game), minced meat from cervids, cereal products and grains and vegetables (especially potatoes and leafy vegetables)	- Data on small game shot with lead ammunition
Methylmercury (MeHg)	7.0	Fish and fish products and shellfish	
Nickel (Ni)	3.0	Plants accumulating nickel, e.g. cocoa products	

Table 12.2-3. Persistent organic pollutants: foods, drinks and/or food supplements for monitoring.

Substance	Total score	Sources	Specific comments
1,2-Bis(2,4,6-tribromophenoxy)ethane (BTBPE)	4.0	Fish and seafood	- Fatty fish and fish liver
Decabromo-diphenyl ethane (DBDPE)	4.0	Fish and seafood, and land-based food such as butter, cheese and eggs	- Composite food such as e.g. fish gratin and fish cakes
Hexabromobenzene (HBB)	4.0	Fish and seafood	
Hexabromocyclododecane (HBCDD)	3.0	Fish and seafood, and land-based food such as butter, cheese and eggs	- Composite food such as e.g. fish gratin and fish cakes
Polybrominated diphenyl ethers (PBDEs) (including Decabromodiphenyl ether (DecaBDE))	3.5	Fish and seafood, and land-based food such as butter, cheese and eggs	- Composite food such as e.g. fish gratin and fish cakes
2,4,6-Tribromophenol (TBP)	4.0	Fish and seafood	
Dechlorane plus (syn-DP and anti-DP)	5.0	Norwegian food in general, food of animal origin are of particular interest	
Dioxins and Dioxin-like PCBs (DL-PCBs)	8.0	Fish and seafood, and land-based food such as butter, cheese and eggs	- Composite food such as e.g. fish gratin and fish cakes
Non-dioxin-like PCBs (NDL-PCBs)	5.5	Fish and seafood, and land-based food such as butter, cheese and eggs	- Composite food such as e.g. fish gratin and fish cakes
Perfluorohexane sulfonic acid (PFHxS), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Perfluoroundecanoic acid (PFUnDA) and Perfluoroheptane sulfonate (PFHpS)	6.5	Drinking water, fish and other seafood	- The LOQ should be lower

Substance	Total score	Sources	Specific comments
Perfluorooctane sulfonate (PFOS), Perfluorooctanoic acid (PFOA)	8.0	Drinking water, fish and other seafood	- Concentrations in drinking water should be analysed
Octamethylcyclotetra-siloxane (D4)	3.5	Fish and seafood	- Pelagic fish have shown higher concentrations of cyclic siloxanes than benthic feeding fish (perch, whitefish and burbot). The concentration of cyclic siloxanes in freshwater fish varies largely between lakes and has been correlated to local sources like the effluent load from wastewater treatment plants
Decamethylcyclopenta-siloxane (D5)	3.5		
Dodecamethylcyclohexa-siloxane (D6)	4.0		

Table 12.2-4. Substances in food contact materials: foods, drinks and/or food supplements for monitoring.

Substance	Total score	Sources	Specific comments
Bisphenol A (BPA)	3.0	All foods packed in food contact materials containing bisphenol A	
Bisphenol S (BPS), Bispenol F (BPF) and Bisphenol AF (BPAF)	6.5	All foods packed in food contact materials containing bisphenol S, F or AF	
Di-butylphthalate (DBP)	3.5	Phthalates are plastic softeners, used in plastic food contact materials, and are therefore present in many types of packaged food. They are also environmental contaminants in foods from many other every day products	
Butyl-benzyl-phthalate (BBP)	3.5		
Bis(2-ethylhexyl)phthalate (DEHP)	3.5		
Di-isononyl phthalate (DINP)	3.5		

Substance	Total score	Sources	Specific comments
Di-isodecyl phthalate (DIDP)	3.5		

Table 12.2-5. Flavourings: foods, drinks and/or food supplements for monitoring.

Substance	Total score	Sources	Specific comments
Caffeine	6.5	All caffeine-containing foods and beverages	

Table 12.2-6. Additives: foods, drinks and/or food supplements for monitoring.

Substance	Total score	Sources	Specific comments
Acesulfame K (E950)	4.5	All foods containing acesulfame K	
Butylated hydroxyanisole (BHA, E320))	4.0	Oil-containing foods, such as potato chips, cake mixes, cereals and dehydrated soups/sauces	- Data from ready to eat foods are needed
Butylated hydroxytoluene (BHT, E321)	5.0	All foods approved to use BHT as an additive. Foods from animals that have eaten feed containing BHT, e.g. farmed fish, milk and eggs	- Data from ready to eat foods are needed
Ethoxyquin (EQ)	6.5	Foods from animals that have eaten feed containing EQ, especially if fish meal has been used as a feed ingredient, e.g. farmed fish	- Fatty products - Transformation products should be included
Sodium and potassium salts of nitrite and nitrate	5.5	Cured meats and other meat products	

Substance	Total score	Sources	Specific comments
Phosphoric acid-phosphates	7.5	Many different foodstuffs, e.g. meat products, fish products, dairy, bakery products, grain-based foods and soft drinks etc.	- Data from ready to eat foods are needed
Sucralose (E955)	3.0	All foods containing sucralose	

Table 12.2-7. Process-induced contaminants: foods, drinks and/or food supplements for monitoring.

Substance	Total score	Sources	Specific comments
Acrylamide	8.0	Biscuits, crackers and crispbreads, bread products, breakfast cereals, coffee, fried potato products, food for infants and young children	
Furan, 2-Methylfuran and 3-Methylfuran	8.5	Brewed coffee, fruit juice, milk-based products, cereal-based products and jarred baby foods	- Commercial foodstuffs as purchased disregarding any further preparation (e.g. coffee powder, juices, jars and cans not heated before consumption) and commercial foodstuffs analysed as consumed after further preparation (e.g. brewed coffee, canned and jarred products heated before consumption)
Glycidyl fatty acid esters (GEs)	8.0	Refined vegetable oil and fish oils	- Fish oils are not included in the EFSA Opinion (for reference, see chapter 8.2.2.2)
3-Monochloropropanediol (3-MCPD) and its fatty esters	5.5	Vegetable oils and fats and derived products such as margarine and similar products	

		Foods for particular nutritional uses infant- and follow on formulae Fine bakery wares, bread and rolls Canned meat (smoked) and canned fish (smoked) Potato- or cereal-based snacks, other fried potato-based products Vegetable oil-containing foods and foods prepared/produced with vegetable oils	
Polycyclic aromatic hydrocarbons (PAHs)	6.0	Barbequed and grilled food, especially over open flame, and blue mussels	- Data from barbecued food prepared using different methods
2-Amino-1-methyl-6-phenylimidazo[4,5-***b***]pyridine (PhIP)	7.0	Meat, especially read meat, but also chicken and fish (fried, grilled or barbequed), in increasing amounts with higher cooking time and temperature	- Data from fried, barbecued and grilled foods prepared using different method
Heterocyclic aromatic amines (HAAs)	7.0		

Table 12.2-8. «Other substances»: foods, drinks and/or food supplements for monitoring.

Substance	**Total score**	**Sources**	**Specific comments**
L-Aspartic acid	4.5	Food supplements	
L-Carnitine and L-Carnithine-L-tartrate	4.0	Food supplements	

Substance	Total score	Sources	Specific comments
Coenzyme Q10 (CoQ10)	3.0	Food supplements	
Conjugated linoleic acids (CLAs)	4.0	Food supplements	
Creatine	3.5	Food supplements	
Curcumin	6.0	Food supplements	
L-Cysteine and L-Cystine	3.5	Food supplements	
Docosahexaenoic acid (DHA)	5.5	Food supplements	
Docosapentaenoic acid (DPA)	3.0	Food supplements	
Eicosapentaenoic acid (EPA)	3.0	Food supplements	
D-Glucurono-γ-lactone	3.0	Energy drinks	
Inositol	3.5	Energy drinks	
Lycopene	6.0	Food supplements	
L-Methionine	4.0	Food supplements	
Piperine	4.5	Food supplements	
Taurine	3.0	Food supplements/energy drinks	
L-Tyrosine	4.5	Food supplements	

Table 12.2-9. Trace elements: foods, drinks and/or food supplements for monitoring.

Substance	Total score	Sources	Specific comments
Iodine	7.0	Seaweed, particularly dried kelp	

12.3 References

- Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02006R0401-20140701.
- Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and polycyclic aromatic hydrocarbons in foodstuffs (Text with EEA relevance). https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02007R0333-20120901.
- Commission Regulation (EU) 2017/644 of 5 April 2017 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 589/2014. https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1495826386026&uri=CELEX:32017R0644.
- Commission Regulation (EC) No 1882/2006 of 19 December 2006 laying down methods of sampling and analysis for the official control of the levels of nitrates in certain foodstuffs. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32006R1882.
- Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food (2016). European Union Reference Laboratory. https://ec.europa.eu/food/sites/food/files/safety/docs/cs_contaminants_sampling_guid-doc-lod-loq.pdf.
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- Guidance Document for the Implementation of Commission Regulation (Eu) No 519/2014 of 16 May 2014 Amending Regulation (Ec) No 401/2006 Laying Down The Methods Of Sampling And Analysis For The Official Control Of The Levels Of Mycotoxins In Food (2014). Endorsed By The Standing Committee On The Food Chain And Animal Health Section Toxicological Safety Of The Food Chain Animal Nutrition.

https://ec.europa.eu/food/sites/food/files/safety/docs/cs_contaminants_sampling_guidance-sampling-final_en.pdf.

- Compilation Of Agreed Monitoring Recommendations As Regards The Presence Of Mycotoxins And Plant Toxins In Food (2014). Summary Report Of The Standing Committee On Plants, Animals, Food And Feed. https://ec.europa.eu/food/sites/food/files/safety/docs/cs_monitoring_recommendations_en.pdf.

13 The ranking of all substances

An overview of the result of the ranking of all included substances, from the highest to the lowest score, based on risk and knowledge gaps, is given in Table 13-1. The table of ranked substances should therefore be read together with the main text, where calculations are included and explanations are given for the scoring.

Table 13-1. Ranking of the included substances.

Substance group	Sub-group	Substance	Total score
Natural toxins	Mycotoxins	T-2 (T2) and HT-2 (HT2) toxins and modified forms	8.5
Process-induced contaminants	Furans	Furan, 2-Methylfuran and 3-Methylfuran	8.5
Natural toxins	Plant toxins	Pyrrolizidine alkaloids (PAs)	8.0
Persistent organic pollutants	Perfluorinated and polyfluorinated alkyl substances	Perfluorooctane sulfonate (PFOS), Perfluorooctanoic acid (PFOA)	8.0
Persistent organic pollutants	Dioxins and Dioxin-like PCBs	Dioxins and Dioxin-like PCBs (DL-PCBs)	8.0
Process-induced contaminants	Esterified 3- and 2-monochloropropane-1,2-diol and glycidyl esters	Glycidyl fatty acid esters (GEs)	8.0
Process-induced contaminants	Acrylamide	Acrylamide	8.0
Metals and metalloids		Lead (Pb)	7.5
Natural toxins	Mycotoxins	Aflatoxins (AFLAs)	7.5
Additives	Phosphates	Phosphoric acid-phosphates	7.5
Metals and metalloids		Methylmercury (MeHg)	7.0
Process-induced contaminants	Hetetrocyclic aromatic amine	2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)	7.0
Process-induced contaminants	Process-induced contaminants	Heterocyclic aromatic amines (HAAs) in general	7.0
Trace elements	Trace element	Iodine	7.0
Flavourings	Flavouring	Caffeine	6.5
Natural toxins	Mycotoxins	Enniatins (ENNs)	6.5
Additives	Synthetic antioxidant	Ethoxyquin (EQ)	6.5
Natural toxins	Plant toxins	Solanine and Chaconine	6.5
Natural toxins	Marine algae toxins	Azaspiracids (AZAs)	6.5
Natural toxins	Marine algae toxins	Tetrodotoxin (TTX) and TTX analogues	6.5
Natural toxins	Freshwater algae toxins	Microcystins (MCs)	6.5
Metals and metalloids		Cadmium (Cd)	6.5

Substance group	Sub-group	Substance	Total score
Metals and metalloids		Inorganic arsenic (As)	6.5
Persistent organic pollutants	Perfluorinated and polyfluorinated alkyl substances	Perfluorohexane sulfonic acid (PFHxS), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Perfluoroundecanoic acid (PFUnDA) and Perfluoroheptane sulfonate (PFHpS)	6.5
Substances in food contact materials	Bisphenols	Bisphenol S (BPS), Bispenol F (BPF) and Bisphenol AF (BPAF)	6.5
Natural toxins	Mycotoxins	Deoksynivalenol (DON) and modified forms	6.0
Natural toxins	Mycotoxins	Alternariol (AOH) and Alternariol methyl ether (AME)	6.0
Natural toxins	Plant toxins	Tropane alkaloids (TAs)	6.0
Process-induced contaminants	Polycyclic aromatic hydrocarbons (PAH)	Polycyclic aromatic hydrocarbons (PAHs)	6.0
«Other substances»		Curcumin	6.0
«Other substances»		Lycopene	6.0
Natural toxins	Plant toxins	Cyanogenic glucosides	5.5
Natural toxins	Mycotoxin	Ochratoxin A (OTA)	5.5
Persistent organic pollutants	Non-dioxin-like PCBs	Non-dioxin-like PCBs (NDL-PCB)	5.5
Process-induced contaminants	Esterified 3- and 2-monochloropropane-1,2-diol (MCPD) and glycidyl esters (GE)	3-Monochloropropanediol (3-MCPD) and its fatty esters	5.5
«Other substances»		Docosahexaenoic acid (DHA)	5.5
Additives	Nitrites and nitrates	Sodium and potassium salts of nitrite and nitrate	5.5
Natural toxins	Plant toxin	Erucic acid	5.0
Persistent organic pollutants	Dechloranes	Dechlorane plus (syn-DP and anti-DP)	5.0
Additives	Synthetic antioxidant	Butylated hydroxytoluene (BHT, E321)	5.0
Metals and metalloids		Aluminium (Al)	4.5
Additives	Sweetener	Acesulfame K (E950)	4.5
«Other substances»		Piperine	4.5
«Other substances»		L-Aspartic acid	4.5
«Other substances»		L-Tyrosine	4.5
Metals and metalloids		Organic arsenic (As)	4.0
Additives	Synthetic antioxidant	Butylated hydroxyanisole (BHA, E320)	4.0
Natural toxins	Plant toxins	Glucosinolates	4.0

Substance group	Sub-group	Substance	Total score
Persistent organic pollutants	Siloxane	Dodecamethylcyclohexasiloxane (D6)	4.0
Persistent organic pollutants	Brominated flame retardant	Hexabromobenzene (HBB)	4.0
Persistent organic pollutants	Brominated flame retardant	Decabromo-diphenyl ethane (DBDPE)	4.0
Persistent organic pollutants	Brominated flame retardant	1,2-Bis(2,4,6-tribromophenoxy)ethane (BTBPE)	4.0
Persistent organic pollutants	Brominated flame retardant	2,4,6-Tribromophenol (TBP)	4.0
«Other substances»		L-Carnithine and L-Carnitine-L-tartrate	4.0
«Other substances»		L-Methionine	4.0
«Other substances»		Conjugated linoleic acids (CLAs)	4.0
Natural toxins	Mycotoxin	Patulin (PAT)	3.5
Persistent organic pollutants	Siloxane	Octamethylcyclotetrasiloxane (D4)	3.5
Persistent organic pollutants	Siloxane	Decamethylcyclopentasiloxane (D5)	3.5
Persistent organic pollutants	Brominated flame retardants	Polybrominated diphenyl ethers (PBDEs), including Decabromodiphenyl ether (DecaBDE)	3.5
Substances in food contact materials	Phthalate	Di-butylphthalate (DBP)	3.5
Substances in food contact materials	Phthalate	Butyl-benzyl-phthalate (BBP)	3.5
Substances in food contact materials	Phthalate	Bis(2-ethylhexyl)phthalate (DEHP)	3.5
Substances in food contact materials	Phthalate	Di-isononyl phthalate (DINP)	3.5
Substances in food contact materials	Phthalate	Di-isodecyl phthalate (DIDP)	3.5
«Other substances»		Inositol	3.5
«Other substances»		L-Cysteine and L-Cystine	3.5
«Other substances»		Creatine	3.5
Natural toxins	Mycotoxins	Zearalenone (ZEN) and modified forms	3.5
Metals and metalloids		Nickel (Ni)	3.0
Persistent organic pollutants	Brominated flame retardant	Hexabromocyclododecane (HBCDD)	3.0

Substance group	Sub-group	Substance	Total score
Substances in food contact materials	Bisphenol	Bisphenol A (BPA)	3.0
Additives	Sweetener	Sucralose (E955)	3.0
«Other substances»		Coenzyme Q10 (CoQ10)	3.0
«Other substances»		D-Glucurono-γ-lactone	3.0
«Other substances»		Taurine	3.0
«Other substances»		Eicosapentaenoic acid (EPA)	3.0
«Other substances»		Docosapentaenoic acid (DPA)	3.0
Metal and metalloids		Chromium (Cr)	3.0

Appendix I

Suggested substances that were not included in this ranking

General reasons for exclusion of substances:

- The ranking method we used was new. We therefore considered it important to test the method thoroughly on a more limited number of different types of substances, and then evalute the method and identify potential needs for revision.
- The time available to perform the ranking was limited. Therefore, it was desirable to reduce the number of substances.

Substance group	Sub-group	Name	Reason for exclusion
Natural toxins	Plant toxins	Furocoumarins	These are classes of several chemical compounds, and to risk rank all was not possible due to the limited time available. Individual substances for inclusion should be identified. Wild mushrom toxins are out of the scope.
		Lectins	
		Phytoestrogens	
		Saponins	
		Toxins in wild mushroms	
Food aditives		Titanium dioxide (E171)	It was not clear to the project group how to rank titanium dioxide, due to the variation in particle size and toxicity of different particles. From EFSA (2018): • The fraction of titanium dioxide nanoparticles measured in E 171 is method-dependent. • There are no set limits for the particle size of titanium dioxide in the EU specifications (Commission Regulation (EU) No 231/2012).
	Dietary emulsifiers		There are several types of dietary emulsifiers, including e.g. stabilisers, thickeners and gelling agents, and to risk rank all was not possible due to the limited time available. Individual substances for inclusion should be identified.
Microplastics			On-going assessment in VKM, could not yet be ranked.
Nanoparticles			It was not possible to rank such a large group of different substances due to the limited time available. Individual substances for inclusion should be identified.