

SEAFOOD^{TOMORROW}



Nutritious, safe and sustainable seafood for consumers of tomorrow

Grant agreement no: 773400

Deliverable 3.5

Consumer's health impact (benefit-to-risk assessment) of solutions

Due date of deliverable: 30/04/2020

Actual submission date: 20/05/2020

Start date of the project: 01/11/2017

Duration: 36 months

Organisation name of lead contractor: RIVM

Revision: v1

Project co-funded by the European Commission within the H2020 Programme	
Dissemination Level	
PU Public	X
PP Restricted to other programme participants (including the Commission Services)	
RE Restricted to a group specified by the consortium (including the Commission Services)	
CO Confidential, only for members of the consortium (including the Commission Services)	



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 773400 (SEAFOOD^{TOMORROW}). This output reflects only the author's view and the European Union cannot be held responsible for any use that may be made of the information contained therein.



Table of Contents

1. Summary.....	5
2. Objective.....	5
3. Background.....	6
4. Materials and methods.....	10
5. Results.....	15
6. Discussion	27
7. Conclusions.....	33
8. References	35
9. Supplements.....	39
10. Appendix.....	48

List of abbreviations

Abbreviation	Meaning
ADI	Acceptable Daily Intake
ARfD	Acute Reference Dose
As	Arsenic
BMDL	Bench Mark Dose level
Cd	Cadmium
CI	Confidence Interval
Cu	Copper
DHA	Docosahexaenoic acid
DNFCS	Dutch National Food Consumption Survey
DNA	DeoxyriboNucleic Acid
DW	Dry Weight
EC	European Commission
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic acid
HBGV	Health Based Guidance Value
Hg	Mercury
I	Iodine
IMTA	Integrated Multi-trophic Aquaculture
InAs	Inorganic Arsenic
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
KAP	Qualityprogramme Agricultural Products (Kwaliteitsprogramma Agrarische Producten)
LB	Lower Bound
LNN	Log-Normal-Normal model
LOD	Level of Detection
LOQ	Level of Quantification
MB	Mean Bound
MCRA	MonteCarlo Risk Assessment
MeHg	Methylmercury
ML	Maximum Limit
MRL	Maximum Residue Level
NEVO	Dutch Food Composition table
NIFES	The Norwegian National Institute of Nutrition and Seafood Research
NVWA	Netherlands Food and Consumer Product Safety Authority (Nederlandse Voedsel- en Warenautoriteit)
OIM	Observed Individual Mean
P50.....P95	50th Percentile.....95th Percentile
Pb	Lead
Pctl	Percentile
PTWI	Provisional Tolerable Weekly Intake
PTMI	Provisional Tolerable Monthly Intake
RfD	Reference Dose
RIVM	The Dutch Institute for Public Health and the Environment
SCF	Scientific Committee on Food
Se	Selenium
UB	Upper Bound
US EPA	United States Environmental Protection Agency
WFSR	Wageningen Food Safety Research
WHO	World Health Organisation
WW	Wet Weight
Zn	Zinc

1. Summary

The consortium SEAFOOD^{TOMORROW} aims to develop innovative environmentally-friendly and transparent seafood, including the production and processing methods, that will improve the production of healthy and safe seafood in Europe. The impacts of these innovative foods on human health, nutrition and safety, are evaluated in this report by the Dutch Institute for Public Health and the Environment (RIVM). Two innovations of the SEAFOOD^{TOMORROW} consortium were included in the main analysis. The first innovation (Task 1.1) was farmed fish (trout, seabream and carp) that was fortified via feed with iodine-rich macroalgae, selenized-yeast and DHA-rich microalgae biomasses. The second innovation (Task 1.2) was seaweed intended for human consumption that was cultivated in proximity of salmon sea-cages (IMTA). The effect of both innovations on human health were assessed by investigating scenarios in which these innovative products were consumed. In these scenarios, current consumption was replaced by consumption of fortified trout, seabream, carp and (partly) seaweed foods and compared with the reference intake.

Using Monte Carlo Risk Assessment software (MCRA), long-term nutrient intake (iodine, selenium, EPA and DHA and heavy metal exposure (total arsenic, inorganic arsenic, lead, mercury and cadmium) for the scenarios were assessed for two European countries i.e. the Netherlands and Portugal. Background diet and concentration data were used to determine the usual intake and exposure.

Results showed that fortified fish has increased levels of iodine, selenium and DHA. Seaweed has relatively high concentrations of iodine, cadmium and arsenic. Exposure to fortified fish did not increase nutrient intake or exposure to heavy metals for the Dutch and Portuguese population. The hypothetical consumption of seaweed foods would increase potential iodine and arsenic intake among Dutch consumers. When compared to health-based guidance values, however, with current low consumption, the higher levels were not of a concern for the Dutch consumers.

Two other innovations developed in SEAFOOD^{TOMORROW} are salt reduction in the production of smoked salmon and fish pate (Task 2.1) and the development of new dishes targeted at pregnant women, youth and the elderly (Task 2.2). The risk and benefit assessment of these two tasks is described in chapter 9. Due to the relatively low consumption of fish pate and smoked salmon in the Netherlands and Portugal, no significant decreases in daily sodium intake for both populations were found. Regarding the new dishes we did not find major differences in average intake of major nutrients when regular foods were replaced by the new developed foods for the specified target populations. Although, assuming best-case assumptions there may be a positive effect on vitamin B and iodine intake in Portuguese seniors due to the consumption of the newly developed mussel soup.

2. Objective

The consortium SEAFOOD^{TOMORROW} aims to develop innovative environmentally-friendly seafood, including the production and processing methods, that will improve the production of healthy and safe seafood in Europe. The claims and impacts on human health, nutrition and safety of cultivated seafoods need to be

validated. This report focusses on the exposure and subsequent benefit-to-risk assessment of the biofortified fish and novel seaweed. These are developed in task 1.1 and 1.2 and described in deliverable D1.2. The aim of this study is to investigate the exposure and subsequent benefits and risks for the Netherlands and Portugal. The selection of countries was decided at the Vigo meeting of SEAFOOD^{TOMORROW}. Dutch and Portuguese data were included, due to pragmatical reasons such as the availability of food consumption data, and because of the variation in fish consumption. Therefore, this report evaluates the impact of fortified fish and seaweed products on iodine, selenium, EPA and DHA and lead, cadmium, arsenic and mercury for the Dutch and Portuguese population.

SEAFOOD^{TOMORROW} developed other innovative foods that will not be assessed in this report, except for the process innovations on fish pate and smoked salmon (task 2.1) and recipes developed to target youth, pregnant women and seniors (task 2.2). The innovations in tasks 1.3 and 1.4 reduce risks either by enabling harvesting such that shellfish are less infected with norovirus or toxins from algal blooms or by detecting toxins with better screening methods. Either way risk will be reduced and nutritional value of the shellfish remains the same. There are only benefits in the form of reduced risk of shellfish poisoning. Therefore, it is not necessary to assess and balance the benefits and risks of those innovations. Work package 2, task 2.1 and 2.2 deal with process innovations. Tasks 2.1 consist of the development of fish pate and smoked salmon, both with a reduced sodium content. In task 2.2, six new recipes, including fish species, were developed for the youth, pregnant women and seniors with specific nutritional targets. Nutritional benefits at the daily intake level of these innovative foods of task 2.1 and 2.2 were evaluated for nutrients (vitamin B12, vitamin D, iodine, omega 3, protein and sodium) and are presented in supplement 1 'Task 2.1' and supplement 2 'Task 2.2'. The microbiological risks of these innovative foods will be dealt with predictive modelling as described in deliverable D3.4. Innovation 2.3 removes contaminants from seafood products, which only have beneficial effects on health. Task 2.4 reduces energy and water in seafood processing, which reduces the environmental burden but has no foreseen effects on health.

3. Background

Considering the growing world population and environmental challenges we are facing, changes in food production and food consumption need to be made. Aquaculture and fisheries can be a key to the future of food production and nutritious food systems and should address these challenges [1, 2]. The current Western dietary patterns are characterized by high consumption of red meat, eggs, processed foods, desserts, sweets and fast-food [3]. A large portion of the Western population do not adhere to the food based dietary guidelines, especially considering fish consumption in many non-Mediterranean countries.

Fish consumption is recommended because fish contain high amounts of beneficial and essential nutrients, such as essential fatty acids (omega n-3 and n-6 fatty acids), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), iodine (I), selenium (Se) and vitamins (B12 and D) [4]. The most important omega n-3 fatty acids from dietary sources include EPA and DHA which is present in fish [5-7]. For the daily intake of EPA and DHA, the Dutch population largely depends on the consumption of fish [8]. In the literature inconsistent data are reported concerning the health benefits of fatty acids [9]. Moderate- and high-quality evidence suggests that

increasing EPA and DHA intake has little or no effect on mortality or cardiovascular health [9]. Furthermore, fish can contain significant amounts of the essential trace element selenium (Se) which is an important constituent of various proteins that are critical for the reproduction, thyroid hormone metabolism, DNA synthesis, and protection from oxidative damage and infections [10, 11]. Iodine (I) is needed for the synthesis of thyroid hormones, involved in regulating the body's metabolism, required for normal growth, and for the development of the brain and nervous system [12, 13].

Although fish is an important contributor to the daily nutrient intake, they are often contaminated with chemical contaminants that have accumulated in the environment. Fish can be a source of harmful contaminants such as polychlorinated biphenyls (PCBs), dioxins, residues of pesticides, and toxic metals. Cadmium (Cd), Lead (Pb) and Methylmercury (MeHg) are amongst others important contaminants that are present in nearly all fish at considerable levels (depending on the type of fish). Heavy metals like Cd, mercury (Hg), (inorganic) As and Pb, are not known to play any metabolic function and can be very toxic to humans, even at very low concentrations [14]. Pb, Hg, As and Cd are dangerous for human health because of their accumulation properties [15]. Heavy metals accumulate in the fatty tissues and internal organs of human body, which may affect the central nervous system [16]. Long-term intake of inorganic arsenic (InAs) has been associated with skin lesions, cardiovascular diseases and various forms of cancer [17]. Pb accumulates in the body and most seriously affects the developing central nervous system in young children [18]. Cd can cause kidney failure and has been statistically associated with an increased risk of cancer [19]. The consumption of fish should be balanced as many people in Western countries could benefit from higher fish consumption, however exposure to toxic heavy metals should be monitored.

Another issue to deal with is that world-wide, overfishing is one of the biggest threats to the health of the seas and their inhabitants. Since the mid-20th century, international efforts to increase the availability and affordability of protein-rich foods led to a considerable expansion of fishing capacity across the world [20]. An important solution against overfishing would be more sustainable fishing and the improvement of aqua culturing methods for fish farming [21]. Innovative solutions could be applied for the cultivation of fish (and the use of seafoods such as seaweed) in order to increase nutrient intake while keeping exposure to contaminants at a minimum. Fortifying fish via feed with beneficial nutrients or the improvement and use of new cultivation techniques for farmed fish, could result in higher nutrient concentration [22].

The SEAFOOD^{TOMORROW} consortium has implemented the biofortification of farmed fish for nutritional beneficial compounds. Currently, the replacement of fishmeal and fish oil by oilseed proteins and vegetable oils in aqua feeds are ongoing in aquaculture. While the effect of new feed sources on n-3 PUFA levels in fish fillets is being investigated, not much attention has been paid to the effect of the biofortification of fish with feed including more nutrients, on micronutrient levels [1]. These nutrients should be restored and may improve consumers health [22]. The SEAFOOD^{TOMORROW} consortium has applied this technique which is shortly described in box 1. If the consumption of fortified fish contributes to human health via increased nutrient intake and decreased exposure to heavy metals is currently unknown.

Box 1. Task 1.1 Biofortification of trout, seabream and carp

*Previously, lab-scale biofortification trials were performed by SEAFOOD^{TOMORROW} and demonstrated that feeds can effectively modulate the composition of fish fillets for bioactive n-3 PUFA, selenium and iodine. Pilot-scale and farm-scale trials have been conducted within the SEAFOOD^{TOMORROW} consortium in order to fortify fish fillet with nutritional compounds. Several biofortification blends, comprising iodine-rich macroalgae, selenized-yeast and DHA-rich microalgae biomasses, were formulated and used to manufacture the experimental feeds to be tested at both pilot- and farm-scale with rainbow trout (*Onchorynchus mykiss*), gilthead seabream (*Sparus aurata*) and common carp (*Cyprinus carpio*). For the fortification blends criteria associated to nutritional value, safety and legal compliance for use in animal feeds and economic factors and costs were taken into account for the definition of the various blends, which varied between the species. Feed for carp was supplemented with iodine-rich macroalgae, selenized yeast and a DHA-rich microalgae and feed for seabream was fortified with iodine-rich macroalgae and selenized yeast. Feed for trout was only fortified with iodine-rich macroalgae. Overall, results from the various pilot-scale biofortification scenarios confirmed that aquafeed supplementation is a highly effective approach to significantly increase the fillets content in iodine, selenium and EPA and DHA (Vera Barbosa, 2020). Based on these results the specifications of the most optimal fortification blends were defined and its efficacy is currently still being validated at large-scale trials (farm-scale) with gilthead seabream, common carp and rainbow trout.*

Besides the fortification of fish, other techniques such as integrated multi-trophic aquaculture (IMTA) have been investigated to innovate aquaculture and the production of sustainable fish and seafoods. Currently, there is high interest in IMTA, which aims to improve production systems towards complete cycles of energy, water and resources [23, 24]. It is a sustainable solution because it involves the utilization of species from different trophic levels, so that 'waste' from one species becomes a resource for the other [23]. The SEAFOOD^{TOMORROW} consortium has applied this technique in order to cultivate salmon and seaweed and has compared the technique with mono-culture (see box 2).

The interest to develop an industrialized cultivation technique for seaweed in Europe is growing rapidly. Seaweed grows easily on large scale in the sea and has several purposes [25, 26]. It is seen as an alternative food with great potential [25, 26], can be used as biofuel, fertilizer, feedstock and in cosmetics and medicinal products [25]. Seaweed as food for human consumption has increased over the past years, however current seaweed consumption is low in Western countries and usually not part of the diet [27, 28]. Seaweed consumption has a positive health effect due to the nutritional composition of essential and beneficial nutrients [12]. It contains low fat and a wide range of important nutrients such as omega n-3 fatty acids, vitamins (A, C, E and B12), I, dietary fibers and antioxidants [27, 29, 30]. Seaweed accumulates high concentrations of I and can be considered as a dietary source for I intake. However, high I intake may lead to I toxicity and can have negative health effects [28]. Globally, the elimination of I deficiency is regarded as a major public health challenge [31, 32]. Previous studies investigating I status in Portuguese pregnant women and school-aged children reported intakes well below the WHO adequacy recommendation [33, 34]. Verkaik-Kloosterman (2017) reported that the I intake among the Dutch population (7-69 years) seems adequate, due to fortification of bread with iodized salt, although it has decreased since the period before 2008 [32].

Seaweed accumulates also high levels of toxic heavy metals such as As, Cd, copper (Cu), zinc (Zn), Hg, and Pb [35, 36] [30]. Long-term exposure to these compounds may lead to a wide range of negative health effects including neurological-, haematological-, gastrointestinal- and cardiovascular impairments, and induce various forms of cancer [17, 19, 37-39]. Values of toxic metal in the majority of edible seaweed are usually below the maximum concentrations allowed for human consumption in most countries [40]. However, for most countries, there is currently no regulation on the maximum levels of heavy metals in seaweeds, except for Cd concentrations in France (0.5 mg/kg as a maximum level for Cd in dried seaweed) [16].

Box 2. Task 1.2 Integrated multi-trophic aquaculture

*SEAFOOD^{TOMORROW} partner, Tarelaks, in the south-western fjords of Norway, have implemented IMTA with salmon and seaweed. They have conducted several pilot-scale trials in order to facilitate optimization of two parameters: (I) vertical and horizontal placement of seaweeds (*Saccharina latissima*) nearby fish farms; (II) deployment/harvesting times, harvesting methods and necessary equipment. The effect of the integrated co-production was closely monitored and validated in comparison with salmon monoculture and regular seaweed cultivation. The harvested seaweed from the different Tarelaks sites were dried and analyzed for nutrient and heavy metal content.*

The consortium SEAFOOD^{TOMORROW} aims to develop innovative environmentally-friendly production and processing methods for seafood in Europe. The impacts on nutrition and food safety of the eco-innovative solutions developed need to be validated through chemical and biological analyses, laboratory assays, predictive modelling and benefit-to-risk assessment.

In this report the health impact of the sustainable, eco-innovative solutions developed by the SEAFOOD^{TOMORROW} consortium will be evaluated. The innovations are: 1) fortification of fish fillets (task 1.1) through alternative feeds for several fish species and 2) seaweed production for human consumption (task 1.2)). The innovative products are assessed on health and risk and focusing on iodine, selenium, EPA and DHA, and lead, cadmium, arsenic and mercury.

In this study we evaluate the impact of the innovations both in terms of nutritional quality as well as food safety by investigating dietary nutrients and heavy metals in two European countries (the Netherlands and Portugal). Therefore, exposure analysis of the heavy metals: arsenic, inorganic arsenic, lead, mercury and cadmium and the nutrients: iodine, selenium, DHA and EPA is performed for scenarios including the innovated fortified fish and seaweed food products. In order to capture the variation type and amount of fish consumption within Europe, different European countries need to be covered. Due to pragmatical reasons i.e. availability of consumption data on a detailed level, and because of the variation in fish consumption, scenarios for the Netherlands and Portugal will be included.

4. Materials and methods

In this chapter we discuss the investigated scenarios and the background data for the Netherlands and Portugal. First, an overview of the different scenarios is given for both the Netherlands and Portugal. Afterwards, an explanation on the used concentrations and calculations for the different scenarios is provided followed by a short description on the Dutch and Portuguese consumption data and the processing of the food composition data for I, Se, EPA and DHA. Finally, the collection and processing of the concentration data on Cd, Pb, InAs, As and Hg will be described.

Scenarios

For the Netherlands and Portugal two scenarios were studied. In the reference scenario (baseline_control_fish), concentrations of interest (I, Se, EPA, DHA, Cd, Pb, InAs, total As and total Hg) of the control trout and seabream fishes that received regular feed were used. In the fortified_fish scenario, concentrations (I, Se, EPA, DHA, Cd, Pb, InAs, total As and total Hg) in trout and seabream were replaced by concentrations of fortified trout and fortified seabream.

In an additional scenario for the Netherlands (seaweedfoods), 10% of the total amount of the consumption of pasta, bacon and lettuce was replaced by seaweed pasta, seaweed bacon and seaweed lettuce. Thus, total pasta, bacon and lettuce consumption in the Dutch population was summed and 10% of the amount was replaced by seaweed pasta, seaweed bacon and seaweed lettuce. Table 1 shows an overview of the different scenarios and also describes the FoodEx2 codes that were linked to trout, seabream, carp, trout, bacon, lettuce and pasta to determine consumption of the different scenarios.

Concentration data for scenarios

Concentration data on nutrients and heavy metals for the innovative fish was previously analyzed by partners of the SEAFOOD^{TOMORROW} consortium and stored in a database. For current study data was derived from the SEAFOOD^{TOMORROW} database [41]. For the seaweedfoods scenario concentration data was derived from additional sources, as described below.

I concentrations in seaweed pasta and seaweed bacon were derived from nutritional information of seaweed food products available on the Dutch market [42, 43]. I concentration for seaweed lettuce was derived from the Dutch Food Composition Table (NEVO 2016/V5) [44] because of lacking data from the consortium.

Cd, Pb, As and Hg in seaweed pasta, seaweed bacon and seaweed lettuce were derived from the mean concentrations of IMTA seaweed and control seaweed cultivated in the consortium (task 1.2). Concentrations for InAs was derived from literature [45]. For concentrations in seaweed lettuce conversion factor of 4.7 was used to convert the dry weight concentrations to wet weight concentrations for Cd, Pb, InAs, As and Hg. It was assumed that seaweed lettuce is eaten fresh and not dried. NEVO reports that wet seaweed consists of 81.6% water and 19.4% other substances, whereas dry seaweed consists of 8.7% water and 91.3% other substances [44]. Hence, the conversion factor $91.3/19.4 = 4.7$.

Table 1. Scenarios

Scenario	Foods	Concentrations levels
Baseline_control_fish	Trout (A029F A029K A029N), seabream (A0FAR and A029V) and carp (A027D)	Concentrations of control trout, seabream and carp.
Fortified_fish	Trout (A029F A029K A029N), seabream (A0FAR and A029V) and carp (A027D)	100% replaced by concentrations of fortified seabream, fortified trout and fortified carp
Scenario for only the Netherlands		
Scenario	Foods	Replacements
Seaweedfoods	Trout (A029F A029K A029N), seabream (A0FAR and A029V) and carp (A027D)	Concentrations of control trout, seabream, and carp
	Pasta (A007D A007E A04LC A007F A007G A007J A007L A007M A007P)	10% replaced by concentrations of seaweed pasta
	Bacon (A022X)	10% replaced by concentrations of seaweed bacon
	Rocket lettuce (A00LN)	10% replaced by concentrations of seaweed lettuce

Population and consumption data

For the Netherlands, population and consumption data was derived from the Dutch National Food Consumption Survey (DNFCS) 2012-2016 for 4313 Dutch participants [46]. This survey aimed to gain insight into the diets of children and adults living in the Netherlands. Participants were derived from consumerpanels and the response rate was 65%. Pregnant and lactating women and people who were institutionalized or those without adequate command of the Dutch language were not included.

Participants were interviewed by telephone or face-to-face, depending on age, by a trained dietitian to assess dietary intake based on two non-consecutive 24-hour dietary recalls. The period between the two 24-hour dietary recalls was circa 4 weeks. Interviewers/dietitians used the GloboDiet system, which is a computer-controlled interview software which enters answers directly in a computer. The reported foods and recipes in the DNFCS 2012-2016 were described according to the GloboDiet methodology [47]. Foods were matched to FoodEx2 classification system [48] and the Dutch National Food Composition Table (NEVO 2016/v 5) [44].

For Portugal, population and consumption data for 5811 Portuguese participants was derived from the National Food and Physical Activity Survey (IAN-AF 2015-2016) which aimed to collect nationwide data on among others dietary habits (food, nutrients, dietary supplements, food safety and insecurity) from the Portuguese from 3 months to 84 years [49]. Participants were derived from the National Health Registry and response rate was 23%. Participants living in collective residences or institutions, living in Portugal for less than one year or non-Portuguese speakers, with diminished physical and/or cognitive abilities that hamper participation and deceased were excluded.

Food consumption data was assessed by two non-consecutive 24-hour dietary recalls by trained fieldworkers (using a computer-assisted tool (CAPI/eAT24)) via face-to-face interview or food diaries depending on age. The period between two recalls was 8 – 15 days. Data was collected during 12 months and incorporated all seasons and weekdays to incorporate seasonal effects and day-to-day variation. Foods were described according to the EFSA FoodEx2 classification system.

Food composition data

Concentration data on Se, I, EPA and DHA was selected from the Dutch food composition table (NEVO) (2016/5) [44]. NEVO is established by the Dutch Institute for Public Health and the Environment (RIVM) and provides information on nutrients expressed per 100 g of the edible part of 2390 generic foods. Food composition data was available for 85% of the Se concentrations; 78% I; 85% EPA; 87% DHA. For remaining foods no concentration data was available and was assumed to be zero. 15% of Se concentrations were analyzed chemically. The remaining concentrations were calculated based on recipes combining the information of different food codes (22%), label type information (21%), calculated by the food industry (18%) or derived from a foreign food composition table or book (16%). I concentrations were chemically analysed (8%), taken from a foreign food composition table or book (28%), calculated based on ingredients (23%), from label information (18%) or derived from the food industry (18%). 83% EPA and 81% DHA concentrations were calculated based on factors (berekend-uit-scores e.g. equivalenten). Foods codes included in NEVO were labelled with FoodEx2 codes and linked to food consumption data.

The Portuguese food composition table did not provide I, Se, DHA or EPA concentrations in foods [50]. Therefore, concentrations were derived from alternative sources. Chemically analyzed I concentrations for 107 foods were derived from WHO report 'Scientific update on the I content of Portuguese foods' by Delgado et al. (2018) which provided data on the I content of Portuguese foods as consumed within, and as representative of, the Portuguese diet [51]. Furthermore, I concentrations were derived from the EFSA occurrence database. A request was submitted to EFSA to provide Portuguese I data from 2014-2018. WHO and EFSA provided I concentration data on 213 Portuguese consumed foods. The remaining I concentrations were linked to I content by the Dutch Food Composition Table (NEVO) (2016/5) [44]. Se, EPA and DHA concentrations of foods were provided by NEVO for all Portuguese consumed foods. NEVO food codes were linked to foods from the Portuguese food consumption survey using the FoodEx2 classification system. In case no FoodEx2 classification code was available, foods were ascribed an average concentration for corresponding FoodEx2 hierarchy. Appendix II provides an overview of concentration data from the different sources to determine the Portuguese food consumption.

Concentration data on heavy metals

For the Netherlands, country-specific concentration data on Pb, Cd, Hg, InAs and total As was derived from the Quality of Agricultural Products (KAP) database, which contains data on contaminants and pesticides in food and feed from the Netherlands Food and Consumer Product Safety Authority (NVWA) and Wageningen Food Safety Research (WFSR). The concentration data derived from the KAP-database included Pb, Cd, MeHg, total Hg, InAs and total As in foods analyzed between 2014-2017. The NVWA inspects only a selected range of foods each year that are suspected to contain considerable levels of contaminants, such as heavy metals. In order to cover the entire diet, concentration data was derived from the European Food and Safety

Authority (EFSA) reports on dietary exposure to heavy metals (Appendix III) [17, 19, 37-39]. These reports include the average mean concentration values on Cd, Pb, InAs, total As, MeHg and total Hg in foods reported to the EFSA by European countries [17, 19, 37-39]. Appendix IV, table 1 shows an overview of foods per substance that were linked to concentration data for the Netherlands.

For Portugal, country-specific concentration data on Cd and total Hg was derived through a direct request send to EFSA. The EFSA occurrence database contained Portuguese data on Cd and Hg in foods analyzed between 2014-2018. However, these data covers a limited range of foods and lacks concentration data on other heavy metals including, Pb, InAs and As. In order to cover the entire diet and to include data on the other missing heavy metals, concentration data was derived from the EFSA reports on dietary exposure to heavy metals (Appendix III) [17, 19, 37-39]. Furthermore, if for a specific food neither Portuguese or EFSA concentration data was available, it was decided to use Dutch country-specific concentration data (NVWA). In appendix IV, table 2 shows an overview of foods per substance that were linked to concentration data for Portugal.

Appendix IV, table 3 shows an overview of foods that lacked concentration data on heavy metals and were not included in the calculations. Out of a total of 255.132 consumption records from the DNFCs-2012-2016 there were 610 records of foods with missing concentration for all the heavy metals. For Portugal out of a total of 411.300 consumption records from the IAN-AF 2015-2016 there were 1450 records of foods with missing concentration data for all heavy metals. The food group "Barley coffee ingredient" and "Mixed coffee imitates" were consumed frequently in Portugal (n of records = 1310). Coffee imitates or replacements usually include drinks made of grains (barley or rye), chicory and sugar beet. The food group "Canned/jarred vegetables" was frequently consumed in the Netherlands (n of records = 538).

Food Mapping for dietary exposure to heavy metals (FoodEx2 coding)

The FoodEx2 food classification and description system is developed by EFSA and used for the description of food and feed matrices within the data collections of different safety domains relevant to EFSA. FoodEx2 consists of descriptions (based on codes) of a large number of individual food and feed items that can be complemented by additional information through the use of facets. The selection of base term codes and accompanying facet codes can be performed with the help of the EFSA Catalogue Browser tool [52]. The use of FoodEx2 codes allows for standardization of data collection and is a helpful tool in quality control [48].

Dutch and Portuguese food consumption data were linked to concentration data using FoodEx2 base term codes without the inclusion of any facets. In order to link the foods from the EFSA reports to FoodEx2 codes, the foods were first divided into different categories based on how the foods were reported: FoodEx level 1, FoodEx level 2 or FoodEx level 3. The food names, as reported in the EFSA reports, were linked to their corresponding food hierarchy code.

The hierarchy codes shown in Appendix V table 1, correspond to foods reported as FoodEx level 1. The hierarchy codes for foods reported as FoodEx level 2 or level 3 are not shown, otherwise the table would become too big and it also serves as an example. However, the principal for linking foods at Foodex level 2 and 3 is similar to that of FoodEx level 1. As example, Grains and grain-based products (FoodEx level 1) is linked to the hierarchy code A.01, Grain milling products (FoodEx level 2) is linked to the hierarchy code A.01.03, Wheat milling products (FoodEx level 3) is linked to the hierarchy code A.01.03.001 and Wheat flour, brown (FoodEx level 4) is linked to the hierarchy code A.01.03.001.001.

Afterwards, the hierarchy codes were linked to their corresponding FoodEx1 codes based on a translation table in the KAP-database. This table was derived from an old EFSA SSD1 catalogue and is comparable to Appendix V, table 1 (only considerably larger). And finally, the FoodEx1 codes were used to link foods to their corresponding FoodEx2 codes based on the EFSA Catalogue MTX (FoodEx2 Matrix) 10.3 (downloadable from the EFSA Catalogue Browser), which contains both FoodEx2 and FoodEx1 codes and thus can be used as a translation table to link the codes together. First the foods reported as FoodEx level 1 were linked, followed by foods reported as level 2 and at the end the foods reported as level 3. This was done in order to avoid the loss of food-specific concentration data.

Foods included in the EFSA reports on Pb, Cd, InAs, total As, MeHg and total Hg (Appendix III, table 1) were linked to their corresponding FoodEx2 code and concentration data as described above. This covered almost all the foods consumed in the Dutch and Portuguese consumption data, except for several miscellaneous foods (Appendix IV, table 3). However, the Scientific Opinion on Arsenic in Food, which includes data on total As is an old article (2009) and used an outdated food classification system (old food names, classifications, several foods (snacks, desserts, composite foods) were not included). Therefore, many FoodEx2 codes could not be linked to the correct concentration data for total As (Appendix IV, table 4).

The FoodEx2 codes with no concentration data were linked to corresponding total As concentration data with the use of queries. Products (linked to FoodEx2 codes) were linked to a subcategory and corresponding concentration data reported in the EFSA report 'Scientific Opinion on Arsenic in Food' (2009).

As example, fish (e.g. carp (A027D), trout (A029F) and Gilthead seabream (A0FAR)) and fish products (e.g. fish fingers (A02KC)) were linked to the concentration of "Fish and fish products", seafood products (e.g. canned seafood (A0BZ5)) to the concentration of "Seafood and seafood products", berries and small fruit (e.g. table grapes (A01DX)) to the concentration of "Berries and small fruits", fruit (e.g. apples (A01DJ)) to the concentration of "Other fruits", etc. Appendix IV, table 4 and 5 are overviews on how products belonging to a specific subcategory were linked to subcategories with corresponding concentration data reported in the EFSA report 'Scientific Opinion on Arsenic in Food' (2009).

Furthermore, foods that were not included into the Scientific Opinion on Arsenic in Food report (2009) like snacks, sauces, baking wares, foods for infant and small children and foods for special nutritional were calculated back from InAs concentrations reported in the EFSA report "Dietary exposure to InAs in the European population" applying a conversion factor of 1.43 (100/70) [17, 38], which is based on the conversion factor of 70% used in the report to calculate inorganic concentration based off the total As concentrations for most of the food categories, except for "Vegetables and vegetable products", "Fish and other seafood" and several products within the category "Composite foods" [17] (Appendix IV, table 5).

Statistical analysis

Dietary nutrient (I, Se, EPA and DHA) intake and exposure to heavy metals (Cd, Pb, Hg, As and InAs) were assessed using the statistical software Monte Carlo Risk Assessment (MCRA), version 8.3 [53]. MCRA is a web based tool to quantify dietary exposure to nutrients or chemicals as a distribution by combining food consumption data with nutrient concentrations or chemicals in foods.

The exposure data (heavy metals) contained samples with concentrations below the limit of detection (LOD or quantification (LOQ). These samples were referred to as non-detect samples and were assigned a concentration equal to ½ LOD or ½ LOQ.

Logistic NormalNormal (LNN) model was applied to assess the long-term exposure, since this model corrects for the within-person's variation in exposure [54]. This approach results in more realistic exposure estimates at the tails of the exposure distribution than without the correction [55]. However, the within-person's variation can only be removed when the daily positive exposure distribution is normally distributed after transformation. If this condition is not met, the use of LNN to assess the long-term exposure might be debatable. Normality can be checked by using the normal quantile-quantile (Q-Q) plot [56]. The Q-Q plots (part of MCRA output) for the calculations of the different scenarios for both the Netherlands and Portugal suggested a normal distribution, justifying the use of LNN to assess the long-term exposure. For the daily positive exposure distribution a logarithmic transformation was used. The correlation between intake frequency and amount was assumed zero.

LNN models were applied for assessment of long-term exposure of I, Se, Cd, Pb, Hg, InAs and total As in for the Netherlands. LNN models were applied for I, Se EPA, Cd, Pb, Hg, InAs and total As in for Portugal. The assumption for normality was not met for DHA intake in the Portuguese consumption data, therefore observed individual means (OIM) are reported.

Calculations were separately performed for the Dutch and Portuguese scenarios. The estimations were based on the total population aged 1-79 years. Cooked amounts of foods were used to estimate the intake of nutrients, raw amounts were used to determine heavy metal exposure. The daily means of foods of individuals were multiplied by concentrations of the nutrients and heavy metals and summed per person per day. Estimates for the Dutch population were weighted for small differences in demographic properties, season, and combination of both consumption days (week or weekend) to make results representative for the Dutch population. For Portugal sampling weights were applied and included the following: (1) initial weights to overcome the different probability of sampling units selection; (2) a second weight to overcome the different probability of individuals selection in each unit, by sex and age (considering the total population, by sex and age groups in the closest recruitment wave); and (3) correction of these initial weights for nonresponse bias.

The applied LNN models corrected for between-person and within-person variation. Daily intake of nutrients were expressed as unit per day and exposure to heavy metals as µg per kg bodyweight per day. Estimated nutrient intakes were reported as mean with 95% confidence interval (CI), 50th-, 25th- and 75th percentile and the 97.5th percentile with 95% CI. The reported percentiles of the long term exposure distribution for heavy metals included the mean, 50th and 95th percentile with 95% CI.

5. Results

In this chapter the results of the fortification of fish (task 1.1) and integrated multi-trophic aquaculture (task 1.2) are described. Furthermore, food consumption and important sources for nutrient intake and heavy metal exposure are described. Finally, the results regarding the control-fish, fortified-fish and seaweed foods scenario will be presented. Results are presented separately for the Netherlands and Portugal.

Concentrations

Nutrient and heavy metal concentrations in fish were analysed in the control fishes and fortified fishes by partners from the SEAFOOD^{TOMORROW} consortium and are presented in table 2. The biofortification of trout

with iodine-rich macroalgae via fish feed, significantly increased I (55 versus 5540 µg per kg) and As (772 versus 810 µg per kg) concentrations in trout, compared to the control trout. Cd (0.10 versus 0.93 µg per kg) and Pb (5.3 versus 18 µg per kg) concentrations decreased compared to the control trout. In seabream, biofortified with iodine-rich macroalgae and selenized yeast, I (67 versus 127 µg per kg), Se (180 versus 273 µg per kg) and InAs (+14.6%) increased compared to the control seabream. Cd (20 versus 10 µg per kg), Hg and As (both -10%) concentrations decreased in the fortified seabream compared to the control. The biofortification of carp with iodine-rich macroalgae, selenized yeast and DHA-rich microalgae led to significant increased concentrations of I (20 versus 190 µg per kg), Se (93 versus 133 µg per kg), EPA (0.05 versus 0.25 g per kg) and DHA (0.39 versus 1.25 g per kg) compared to control carp. Furthermore, Hg (+67%) and As (+268%) increased in fortified carp compared to the control carp, whereas, InAs (-12.5%) concentration decreased compared to the control.

Limited concentration data was available in the SEAFOOD^{TOMORROW} database regarding the IMTA farmed salmon and IMTA seaweed and monoculture (control/NON-IMTA) farmed salmon and seaweed. Appendix I provides an overview of concentration data for IMTA and non-IMTA salmon. Se and As concentrations decreased in IMTA farmed salmon compared to the control salmon. Table 3 provides concentration data for seaweed, regular foods to replace (pasta, bacon and lettuce) and for seaweed foods. IMTA seaweed contained 443 µg Cd, 40333 µg As and 111 µg Pd per kg (WW). No measured concentrations data was available for nutrients in IMTA or NON-IMTA seaweed. Seaweed pasta, seaweed bacon and seaweed lettuce contained 29500 µg, 163000 µg and 75530 µg I per kg, respectively. Cd, As, InAs and Pb concentrations were significantly higher in seaweed pasta, seaweed bacon and seaweed lettuce compared to the regular foods. Hg concentrations in novel seaweed foods was lower compared to the regular foods.

Table 2. Iodine, selenium, EPA, DHA, cadmium, mercury, total arsenic, inorganic arsenic and lead concentrations in unit per kg of control and fortified trout, seabream and carp (in wet weight).

	Control Trout	Fortified Trout	Control seabream	Fortified seabream	Control carp	Fortified carp
	PSCT	PST2	PSCS	PSS2	PSCC	PSC3
Nutrients						
Iodine (µg/kg)(WW)	54.67 ^a	5540.00 ^b	66.67 ^c	126.67 ^d	20.00 ^e	190.00 ^f
Selenium (µg/kg)(WW)	210.00 ^a	217.33 ^b	180.00 ^c	273.33 ^d	93.33 ^e	133.33 ^f
EPA (g/kg) (WW)	N/A	N/A	2.01 ^c	1.86 ^d	0.05 ^e	0.25 ^f
DHA (g/kg)(WW)	N/A	N/A	3.30 ^c	3.47 ^d	0.39 ^e	1.25 ^f
Heavy metals						
Cadmium (µg/kg) (WW)	0.93 ^a	0.10 ^b	20.00 ^c	10.00 ^d	10.00 ^e	10.00 ^f
Total mercury (µg/kg) (WW)	32.33 ^a	32.23 ^b	96.67 ^c	86.67 ^d	20.00 ^e	33.33 ^f
Total arsenic (µg/kg) (WW)	772.67 ^a	810.00 ^b	1803.33 ^c	1616.67 ^d	73.33 ^e	270.00 ^f

Inorganic arsenic (µg/kg) (WW)	1.30 ^a	N/A ^b	8.23 ^c	9.43 ^d	2.40 ^e	2.10 ^f
Lead (µg/kg) (WW)	18.00 ^a	5.33 ^b	76.67 ^c	76.67 ^d	83.33 ^e	70.00 ^f
a Mean concentration control trout 'PSCT' b Mean concentration fortified trout 'PST2' c Mean concentration control seabream 'PSCS' d Mean concentration fortified seabream 'PSS2' e Mean concentration control carp 'PSCC' f Mean concentration fortified carp 'PSC3'						

Table 3. Iodine, selenium, EPA, DHA, cadmium, mercury, total arsenic, inorganic arsenic and lead concentrations in unit per kg of seaweed, pasta, bacon, lettuce and novel seaweed foods.

	Control Non- IMTA macro algae	IMTA macro algae	Pasta	Seaweed pasta	Bacon	Seaweed bacon	Lettuce	Seaweed lettuce
Nutrients								
Iodine (µg/kg)	N/A	N/A	9.50 ^b	29500.00 ^d (WW)	136.40 ^b	163000.00 ^f (DW)	0 ^b	75530.00 ^b (WW)
Selenium (µg/kg)	N/A	N/A	N/A	N/A	86.70 ^b	N/A	0 ^b	N/A
EPA (mg/kg)	N/A	N/A	N/A	N/A	0.00 ^b	N/A	0 ^b	N/A
DHA (mg/kg)	N/A	N/A	N/A	N/A	0.08 ^b	N/A	0 ^b	N/A
Heavy metals								
Cadmium (µg/kg)	N/A	443.33 ^a (DW)	13.90 ^c	443.33 ^a (DW)	7.37 ^c	443.33 ^a (DW)	36.40 ^c	49.33 ^{a,g} (WW)
Mercury (µg/kg)	N/A	<LOQ (0.90) ^a (DW)	9.70 ^c	<LOQ (0.90) ^a (DW)	2.90 ^c	<LOQ (0.90) ^a (DW)	2.10 ^c	<LOQ (0.90) ^{a,g} (WW)
Arsenic (µg/kg)	N/A	40333.33 ^a (DW)	20.85 ^c	40333.33 ^a (DW)	15.30 ^c	40333.33 ^a (DW)	19.85 ^c	8581.56 ^{a,g} (WW)
Inorganic arsenic (µg/kg)	N/A	N/A ^a	15.20 ^c	50.00 ^e (DW)	10.80 ^c	50.00 ^e (DW)	12.90 ^c	10.64 ^{e,g} (WW)
Lead (µg/kg)	N/A	111.43 ^a (DW)	8.00 ^c	111.43 ^a (DW)	11.00 ^c	111.40 ^a (DW)	30.00 ^c	23.71 ^{a,g} (WW)

^a Mean concentrations IMTA macroalgae (SeafoodTomorrow data)

^b Mean concentrations NEVO

^c Mean concentrations EFSA

^d Concentration of seaweed pasta ('I Sea Pasta') reported by the company Seamore on their website

^e Mean concentration of heavy metals in *Saccharina latissimi* measured by The Norwegian National Institute of Nutrition and Seafood Research (NIFES)

^f Concentration of seaweed bacon ('I Sea Bacon') reported by the company Seamore on their website

^g Concentrations were divided by a conversion factor of 4.7 for converting dry weight (DW) to wet weight (WW)

Consumption

Among Dutch adults and children aged 1 to 79 years, the average food consumption over two consumption days was estimated at 3.0 kg per day of which 1.9 kg beverages. Figure 1 shows the average food consumption in gram per day per food group. The average fish consumption in the Netherlands was low with 16 g per person per day. Most consumed fishes in the Netherlands were processed fish (e.g. fish fingers), salmon, pike perch, cod and herring.

The average consumption of trout was 0.1 g per day, carp and seabream were not consumed in the Netherlands. The average consumption was 2.5 g bacon, 1 g lettuce and 24 g pasta per person (table 4). Table 5 presents the average consumption of consumers only and shows that the average consumption of trout was 48 g per day among consumers only.

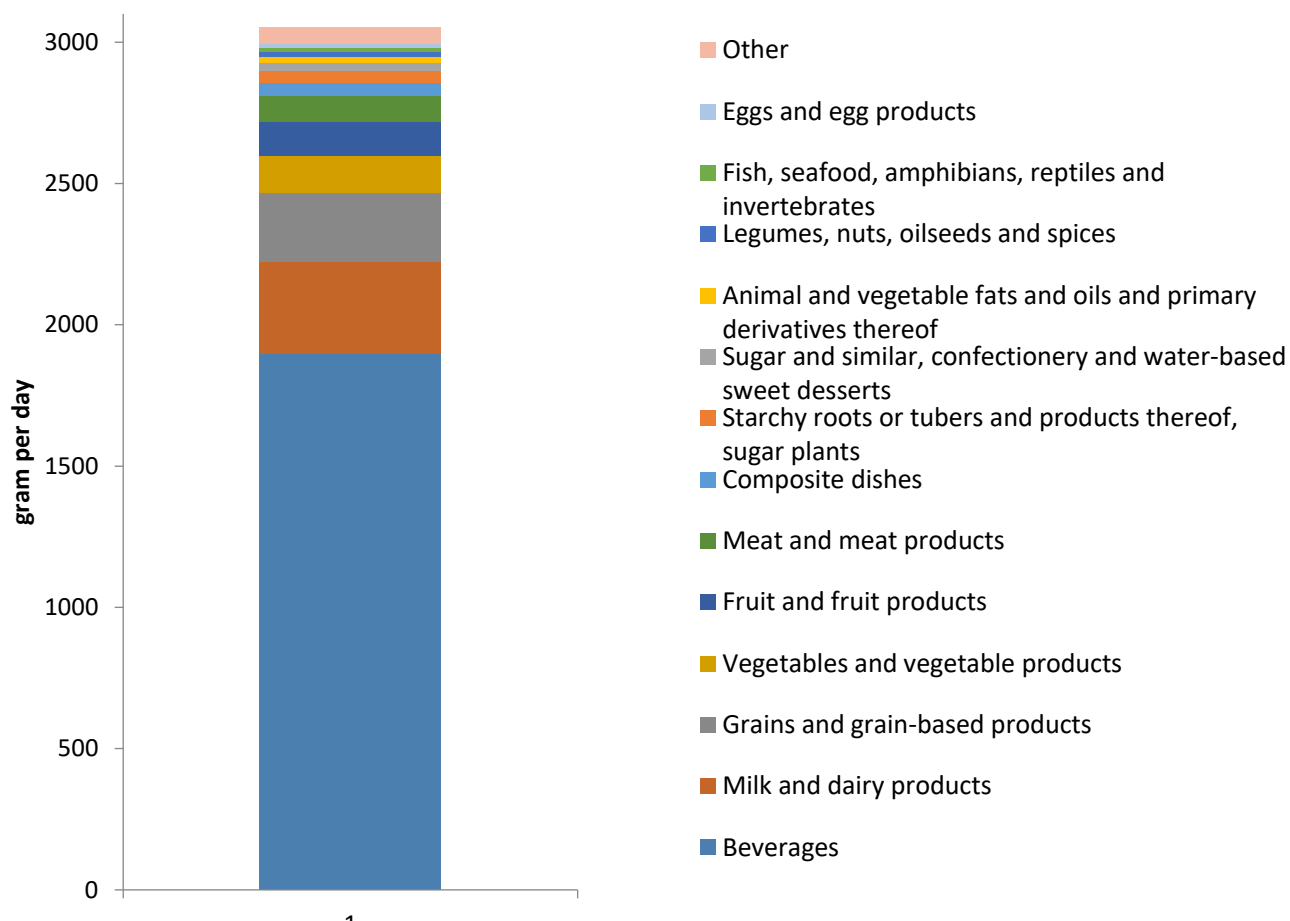


Figure 1. The average food consumption per day in grams by food groups for the Netherlands

Table 4. The consumption of trout, seabream, carp, pasta, bacon and lettuce in grams per day in the Netherlands.

	N	Mean	Std Dev	5th Pctl	25th Pctl	50th Pctl	75th Pctl	95th Pctl
Trout	4313	0.09	2.26	0.00	0.00	0.00	0.00	0.00
Seabream	4313	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Carp	4313	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pasta	4313	24.44	50.27	0.00	0.00	0.00	28.71	120.00
Bacon	4313	2.45	7.65	0.00	0.00	0.00	0.00	17.84
Lettuce	4313	0.98	5.48	0.00	0.00	0.00	0.00	4.00

Table 5. The consumption of trout, seabream, carp, pasta, bacon and lettuce in grams per day among consumers only in the Netherlands.

	N	Mean	Std Dev	5th Pctl	25th Pctl	50th Pctl	75th Pctl	95th Pctl
Trout	7	47.74	22.89	30.00	30.00	42.08	50.00	100.50
Pasta	1514	69.21	63.90	7.73	23.63	55.00	92.41	182.55
Bacon	685	14.29	13.65	1.36	6.00	10.80	18.75	37.50
Lettuce	204	14.68	18.79	1.65	3.83	9.90	20.00	49.00

In Portugal, the average food consumption over two consumption days was approximately 2.5 kg of which 1.3 kg beverages for adults and children, aged 3 months to 84 years (figure 2). The average consumption of fish was 43 g per person per day. Fishes that were most consumed among the Portuguese population were hakes, cod, salmon, tuna, sardine and mackerel.

Table 6 and table 7 show the daily average consumption for foods of interest for the entire study population and for consumers only. The daily average consumption over two consumption days was 0.6 g per day for seabream and 0.12 g per day for trout among the Portuguese. Carp was not consumed. Portuguese consumed on average 0.6 g bacon, 0.2 g lettuce and 27 g pasta per day. Among consumers only, the consumption of trout and seabream was 36 g and 20 g per day, respectively.

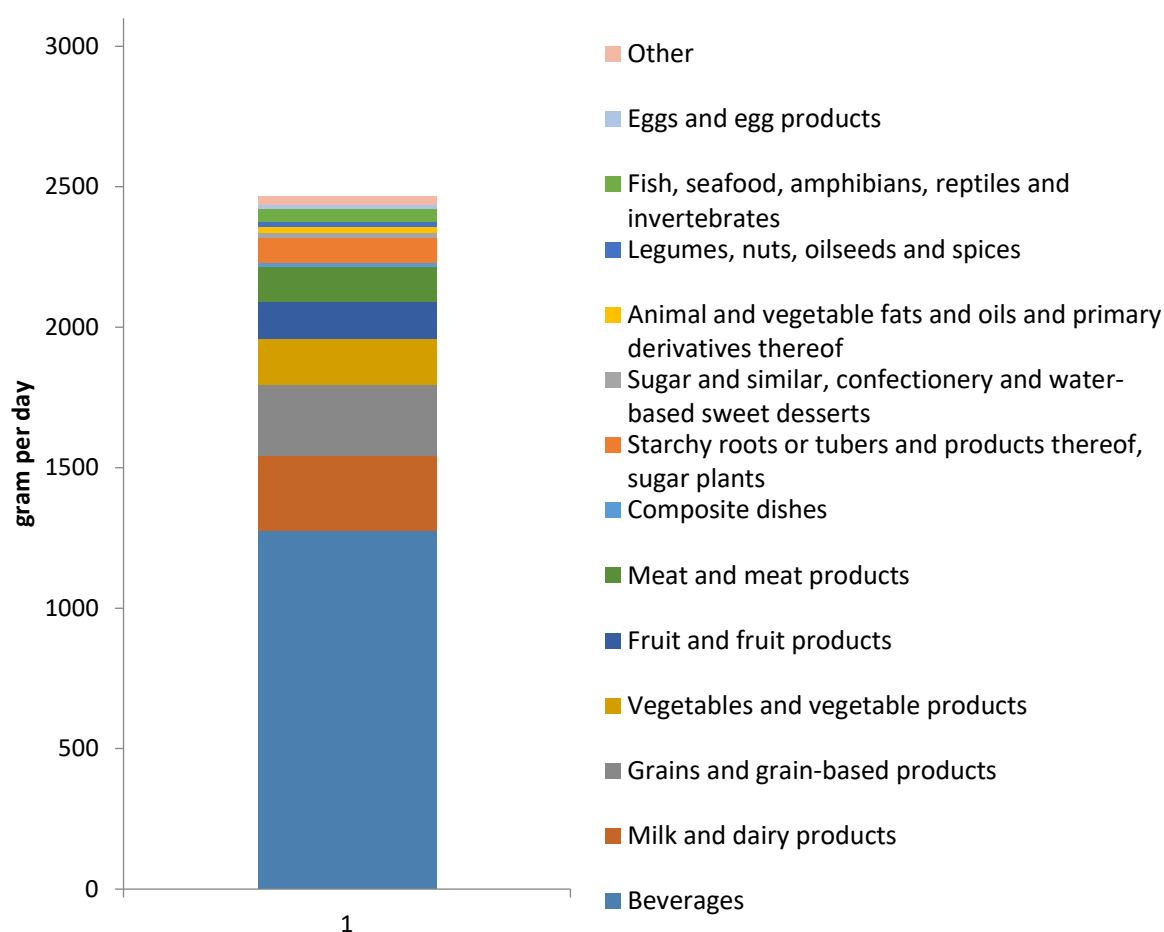


Figure 2. The average food consumption per day in grams by food groups for Portugal

Table 6. The consumption of trout, seabream, carp, pasta, bacon and lettuce in grams per day for Portugal

	N	Mean	Std Dev	5th Pctl	25th Pctl	50th Pctl	75th Pctl	95th Pctl
Trout	5811	0.12	3.16	0.00	0.00	0.00	0.00	0.00
Seabream	5811	0.64	5.30	0.00	0.00	0.00	0.00	0.00
Carp	5811	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pasta	5811	27.14	67.58	0.00	0.00	0.00	37.30	112.07
Bacon	5811	0.59	4.97	0.00	0.00	0.00	0.00	1.52
Lettuce	5811	0.21	2.18	0.00	0.00	0.00	0.00	0.00

Table 7. The consumption of trout, seabream, carp, pasta, bacon and lettuce in grams per day among consumers only for Portugal

	N	Mean	Std Dev	5th Pctl	25th Pctl	50th Pctl	75th Pctl	95th Pctl
Trout	9	33.51	33.51	45.14	15.40	16.82	21.07	37.88
Seabream	146	20.30	13.40	6.41	15.74	20.45	25.51	33.47
Pasta	2739	61.95	77.13	13.31	27.97	44.48	73.44	166.67
Bacon	413	6.99	15.82	0.37	0.90	2.34	9.89	25.00
Lettuce	164	7.11	8.50	1.86	3.95	4.88	8.00	22.32

Sources

The contribution of most important food groups to nutrient intake and exposure to heavy metals in the Dutch diet can be found in figure 3. Although the low observed fish consumption in the Netherlands, fish is important especially for intakes of EPA (69%) and DHA (87%) and contributes for 4% and 13% to daily I and Se intake, respectively. Seaweed (nori) was consumed by two individuals in low amounts and contributed with 0.1% to daily I intake. I was mainly derived from grains and grain-based foods, and milk and dairy (products). Bread and milk contributed the most as individual foods to daily I intake. The most important sources for Se intake were meat and meat products, grains and grain-based products and milk and dairy products.

The food group fish and other seafood was important for the exposure to total As (cod, giant tiger prawn, shrimps and prawns, farmed salmon and breaded fish fingers) and total Hg (cod, canned tuna, tuna, pangas catfishes and farmed salmon) with 54% and 28 %, respectively. The contribution of fish and other seafood towards exposure to Pb (1%), Cd (4%) and InAs (2%) was low. Seaweed was consumed in low amounts and contributed 0.8% of total As and 0.1% to Cd exposure. For Cd the most important sources included grains and grain-based products (23%), vegetables and vegetable products (16%) and starchy roots and tubers (12%). For Pb the most important sources included non-alcoholic beverages (rooibos tea, tea infusions, fermented and non-fermented tea, coffee and soft drinks) (20%), grains and grain-based products (13%), milk and dairy products (11%) and drinking water (tap water) (10%). Important sources for InAs included non-alcoholic beverages (rooibos tea, tea infusions, fermented and non-fermented tea, coffee and soft drinks) (28%), grains and grain-based products (20%) and milk and dairy products (12%). Besides fish, important contributors for total Hg include non-alcoholic beverages (herbal tea, rooibos tea, tea infusions, fermented and non-fermented tea, coffee and soft drinks) (41%).

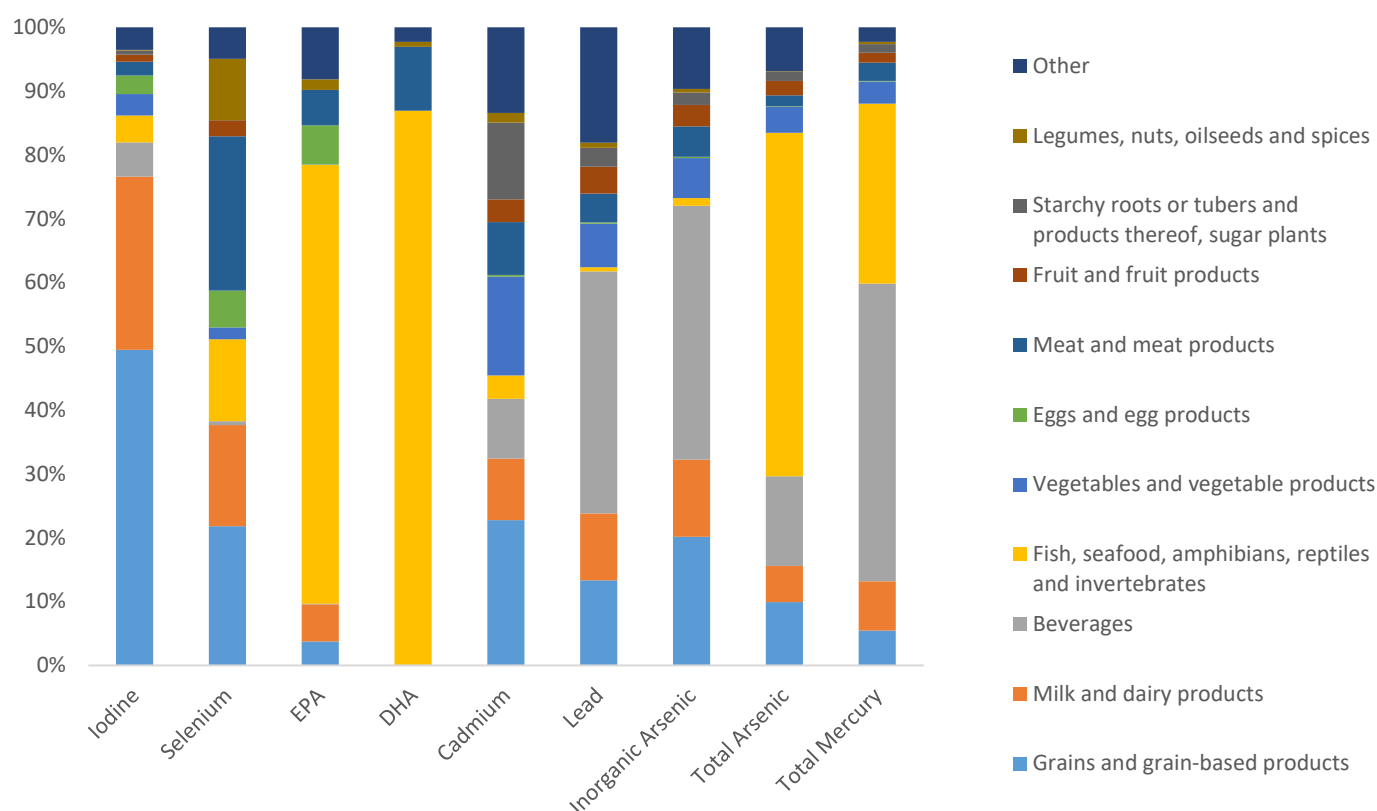


Figure 3. Contribution in percentage of most important food groups to daily nutrients and exposure to heavy metals for the Dutch population

The contribution of most important food groups to nutrient intake and exposure to heavy metals in the Portuguese diet can be found in figure 4. Among the Portuguese, fish and seafood products (salted cod, salmon, sardine, hakes) were most important especially for intakes of EPA and DHA with 85% and 91%, respectively. Fish and seafood products (e.g. salted cod) contributed with 35% to daily Se and with 18% to daily I intake. Grain and grain-based products were most important to determine I intake (50%) and contributed significantly to Se intake (21%). Meat and meat products contributed with 31% to daily Se intake. Fish and seafoods were the most important sources for total As (hakes, salted cod, octopus, canned tuna, salmon and squids) and total Hg (salted cod, hakes, canned tuna, tuna, salmon and scabbardfishes) with 73% and 76%, respectively. The contribution of fish and seafoods towards Cd (12%), Pb (3.5%) and InAs (4.4%) was low. Seaweed was consumed in low amounts and contributed 0.5% of total As and 0.1% to Cd exposure. For Cd the most important sources included vegetables and vegetable products (19%), grains and grain-based products (18%) and starchy roots and tubers (15%). For Pb the most important sources included vegetables and vegetable products (15.4%), grains and grain-based products (15%), milk and dairy products (14.6%) and drinking water (9%). Important sources for InAs included grains and grain-based products (26%), drinking water (15%), milk and dairy products (15%) and vegetables and vegetable products (9%).

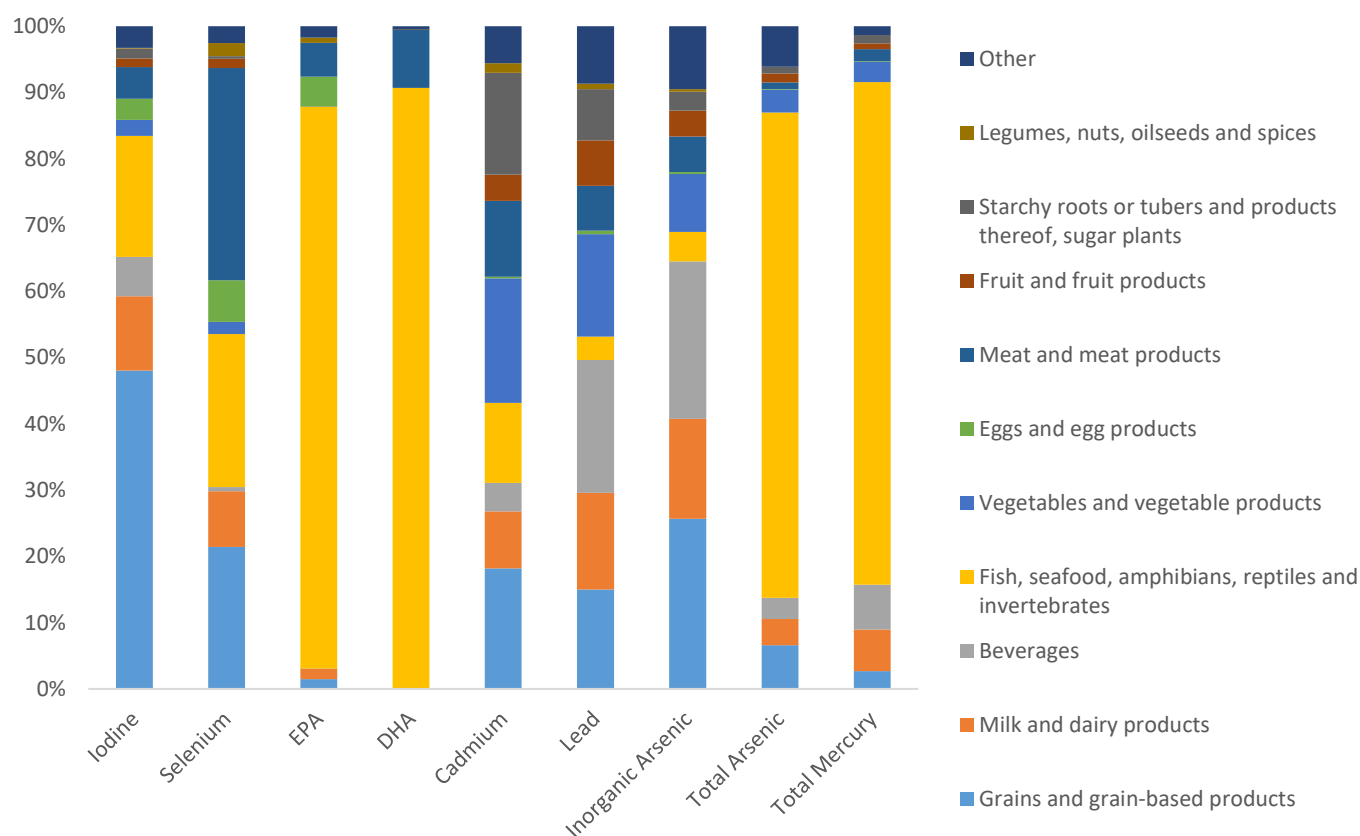


Figure 4. Contribution in percentage of most important food groups to daily nutrient intake and exposure to heavy metals for the Portuguese population

Scenarios

Estimated nutrient intake for the current dietary pattern and scenarios for Dutch children and adults aged 1 to 79 years are shown in table 8. In the baseline_control_fish scenario the average estimated intake over two consumption days was 171 µg I (95% confidence interval (CI) of 168-173 µg) and 40 µg Se (95% CI 40-41 µg) among the Dutch population. Consumption of fortified trout in the fortified_fish scenario did not change I intake compared to control trout in the baseline_control_fish scenario.

The consumption of seaweed bacon, seaweed pasta and seaweed lettuce Pb to an approximately 60% higher I intake compared to the baseline scenario (baseline_control_fish). In the seaweedfoods scenario, the mean I intake was estimated at 273 µg per person per day with a 95% CI of µg and 282 µg per day. Seaweed pasta contributed with 25%, seaweed lettuce with 3% and seaweed bacon with 14% to estimated I intake. The estimated intake of Se remained similar when control fish was replaced by the fortified fish in the fortified_fish scenario.

Table 9 shows the mean exposure (in µg/kg b.w./day) of the total population in the Netherlands for Cd, Pb, InAs, total As and total Hg for the three investigated scenarios. In the baseline_control_fish scenario the Dutch were on average exposed to 0.29 µg Cd, 0.68 µg Pb, 0.34 µg InAs, 0.75 µg total As and 0.10 µg total Hg per kg/b.w. per day. The findings for the fortified_fish scenario indicated that the consumption of fortified trout and fortified seabream did not significantly change the exposure to Cd, Pb, and Hg compared to the baseline_control_fish scenario. In the seaweedfoods scenario, the consumption of seaweed pasta, seaweed

bacon and seaweed lettuce led to a significant increase in total As compared to the baseline_control_fish scenario. Mean exposure to total As increased from 0.75 µg (95% CI 0.71-0.79) to 2.02 µg per kg/b.w per day (95% CI 1.89-2.13) in the seaweedfoods scenario. Although there is a significant increase in total As, the exposure to InAs in the seaweedfoods scenario remained similar compared to the baseline_control_fish scenario.

Table 8. Daily average nutrient intake (µg/day) for scenarios for the Dutch population aged 1 to 79 years.

	Mean (LNN)	95% Confidence interval	50th Pctl	25th Pctl	75th Pctl	97.5th Pctl	95% Confidence interval
Iodine (µg/day)							
Baseline_control_fish	171	168-173	163	130	203	307	297-314
Fortified_fish	172	168-173	163	130	203	309	298-317
Seaweedfoods	273	269-282	252	193	331	554	523-578
Selenium (µg/day)							
Baseline_control_fish	40	40-41	38	30	48	76	72-78
Fortified_fish	40	40-41	38	30	48	76	72-78

Table 9. Mean exposure (µg/kg b.w/day) to heavy metals for scenarios for the Dutch population aged 1-79 years.

	Mean (LNN)	95% Confidence interval	50th Pctl	95% Confidence interval	95th Pctl	95% Confidence interval
Cadmium (µg/kg bw/day)						
Baseline_control_fish	0.29	0.28-0.30	0.27	0.26-0.27	0.52	0.50-0.54
Fortified_fish	0.29	0.28-0.30	0.27	0.26-0.27	0.52	0.50-0.54
Seaweedfoods	0.30	0.29-0.30	0.27	0.27-0.28	0.53	0.51-0.55
Lead (µg/kg bw/day)						
Baseline_control_fish	0.68	0.67-0.69	0.63	0.62-0.64	1.20	1.15-1.23
Fortified_fish	0.68	0.67-0.69	0.63	0.62-0.64	1.20	1.15-1.23
Seaweedfoods	0.69	0.68-0.70	0.64	0.63-0.65	1.21	1.17-1.25
Inorganic arsenic (µg/kg bw/day)						
Baseline_control_fish	0.34	0.34-0.35	0.31	0.31-0.32	0.63	0.61-0.65
Fortified_fish	0.34	0.34-0.35	0.31	0.31-0.32	0.63	0.61-0.65
Seaweedfoods	0.34	0.34-0.35	0.31	0.31-0.32	0.63	0.61-0.66
Total arsenic (µg/kg bw/day)						
Baseline_control_fish	0.75	0.71-0.79	0.67	0.64-0.70	1.45	1.34-1.58
Fortified_fish	0.75	0.71-0.79	0.67	0.64-0.70	1.45	1.34-1.57
Seaweedfoods	2.02	1.89-2.13	1.68	1.59-1.77	4.60	4.20-4.89
Total mercury (µg/kg bw/day)						
Baseline_control_fish	0.10	0.10-0.11	0.09	0.09-0.1	0.20	0.19-0.21
Fortified_fish	0.10	0.10-0.11	0.09	0.09-0.1	0.20	0.19-0.21
Seaweedfoods	0.11	0.10-0.11	0.09	0.09-0.1	0.20	0.19-0.21

In Table 10 the daily average I, Se, EPA and DHA intake among the Portuguese population can be found. Among Portuguese children and adults aged 3 months to 84 years, the average intake over two consumption days was 140 µg I (95% CI 137-143 µg), 48 µg Se (95% CI 47-48 µg), 150 mg EPA (95% CI 170-200 mg) and 265 mg DHA (95% CI 227-245 mg) for current consumption pattern (baseline_control_fish scenario). The consumption of fortified trout and fortified seabream in the fortified_fish scenario did not significantly change estimated I, Se, EPA or DHA intake among the Portuguese compared to the baseline_control_fish scenario. In the fortified_fish scenario the consumption of fortified trout contributed with 0.3% and fortified seabream with 0.1% to daily I intake. Furthermore, the consumption of fortified seabream contributed with 0.4% to daily Se intake in the fortified_fish_scenario.

Table 10. Daily average nutrient intake (µg/day) for scenarios for the Portuguese population aged 3 months to 84 years.

	Mean (LNN)	95% Confidence interval	50th Pctl	25th Pctl	75th Pctl	97.5th Pctl	95% Confidence interval
Iodine (µg/day)							
Baseline_control_fish	140	137-143	129	98	170	287	277-297
Fortified_fish	140	138-143	129	98	170	287	277-297
Selenium (µg/day)							
Baseline_control_fish	48	47-48	45	35	58	94	90-97
Fortified_fish	48	47-49	45	35	58	94	90-97
EPA (mg/day)							
Baseline_control_fish	188	170-200	121	65	228	758	665-835
Fortified_fish	187	170-199	121	65	227	757	664-833
DHA (mg/day)							
Baseline_control_fish ^a	265	227-245	127	39	329	1216	1133-1306
Fortified_fish ^a	265	227-245	127	39	329	1216	1133-1306
a The Observed Individual Means (OIM) were calculated instead of Log-Normal-Normal (LNN) based on the normal distribution of the Q-Q plots.							

Table 11 shows the mean exposure (in µg/kg b.w./day) of the total population in Portugal for Cd, Pb, InAs, total As and total Hg for the two investigated scenarios. The mean exposure over two days for the Portuguese was 0.33 µg Cd, 0.57 µg Pb, 0.37 µg InAs, 2.02 µg total As and 0.18 µg total Hg per kg/b.w. per day in the baseline_control_fish scenario. The consumption of fortified trout or fortified seabream in the fortified_fish scenario did not significantly change the estimated exposures compared to the baseline_control_fish scenario.

Table 11. Mean exposure (µg/kg b.w/day) to heavy metals for scenarios for the Portuguese population ages 3 months to 84 years.

	Mean (LNN)	95% Confidence interval	50th Pctl	95% Confidence interval	95th Pctl	95% Confidence interval
Cadmium (µg/kg b.w/day)						
Baseline_control_fish	0.33	0.31-0.35	0.28	0.26-0.29	0.71	0.66-0.76
Fortified_fish	0.33	0.31-0.35	0.28	0.26-0.29	0.71	0.66-0.76
Lead (µg/kg b.w/day)						
Baseline_control_fish	0.57	0.56-0.59	0.50	0.49-0.51	1.19	1.15-1.24
Fortified_fish	0.57	0.56-0.59	0.50	0.49-0.51	1.19	1.15-1.24
Inorganic arsenic (µg/kg b.w/day)						
Baseline_control_fish	0.37	0.36-0.38	0.32	0.31-0.33	0.78	0.75-0.81
Fortified_fish	0.37	0.36-0.38	0.32	0.31-0.33	0.78	0.75-0.81
Total arsenic (µg/kg b.w/day)						
Baseline_Control_fish	2.02	1.94-2.1	1.62	1.55-1.69	4.86	4.57-5.16
Fortified_fish	2.02	1.94-2.1	1.61	1.55-1.69	4.82	4.53-5.11
Total mercury (µg/kg b.w/day)						
Baseline_control_fish	0.18	0.17-0.19	0.15	0.14-0.16	0.41	0.37-0.44
Fortified_fish	0.18	0.17-0.19	0.15	0.14-0.16	0.41	0.37-0.44

6. Discussion

In this study we evaluated the nutritional (benefits) intake and chemical (risks) exposure of the consumption of farmed trout and seabream, fortified with blends of iodine-rich macroalgae, selenised-yeast and DHA-rich microalgae biomasses as well as novel seaweed foods containing the seaweed *Saccharina latissima* cultivated in proximity of salmon sea-cages (IMTA). Fortified trout contained higher concentrations of I and total As and lower concentrations of Cd and Pb compared to the control fish. Fortified seabream contained higher concentrations of I and Se and lower concentrations of Cd, Hg and total As. Due to the low consumption of trout and seabream in the Netherlands and Portugal, estimated nutrient intake and exposure to heavy metals did not change in the Dutch and Portuguese population. Given current consumption patterns, the consumption of fortified trout and fortified seabream did not affect consumers health in the Netherlands and in Portugal. The seaweed (*Saccharina latissima*) cultivated in proximity of salmon sea-cages contained higher concentrations of Cd and total As compared to regular seaweed cultivation. Seaweed foods (pasta, bacon and lettuce) contained significantly higher concentrations of I compared to the I composition of regular foods. The replaced of regular pasta, bacon and lettuce by seaweed foods with 10%, led to an significant increase in I intake and exposure to total As compared to the baseline scenario in the Netherlands. Thus, by increased consumption of novel seaweed foods, higher intakes of I and total As are expected and concentrations should be monitored in these type of foods and also the consumption levels.

Scenarios

In this benefit-to-risk analysis, the reference scenario was based on the current food intake derived from the Dutch and Portuguese national food consumption survey. Based on current consumption patterns we constructed scenarios. We assumed that trout and seabream were eaten in the same quantities as current pattern but with every trout and seabream replaced by the fortified trout and fortified seabream. Because carp was not consumed in the Netherlands nor Portugal, we did not included carp. Furthermore, in an additional scenario for the Netherlands we assumed that 10% of the current consumption of lettuce, pasta and bacon was replaced with a similar seaweed food. Given current consumption of seaweed in the Netherlands (approximately 30 and 50 grams of seaweed were consumed by two different individuals), the scenarios could be considered as worst-case scenarios. In reality, the replacement of 10% consumption of lettuce, pasta and bacon with seaweed products in the Netherlands is very optimistic as consumption of seaweed products is currently relatively low in the Netherlands. Even though consumption patterns do shift over time, it will take considerable time, if ever, before consumption of seaweed products takes over 10% of the market share of lettuce, pasta and bacon. However, by developing a worst-case scenario we can estimate benefits and risks that may be expected.

Fortified trout and fortified seabream (T1.1)

Significant differences were observed between nutrient and contaminants concentrations in fortified trout and fortified seabream compared to concentrations in the baseline control trout and seabream. For several nutrients and heavy metals, we can conclude that the fortification of fish led to a healthier fish, e.g. Se and I concentrations were enhanced in all the species. The effectiveness of fortification fish was previously

investigated [57-59]. However, Kwasek et al. (2020), states in a review, where several studies were discussed regarding the fortification of fish feed with Se, EPA or DHA, that the literature regarding fortification of fish feed with nutrients essential for humans is scarce [1]. We did not find any differences in intake for the nutrients or heavy metals on a population level between the control fish scenario and scenario where concentrations of seabream, carp and trout were replaced by concentrations of the fortified fishes. Because of the low frequency and amount of consumption of trout in the Netherlands (0.1 g per day) and no consumption of seabream in both the Netherlands and Portugal, we did not find any differences on population level. When comparing the baseline scenario and the fortified fish scenario to the dietary HBGVs and BMDLs established by JECFA and EFSA for heavy metals (Appendix VI, table 2), it shows for both countries that the mean exposure for Cd (total population) in both scenario's is below the TWI (25 µg/kg bw/month) reported by JECFA. For Pb the mean exposure is below the BMDL10 (0.63 µg/kg bw per day based on a 10% increase in incidence for chronic kidney disease) for the Portuguese population and above the BMDL10 for the Dutch population. For both countries the mean exposure to total As and InAs are both below the BMDL0.5 (3 µg/kg bw/day based on 0.5 % increased incidence of lung cancer) reported by JECFA. The mean exposure to Hg is below the TWI of 4 µg/kg bw per week established by JECFA.

It can be argued if the inclusion of farmed trout, seabream and carp and Dutch and Portuguese food consumption data were most suitable to assess the impact of the innovations. To start, there are various factors that can influence fish consumption behavior [60]. In various studies on consumer beliefs [61, 62], consumers generally appeared to be rather poorly aware of the fish they consume to be farmed or wild. In these studies health involvement was found to be a strong predictor of the attitudes towards fish consumption [60]. Different types of consumers across Europe may chose for farmed and wild fish based on their involvement in health issues and their attitudes towards fish consumption. Although in literature no significant difference between farm and wild fish can be found on exceeding European safety regulations [63], in general farmed fish is perceived to be less affected by marine pollution, heavy metals and parasites by consumers. However, on the contrary, wild fish was considered to have healthier feeding, to contain fewer antibiotics and to be fresher, healthier, less handled and more natural [61, 62]. Consumer beliefs related to quality were in favour of wild fish, while those related to availability and price were in favour of farmed fish. Significant differences were observed in the perception of both kinds of fish depending on the consumers' objective knowledge about fish, level of education, age, ethics and ethical beliefs [61]. The nutritional composition of farmed fish is associated with the nutritional composition of the aquatic feed and unlocks the possibility to tailor fish composition with healthy and valuable nutrients. Also, the health of the fish is dependent on the type of cultivation technique(s) used to cultivate the farmed fish [22]. The fortification blends and cultivation techniques used in the SEAFOOD^{TOMORROW} consortium could be important factors to improve the current consumer beliefs on farmed fish. In addition to the choice of species, it can be questioned if food composition data exist that distinguishes between wild and farmed fish and is suitable to evaluate the health benefits and risks of (farmed) fishes.

Furthermore, the type of farmed fishes included in the innovations can be argued. Seabream, trout and carp were initially chosen because they are common farmed fishes and can be fed with fortified feed in order to change nutrient composition in the fish fillets [64]. Also, these three fishes are often eaten in European countries, such as Italy for seabream, Denmark for salmon and trout and Poland for carp [65]. However, the

Dutch consume mostly salmon (wild and cultivated), Alaska pollack (wild), tilapia (cultivated) and pangasius (cultivated) [66]. And the Portuguese consume mainly cod, tuna, hake, mackerel and sardine based on the Portuguese food consumption data and the relative house-hold consumption of the main species in Portugal assuming a per capita consumption for Portugal of 55 kg from FAO for 2005 [67]. Perhaps the inclusion of other European countries that consume high(er) amounts of trout and seabream (and carp, salmon) such as Italy, Norway, Denmark and Poland [65] would have resulted in more representative food consumption data for the whole of Europe. These countries could be included in a follow-up study.

Based on our results on the dietary exposure to heavy metals, we advise that dietary exposure to Pb and MeHg should be closely monitored in European countries [7, 18]. Literature indicates that especially children and toddlers are possible risk groups for adverse health effects by excessive exposure to Pb and MeHg. It is well-known that Pb accumulates in the body and most seriously affects the developing central nervous system in young children [7, 18]. Multiple BMDLs for neurotoxicity and increased incidence of chronic kidney disease have been established by EFSA for different age groups (Appendix VI, table 2) [18]. Over the past decades legislative measures have been gradually introduced to reduce exposure by removing lead from paint, food cans, water pipes and petrol. The Netherlands has a mean dietary exposure that is above the BMDL10 for chronic kidney disease (Appendix VI, table 2). It has to be noted that the consumption of fish has very limited influence on dietary Pb exposure. Important dietary sources of Pb include grains and grain-based products, drinking water, non-alcoholic beverages and milk and dairy products. Also, it is important to note that for children ingestion of soil and dust can be important contributors of exposure to Pb as well [18].

For MeHg a Tolerable Weekly Intake (TWI) of 1.6 µg/kg bw per day is established by JECFA [68]. MeHg, abundantly present in (fatty) fish, is considered a highly toxic organic compound of Hg, given its readily and easy absorption by the gastrointestinal tract and its effect as a neurotoxin that may adversely affect the development of the brain and nervous system [69]. The major dietary contributors for adults include tuna, swordfish, cod, whiting and pike and to a lesser extent salmon/trout, bream, bass and flatfishes. The important contributors for children and toddlers include the same previously mentioned species, with the addition of hake [7].

MeHg concentrations were not chemically analyzed in task 1.1 or 1.2 and therefore not assessed in this report. However, MeHg concentrations were calculated back from the total Hg concentrations reported in task 1.1 based on %proportion (MeHg/Total Hg) reported in the EFSA report 'Scientific Opinion on the risk for public health related to the presence of Hg and MeHg in food' [60]. The %proportion (MeHg/Total Hg) are 93.0%, 82.0% and 86.8% for trout, gilthead seabream and common carp, respectively [60] (Appendix VII, table 1). Additional analysis show that the mean exposure to MeHg for young Portuguese children (1-9 year old) was 0.237 µg/kg bw/day for the fortified fish scenario, which is slightly above the TWI of 1.6 µg/kg bw/week reported by the JECFA (Appendix VI). The mean exposure to MeHg for the total Portuguese population (0.121 µg/kg bw/day) was below the TWI. The mean exposure to MeHg from seaweed (*Saccharina latissima*) was considered to be negligible as the concentration of total Hg in IMTA-MA (task 1.2) was reported to be <LOQ (0.9) µg/kg dry weight. Mean exposure to total Hg (calculated for the total population) in the Netherlands (0.10-0.11 µg/kg bw/day) and Portugal (0.18 µg/kg bw/day) for the reported

scenarios (the seaweedfoods scenario included for the Netherlands only) were well below the TWI of 4 µg/kg bw/week reported by the JECFA.

Integrated multi-trophic aquaculture (T1.2)

We were not able to analyze the impact of integrated multi-trophic aquaculture (IMTA) salmon and salmon monoculture on consumption level because the SEAFOOD^{TOMORROW} database did not provide sufficient concentration data. For this reason, we did not include salmon in our analysis.

The SEAFOOD^{TOMORROW} database provides us with data on heavy metals measured in seaweed (IMTA and non-IMTA) but not with nutrient concentration data. We were able to evaluate the effect of seaweed food consumption in the Netherlands using nutrient composition data of seaweed from the Dutch food consumption table and actual products on the Dutch market. Portugal was excluded from this analysis because seaweed foods were not yet available on the Portuguese market (this was discussed during the 24M Porto meeting).

In the seaweed foods scenario, we replaced regular pasta, bacon and lettuce by 10% of novel seaweed foods, available on the Dutch market and we found increased exposures of I and As. As we mentioned earlier, this is a worst-case scenario which enabled us to investigate the benefits and risks that can be expected over time. Lately, seaweed has raised more public awareness because of several reasons; it rapidly grows in the sea, it has multifunctional use (as fuel, feed or as food). Considering food security and global sustainability the challenges, it can be expected that the consumption of seaweed increases in Europe [27]. Consumption of seaweed in Europe is very low, daily seaweed consumption per person in Japan is ten fold higher and has remained relatively consistent over the last 40 years (4.3 g/day in 1955 and 5.3 g/day in 1995, dry weight) [70]. It was estimated by Zava and Zava (2011) that the average Japanese I intake, largely from seaweed consumption, based on dietary records, food surveys, urine I analysis and seaweed I content, was 1,000-3,000 µg/day [12]. The I intake of the Japanese, was only derived from seaweed and is not considered as a health risks, therefore it can be questioned if the maximum level of 600 µg per day is realistic.

We estimated in our scenario an average intake of 273 µg I per person per day in the Netherlands. In the seaweed foods scenario, seaweed pasta contributed with 25%, seaweed lettuce with 3% and bacon with 14% to total daily I intake. Although the concentration of I was lower in seaweed pasta compared to seaweed lettuce and seaweed bacon, pasta products were more frequently and in higher amounts consumed in the Netherlands and have therefore a higher contribution towards daily I intake. For the interpretation of the results it should be noted that estimation of I intake is rather complex. Besides the large variation of I concentrations in foods and the uncertain use of iodized salt, I intake estimation in diets is often an underestimation [71]. I intake is usually estimated from several sources (supplements, household salt (iodized salt) and (a part of) iodized salt used in manufactured foods (processed foods)) but not included in this study. If the population is at risk of high I intake due to the substitution of seafood products further research should be undertaken to assess I intakes, by preferably 24h urinary study. It has to be noted that seaweed contains also high levels of natrium which has a negative health effect on human health in high quantities[30]. We did not assess the impact of natrium on human health.

Considering the extreme cases in the upper tail, we found a 97.5th percentile intake of 554 µg I per day in the Dutch population with an 95% confidence interval (CI) of 523 – 578 µg per day. Currently, the tolerable upper intake level of I intake is set at 600 µg I per person per day for males and females (including pregnant

or lactating) aged 18 to ≥ 75 years old [72]. Although we estimated that I intake increased due to consumption of seaweed foods in our scenario, the extreme cases in the upper tail stay below the daily tolerable upper intake level set by EFSA. For children aged 1 to 17 years old, the tolerable upper intake level on daily basis ranges from 200 to 500 μg . Therefore, based on current estimation of included I sources and compared to the population mean (274 μg) children aged 1-17 years are exceeding the tolerable upper intake level (200 to 450 μg). Further research, such as the estimation of I intake derived from all I sources and the assessment of I concentrations via urinary studies, are needed to investigate if risks to health occur in this population.

When comparing the Dutch exposure to heavy metals from the baseline scenario and of the novel seaweed food scenario the dietary HBGVs and BMDLs established by JECFA and EFSA for heavy metals (Appendix VI, table 2) it can be seen for Cd that the mean exposure (total population) for the scenarios is below the TMI of 25 $\mu\text{g}/\text{kg}$ bw/month reported by JECFA. For Pb the mean exposure is slightly above the BMDL10 of 0.63 $\mu\text{g}/\text{kg}$ bw per day, which is based on a 10% increase in incidence for chronic kidney disease. This is relevant for men and women from 18 years of age. However, it has to be noted that this is due to the high baseline exposure to Pb and that the increased exposure to seaweed caused no significant differences when comparing to the baseline scenario. The mean exposure to Hg is below the TWI of 4 $\mu\text{g}/\text{kg}$ bw per week established by the JECFA. The mean exposure to total As and InAs are both below the BMDL0.5 of 3 $\mu\text{g}/\text{kg}$ bw/day reported by the JECFA which is based on a 0.5 % increased incidence of lung cancer. However, it has to be noted that for total As the 95th percentile for the seaweed foods scenario does exceed the BMDL0.5 of 3 $\mu\text{g}/\text{kg}$ bw/day. Furthermore, total As exposure increased (2.69 fold) when 10% of pasta, bacon and lettuce consumption (in amounts) was replaced by novel seaweed foods in the Netherlands. This increase is most likely due to the fact that the seaweed *Saccharina latissima* contains high amounts of total As (average of 40333.33 $\mu\text{g}/\text{kg}$ (DW) was reported in task 1.2). With this worst-case scenario the daily mean exposure to total arsenic remained below the BMDL0.5 of 3 $\mu\text{g}/\text{kg}$ b.w. per day for InAs, set by the JECFA (Appendix VI, table 2) [68]. Despite the high increase in exposure to total As, the exposure to InAs remained constant when comparing the baseline scenario with the seaweedfoods scenario. In the seaweedfoods scenario, we used total As concentrations for IMTA macroalgae that were chemical analysed in the consortium. However, InAs was not chemically analyzed and instead we used literature values for the calculation of InAs exposure (see table 3). Based on toxicokinetic studies, InAs is the most toxic form of As. The JECFA has established a BMDL0.5 of 3 $\mu\text{g}/\text{kg}$ b.w. per day for InAs based on lung and urinary cancer studies [68]. The fact that the exposure to InAs remains constant after the substitution of seaweed foods is explained because *Saccharina latissima* contains relatively low concentrations of InAs. The Norwegian National Institute of Nutrition and Seafood Research (NIFES) reported for InAs a concentration of 50 $\mu\text{g}/\text{kg}$ dryweight [45] when compared to concentrations of total As levels of 40333.33 $\mu\text{g}/\text{kg}$ dryweight found in *Saccharina latissima* reported by the consortium. Furthermore, about 90% of total As in seaweeds consists of organic As, especially arsenosugars which are known to have very little toxicity [16]. Based on all these findings it is safe to assume that the significant increase in dietary exposure to total As in the seaweedsfoods scenario is mostly due to the increase in organic As intake (and not inorganic intake).

Due to the nutritional aspects of seaweed, consumption of seaweed can be advised, however, our study suggests that (partial) replacement of common food products need to be done carefully and safety regulations are needed. For As, Cd and Pb, maximum levels (MLs) for various foodstuffs are established

under commission regulation (EC) No 1881/2006. However, currently no MLs are established for these substances in seaweed. With exception for the MLs established under this regulation for food supplements consisting exclusively or mainly of seaweed. For Hg in algae and prokaryotic organisms a maximum residue level (MRL) of 0.01 mg/kg is established according to EC regulations No 396/2005. For Food additives based on seaweed, specification are laid down in the annexes of regulation (EU) No 231/2012 [73]. When more seaweed products enter the European market, it would be wise to establish maximum levels (MLs) for As, Cd and Pb in those seaweeds and seaweed products.

We summarize the evidence from this report on the consumption of fortified fish and novel seaweed food. The 95th percentile of I intake was slightly below the current daily tolerable upper level (UL) in the seaweed foods scenario. In addition, the 95th percentile of the mean exposure to total As for the Netherlands in the seaweed foods scenario exceeded the BMDL0.5 of 3 µg/kg bw/day for InAs set by the JECFA. However, because there was no significant increase in InAs exposure for this scenario and about 90% of total As in seaweeds consists of organic As, it is safe to assume that InAs exposure remains below the BMDL0.5. Further research is advised to estimate I and As intake more accurately according to all sources. I can be measured in a urine and As in hair and fingernails. These methods are far more sophisticated in measuring the intake of I or exposure to As [38, 74].

Strengths and limitations

This study has strengths and limitations that should be addressed. To the best of our knowledge, this is the first study that assesses the benefits and risks on human health of the modelled consumption of fortified fish and novel seaweed foods including background diet. This is a major strength because a given product (e.g. biofortified fish) is not consumed in isolation but is incorporated in a diet which is already associated with a certain exposure to nutrients and contaminants. Although many studies have been done to identify the nutrient requirements needed to maximize the health and growth of fish, virtually no studies have been done to directly assess human health benefits of enhancing the nutritional content of fish through feeds [1]. It has been investigated that the consumption of fortified fish contributes to an increased daily reference intake (DRI) for nutrients (e.g. when 150 g of fortified fish is consumed), however it was not yet investigated including background diet and actual intakes [57].

There are some limitations that need to be considered. First, the availability of data from the SEAFOOD^{TOMORROW} consortium was limited. We aimed to replace all concentrations and heavy metals for fish and seaweed with analyzed data from the SEAFOOD^{TOMORROW} database. For several nutrients and heavy metals, the consortium did not provide data and we used proxy values obtained from the literature. Besides, only pilot-scale data was available. Unfortunately, farm-scale data was lacking at time of analysis. Regarding our outcomes compared to other outcomes of the SEAFOOD^{TOMORROW} consortium, it has to be noted that in the study by Vera Barbosa et al. (2020) looked at the differences between median values between the different blends and fillets of the fortified fish. Whereas in the current study we calculated values based on the mean concentrations. This might result in slight differences between conclusions drawn from the results. Secondly, the quality of nutritional composition and heavy metal data used and the underlying assumptions may influence our study results. In our study, nutrients and heavy metals of background diet were estimated with data from the Dutch food composition table [44] and NVWA, EFSA and WHO [51] reports. Although, we

systematically managed to cover all important food groups with data, we used different sources. The Dutch food composition table did not cover nutrient concentrations for all foods consumed and therefore several values were set to zero. Ideally, all concentrations would be measured. However, for all foods that we expected to contain levels of the relevant substances, concentrations were covered.

Dutch food composition data was used to estimate I (partly), Se, DHA and EPA intake in the Portuguese diet because these data were not available in the Portuguese food composition table. Therefore, values for the Portuguese intake could differ. Nevertheless, we do not expect that concentrations in Portuguese foods differ significantly regarding their nutrient content, except for I intake. In the Dutch food composition table it is assumed that part of the bread was manufactured with iodized salt. The usage of iodized salt is voluntary in Portugal and there are currently no regulations regarding the concentration of I and I in iodized salt. The estimation of I intake derived from bread might therefore be an overestimation in the Portuguese diet.

The concentration data obtained from the EFSA reports were based on values reported as mean and/or Mean Bound (MB). These values are based on concentrations reported across all of Europe and are not country-specific. The EFSA reports can include concentration data from countries with relatively high contamination levels, and therefore may be less accurate for countries like the Netherlands and Portugal. Also, in some cases the concentration data used from the EFSA reports are older than the monitoring data. Ideally, all concentration data used for the calculations should have covered the same period as the monitoring data (2012-2016 for the Netherlands and 2015-2016 for Portugal). As example, the EFSA report 'Scientific Opinion on Arsenic in Food' (2009) uses an old classification system, which does not cover all current FoodEx2 food subcategories. Which makes it difficult to correctly link all FoodEx2 codes to the concentration data. Furthermore, this article only reported concentration data at subcategory level and not at product-specific level. As a result, the concentration data used is less detailed compared to if more product specific data was reported (e.g. FoodEx level 3 or 4).

At last, for both the Netherlands and Portugal it is possible that under- and overreporting of foods and therefore actual means might have been affected [46, 75]. However, the scope of this study was to determine whether the consumption of innovative solutions would lead to an increase in daily intake of I, Se, EPA and DHA intake and changes in the exposure to Cd, Pb, InAs, As and Hg on population level.

7. Conclusions

To conclude, we evaluated the impact of the hypothetical consumption of biofortified fish with blends of I-rich macroalgae, selenized-yeast and DHA-rich microalgae biomasses and novel seaweed products, consisting of seaweed for human consumption cultivated in proximity of salmon sea-cages, on the nutritional and chemical intake of the Dutch and Portuguese population. Fortified trout contained higher concentrations of I and total As and lower concentrations of Cd and Pb compared to the control fish. Fortified seabream contained higher concentrations of I and Se and lower concentrations of Cd, Hg and total As. Based on our findings we can conclude that although the different fortified feeds led to significant differences in I, Se, EPA, DHA, Cd, Pb, Hg, InAs and total As in the fortified fish fillets it did not lead to a noticeable difference in intake on population level for the Netherlands and Portugal due to low consumption of specified fishes. I intake increased in the Netherlands due to the hypothetical consumption of novel seaweed foods (lettuce, bacon

and pasta) but stayed within safety limits. The exposure to total arsenic increased due to the hypothetical consumption of seaweed foods. Although the mean exposure towards total arsenic stayed below the maximum for InAs, the 95th percentile exceeded the BMDL0.5 of 3 µg/kg bw/day set by the JECFA for InAs based on hypothesised consumption. However, because there was no significant increase in InAs exposure for this scenario and about 90% of total As in seaweeds consists of organic As it is safe to assume that dietary InAs exposure remains below the BMDL0.5.

Higher intakes of I and exposure to As are expected in case of increased consumption of novel seaweed foods and should be monitored in the food as well as the food consumption level. Further research is needed to determine accurate concentrations of I and As in the seaweed foods and diets. Moreover, accumulated I and As intake can be measured more accurately in urinary and hair studies.

8. References

1. Kwasek, K., A.L. Thorne-Lyman, and M. Phillips, *Can human nutrition be improved through better fish feeding practices? a review paper*. Critical Reviews in Food Science and Nutrition, 2020: p. 1-14.
2. Msangi, S., et al., *Fish to 2030: prospects for fisheries and aquaculture*. World Bank Report, 2013. **83177**(1): p. 102.
3. Afshin, A., et al., *Health effects of dietary risks in 195 countries, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017*. The Lancet, 2019. **393**(10184): p. 1958-1972.
4. Gezondheidsraad, *Richtlijnen goede voeding 2015*. 2015: Gezondheidsraad.
5. Augood, C., et al., *Oily fish consumption, dietary docosahexaenoic acid and eicosapentaenoic acid intakes, and associations with neovascular age-related macular degeneration*. The American Journal of Clinical Nutrition, 2008. **88**(2): p. 398-406.
6. Pieniak, Z., W. Verbeke, and J. Scholderer, *Health-related beliefs and consumer knowledge as determinants of fish consumption*. Journal of human nutrition and dietetics, 2010. **23**(5): p. 480-488.
7. Committee, E.S., *Statement on the benefits of fish/seafood consumption compared to the risks of methylmercury in fish/seafood*. EFSA journal, 2015. **13**(1): p. 3982.
8. Gezondheidsraad, *Eicosapentaenzuur en docosahexaenzuur – Achtergronddocument bij Richtlijnen goede voeding 2015*. 2015: Gezondheidsraad.
9. Abdelhamid, A.S., et al., *Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease*. Cochrane Database of Systematic Reviews, 2018(11).
10. Ellis, D.R. and D.E. Salt, *Plants, selenium and human health*. Current opinion in plant biology, 2003. **6**(3): p. 273-279.
11. Rayman, M.P., *Selenium and human health*. The Lancet, 2012. **379**(9822): p. 1256-1268.
12. Zava, T.T. and D.T. Zava, *Assessment of Japanese iodine intake based on seaweed consumption in Japan: a literature-based analysis*. Thyroid research, 2011. **4**(1): p. 14.
13. Zimmermann, M.B., P.L. Jooste, and C.S. Pandav, *Iodine-deficiency disorders*. The Lancet, 2008. **372**(9645): p. 1251-1262.
14. Olmedo, P., et al., *Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers*. Environment international, 2013. **59**: p. 63-72.
15. Tressou, J., et al., *Probabilistic exposure assessment to food chemicals based on extreme value theory. Application to heavy metals from fish and sea products*. Food and Chemical Toxicology, 2004. **42**(8): p. 1349-1358.
16. Chen, Q., et al., *Distribution of metals and metalloids in dried seaweeds and health risk to population in southeastern China*. Scientific reports, 2018. **8**(1): p. 1-7.
17. Authority, E.F.S., *Dietary exposure to inorganic arsenic in the European population*. EFSA Journal, 2014. **12**(3): p. 3597.
18. Authority, E.F.S., *Lead dietary exposure in the European population*. EFSA Journal, 2012. **10**(7): p. 2831.
19. Authority, E.F.S., *Cadmium dietary exposure in the European population*. Efsa Journal, 2012. **10**(1): p. 2551.
20. Bell, J.D., R.A. Watson, and Y. Ye, *Global fishing capacity and fishing effort from 1950 to 2012*. Fish and Fisheries, 2017. **18**(3): p. 489-505.
21. Cummins, A., *The Marine Stewardship Council: A multi-stakeholder approach to sustainable fishing*. Corporate Social Responsibility and Environmental Management, 2004. **11**(2): p. 85-94.
22. Ribeiro, A.R., et al., *Farmed fish as a functional food: perception of fish fortification and the influence of origin—insights from Portugal*. Aquaculture, 2019. **501**: p. 22-31.

23. Handå, A., et al., *Seasonal-and depth-dependent growth of cultivated kelp (Saccharina latissima) in close proximity to salmon (Salmo salar) aquaculture in Norway*. Aquaculture, 2013. **414**: p. 191-201.
24. Troell, M., et al., *Ecological engineering in aquaculture—potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems*. Aquaculture, 2009. **297**(1-4): p. 1-9.
25. Stévant, P., et al., *Biomass soaking treatments to reduce potentially undesirable compounds in the edible seaweeds sugar kelp (Saccharina latissima) and winged kelp (Alaria esculenta) and health risk estimation for human consumption*. Journal of applied phycology, 2018. **30**(3): p. 2047-2060.
26. Forbord, S., et al., *Development of Saccharina latissima (Phaeophyceae) kelp hatcheries with year-round production of zoospores and juvenile sporophytes on culture ropes for kelp aquaculture*. Journal of applied phycology, 2012. **24**(3): p. 393-399.
27. Brown, E.M., et al., *Seaweed and human health*. Nutrition Reviews, 2014. **72**(3): p. 205-216.
28. Bouga, M. and E. Combet, *Emergence of seaweed and seaweed-containing foods in the UK: focus on labeling, iodine content, toxicity and nutrition*. Foods, 2015. **4**(2): p. 240-253.
29. Cornish, M.L., A.T. Critchley, and O.G. Mouritsen, *Consumption of seaweeds and the human brain*. Journal of Applied Phycology, 2017. **29**(5): p. 2377-2398.
30. Circuncisão, A.R., et al., *Minerals from macroalgae origin: Health benefits and risks for consumers*. Marine drugs, 2018. **16**(11): p. 400.
31. Costa Leite, J., et al., *Iodine status and iodised salt consumption in Portuguese school-aged children: the iogeneration study*. Nutrients, 2017. **9**(5): p. 458.
32. Verkaik-Kloosterman, J., et al., *Decreased, but still sufficient, iodine intake of children and adults in the Netherlands*. British Journal of Nutrition, 2017. **117**(7): p. 1020-1031.
33. Limbert, E., et al., *Iodine intake in portuguese school children*. Acta medica portuguesa, 2012. **25**(1): p. 29-36.
34. Limbert, E., et al., *Iodine intake in Portuguese pregnant women: results of a countrywide study*. European journal of endocrinology, 2010. **163**(4): p. 631.
35. Kim, H.S., Y.J. Kim, and Y.R. Seo, *An overview of carcinogenic heavy metal: molecular toxicity mechanism and prevention*. Journal of cancer prevention, 2015. **20**(4): p. 232.
36. Baker, D.H., *Iodine toxicity and its amelioration*. Experimental Biology and Medicine, 2004. **229**(6): p. 473-478.
37. Chain, E.P.o.C.i.t.F., *Scientific Opinion on lead in food*. EFSA Journal, 2010. **8**(4): p. 1570.
38. Chain, E.P.o.C.i.t.F., *Scientific Opinion on arsenic in food*. EFSA Journal, 2009. **7**(10): p. 1351.
39. Chain, E.P.o.C.i.t.F., *Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food*. EFSA Journal, 2012. **10**(12): p. 2985.
40. Biancarosa, I., et al., *Chemical characterization of 21 species of marine macroalgae common in Norwegian waters: benefits of and limitations to their potential use in food and feed*. Journal of the Science of Food and Agriculture, 2018. **98**(5): p. 2035-2042.
41. SeafoodTomorrow. *T3.2 Database*. 2020 [12-2-2020]; Available from: <https://docs.google.com/spreadsheets/d/1W5jylG61ePthG4LPrtB1MzS3mE9JL-J5MU3XS7PxGUo/edit#gid=1071568862>.
42. Seamorefood. *Seamore I sea bacon*. 2020 [cited 12-2-2020; Available from: <https://seamorefood.com/health-matrix/i-sea-bacon/>.
43. Seamorefood. *Seamore I sea pasta*. 2020 [12-2-2020]; Available from: <https://seamorefood.com/health-matrix/i-sea-pasta/>.
44. The National Institute for Public Health and the Environment, *NEVO-online version 2016/5.0*. 2016: Bilthoven.

45. Duinker, A., et al., *Potential risks posed by macroalgae for application as feed and food—a Norwegian perspective*. National Institute of Nutrition and Seafood Research: Trondheim, Norway, 2016: p. 1-24.
46. Van Rossum, C., et al., *The diet of the Dutch: Results of the first two years of the Dutch National Food Consumption Survey 2012-2016*. RIVM letter report 2016-0082, 2016.
47. Slimani, N., et al., *Standardization of the 24-hour diet recall calibration method used in the European Prospective Investigation into Cancer and Nutrition (EPIC): general concepts and preliminary results*. European journal of clinical nutrition, 2000. **54**(12): p. 900-917.
48. Authority, E.F.S., *The food classification and description system FoodEx 2 (revision 2)*. EFSA Supporting Publications, 2015. **12**(5): p. 804E.
49. Lopes, C., et al., *National food, nutrition, and physical activity survey of the Portuguese general population (2015-2016): protocol for design and development*. JMIR research protocols, 2018. **7**(2): p. e42.
50. Dias, M.G. and L. Oliveira, *Food Composition Database Activities Portugal*. 10th International Graduate Course on Production and Use of Food Composition Data in Nutrition-FoodComp, 16-26 October 2011, 2011.
51. Delgado, I., et al., *Scientific update on the iodine content of Portuguese foods*. 2018.
52. Authority, E.F.S. and S. Ioannidou, *EFSA Catalogue Browser User Guide*. EFSA Supporting Publications, 2019. **16**(11): p. 1726E.
53. De Boer, W., et al., *MCRA 8.2 a web-based program for Monte Carlo Risk Assessment*. Reference Manual. December, 2016.
54. van der Voet, H., et al., *The MCRA model for probabilistic single-compound and cumulative risk assessment of pesticides*. Food and Chemical Toxicology, 2015. **79**: p. 5-12.
55. Dodd, K.W., et al., *Statistical methods for estimating usual intake of nutrients and foods: a review of the theory*. Journal of the American Dietetic Association, 2006. **106**(10): p. 1640-1650.
56. de Boer, W.J., et al., *Comparison of two models for the estimation of usual intake addressing zero consumption and non-normality*. Food Additives and Contaminants, 2009. **26**(11): p. 1433-1449.
57. Ribeiro, A.R., et al., *Natural fortification of trout with dietary macroalgae and selenised-yeast increases the nutritional contribution in iodine and selenium*. Food Research International, 2017. **99**: p. 1103-1109.
58. Ribeiro, A.R., et al., *Dietary macroalgae is a natural and effective tool to fortify gilthead seabream fillets with iodine: Effects on growth, sensory quality and nutritional value*. Aquaculture, 2015. **437**: p. 51-59.
59. Valente, L.M., et al., *Iodine enrichment of rainbow trout flesh by dietary supplementation with the red seaweed *Gracilaria vermiculophylla**. Aquaculture, 2015. **446**: p. 132-139.
60. Altintzoglou, T., et al., *Association of health involvement and attitudes towards eating fish on farmed and wild fish consumption in Belgium, Norway and Spain*. Aquaculture International, 2011. **19**(3): p. 475-488.
61. Claret, A., et al., *Consumer beliefs regarding farmed versus wild fish*. Appetite, 2014. **79**: p. 25-31.
62. Verbeke, W., et al., *Consumer perception versus scientific evidence of farmed and wild fish: exploratory insights from Belgium*. Aquaculture International, 2007. **15**(2): p. 121-136.
63. Fallah, A.A., et al., *Comparative study of heavy metal and trace element accumulation in edible tissues of farmed and wild rainbow trout (*Oncorhynchus mykiss*) using ICP-OES technique*. Microchemical Journal, 2011. **98**(2): p. 275-279.
64. Goldberg, R. and R. Naylor, *Future seascapes, fishing, and fish farming*. Frontiers in Ecology and the Environment, 2005. **3**(1): p. 21-28.
65. Commission, E., *The EU fish market*. 2018.

66. Seves, S.M., et al., *Are more environmentally sustainable diets with less meat and dairy nutritionally adequate?* Public Health Nutrition, 2017. **20**(11): p. 1-13.
67. Almeida, C., V. Karadzic, and S. Vaz, *The seafood market in Portugal: Driving forces and consequences.* Marine Policy, 2015. **61**: p. 87-94.
68. Joint, F. and W.E.C.o.F. Additives, *Safety evaluation of certain food additives and contaminants.* 2002.
69. Cardoso, C., et al., *Methylmercury risks and EPA+ DHA benefits associated with seafood consumption in Europe.* Risk Analysis: An International Journal, 2010. **30**(5): p. 827-840.
70. Matsumura, Y., *Nutrition trends in Japan.* Asia Pacific journal of clinical nutrition, 2001. **10**: p. S40-S47.
71. Verkaik-Kloosterman, J., P. van't Veer, and M.C. Ocké, *Simulation model accurately estimates total dietary iodine intake.* The Journal of nutrition, 2009. **139**(7): p. 1419-1425.
72. EFSA Panel on Dietetic Products, N. and Allergies, *Scientific opinion on dietary reference values for iodine.* EFSA Journal, 2014. **12**(5): p. 3660.
73. COMMISSION, E., *COMMISSION RECOMMENDATION (EU) 2018/464 of 19 March 2018 on the monitoring of metals and iodine in seaweed, halophytes and products based on seaweed.* 2018.
74. Vejbjerg, P., et al., *Estimation of iodine intake from various urinary iodine measurements in population studies.* Thyroid, 2009. **19**(11): p. 1281-1286.
75. Magalhães, V., et al., *Characterizing energy intake misreporting and its effects on intake estimations, in the Portuguese adult population.* Public Health Nutrition, 2019: p. 1-10.
76. van Rossum, C.T., et al., *Dutch National Food Consumption Survey 2007-2010: Diet of children and adults aged 7 to 69 years.* 2011.

9. Supplements

Supplement 1 – Task 2.1 ‘Fish pate and smoked salmon with reduced sodium level’

Supplement 2 – Task 2.2 ‘The effects of the consumption of the innovative foods for youth, pregnant women and seniors’

Supplement 1 – Task 2.1 ‘Fish pate and smoked salmon with reduced sodium level’

In task 2.1 of the SEAFOOD^{TOMORROW} consortium two seafood products with a sodium reduction of approximately 25% without compromising safety or quality were developed. The two products consisted of fish pate and smoked salmon. Common table salt (sodium chloride) was replaced by Saltwell for fish pate. Three different samples were measured for the fish pate: Sample A (100% NaCl), Sample B (60% NaCl, 40% Saltwell) and Sample C (20% NaCl, 80% Saltwell). Saltwell is a natural salt originating from the Chilean desert and contains a mixture of sodium chloride and potassium chloride. For smoked salmon, partial substitution of sodium chloride by potassium chloride food grade was applied to reduce sodium content (combined with four smoking treatments (F1-F4) (cold smoking with wood (F1) or liquid (F3) and hot smoking with wood (F2) or liquid (F4) and three salt treatments (Control, T25 and T50)). In comparison to the control treatment a sodium reduction was obtained for several combinations of treatments: T25+F1, 22% reduction; T25+F2, 33% reduction, T50+F1, 50% reduction; T50+F2, 49% reduction; T50+F3, 37% reduction and T50+F4, 36% reduction.

Fish pate was not consumed in the Netherlands nor in Portugal. A comparable food (meat pate) was used to estimate the effect of the replacement of fish pate consumption on daily sodium intake. 10% of the consumption of meat pate was replaced by fish pate. Sodium concentration of fish pate, sample C (20% NaCl, 80% Saltwell), was used because it had the highest sodium reduction. 10% of the consumption of smoked salmon in the Netherlands and Portugal was replaced by smoked salmon with reduced sodium concentrations to estimate the effect of sodium reduction on the daily intake. A set of combinations of treatments were used as comparison (F1+T25, F2+T25, F1+T50 and F2+T50)

The daily average sodium intake was 2418 mg and 2962 mg for the Dutch and Portuguese, respectively. Table 1 and 2 show the daily consumption of fish pate and smoked salmon. Table 3 and 4 display the corresponding sodium intake for the Netherlands and Portugal. The average consumption of meat pate (as a proxy for fish pate) was 2.47 g per day and among consumers only (n=620) 20 g per day in the Netherlands (table 1). The consumption of meat pate was on average 0.002 g per day in Portugal and among consumers only (n=2) 6 g per day (table 2). Fish pate with reduced sodium concentrations contains 400 mg sodium per 100 g (control fish pate: 510 mg sodium) and meat pate contains 779 mg sodium per 100 g. Sodium intake derived from meat pate was 0.8% of total sodium intake in the Netherlands. Compared with the reference of meat pate consumption, replacement with fish pate reduced the daily sodium intake with 0.4% on average in the Dutch population.

Consumption of smoked salmon was 1.32 g per day and among consumers only (n=160) 27 g per day in the Netherlands (table 1). Smoked salmon consumption in Portugal was 0.02 g per day and among consumers only (n=11) 8 g per day (table 2). Smoked salmon treated with F1+T25 and F2+T25 contained 790 and 730 mg sodium per 100 g, respectively. Smoked salmon treated with F1+T50, and F2+T50 contained 500 mg and 550 mg sodium. Regular smoked salmon contained 1178 mg sodium per 100 g (control smoked salmon contained approximately 1000 mg per 100 g). The consumption of smoked salmon contributed with 0.06% to daily sodium intake in the Netherlands. Replacement with the innovated smoked salmon, treated with different methods, contributed less, ranging from 0.03% to 0.04% to daily sodium intake. The consumption of fish pate and smoked salmon in Portugal was too low. The consumption of both foods, fish pate and smoked salmon, contributed less than 0.0001% to daily sodium intake when foods were replaced.

Conclusion: fish pate and smoked salmon were developed with reduced sodium content. In this analysis the aim was to estimate the reduction in daily sodium intake for the Dutch and Portuguese population when

comparable food was replaced by fish pate and smoked salmon with reduced sodium content. Both, fish pate and smoked salmon, were consumed in small amounts in both populations. Due to the relatively low consumption of fish pate and smoked salmon in the Netherlands and Portugal, we did not find significant decreases in daily sodium intake for both populations.

Table 1. The consumption of pate and smoked salmon in grams per day for the Dutch populations and consumers only.

	N	Mean	Std Dev	5th Pctl	25th Pctl	50th Pctl	75th Pctl	95th Pctl
Pate (g/d)	4313	2.47	8.27	0.00	0.00	0.00	0.00	19.73
Smoked salmon (g/d)	431	1.32	8.00	0.00	0.00	0.00	0.00	0.00
Consumers only								
Pate (g/d)	620	19.76	13.35	5.00	9.73	15.30	25.00	48.48
Smoked salmon (g/d)	160	26.79	28.64	2.13	8.46	20.00	38.50	77.00

Table 2. The consumption of pate and smoked salmon in grams per day for the Portuguese population and consumers only.

	N	Mean	Std Dev	5th Pctl	25th Pctl	50th Pctl	75th Pctl	95th Pctl
Pate (g/d)	5811	0,002	0.14	0.00	0.00	0.00	0.00	0.00
Smoked salmon (g/d)	5811	0,02	0,51	0,00	0,00	0,00	0,00	0,00
Consumers only								
Pate (g/d)	2	5.95	3.55	5.28	5.28	5.28	5.28	12.50
Smoked salmon (g/d)	11	8.01	3.65	5.05	5.05	10.09	10.09	10.09

Table 3. Daily average sodium intake for the Dutch population consuming fish pate and smoked salmon with reduced sodium levels.

Sodium per 100 g	Sodium per 100g	Sodium/SFT food total Dutch population (% of daily intake)	Sodium/regular food total Dutch population (% of daily intake)
Fish pate Sample C(400 mg)	Meat pate (779 mg)	0.99 (0.04%)	1.92 (0.08%)
Smoked salmon Sample F1+T25(790 mg)	Smoked salmon (1178 mg)	1.04 (0.04%)	1.55 (0.06%)
Smoked salmon Sample F2+T25(730 mg)	Smoked salmon (1178 mg)	0.96 (0.04%)	1.55 (0.6%)
Smoked salmon Sample F1+T50(500 mg)	Smoked salmon (1178 mg)	0.66 (0.03%)	1.55 (0.6%)
Smoked salmon Sample F2+T50(550 mg)	Smoked salmon (1178 mg)	0.73 (0.03%)	1.55 (0.6%)

Table 4. Daily average sodium intake for the Portuguese population consuming fish pate and smoked salmon with reduced sodium levels.

Sodium per 100 g	Sodium per 100g	Sodium/SFT food total Portuguese population (% of daily intake)	Sodium/regular food total Portuguese population (% of daily intake)
Fish pate Sample C(400 mg)	Meat pate (779 mg)	0.00 (0.000%)	0.00 (0.000%)
Smoked salmon Sample F1+T25(790 mg)	Smoked salmon (1178 mg)	0.02 (0.0005%)	0.02 (0.0008%)
Smoked salmon Sample F2+T25(730 mg)	Smoked salmon (1178 mg)	0.01 (0.0005%)	0.02 (0.0008%)
Smoked salmon Sample F1+T50(500 mg)	Smoked salmon (1178 mg)	0.01 (0.0003%)	0.02 (0.0008%)
Smoked salmon Sample F2+T50(550 mg)	Smoked salmon (1178 mg)	0.01 (0.0004%)	0.02 (0.0008%)

Supplement 2 – Task 2.2 ‘The effects of the consumption of the innovative foods for youth, pregnant women and seniors’

In task 2.2. of the SEAFOOD^{TOMORROW} consortium six recipes containing fish were developed for three specific target populations: youth, pregnant women and seniors. The recipes included sustainable overlook species, to promote their consumption and open the market to fish species other than the usual high in-demand species, which are often overfished. Recipes for the youth included fish sausage with vegetables and fish balls with puree and recipes for pregnant women included fish roulade and fish fillet with wheat salad. The recipes for seniors included mussel soup and fish balls with vegetables and sauce. Recipes were optimized to reach nutritional targets. Recipes for youth focused on omega 3 and vitamin D, recipes for pregnant women on omega 3, vitamin D and iodine and recipes for seniors on vitamin D, B12, low salt and high protein.

In order to estimate the effect of the consumption of the new developed recipes (dishes), consumed foods in the Dutch and Portuguese consumption data were replaced by foods included in the recipes for the three specific target groups. Youth consisted of children aged ≤ 10 years and seniors were aged ≥ 60 years. Pregnant women were included in the Portuguese data. As a proxy for pregnant women, Dutch women aged 20 to 40 years were used due to lack of pregnant women in the survey.

Food consumption data did not include composite dishes such as soups or meals. Therefore, singular foods were replaced. The average amount of the consumption of soup per day was derived from the previous Dutch food consumption survey 2007-2010 [76] for children (9 to 18 years) and adults (19 to 69 years) as the latest food survey of 2012-2016 did not include composite dishes such as soup. Soup consumption per day among the Portuguese was obtained from National Food, Nutrition and Physical Activity Survey, IAN-AF 2015–2016 [49]. The consumption of meat balls was replaced by fish balls, meat sausage by fish sausage, schnitzel by fish filet and soup by mussel soup. For all foods it was hypothesized that 10% of the consumption of regular foods was replaced by the food included in the new developed recipes.

Concentration data of baseline foods and the new recipes can be found in table 1. Chemical analysis was conducted to measure vitamin B12 in the new developed dishes. Fish balls for youth contained 0.72 μg vitamin B12, fish sausage for youth 1.52 μg vitamin B12, fish roulade 1.47 μg vit B12 and fish fillet 0.46 μg vitamin B12, fish soup for seniors contained 1.90 μg vitamin B12 and fish balls for seniors contained 1.24 μg vitamin B12. Measured vitamin B12 concentrations were lower in all dishes compared to foods derived from the Dutch Food Composition table [44], except for fish sausage. Chemical analyses of the other nutritional components were not available. Concentrations of baseline foods were derived from the Dutch Food Composition Table [44] as the average of all comparable foods that were consumed in the Netherlands or in Portugal. For soup, the average of all soups available in the composition table was used. For concentrations for the new recipes (except for vitamin B because it was measured) there were hypothesized beneficial changes (+10% increased content per 100 gram for vitamin D, iodine, omega 3 fatty acids (EPA+DHA) and protein, while a 10% decrease in sodium per 100 gram (Table 1). Concentration data for mussel soup was modelled with 23% mussels and 73% (average) soup, as provided by NEVO recipes [44].

Differences in estimated nutrient intake derived from the developed foods were presented as percentage (%) of the daily intake compared with the reference. Daily intake of nutrients were derived from several sources: vitamin B12, vitamin D, protein and salt content were calculated for the specified target population, using the Dutch food consumption survey and the Dutch Food Composition Table [44]. Daily mean iodine and omega 3 fatty acids (EPA+DHA) intake for the Netherlands and Portugal were calculated based on data of the main report. Portuguese IAN report [49] provided data on daily intake of vitamin B12, vitamin D,

protein and sodium for youth ($\leq 10y$), adolescent women (as a proxy for pregnant women) and elderly (age not specified).

Table 1. Vitamin B12, vitamin D, iodine, omega 3, protein and sodium concentrations per 100 gram of foods for the Netherlands and Portugal, and concentrations of the six recipes.

	Vitamin B12 (μg)	Vitamin D (μg)	Iodine (μg)	Omega 3 (g)	Protein (g)	Sodium (mg)
Concentrations per foods for the Netherlands						
Meat balls	3.95	1.35	21.64	0.51	24.58	376.36
Meat sausage	1.10	0.77	2.56	0.03	17.29	626.19
Meat roulade	-	-	-	-	-	-
Schnitzel	0.46	0.30	1.48	0.03	20.73	394.19
Soup	0.06	0.09	1.02	0.002	2.15	300.10
Concentrations per foods for Portugal						
Meat balls	1.71	0.66	3.20	0.10	18.42	340.58
Meat sausage	1.39	0.71	3.62	0.13	16.95	1032.00
Meat roulade	-	-	-	-	-	-
Schnitzel	0.46	0.41	2.25	0.06	20.06	153.88
Soup	0.06	0.09	1.02	0.002	2.15	300.10
Concentrations of new recipes						
Fish balls for youth	0.72 ^a	1.49	23.80	0.56	27.04	338.73
Fish sausage	1.52 ^a	0.85	2.82	0.04	19.02	563.57
Roulade fish	1.47 ^a	-	-	-	-	-
Fish fillet	0.50 ^a	0.33	1.62	0.03	22.80	354.77
Fish Soup	1.90 ^a	0.06	29.42	0.15	5.53	308.08
Fish balls for seniors	1.24 ^a	1.49	23.80	0.56	27.04	338.73
^a Analyzed concentrations						

The consumption of foods for the target population (youth, pregnant women and seniors), as well as the consumption for consumers only, is presented in table 2 and 3 for the Dutch population and in table 4 and 5 for the Portuguese population.

Table 2. The consumption of meat balls, sausage, roulade and soup in grams per day for Dutch youth aged ≤ 10 years, women 20 to 40 years and seniors aged ≥ 60 years.

	N	Mean	Std Dev	5th Pctl	25th Pctl	50th Pctl	75th Pctl	95th Pctl
Meat balls (youth)	1390	0.05	0.58	0.00	0.00	0.00	0.00	0.00
Meat sausage (youth)	1390	3.24	7.22	0.00	0.00	0.00	0.00	28.00
Roulade (pregnant)	333	-	-	-	-	-	-	-
Schnitzel (pregnant)	333	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Soup (seniors ^a)	2106	63.80	-	-	-	-	-	-
Meat balls (seniors)	814	0.42	4.18	0.00	0.00	0.00	0.00	0.00

^a derived from Dutch food consumption survey 2007 – 2010 for adults aged 19 to 69 years.

Table 3. The consumption of meat balls, sausage, roulade and soup in grams per day for consumers only, for Dutch youth aged ≤10 years, women 20 to 40 years and seniors aged ≥ 60 years.

	N	Mean	Std Dev	5th Pctl	25th Pctl	50th Pctl	75th Pctl	95th Pctl
Meat balls (youth)	5	15.44	3.19	9.08	11.36	16.20	16.20	23.20
Meat sausage (youth)	135	34.16	12.71	7.48	18.75	28.00	49.00	75.00
Roulade (pregnant)	-	-	-	-	-	-	-	-
Schnitzel (pregnant)	-	-	-	-	-	-	-	-
Soup (seniors)	-	-	-	-	-	-	-	-
Meat balls (seniors)	8	29.43	18.76	12.50	15.20	30.83	46.00	56.00

Table 4. The consumption of meat balls, sausage, roulade and soup in grams per day for Portuguese youth aged ≤ 10, pregnant women and seniors aged ≥ 60 years.

	N	Mean	Std Dev	5th Pctl	25th Pctl	50th Pctl	75th Pctl	95th Pctl
Meat balls (youth)	1403	1.13	5.18	0.00	0.00	0.00	0.00	0.00
Meat sausage (youth)	1403	0.97	4.55	0.00	0.00	0.00	0.00	0.00
Roulade (pregnant)	-	-	-	-	-	-	-	-
Schnitzel (pregnant)	96	1.70	13.22	0.00	0.00	0.00	0.00	7.83
Soup (seniors ^a)	-	199	-	-	-	-	-	-
Meat balls (seniors)	1034	0.14	4.06	0.00	0.00	0.00	0.00	0.00

^a derived from the Portuguese food consumption survey 2016.

Table 5. The consumption of meat balls, sausage, roulade and soup in grams per day for consumers only, for Portuguese youth ≤ 10 years, pregnant women and seniors aged ≥ 60 years.

	N	Mean	Std Dev	5th Pctl	25th Pctl	50th Pctl	75th Pctl	95th Pctl
Meat balls (youth)	40	32.57	12.64	12.04	20.87	36.13	36.13	41.75
Meat sausage (youth)	41	23.04	15.80	2.091	12.50	21.45	25.00	60.52
Roulade (pregnant)	-	-	-	-	-	-	-	-
Schnitzel (pregnant)	4	30.76	40.54	7.835	7.835	47.98	47.98	47.98
Soup (seniors)	-	-	-	-	-	-	-	-
Meat balls (seniors)	4	46.59	11.13	48.17	48.17	48.17	48.19	48.17

Table 6 shows the daily intake mean of nutrients for the Dutch population as well as the change in intake (in percentage) for the specified target population, e.g. youth, (pregnant) women and seniors, due to the replacement of 10% of the consumption of the developed foods. Results of the target nutrients, as proposed in task 2.2, will be discussed. The two recipes for fish balls and fish sausage, that were developed for youth (≤ 10 y) aimed at increasing daily vitamin D and omega 3 fatty acids intake. The mean intake was 2.43 μg vitamin D and 76 mg omega 3 for Dutch youth. Daily mean consumption of meat balls and sausage among Dutch youth was 0.05 g and 3.24 g, respectively (see table 2). The replacement of the consumption of 10% fish balls or fish sausage did not increase the average daily intake of vitamin D or omega 3 fatty acids. Recipes for (pregnant) women contained fish roulade and fish fillet, aiming to increase vitamin D, omega 3 fatty acids and iodine intake. Roulade or schnitzel was not consumed, therefore estimates are not available. Fish soups and fish balls for adults were developed aiming to increase vitamin B12, vitamin D and protein intake and reduce sodium intake for seniors. Daily mean intake of vitamin B12 and vitamin D in adults were 5.09 μg and 3.7 μg , respectively. The average daily protein intake was approximately 81 g and mean sodium intake was almost 2400 mg. It was assumed that seniors consumed 64 g soup and 0.42 g fish balls daily. The hypothetical replacement of soup consumed in the reference situation with 10% fish soup for the senior population increased the mean intake of vitamin B12 with 2% and iodine intake with 1%. The replacement of fish balls did not change the daily intake of nutrients among seniors.

Table 6. Daily intake of nutrients and corresponding change in intake in percentage (%) due to replacement of foods for Dutch youth, pregnant women and seniors.

	Youth (n=1390)	Fish balls (%)	Fish sausage (%)	Pregnant women (n=333)	Fish roulade (%)	Fish fillet (%)	Seniors (n=814)	Fish soup (%)	Fish balls (%)
Vitamin B12	2.85 μg	-0.0061	0.0478	3.82 μg	-	-	5.09 μg	2.3017	-0.0222
Vitamin D	2.43 μg	0.0003	0.0103	2.55 μg	-	-	3.7 μg	-0.0412	0.0015
Iodine	144 μg	0.0001	0.0006	158 μg	-	-	170 μg	1.0661	0.0005
Omega 3	76 mg	0.0000	0.0000	195 g	-	-	261 mg	0.0036	0.0000
Protein	51.2 g	0.0003	0.0110	72.56 g	-	-	80.71 g	0.2668	0.0013
Sodium	1606 mg	-0.0001	-0.0126	2233 mg	-	-	2396 mg	0.0213	-0.0007

Table 7 shows the daily mean intake of nutrients for the Portuguese population as well as the change in intake (in percentage) for the specified target populations, e.g. youth, (pregnant) women and seniors. The average daily mean intake of vitamin D and omega 3 fatty acids for Portuguese youth (≤ 10 y) was 22 mg and 276 mg, respectively. Median vitamin D intake was 4.9 mg per day. Portuguese youth consumed on average daily 1.13 g meat balls and 0.97 g meat sausage (see table 4). No changes were observed for vitamin D or omega 3 fatty acid intake due to the replacement of meat balls and sausage with fish balls or fish sausage. Daily mean intake of vitamin D, omega 3 fatty acids and iodine was 4.4 μg , 321 mg and 124 μg for Portuguese women. Roulade was not consumed by Portuguese pregnant women. Schnitzel consumption was 1.70 g per day. The replacement of 10% schnitzel consumption with fish fillet did not increase daily vitamin D, omega 3 or iodine intake. Recipes for seniors were focused on increased vitamin B12, vitamin D, protein intake and decreased sodium intake. Mean intake for seniors was 4 μg vitamin B12, 5.5 μg vitamin D, 77 g protein and 2778 mg sodium. The consumption of meat balls was on average 0.14 g for seniors. Replacement of meat balls with fish balls did not influence daily intakes. It was assumed that the daily consumption of soup was 199 g among seniors. The replacement of 10% soup by mussel soup increased vitamin B12 intake with 9%. Moreover, iodine intake increased with 4% and protein intake with almost 1% in Portuguese seniors.

Table 7. Daily intake of nutrients and corresponding change in intake in percentage (%) due to replacement of foods for Portuguese youth, pregnant women and seniors.

	Youth	Fish balls (%)	Fish sausage (%)	Pregnant women	Fish roulade (%)	Fish fillet (%)	Seniors	Fish soup (%)	Fish balls (%)
Vitamin B12	3.6 µg	-0.0310	0.0035	4.4 µg	-	0.0015	4 µg	9.1358	-0.0016
Vitamin D	22 µg	0.0003	0.0003	5.7 µg	-	0.0012	5.5 µg	-0.0864	0.0002
Iodine	142 µg	0.0003	0.0002	124 µg	-	0.0003	139 µg	4.0668	0.0000
Omega 3	276 mg	0.0000	0.0000	321 mg	-	0.0000	461 µg	0.0064	0.0000
Protein	68 g	0.0031	0.0024	79.7 g	-	0.0043	76.6 g	0.8767	0.0003
Sodium	2151 mg	-0.0018	-0.0047	2679 mg	-	-0.0010	2778 mg	0.0572	-0.0002

Conclusion: In this study we hypothetically replaced 10% of the consumption of regular food with new developed SEAFOOD^{TOMORROW} recipes, including fish, for youth, pregnant women and seniors. Due to the low consumption of the foods at reference, combined with the lower vitamin B12 concentration in the new developed foods, we did not find any large differences when regular foods were replaced by the new foods for vitamin B12. We did find an increase in vitamin B and iodine intake in Portuguese seniors due to the consumption of mussel soup. Therefore, the consumption of soups by Portuguese seniors may serve as a method to increase this nutrient intake among seniors. For other nutrients, taking a hypothetical 10% increased concentrations of vitamin D, iodine, omega 3 and protein, and decreased content of sodium in the new developed foods, we did not find major differences in average intake when regular foods were replaced by the new developed foods for the specified target populations.

10. Appendix

Appendix I – Table 1. Concentrations of nutrients and heavy metals ($\mu\text{g}/\text{kg}$) in non-IMTA and IMTA salmon (task 1.2).

Appendix II – Table 1. Numbers of Portuguese foods (total $N=1580$) with concentration data of the WHO, EFSA or NEVO.

Appendix III – Table 1. Overview of the European Food Safety Authority (EFSA reports) used per heavy metal for the concentration data.

Appendix IV - Table 1. Amount of foods with concentration data derived from NVWA or EFSA for the Dutch population. Total amount of foods in the Dutch consumption data is 925 (based on FoodEx2 codes with only the base term without the addition of any facets).

Table 2. Amount of foods with concentration data derived from ASAE/DGAV/INSA or EFSA for the Portuguese population. Total amount of foods in the Dutch consumption data is 828 (based on FoodEx2 codes with only the base term without the addition of any facets).

Table 3. Overview products that were not linked to any concentration data due to missing data. The Dutch consumption data contains a total of 255.132 records and the Portuguese consumption data contains a total of 411.300 records.

Table 4. Comparison between new and old FoodEx classification categories used.

Table 5. Overview on how products were linked to total arsenic concentration data. Products that are listed under a subcategory were linked to a subcategory with corresponding concentration data reported in the EFSA report 'Scientific Opinion on Arsenic in Food' (2009).

Appendix V - Table 1. Example of translation table used to link products to corresponding Hierarchy codes and FoodEx1 codes. The FoodEx1 codes were used to link the products further to FoodEx2 codes. The table only shows the hierarchy codes for products reported as FoodEx level 1.

Appendix VI - Table 1. EFSA recommended daily intakes for EPA, DHA, iodine and selenium for different age groups groups.

Table 2. Dietary HBGVs and BMDLs: Heavy metals. Tolerable daily intake (TDI), Tolerable weekly intake (TWI) or BenchMark Dose Level (BMDL)

Appendix VII – Table 1. Mean exposure ($\mu\text{g}/\text{kg}$ b.w/day) to methylmercury for the total Dutch and Portuguese population.

Appendix I

Table 1. Concentrations of nutrients and heavy metals (µg/kg) in non-IMTA and IMTA salmon (task 1.2).

Nutrients	Non-IMTA salmon ^a	IMTA salmon ^b
Iodine (µg/kg)(WW)	N/A ^c	N/A ^c
EPA (g/kg) (WW)	N/A ^c	N/A ^c
DHA (g/kg)(WW)	N/A ^c	N/A ^c
Selenium (µg/kg)(WW)	20.00	16.70
Heavy metals		
Cadmium (µg/kg) (WW)	<LOQ (0.60)	<LOQ (0.60)
Total mercury (µg/kg) (WW)	<LOQ (0.90)	<LOQ (0.90)
Methylmercury (µg/kg) (WW)	N/A ^c	N/A ^c
Total arsenic (µg/kg) (WW)	10666.70	100000.00
Inorganic arsenic (µg/kg) (WW)	N/A ^c	N/A ^c
Lead (µg/kg) (WW)	<LOQ (0.12)	<LOQ (0.12)

^a Mean concentrations of non-IMTA salmon1 (task 1.2) from SeafoodTomorrow google spreadsheet

^b Mean concentrations of IMTA salmon1 (task 1.2) from SeafoodTomorrow google spreadsheet

^c Not Available

Appendix II

Table 1. Numbers of Portuguese foods (total N=1580) with concentration data of the WHO, EFSA or NEVO.

Selenium, EPA and DHA (N)	Iodine (N)	Source
0	213	WHO ^a , EFSA report ^b
227	227	Directly linked to NEVO ^c
866	703	Linked via foodex2 hierarchy level 6 ^c
41	29	Linked via foodex2 hierarchy level 5 ^c
137	112	Linked via foodex2 hierarchy level 4 ^c
172	162	Linked via foodex2 hierarchy level 3 ^c
75	75	Linked via foodex2 hierarchy level 2 ^c
48	45	Linked via foodex2 hierarchy level 1 ^c
14	14	None ^d
a[51] b[72] c[44] d Concentrations were assumed to be zero.		

Appendix III

Table 1. Overview of the European Food Safety Authority (EFSA reports) used per heavy metal for the concentration data.

Heavy metal	Source
Lead	EFSA Journal 2012;10(7):2831
Cadmium	EFSA Journal 2012;10(1):2551
Total Mercury	EFSA Journal 2012;10(12):2985
Methylmercury	EFSA Journal 2012;10(12):2985, EFSA Journal 2015;13(1):3982
Inorganic Arsenic	EFSA Journal 2014;12(3):3597
Total Arsenic	EFSA Journal 2009; 7(10):1351

Appendix IV

Table 1. Amount of foods with concentration data derived from NVWA or EFSA for the Dutch population. Total amount of foods in the Dutch consumption data is 925 (based on FoodEx2 codes with only the base term without the addition of any facets).

Substance	Number of foods (N)	Total of foods (N)	Source
Cadmium	40	920	NVWA ^c
	919		EFSA report: 'Cadmium dietary exposure in the European population' (2012)
Lead	40	920	NVWA
	921		EFSA report: 'Lead dietary exposure in the European population' (2012)
Total Arsenic	25	918	NVWA
	908 ^b		EFSA report: 'Scientific Opinion on Arsenic in Food' (2009)
Inorganic arsenic	6	917	NVWA
	916		EFSA report: 'Dietary exposure to inorganic arsenic in the European population' (2014)
Total Mercury	39	920	NVWA
	918		EFSA report: 'Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food' (2012)
Methylmercury ^a	7	48	NVWA
	46		EFSA report: 'Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food' (2012) + 'Statement on the benefits of fish/seafood consumption compared to the risks of methylmercury in fish/seafood' (2015)

a Methylmercury is monitored only in fish + seafood

b 39 foods were calculated back from inorganic arsenic concentrations based on conversion factor of 1.43

c The Dutch Food Safety Authority monitors contaminants in food and feed

Table 2. Amount of foods with concentration data derived from ASAE/DGAV/INSA or EFSA for the Portuguese population. Total amount of foods in the Portuguese consumption data is 828 (based on FoodEx2 codes with only the base term without the addition of any facets).

Substance	Number of foods (N)	Total of foods (N)	Source
Cadmium	90	828	ASAE/DGAV/INSA ^d
	828		EFSA report: 'Cadmium dietary exposure in the European population' (2012)
Lead	0	828	ASAE/DGAV/INSA
	828		EFSA report: 'Lead dietary exposure in the European population' (2012)
Total Arsenic	0	810	ASAE/DGAV/INSA
	810 ^b		EFSA report: 'Scientific Opinion on Arsenic in Food' (2009)
	10 ^c		NVWA
Inorganic arsenic	0	825	ASAE/DGAV/INSA
	825		EFSA report: 'Dietary exposure to inorganic arsenic in the European population' (2014)
Total Mercury	57	825	ASAE/DGAV/INSA
	825		EFSA report: 'Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food' (2012)
Methylmercury ^a	0	75	ASAE/DGAV/INSA
	75		EFSA report: 'Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food' (2012) + 'Statement on the benefits of fish/seafood consumption compared to the risks of methylmercury in fish/seafood' (2015)

a Methylmercury is monitored only in fish + seafood

b 78 foods were calculated back from inorganic arsenic concentrations based on conversion factor of 1.43

c 10 foods were based on NVWA data as no concentration data from Portugal or EFSA was available

d ASAE/DGAV/INSA are Portuguese institutes that monitor contaminants in food and feed

Table 3. Overview products that were not linked to any concentration data due to missing data. The Dutch consumption data contains a total of 255.132 records and the Portuguese consumption data contains a total of 411.300 records.

Product Name	FoodEx2 code	Compound(s)	Number of records NL (N)	Number of records PT (N)
Fish roe	A02EM	Total Arsenic	0	12
Cod roe	A02EP	Total Arsenic	0	7
Limpet	A02GV	Total Arsenic	0	22
Snails	A02LK	Total Arsenic	0	52
Water snails, conches and whelks	A02GX	Total Arsenic	0	2
Dried mushrooms	A00TY	Cadmium, lead, inorganic arsenic, total arsenic and total mercury	1	0
Coffee imitate ingredients	A03GS	Inorganic arsenic, total arsenic	0	45
Minor imitate coffee ingredients	A03GX	Inorganic arsenic, total arsenic	1	0
Barley coffee ingredient	A03HA	Inorganic arsenic, total arsenic	0	705
Mixed coffee imitates	A03HD	Inorganic arsenic, total arsenic	3	605
Textured fungal proteins	A03TG	Inorganic arsenic, total arsenic	13	0
Canned/jarred vegetables	A0ETQ	Cadmium, lead, inorganic arsenic, total arsenic and total mercury	538	0
Candied or sugar preserved vegetables	A0ETS	Cadmium, lead, inorganic arsenic, total arsenic and total mercury	27	0
Marinated / pickled fish	A0F0P	Cadmium, lead, inorganic arsenic, total arsenic and total mercury	26	0
Marinated / pickled seafood	A0EZA	Cadmium, lead, inorganic arsenic, total arsenic and total mercury	1	0

Table 4. Comparison between new and old FoodEx classification categories used.

Main food category (FoodEx level 1)	Food category present in EFSA Scientific Opinion on Arsenic in Food (2009)
01. Grains and grain-based products	01. Cereal and cereal products
02. Vegetables and vegetable products (including fungi)	04. Vegetables, nuts and pulses
03. Starchy roots and tubers	05. Starchy roots and potatoes
04. Legumes, nuts and oilseeds	04. Vegetables, nuts and pulses
05. Fruit and fruit products	06. Fruits
06. Meat and meat products (including edible offal)	10. Meat, meat products and offal
07. Fish and other seafood (including amphibians, reptiles, snails and insects)	11. Fish and seafood
08. Milk and dairy products	13. Milk and dairy based products
09. Eggs and egg products	12. Eggs
10. Sugar and confectionary	02. Sugar and sugar products
11. Animal and vegetable fats and oils	03. Fats (animal and vegetable)
12. Fruit and vegetable juices	07. Juices, soft drinks and bottled water
13. Non-alcoholic beverages (excepting milk based beverages)	08. Coffee, tea and cocoa
14. Alcoholic beverages	09. Alcoholic beverages
15. Drinking water (water without any additives except carbon dioxide; includes water ice for consumption)	15. Tap water + 07. Juices, soft drinks and bottled water
16. Herbs, spices and condiments	14. Miscellaneous products and products for special dietary use (only partially covered)
17. Food for infants and small children	14. Miscellaneous products and products for special dietary use (only partially covered)
18. Products for special nutritional use	Not included
19. Composite food (including frozen products)	Not included
20. Snacks, desserts, and other foods	Not included

Table 5. Overview on how products were linked to total arsenic concentration data. Products that are listed under a subcategory were linked to a subcategory with corresponding concentration data reported in the EFSA report ‘Scientific Opinion on Arsenic in Food’ (2009).

Main category	Subcategory (FoodEx2 level 2)	Subcategory present in EFSA Scientific Opinion on Arsenic in Food (2009)
01. Grains and grain-based products	Grains for human consumption	Cereal grains excluding rice, Rice grains and Bran and germ
	Grain milling products	Cereal products, excluding rice based products, Rice based products
	Bread and rolls	Cereal products, excluding rice based products
	Pasta (raw)	Cereal products, excluding rice based products
	Breakfast cereals	Cereal products, excluding rice based products, Rice based products
	Fine bakery wares	Cereal products, excluding rice based products, Rice based products
02. Vegetables and vegetable products (including fungi)	Root vegetables	Root vegetables
	Bulb vegetables	Vegetables, nuts, pulses (except soups)
	Fruiting vegetables	Vegetables, nuts, pulses (except soups)
	Brassica vegetables	Brassica vegetables
	Leaf vegetables	Leafy vegetables
	Legume vegetables	Vegetables, nuts, pulses (except soups)
	Stem vegetables (fresh)	Stem vegetables
	Sugar plants	Vegetables, nuts, pulses (except soups)
	Sea weeds	Algae as food
	Tea and herbs for infusions (solid)	Tea and other infusions (Powder or dry leaves)
	Cocoa beans and cocoa products	Cocoa (Powder or cocoa bean)
	Coffee beans and coffee products (solid)	Coffee (Powder)
	Coffee imitates (solid)	Not reported

	Vegetable products	Vegetable soups, Vegetables, nuts, pulses (except soups), Other vegetables and vegetable products
	Fungi, cultivated	Mushrooms
	Fungi, wild, edible	Mushrooms
	Dried mushrooms	Not reported
03. Starchy roots and tubers	Potatoes and potato products	Peeled potatoes
	Other starchy roots and tubers	Conversion factor of 1.43 used (inorganic arsenic) ^a
04. Legumes, nuts and oilseeds	Legumes, beans, green, without pods	Pulses (Legumes)
	Legumes, beans, dried	Pulses (Legumes)
	Tree nuts	Nuts
	Oilseeds	Oilseeds
	Other seeds	Oilseeds
05. Fruit and fruit products	Citrus fruits	Other fruits
	Pome fruits	Other fruits
	Stone fruits	Other fruits
	Berries and small fruits	Berries and small fruits
	Oilfruits	Other fruits
	Miscellaneous fruits	Other fruits
	Dried fruits	Dried fruits
	Jam, marmalade and other fruit spreads	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Other fruit products (excluding beverages)	Other fruits
06. Meat and meat products (including edible offal)	Livestock meat	Bovine, sheep and, goat and pig meat
	Poultry	Poultry meat
	Game mammals	Game meat
	Game birds	Game meat
	Mixed meat	Other meat
	Edible offal, farmed animals	Edible offal and offal products
	Preserved meat	Meat based preparations
	Sausages	Processed meat products

	Meat specialties	Meat based preparations
	Pastes, pâtés and terrines	Processed meat products
	Meat imitates	Meat based preparations
07. Fish and other seafood (including amphibians, reptiles, snails and insects)	Fish meat	Fish and fish products
	Fish products	Fish and fish products
	Fish offal	Not included in calculations
	Crustaceans	Crustaceans
	Water molluscs	Bivalve molluscs
	Amphibians, reptiles, snails, insects	Not reported
08. Milk and dairy products	Liquid milk	Milk and dairy drinks
	Milk based beverages	Milk and dairy drinks
	Concentrated milk	Milk and dairy drinks
	Whey and whey products	Dairy based products
	Cream and cream products	Dairy based products
	Fermented milk products	Dairy based products
	Milk derivatives	Milk and dairy drinks
	Cheese	Cheese
	Milk and milk product imitates	Milk and dairy drinks
09. Eggs and egg products	Eggs, fresh	Total for Eggs
	Eggs, powder	Total for Eggs
10. Sugar and confectionary	Sugars	Other sugar and sugar products
	Sugar substitutes	Other sugar and sugar products
	Chocolate (cocoa) products	Chocolate and chocolate based products
	Confectionery (non-chocolate)	Other sugar and sugar products
	Dessert sauces	Chocolate and chocolate based products
	Molasses and other syrups	Other sugar and sugar products
	Honey	Other sugar and sugar products
11. Animal and vegetable fats and oils	Animal fat	Animal fats and oils
	Fish oil	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Vegetable fat	Vegetable fats and oils
	Vegetable oil	Conversion factor of 1.43 used (inorganic arsenic) ^a

	Fats of mixed origin	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Margarine and similar products	Butter
12. Fruit and vegetable juices	Fruit juice	Fruit juices
	Concentrated fruit juice	Fruit juices
	Fruit nectar	Fruit juices
	Mixed fruit juice	Fruit juices
	Dehydrated/powdered fruit juice	Fruit juices
	Vegetable juice	Vegetables juices
	Mixed vegetable juice	Vegetables juices
	Mixed fruit and vegetable juice	Fruit and vegetable juices
13. Non-alcoholic beverages (excepting milk based beverages)	Soft drinks	Soft drinks
	Tea (infusion)	Coffee, tea, cocoa expressed as liquid
	Coffee (beverage)	Coffee, tea, cocoa expressed as liquid
	Coffee imitates beverage	Not reported
	Cocoa beverage	Coffee, tea, cocoa expressed as liquid
14. Alcoholic beverages	Beer and beer-like beverage	Beer and substitutes
	Wine	Wine and substitutes
	Fortified and liqueur wines	Other alcoholic beverages and substitutes
	Wine-like drinks	Wine and substitutes
	Liqueur	Other alcoholic beverages and substitutes
	Spirits	Other alcoholic beverages and substitutes
	Alcoholic mixed drinks	Other alcoholic beverages and substitutes
15. Drinking water (water without any additives except carbon dioxide; includes water ice for consumption)	Tap water	Total for Tap water
	Bottled water	Bottled water
	Water ice (for consumption)	Conversion factor of 1.43 used (inorganic arsenic) ^a
16. Herbs, spices and condiments	Herbs	Fresh herbs, Dry herbs
	Spices	Spices
	Herb and spice mixtures	Conversion factor of 1.43 used (inorganic arsenic) ^a

	Seasoning or extracts	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Condiment	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Dressing	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Chutney and pickles	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Savoury sauces	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Flavourings or essences	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Baking ingredients	Conversion factor of 1.43 used (inorganic arsenic) ^a
17. Food for infants and small children	Infant formulae, powder	Infant formulae and follow-on formulae
	Infant formulae, liquid	Infant formulae and follow-on formulae
	Follow-on formulae, powder	Infant formulae and follow-on formulae
	Follow-on formulae, liquid	Infant formulae and follow-on formulae
	Cereal-based food	Cereal based infant and follow-on formulae
	Ready-to-eat meal	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Yoghurt, cheese and milk-based dessert	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Fruit juice and herbal tea	Conversion factor of 1.43 used (inorganic arsenic) ^a
18. Products for special nutritional use	Food for weight reduction	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Dietary supplements	Conversion factor of 1.43 used (inorganic arsenic)
	Food for sports people	Conversion factor of 1.43 used (inorganic arsenic)
	Dietetic food for diabetics	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Medical food	Conversion factor of 1.43 used (inorganic arsenic) ^a
19. Composite food (including frozen products)	Cereal-based dishes	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Rice-based meals	Conversion factor of 1.43 used (inorganic arsenic) ^a

	Potato based dishes	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Beans-based meals	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Meat-based meals	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Fish and seafood based meals	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Vegetable-based meals	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Egg-based meal (e.g. omelette)	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Mushroom-based meals	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Ready to eat soups	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Prepared salads	Conversion factor of 1.43 used (inorganic arsenic) ^a
20. Snacks, desserts, and other foods	Snack food	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Ices and desserts	Conversion factor of 1.43 used (inorganic arsenic) ^a
	Other foods	Conversion factor of 1.43 used (inorganic arsenic) ^a
<p>a. Conversion factor based of EFSA report 'Dietary exposure to inorganic arsenic in the European population' [EFSA, 2014]. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2014.3597</p>		

Appendix V

Table 1. Example of translation table used to link products to corresponding Hierarchy codes and FoodEx1 codes. The FoodEx1 codes were used to link the products further to FoodEx2 codes. The table only shows the hierarchy codes for products reported as FoodEx level 1.

FoodEx 1 Code	Product name (reported as FoodEx level 1)	Hierarchy code
A.01.000001	Grains and grain-based products	A.01
A.01.000317	Vegetables and vegetable products (including fungi)	A.02
A.01.000467	Starchy roots and tubers	A.03
A.01.000486	Legumes, nuts and oilseeds	A.04
A.01.000544	Fruit and fruit products	A.05
A.01.000727	Meat and meat products (including edible offal)	A.06
A.01.000876	Fish and other seafood (including amphibians, reptiles, snails and insects)	A.07
A.01.000948	Milk and dairy products	A.08
A.01.001252	Eggs and egg products	A.09
A.01.001267	Sugar and confectionary	A.10
A.01.001346	Animal and vegetable fats and oils	A.11
A.01.001394	Fruit and vegetable juices	A.12
A.01.001470	Non-alcoholic beverages (excepting milk based beverages)	A.13
A.01.001534	Alcoholic beverages	A.14
A.01.001573	Drinking water (water without any additives except carbon dioxide; includes water ice for consumption)	A.15
A.01.001580	Herbs, spices and condiments	A.16
A.01.001715	Food for infants and small children	A.17
A.01.001748	Products for special nutritional use	A.18
A.01.001789	Composite food (including frozen products)	A.19
A.01.001877	Snacks, desserts, and other foods	A.20

Appendix VI

Table 1. EFSA recommended daily intakes for EPA, DHA, iodine and selenium for different age groups groups.

Nutrient	Age	Gender	Adequate Daily Intake	Upper level
(EPA, DHA)	7-11 months	Both genders	100 mg/day DHA	ND
	1 year	Both genders	100 mg/day DHA	ND
	2-3 years	Both genders	250 mg/day DHA +EPA	ND
	4-17 years	Both genders	250 mg/day DHA +EPA	ND
	≥ 18 years	Both genders	250 mg/day DHA +EPA	ND
	≥ 18 years	Female, pregnant	(+) 100–200 mg/day DHA	ND
	≥ 18 years	Female, lactating	(+) 100–200 mg/day DHA	ND
Iodine	7-11 months	Both genders	70 µg/day	ND
	1-3 years	Both genders	90 µg/day	200 µg/day
	4-6 years	Both genders	90 µg/day	250 µg/day
	7-10 years	Both genders	90 µg/day	300 µg/day
	11-14 years	Both genders	120 µg/day	450 µg/day
	15-17 years	Both genders	130 µg/day	500 µg/day
	≥ 18 years	Both genders	150 µg/day	600 µg/day
	≥ 18 years	Female, pregnant	200 µg/day	600 µg/day
	≥ 18 years	Female, lactating	200 µg/day	600 µg/day
Selenium	7 months -6 years	Both genders	15 µg/day	NA
	7-9 years	Both genders	20 µg/day	NA
	10-14 years	Both genders	35 µg/day	NA
	15-17 years	Both genders	70 µg/day	NA
	≥ 18 years	Both genders	70 µg/day	NA
	≥ 18 years	Female, pregnant	70 µg/day	NA
	≥ 18 years	Female, lactating	70 µg/day	NA

Table 2. Dietary HBGVs and BMDLs: Heavy metals. Tolerable daily intake (TDI), Tolerable weekly intake (TWI) or BenchMark Dose Level (BMDL).

Compound	Type	Value	Unit	Source
Cadmium	(P)TWI	2.5	µg/kg bw per week	EFSA, 2009
	(P)TMI	25	µg/kg bw per month	JECFA, 2013
Lead	BMDL01	0.5(c), 0.54(d) and 1.5(e)	µg/kg bw per day	EFSA, 2012
	BMDL10	0.63(f)	µg/kg bw per day	EFSA, 2012
Inorganic Arsenic	BMDL0.5 (a)	3	µg/kg bw per day	JECFA, 2011 EFSA, 2014
Total Arsenic	BMDL0.1 (b)	0.3-8	µg/kg bw per day	EFSA, 2014
Methyl Mercury	(P)TWI	1.6	µg/kg bw per week	JECFA, 2007
	(P)TWI	1.3	µg/kg bw per week	EFSA, 2015
Total Mercury	(P)TWI	4	µg/kg bw per week	JECFA, 2011 EFSA, 2015

a JECFA identified a benchmark dose lower confidence limit for a 0.5 % increased incidence of lung cancer (BMDL0.5) of 3.0 µg/kg b.w. per day (EFSA, 2014)

b The EFSA CONTAM panel reported a benchmark dose lower confidence limit for a 0.1 % increased incidence for risk of cancer of the lung, skin and bladder, as well as skin lesions between 0.3 and 8 µg/kg b.w. per day (EFSA, 2014)

c The BMDL01 for a 1% increase in incidence for neurotoxicity of 0.50 µg/kg bw per day is relevant for children up to and including 7 years of age (EFSA, 2012)

d The BMDL01 for a 1% increase in incidence for neurotoxicity of 0.54 µg/kg bw per day is relevant for the foetus via the lead intake by the mother (EFSA, 2012)

e The BMDL01 for a 1% increase in incidence for neurotoxicity of 1.5 µg/kg bw per day are relevant for men and women from 18 years of age (EFSA, 2012)

f The BMDL10 for a 10% increase in incidence for chronic kidney disease of 0.63 µg/kg bw per day are relevant for men and women from 18 years of age (EFSA, 2012)

Appendix VII

Table 1. Mean exposure ($\mu\text{g/kg b.w/day}$) to methylmercury for the Dutch and Portuguese population.

	Mean (LNN)	95% Confidence interval	50th Pctl	95% Confidence interval	95th Pctl	95% Confidence interval
Netherlands (total population)^a						
Baseline_control_fish	0.04	0.03-0.05	0.02	0.01-0.02	0.14	0.09-0.18
Fortified_fish	0.04	0.03-0.05	0.02	0.01-0.03	0.13	0.08-0.18
Portugal (total population)^a						
Baseline_control_fish	0.12	0.12-0.13	0.10	0.10-0.11	0.27	0.24-0.30
Fortified_fish	0.12	0.12-0.13	0.10	0.10-0.11	0.27	0.24-0.30
Portugal (children 1-9 yr old)^a						
Baseline_control_fish	0.24	0.22-0.26	0.23	0.20-0.25	0.44	0.36-0.55
^a MeHg concentrations were calculated back from the total Hg concentrations reported in task 1.1 based on %proportion (MeHG/Total Hg) reported in the EFSA report 'Scientific Opinion on the risk for public health related to the presence of Hg and MeHg in food' [60]. The %proportion (MeHG/Total Hg) are 93.0%, 82.0% and 86.8% for trout, gilthead seabream and common carp, respectively						