Superior Health Council

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Arsenic and other elements in algae and dietary supplements based on algae

In this scientific advisory report on public health policy, the Superior Health Council of Belgium provides a risk assessment of As, Cd, Pb, Hg in algae and marine products for the general population, but also for vegetarians and consumers of food supplements derived from algae.

It would like to provide to public health authorities and heavy consumers of algae (21 g of the products as sold - i.e. not hydrated before consumption), specific recommendations and attention to the toxic effect for their health (especially with seaweed salads, algae belonging to the Hijiki species - *Hisikia fusiforme)*. Pb and more particularly Cd consumption is far from being negligible in this context too.

Version validated by the Board on
1 April 2015[1]

SUMMARY

Algae and other marine products are known to generally contain high levels of Arsenic (As) species (organic and inorganic) as well as other contaminants such as Cadmium (Cd), Lead (Pb) and Mercury (Hg). Some algae products are sporadically consumed as such (e.g. seaweed salads) by the general population but it seems that some segments of the population, such as the vegetarians, are preferential consumers of algae (products). In addition, people with particular deficiencies (e.g. Iodine) or with other different needs (e.g. weight loss or detoxification) are quite regular consumers of a variety of food supplements (FS) derived from algae and, hence, potentially rich in As species.

In order to better evaluate the risks posed specifically for the regular consumers of such foodstuffs and FS, a risk assessment has been carried out. First of all, a literature study has been performed in order to gather all relevant information about the toxicity of the various As species (organic and inorganic) that can be found in marine products, in general, and in algae, more specifically. Then, contamination data were gathered from the Belgian food agency control program as well as from the general scientific literature. Due to a lack of formal consumption data, several intake scenarios were elaborated in order to assess the exposure by the concerned people and to perform the risk characterization, taking into account the oral background exposure of the general population and the additional exposure for some target groups such as vegetarians and FS consumers.

From this assessment, it appears that consumers of algae-based FS are not exposed to a substantial additional intake of the most toxic As species, iAs (inorganic As - i.e. arsenate, As^{V} and arsenite, As^{III}). “Normal” consumers of algae products (i.e. not more than 7 g/day) seem to be exposed to a relative low risk whilst the heavy consumers of algae, such as seaweed salads,

[1] The Council reserves the right to make minor typographical amendments to this document at any time. On the other hand, amendments that alter its content are automatically included in an erratum. In this case, a new version of the advisory report is issued.

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could be at risk when their daily consumption reaches levels as high as 21 g of the products as sold (i.e. not hydrated before consumption). Algae belonging to the Hijiki species (*Hisikia fusiforme)* generally contain larger amounts of iAs than other species; therefore, consumers of such algae are exposed to iAs levels that have a potentially high impact on their health. Additionally, the probable presence of other toxic elements, such as heavy metals, in algae can also have a negative impact on the consumers' health.

Keywords

Keywords	**Mesh terms***	**Sleutelwoorden**	**Mots clés**	**Stichworte**
Food legislation	Legislation, Food	Voedingswetgeving	Législation, nutrition	Gesetzgebung
Food supplement	Dietary supplement	Voedingssupplement	Complément alimentaire	Nahrungsergänzungsmittel
Food safety	Food Safety	Voedselveiligheid	Sécurité alimentaire	
Risk assessment	Risk assessment	Risicoanalyse	Evaluation des risques	Risikobewertung
Heavy metals	Heavy metals	Zware metalen	Métaux lourds	
Arsenic	Arsenic	Arseen	Arsénique	
Cadmium	Cadmium	Cadmium	Cadmium	
Lead	Lead	Lood	Plomb	
Mercury	Mercury	Kwik	Mercure	
Algae	Algae	Algen	Algues	

** MeSH (Medical Subject Headings) is the NLM controlled vocabulary thesaurus used for indexing articles for PubMed.*

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ABBREVIATIONS AND SYMBOLS

ADI	Acceptable daily intake
ALARA	As low as reasonably achievable
ARfD	Acute reference dose
AAS	Atomic absorption spectrometry
As	Arsenic
As^{III}	Arsenite
As^{V}	Arsenate
AsB	Arsenobetaine
AsC	Arsenocholine
As3MT	Arsenic-methyltransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BMDL	Benchmark dose lower confidence limit
Cd	Cadmium
CONTAM	EFSA Panel on Contaminants in the food chain
DG4	Directorate General Animal, Plant and Food
DM	Dry mass
DMA^{III}	Dimethylarsonous acid
DMA^{V}	Dimethylarsinic acid
$DMAA^{V}$	Dimethylarsinoyl acetic acid
$DMAE^{V}$	Dimethylarsinoylethanol
EFSA	European Food Safety Authority
FASFC	Federal Agency for the Safety of the Food Chain
FS	Food supplements
GSH	Glutathione
HBVG	Health Based Guidance Values
HG	Hydride generation
Hg	Mercury
HPLC-ICP-MS	High Performance Liquid Chromatography (for the separation of species), with Inductively Coupled Plasma Mass Spectrometry
IARC	International Agency for Research on Cancer
iAs	inorganic arsenic
ICP-OES	Inductively coupled plasma atomic emission spectrometry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Median lethal dose
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
LOQ	Limit of quantification
MA	Monomethylarsonic acid
MB	Middle bound
MMA^{III}	Methylarsonous acid
MMA^{V}	Methylarsonic acid
MRL	Minimal risk level
MoE	Margin of exposure
NAOEL	No observable adverse effect level
Pb	Lead
(P)TWI	(provisional) Tolerable weekly intake
SAM	S-adenosylmethionine
Se	Selenium
SHC	Superior Health Council

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tAs	total arsenic
TDI	Tolerable daily intake
TMAO	trimethylamine-N-oxide

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1. INTRODUCTION AND ISSUES

In July 2000, the Superior Health Council (SHC) issued an advisory report (SHC 6976) on the heavy metals Cd, Pb, As and Hg in dietary supplements. At the time, the SHC drew the following conclusion:

- The regular intake of dietary supplements with the high levels (15 mg/kg for Pb or 4.3 mg/kg for As) that were measured at the time could entail a genuine risk for consumers;
- Setting maximum levels would focus the manufacturers' attention on raw material selection and product monitoring;
- The use of standards based on ALARA (as low as reasonably achievable) values was the most acceptable.

On the basis of this advisory report, maximum levels were set for metals in dietary supplements, including a maximum level of 1 mg (total Arsenic) tAs per product kg for dietary supplements in the Royal Decree of 14 June 2002 laying down maximum levels for contaminants, including heavy metals in FS.

In the meantime, the national maximum levels for Cd, Hg and Pb have been replaced by the European maximum levels in Regulation (EC) No 1881/2006. The maximum levels for As have not been harmonised at the European level yet. This harmonisation process is currently at its first stage, and discussions are ongoing about maximum levels for iAs in rice and certain rice products. In the EU, it was decided that no maximum level could be set yet for As in algae due to a lack of data and difficulties with the analytical methodology. The available data also showed that the levels of As vary significantly. In addition, some Member States have preferred to address this issue through consumption advice since their consumption by an average consumer is limited. In the past, a lack of data also made it impossible to set maximum levels for Cd and Pb in algae.

The national maximum level for As in dietary supplements is now being questioned by several parties.

The SHC has therefore been requested by the Directorate General Animal, Plant and Food (DG4) to issue an advisory report on the speciation forms of As in dietary supplements that are relevant for public health as well as on the possible risks attendant on a regular or heavy consumption of algae. The SHC has also been asked, if possible, to provide consumption-advice for algae as well as recommendations for further research that would make it possible to carry out a more comprehensive risk assessment for As in dietary supplements and metals in algae. More specifically, the following questions were put to the SHC:

"As in dietary supplements: an evaluation of the risks posed by As for the general population and/or for specific population groups due to the consumption of dietary supplements and/or certain subcategories of dietary supplements (e.g. based on oil, algae, fish products and clay). Which forms of As need to be taken into consideration: only iAs or are there organic forms that are also liable to have adverse effects on public health and how toxic are they compared to iAs? Based on current data, does the SHC expect it to be possible for these organic forms or some of these organic forms to be ingested in amounts that are liable to be harmful to public health when average dietary-supplement consumers or certain sensitive subpopulations take dietary supplements and/or certain dietary-supplement subcategories?

Algae: what is the assessment of the risks posed by As, as well as by other metals such as Cd, Pb and Hg for the general population and for specific population groups when algae/seaweed and their derivatives are consumed on a regular basis or in high amounts? Please make assumptions as regards the use of these products by Belgian consumers. What are the algae and/or seaweeds that are liable to entail unacceptably high risks for the general population or any

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specific population groups? What consumption-advice can be offered to reduce these risks to an acceptable level?

Research questions: recommendations arising from this report for further research that would make it possible to carry out a more comprehensive risk assessment for As in dietary supplements and algae."

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2. CONCLUSION AND RECOMMENDATIONS

2.1 Conclusions

Food supplements (FS) derived from algae as well as edible algae used as foodstuffs are generally contaminated by several As species such as iAs and organic species. Some of the organic species seem to be toxicologically relevant but no Health Based Guidance Values (HBGV) are available for risk characterization. Some other elements such as Cd, Pb and Hg have also been reported to be present in edible algae.

Several scenarios were constructed in order to assess the potential short and long term risks for some groups of the Belgian population that are known to consume such foodstuffs.

For the FS, the daily exposure to total As and iAs has been estimated from the label instructions (amount ingested), on the one hand, and from the analytical data on As contamination, on the other hand. For the edible algae, the estimation of the exposure to As, as well as to Cd, Pb and Hg, was based on some nutritionists' consumption advices, as given to vegetarians (average and worst case amounts ingested daily) and from available contamination data.

The results show that, for the FS, the estimated exposure to iAs is relatively low compared to the background intake of the Belgian general population (i.e. 0.11 µg iAs/kg bw/d), and that the short term risks could be considered as low (less than 1 % of the HBGV). As to the long term exposure, it appears that the Margin of Exposure (MoE, i.e. the ratio between the Benchmark Lower Dose confidence Limit for cancer effects ($BMDL_{01}$) and the exposure considered with the selected scenario) ranges from 40 to > 7747. However, it must be noted that the exposure to iAs via FS comes on top of the background exposure to iAs due to other foodstuffs (i.e. 0.11 µg/kg bw/d), which is relatively high compared to the lowest proposed $BMDL_{01}$ (0.30 µg iAs/kg bw/d), so that the MoE is already very low (2.73).

Exposure to iAs linked to the consumption of edible algae belonging to the red algae (*Rhodophyta*) such as *Nori*, and to the brown algae (*Phacophyta*) such as *Arame*, *Kombu* and *Wakame* is estimated to range from one third to 100 % of the background intake of iAs. In terms of acute toxicity, this intake is still low (max 2 % of the HBGV) but, for the long term effects, this intake can be worrying by reducing the MoE by a factor 2 when the cumulated intake for all foodstuffs is considered/taken into account. As to the specific consumption of *Hijiki* algae (i.e. *Hisikia fusiforme* belonging to the brown algae, *Phacophyta*), the assessment using the same scenarios as for the other algae, leads to the conclusion that iAs intake is expected to largely exceed the background intake (from about 50 to 150 times). Both short and long term toxic effects are expected to occur at such levels of exposure.

Exposure to other elements (Cd, Pb and Hg) linked to the consumption of edible algae has been estimated using the same scenarios as for As. This assessment demonstrates that the potential intake of Pb and more particularly of Cd is far from being negligible with some deleterious effects to be expected on the long term considering the other sources of dietary exposure.

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2.2 Recommendations

2.2.1 Recommendations for risk management

The current legislation is based on the determination of tAs whilst Health Based Guidance Values are only available for iAs. The current legislation needs to be adapted because there is no direct link between tAs and iAs in algae products.

Taken into account the above mentioned uncertainties related to the lack of toxicological information of many chemical species other than the iAs, the SHC recommends to set legal maximum levels based on "tAs, reduced by AsB" (cf. Feldman *et al*., 2011) instead of "tAs", as it is the case in current legislation.

Furthermore, the SHC recommends the competent bodies to take appropriate measures at national level (and by extension at European level) according to the findings of this report.

2.2.2 Recommendations for consumers

- Consumption of Hijiki has to be avoided;
- Restrain the consumption of other edible algae to 7 g (i.e. half a spoon of dried material) per day;
- Be aware that a normal diet does already provide a relatively high exposure to iAs;
- Since As is known to leach (partly) to the cooking liquid, it is advised not to consume the cooking liquid;
- Consumption of (food supplement based on) algae is not recommended for children and pregnant women;
- Consumers should avoid large cumulated intake of foodstuffs, substantially contributing to iAs exposure such as rice, algae and derived products.

2.2.3 Recommendations for research

- A comparison of iAs results in algae obtained by different analytical methods is necessary to confirm the trueness of the results;
- Toxicology of arsenosugars and arsenolipids, identification and quantitative analysis of all As species to be covered by the legislation;
- Risks related to As (and other heavy metals) due to the consumption of clay minerals (a.o. as "detoxification");
- Consumption data and/or total diet study with focus on the foodstuffs as consumed (cooked or rehydrated) in order to better assess the intake of As and other toxic elements.

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3. FURTHER DETAILS AND ARGUMENTATION

3.1 Methodology

After having analysed the request, the Board and working group Chair identified the necessary areas of expertise. The working group experts provided a general and an *ad hoc* declaration of interests and the Committee on Professional Conduct assessed the potential risk of conflicts of interest.

This advisory report is based on a review of the relevant literature as well as on a risk evaluation which estimated the consumers' exposure based on available consumption and contamination data, gathered in different realistic scenarios of consumers' exposure. First of all, a literature study has been performed in order to gather all relevant information about the toxicity of the various As species (organic and inorganic) that can be found in marine products, in general, and in algae, more specifically. Then, contamination data were gathered from the Belgian food agency control program as well as from the general scientific literature. Due to a lack of formal consumption data, several intake scenarios were elaborated in order to assess the exposure by the concerned people and to perform the risk characterization, taking into account the oral background exposure of the general population and the additional exposure for some target groups such as vegetarians and food supplement consumers.

In order to provide an answer to these questions, an *ad hoc* working group was established. The areas of expertise of the members of this working group are those listed in the table in Section 6.

Once the draft advisory report was approved by the *ad hoc* working group and by the standing working group tasked with NHFS, it was ultimately validated by the Board.

3.2 Elaboration

3.2.1 General context

As, as well Cd, Pb and Hg, are food contaminants for which a risk assessment has already been carried out both at the Belgian level (CSS-HGR, 2000; As: SPECAS 2010; SHC, 2000; Cd: Sci Com, 2009a; Vromman *et al*., 2010; Pb: Sci Com, 2009b and 2011) and at the international level, especially by the European Food Safety Authority (As: EFSA 2009a, 2014a; Cd: EFSA 2009b, 2012a; Hg: EFSA 2012c, 2014b, 2015; Pb: EFSA 2010, 2012b).

These different assessments have revealed that, taking into account toxicological reference values {e.g. « *health based guidance values* » (HBVG), such as tolerable daily intake (TDI), acceptable daily intake (ADI), acute reference dose (ARfD) or « reference points », such as no observable adverse effect level (NOAEL), benchmark dose lower confidence limit (BMDL)}[2], the level of consumer exposure to these contaminants is usually quite high, with a low or even non-existent safety margin for specific target groups (children, heavy consumers of certain foodstuffs).

Moreover, changing dietary trends could play a part in narrowing down the gap between consumer exposure and the toxicological reference values for certain contaminants even further. Thus, the use of algae-based dietary supplements as well as the consumption of seaweed for certain specific dietary purposes could lead to the excessive exposure of certain consumers to iAs, as well as to other elements (Cd, Hg and Pb).

[2] cf. paragraph 3.2.3.1.1.c

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A random screening of food products based on algae (FS and edible algae) available on the Belgian market shows that algae are used in a wide range of products. Algae-based FS are marketed to fulfill different needs, such as improvement of the gastrointestinal transit, deficiency of iodine and other minerals. Edible algae, on the other hand, are mainly sold as vegetarian or vegan food products or as ingredients for Asian food preparations. Most of these algae preparations (biscuits, spice mixtures, bread spreads, etc.) contain other (other than algae) ingredients than algae as well and are ready for instant consumption, while others, mainly those purely based on algae, are intended to be consumed after processing (hydration, cooking).

3.2.2 Scope

A risk assessment was carried out specifically on the potential contribution of algae-based dietary supplements as well as the direct consumption of algae, with a particular emphasis on iAs. Incidentally, a first attempt to assess the risks associated with the presence of Cd, Hg and Pb in algae was also undertaken within the limits of the available data. Yet it should be noted that, due to insufficient data, this advisory report does not concern exposure to As, Cd, Pb and Hg resulting from the ingestion of certain clay preparations, e.g. those used as FS.

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3.2.3 Risk assessment

3.2.3.1 Hazard identification and characterisation

As sources in the food chain can be natural (depending on soil and water composition) or anthropogenic. Anthropogenic sources are multitudinous and highly variable. These include current or past industrial or urban pollution, the use of fertilizers and organic manures contaminated with As, historical use of pesticides and biocides (wood preservatives), and contamination of irrigation water.

Different biologically relevant As species have been identified in natural samples including:

- Inorganic compounds:
  - arsenate (As^{V}) and arsenite (As^{III}).

- Organic As compounds:
  - methylated As compounds: methylarsonic acid (MMA^{V}), dimethylarsinic acid (DMA^{V}), methylarsonous acid (MMA^{III}) and dimethylarsonous acid (DMA^{III})[3];
  - arsenobetaine (AsB) and arsenocholine (AsC);
  - arsenosugars;
  - arsenolipids.

The predominant dietary source of As is seafood, followed by rice. While seafood contains the greatest total amount of As, in fish and shellfish it is mostly present in an organic form of As called AsB, which is much less harmful than iAs. Some seaweeds may contain high levels of inorganic forms or other organic compounds (such as arsenosugars) that may be more harmful.

Although As forms, under reducing conditions, chemical species with the As atom in oxidation state -3 and +3, the most stable As species found under normal environmental conditions contain the As atom in oxidation state +5. Consequently, the vast majority of As species found in organisms and in foods also contain As in oxidation state +5 (EFSA, 2009a).

Toxicity, bioaccumulation and transport properties vary according to the type of As compound (Niegel *et al*., 2010). Whereas knowledge on the iAs compounds and AsB has accumulated over the years, data on the toxicity of arsenosugars, arsenolipids and thioarsenicals is still limited.

[3] Except for biochemical and toxicological studies of specific As compounds, the valency of MMA and DMA is usually not specified. The analysis of MMA^{III} and DMA^{III} has become possible only recently. In this report, the terms MMA and DMA are used as cited in the original papers. Where MMA and DMA are measured in foods, they have been measured as the pentavalent form. Where biological samples have been analysed, it is assumed that MMA and DMA refer to total [MMA^{III} + MMA^{V}] and total [DMA^{III} + DMA^{V}], respectively (FAO/WHO, 2011).

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3.2.3.1.1 Inorganic arsenic compounds

The toxicokinetic and toxicodynamic properties of iAs compounds have been extensively described and reviewed in literature. A brief overview, mainly based on the EFSA opinions of 2009 (EFSA, 2009a) and 2014 (EFSA, 2014a) and associated references, is provided below.

A. <u>Toxicokinetic data on inorganic arsenic compounds</u>

IAs compounds are readily absorbed after oral exposure, although absorption can be influenced by (i) the solubility of the arsenical compound, (ii) the presence of other food constituents and nutrients in the gastrointestinal tract and (iii) by the food matrix itself. Once absorbed, iAs is widely distributed to almost all organs and extensively transformed. Biotransformation of iAs in mammals includes reduction of pentavalent As to trivalent As and methylation of trivalent As (DMA^{III}, MMA^{III}) with formation of MMA^{V} and DMA^{V}. Most of the iAs is excreted via urine as DMA^{V} (EFSA, 2009a, 2014a - Figure 1).

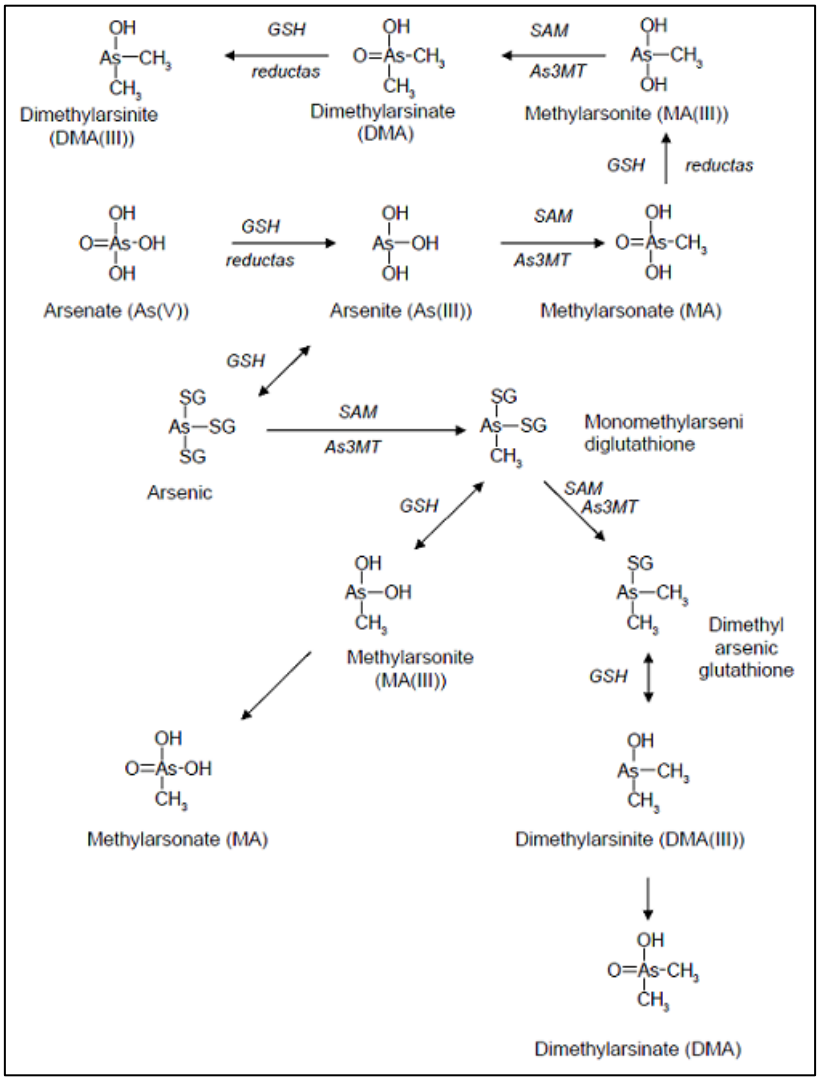

Figure 1 *Proposed metabolic pathways of iAs in mammals (in EFSA opinion 2009 adapted from). SAM: S-adenosylmethionine; As3MT: arsenic-methyltransferase; GSH: glutathione.*

B. Toxicodynamic data on inorganic arsenic compounds

Inorganic As does not only display high acute toxicity, it is also classified as a human carcinogen, both via ingestion and inhalation routes (IARC, 1987, 2012). Although both forms of iAs are potentially harmful, trivalent As is considered more harmful than the pentavalent forms (FAO/WHO, 2011).

Acute toxicity

Acute exposure to iAs may have deleterious effects. Available median lethal dose (LD_{50}) values for iAs are 15 to 145 mg/kg bw for As^{III} in the rat, 26-39 mg/kg bw for As^{III} in the mouse and 112-175 mg/kg bw for As^{V} in the rat (ATSDR, 2007). The variability in LD50 values can be attributed to differences in species, strain, specific compound, and testing laboratory. Most deaths occurred within 1 day of exposure, but details regarding cause of death were not generally reported. Data on lethality from subacute exposure studies (< 2 weeks) in animals are relatively sparse (EFSA, 2009a).

Reports on the acute and subacute exposure in humans show that almost all physiological systems of the body can be affected including the gastro-intestinal, cardiovascular, renal and nervous systems and to a lesser extent, the respiratory system, liver, skin and hematologic system (EFSA, 2009a). The Agency for Toxic Substances and Disease Registry (ATSDR) reported a lethal dose in humans after acute ingestion of 100-300 mg (1-5 mg As/kg bw). For subchronic exposure, the LOAELs (lowest observed adverse effect level) in humans range approximately between 0.05 and 0.1 mg/kg bw/day (ATSDR, 2007).

Carcinogenicity

Inorganic As has been evaluated on a number of occasions by the International Agency for Research on Cancer (IARC). In 1987, IARC has classified iAs in group 1 as human carcinogen (IARC, 1987). In 2004, IARC concluded that As in drinking-water causes cancers of the urinary bladder, lung and skin and that the evidence was “limited” for cancers of the kidney, liver and prostate (IARC, 2004). In its opinion of 2012, IARC confirmed that As and iAs compounds are carcinogenic to humans (Group 1) (IARC, 2012).

To date the underlying molecular mechanisms of iAs-induced carcinogenicity are not completely identified. Evidence from a wide range of studies, however, has led to the conclusion that As compounds do not react directly with DNA (FAO/WHO, 2011). Established mechanistic events are oxidative DNA damage, genomic instability, aneuploidy, gene amplification, epigenetic effects and DNA-repair inhibition leading to mutagenesis (Straif *et al*., 2009).

Reproductive/developmental toxicity

There is emerging evidence of a negative impact of iAs on foetal and infant development (i.e. reduced birth weight). More data on the dose-response relationships and critical exposure times are required (EFSA, 2009a).

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C. Toxicodynamic data on organic metabolites of inorganic arsenic

Interestingly, data from *in vitro* and *in vivo* studies indicate that organic As species (e.g. mono- and dimethylated trivalent and pentavalent arsenicals) formed during metabolism contribute to iAs induced toxicity (FAO/WHO, 2011; EFSA, 2009a; Rehman & Naranmandura, 2012). MMA^{III} and DMA^{III} were even more cytotoxic in cell cultures than their parent compounds As^{III} and As^{V} (Petrick *et al*., 2000; Mass *et al*., 2001). Based on these results, the order of toxicity is expected to be $MMA^{III} > DMA^{III} = As^{III} > As^{V} > MMA^{V} > DMA^{V}$ (FAO/WHO, 2011).

Recently, IARC classified both DMA and MMA as "possibly carcinogenic to humans" (Group 2B) on the basis of sufficient evidence of cancer caused by DMA in experimental animals, and the extensive metabolism of MMA to DMA (IARC, 2012). As will be discussed later, these metabolites are also formed after intake of arsenosugars and arsenolipids.

D. Establishment of Health Based Guidance Values for inorganic arsenic compounds

Acute effects

The ATSDR (2007) has derived a minimal risk level (MRL) of 0.005 mg As/kg bw/day for acute-duration (14 days or less) oral exposure to iAs based on the results of a Japanese study (Mizuta *et al*., 1956). As exposure through the intake of contaminated soy sauce was estimated to be 3 mg/day during 2-3 weeks. For derivation of the acute oral MRL, facial edema and gastrointestinal symptoms (nausea, vomiting, diarrhea), which were characteristic of the initial poisoning and then subsided, were considered to be the critical effects. The MRL of 0.005 mg As/kg bw/day was calculated by applying an uncertainty factor of 10 (10 for use of a LOAEL and 1 for human variability) to the LOAEL of 0.05 mg As/kg bw/day (ATSDR, 2007).

Chronic effects

In 1989, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a provisional tolerable weekly intake (PTWI) of 15 µg/kg bw for iAs (FAO/WHO, 1989). The PTWI was however withdrawn by JECFA in 2010 as more recent studies indicated that iAs caused cancer of the lung and urinary tract in addition to skin cancer, at exposures levels lower than the PTWI. Based on epidemiological studies, JECFA identified a benchmark dose lower confidence limit for 0.5 % increased incidence of lung cancer ($BMDL_{0.5}$) of 3.0 µg/kg bw/day (FAO/WHO, 2011).

In 2009, the EFSA Panel on Contaminants in the food chain (CONTAM Panel) had already concluded that the PTWI of 15 µg/kg bw was no longer appropriate. In their scientific opinion, the CONTAM Panel modelled the dose-response data from key epidemiological studies. Furthermore, other reported dose-response modelling results were also considered. Due to the uncertainties in the exposure in the key epidemiological studies, the CONTAM Panel identified a range of values for the 95 % lower confidence limit of the benchmark dose of 1 % extra risk ($BMDL_{01}$) for each endpoint (Table 1) (EFSA, 2009a). The lowest $BMDL_{01}$ values were found for lung cancer. These data were obtained from a relatively small study which had however the advantage that the nutritional and genetic background of the population were probably more similar to that of EU populations than those of the rural Asian populations, for which most of the epidemiological data are available. The CONTAM panel noted that the association between iAs exposure and the incidence of lung cancer was much stronger in smokers. This observation is consistent with iAs being a co-carcinogen. However, the CONTAM panel could not determine whether there would be residual confounding after adjustment for smoking. In contrast, the data for skin lesions were from larger populations and showed a high degree of consistency between studies. These data should however be considered with care. Indeed, As exposure is considered to be a necessary but not sufficient cause of dermal lesions. As most of the observations of

dermal lesions originate from rural Asian communities with high levels of As in the water, they could have been influenced by other factors such as nutritional status. The CONTAM Panel therefore concluded that the overall range of $BMDL_{01}$ values of 0.3 to 8 µg/kg bw/day should be used instead of a single reference point in the risk characterisation for iAs (EFSA, 2009a).

Table 1 *Summary of potential reference points for iAs (EFSA, 2009a)*

Endpoint	Population	Reference point µg/L water	Reference point µg/kg b.w. per day
Dermal lesions	Bangladesh (Ahsan et al., 2006)	$BMCL_{01}$: 23[(a)]	$BMDL_{01}$: 2.2-5.7[(b)]
Dermal lesions	Mongolia (Xia et al., 2009)	$BMCL_{01}$: 0.3[(a)]	$BMDL_{01}$: 0.93-3.7[(b)]
Lung cancer	Chile (Ferreccio et al., 2000)	$BMCL_{01}$: 14 (NRC, 2001)	$BMDL_{01}$: 0.34-0.69[(c)]
Bladder cancer	North East Taiwan (Chiou et al., 2001)	$BMCL_{01}$: 42 (NRC, 2001)	$BMDL_{01}$: 3.2-7.5[(b)]
Skin cancer	USA (New Hampshire) (Karagas et al., 2002)	Change point[(d)]: 1-2	Change point: 0.16-0.31[(c)]
Bladder cancer	USA (New Hampshire) (Karagas et al., 2004)	Change point: *ca.* 50	Change point: 0.9-1.7[(c)]

b.w.: body weight; $BMCL_{01}$: 95 % lower confidence limit of the benchmark concentration of 1 % extra risk; $BMDL_{01}$: 95 % lower confidence limit of the benchmark dose of 1 % extra risk

(a): Calculated by CONTAM Panel for this opinion

(b): Extrapolated from the $BMCL_{01}$ assuming 3-5 L water and 50-200 µg/day inorganic arsenic in food per day, 55 kg b.w. (see Section 8.4.1.1)

(c): Extrapolated from the $BMCL_{01}$ assuming 1-2 L water and 10-20 µg/day inorganic arsenic in food consumed per day, 70 kg b.w. (see Section 8.4.1.1)

(d): The maximum likelihood change point before the trend becomes significant, which provides an indication of a no effect level rather than a BMDL (see Section 8.3.3.1)

3.2.3.1.2 Organoarsenic compounds

At present, the physiological significance and the biochemical role of the organic As compounds remain unclear. One hypothesis considers the formation of these compounds as a detoxification and elimination process (Edmonds *et al*., 1993; Edmonds & Francesconi, 1983) following the intake of inorganic species which naturally occur in seawater and marine sediments (Edmonds & Francesconi, 1983; 1987). The adsorption may be due to the inability of phosphate transporters to differentiate between the structurally similar As^{V} and phosphate in the marine environment (Andrea, 1978; Sele *et al*., 2012).

A. <u>Arsenobetaine</u>

In AsB, As is oxidised and has four stable carbon bonds, which are enzymatically and thermally hard to break (Feldmann & Krupp, 2011).

<u>Toxicokinetic data</u>
AsB is rapidly excreted in human urine in its unchanged form (Vahter, 1994). Two additional toxicokinetic studies however showed that small amounts of AsB may be retained in the body of rabbits (Vahter, 1983) or humans (Newcombe *et al*., 2010). In contrast to iAs, AsB does not accumulate in hair (Raab *et al*., 2005; Yanez *et al*., 2005).

<u>Toxicodynamic data</u>
Based on its high LD_{50} value in mice (› 10 000 mg/kg), acute toxicity of AsB is considered to be negligible (Kaise & Fukui, 1994). The lack of toxicity of AsB has been associated with the presence of the four stable As-C bonds. This hypothesis is further supported by the observation that two structurally related compounds (i.e. tetramethylarsonium and AsC) do not induce toxicity (Kaise & Fukui, 1994).

B. <u>Arsenosugars</u>

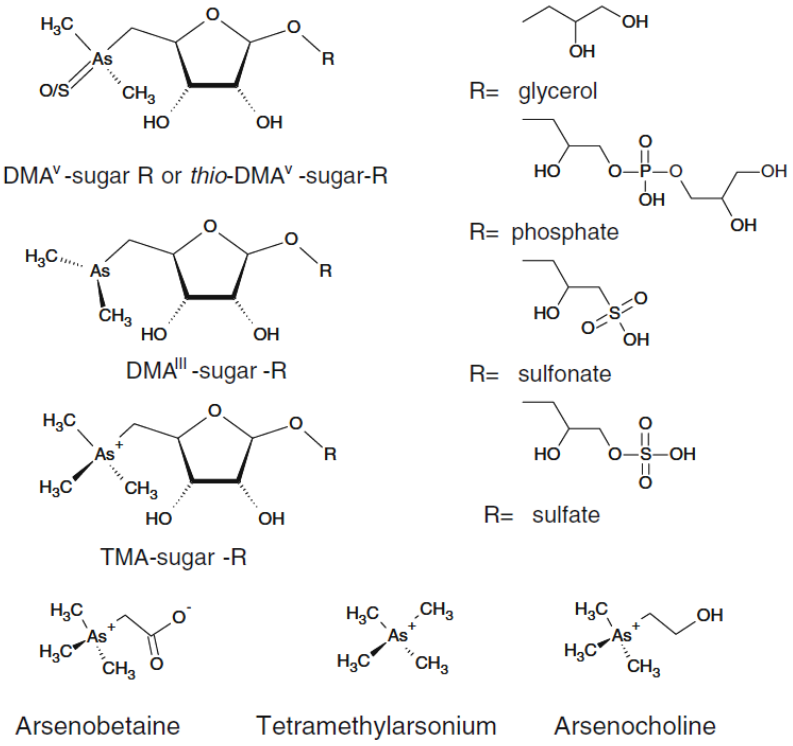


Figure 2 *General structures of arsenosugars compared to other organoarsenicals in which As forms four carbon bounds (Feldmann & Krupp, 2011)*

The term arsenosugars covers a range of carbohydrate compounds containing As. Among the more than fifteen different arsenosugars that have been identified in the marine environment, the dimethylarsinoyl ribosides are the most represented (Edmonds & Francesconi, 2003). These compounds contain pentavalent As which is bound to two methyl groups, to oxygen and to the D-ribose derivatives at the C5 position (Andrewes *et al*., 2004). Recently, thio-arsenosugars have been identified in which the oxygen is replaced by sulphur (Soeroes *et al*., 2005; Kahn *et al*., 2005). Arsenosugars can also be present in the form of trimethylarsonium compounds. As these compounds are structurally similar to AsB and are only found in traces, they are not considered of toxicological concern.

With the exception of one trimethylated arsenosugar (Shibata & Morita, 1988), all arsenosugars identified so far are water-soluble. Together with the non-toxicity of AsB, this has led to the assumption that organoarsenic compounds are benign. Although intake of arsenosugars is not associated with any known acute toxic effects, little is known about their toxicity and the possibility that arsenosugars induce chronic toxicity cannot be excluded (Feldmann & Krupp, 2011). First, arsenosugars may directly exhibit toxic effects. Alternatively, arsenosugars may exert chronic toxicity through metabolism into other arsenicals (Andrewes *et al*., 2004). Indeed, in contrast to AsB, arsenosugars appear to be less inert and susceptible to metabolisation. Consequently, both the toxicity of the arsenosugars as such and their metabolites needs to be discussed/investigated.

Toxicokinetic data

Arsenosugars are readily taken up and completely metabolised before excretion. Based on the results of studies with human volunteers (Francesconi *et al*., 2002; Le XC *et al*., 1994; Ma & Le XC, 1998; Wei *et al*., 2003), DMA^V is considered to be the major metabolite after consumption of arsenosugars or arsenosugar-containing meals. As arsenosugars do not decompose during the cooking process of seaweed (Wei *et al*., 2003) nor in the acidic environment of the stomach (Le XC *et al*., 1994; Gamble *et al*, 2002), either enzymatic or microbial activity appears to be responsible for the formation of DMA^V. Based on the low intestinal bioavailability of arsenosugars assessed in a Caco-2 model, formation of arsenosugar metabolites prior to intestinal absorption has been proposed (Leffers *et al*., 2013a). This hypothesis was supported by the high intestinal bioavailability of arsenosugar metabolites compared to arsenosugars in the same *in vitro* model (Leffers *et al*., 2013b). Interestingly, two additional studies revealed enormous differences in urinary recovery after the intake of an arsenosugar-containing meal (Le *et al*., 1994) or a synthesised arsenosugar (Raml *et al*., 2009) indicating that there is an intrinsic difference in arsenosugar metabolism. These interindividual differences in arsenosugar metabolism may be related to genetic polymorphisms in the metabolic enzymes or differences in microbiota. Other As metabolites that have been identified so far include dimethylarsinoyl acetic acid ($DMAA^V$) or dimethylarsinoylethanol ($DMAE^V$) and thio-analogues e.g. thio-DMA^V, thio-$DMAE^V$ and thio-$DMAA^V$. At present, the mechanism by which these thioarsenicals are formed remains unclear (Bu *et al*., 2011; Pinyayev *et al*., 2011; Yoshida *et al*., 2003). Thioarsenicals may be generated directly from oxyarsenicals by exchanging an O atom for an S atom, either by microbial activity (Kubachka *et al*., 2009; Pinyayev *et al*., 2011; Rubin *et al*., 2014) or by biochemical process inside living mammalian cells (Naranmandura *et al*., 2013). Although trivalent arsenosugars have not yet been detected *in vivo*, the possibility that pentavalent arsenosugars ingested in seaweed will be metabolized to trivalent arsenosugars cannot be excluded (Andrewes *et al*., 2004). However, these trivalent arsenocompounds are highly reactive and thus difficult to detect analytically.

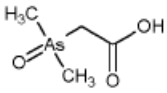
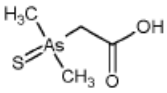
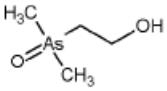
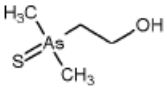
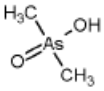
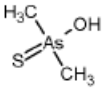
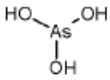
Structure	Name	Abbreviation
	oxo-dimethylarsenoacetic acid	oxo-DMAA^{V}
	thio-dimethylarsenoacetic acid	thio-DMAA^{V}
	oxo-dimethylarsenoethanol	oxo-DMAE^{V}
	thio-dimethylarsenoethanol	thio-DMAE^{V}
	dimethylarsinic acid	DMA^{V}
	thio-dimethylarsinic acid	thio-DMA^{V}
	arsenite (arsenious acid)	iAs^{III}

Figure 3 *Chemical structures, names, and abbreviations of seven As species related to the metabolism of arsenosugars (Leffers et al*., 2013a)

No data on As accumulation in human tissues after prolonged arsenosugar exposure is currently available (Feldmann & Krupps, 2011). However, results from studies with seaweed-eating sheep indicate the intake of seaweed results in similar bioconcentration as expected from iAs ingestion (Feldmann *et al*., 2000). The concentration of As in the seaweed, i.e. *Laminaria spp*, amounted to 50-100 mg/kg. Almost 80 % of the total As was present as arsenosugars, whereas only trace amounts of iAs were found (Beresford *et al*., 2001). Unfortunately, As speciation in the different tissues (i.e. kidney, liver and muscle) was not determined (Feldmann & Krupps, 2011). In an additional study investigating accumulation of As in the wool and the horn, As was speciated and DMA^{V} was identified as the main extractable As (Caumette *et al*., 2007; Raab *et al*., 2002).

Toxicodynamic data

As discussed previously, both the toxicity of the arsenosugars and their metabolites need to be investigated as metabolism may dramatically increase the toxicological potential of arsenosugars (Feldmann & Krupps, 2011). Studies towards the toxic effects of arsenosugars are however complicated by the fact that pure arsenosugars are difficult to obtain in sufficient quantities for toxicity testing (Andrewes *et al*., 2004). Also for metabolites other than DMA^{V}, toxicity data are scare.

Arsenosugars – To date, no cytotoxic effects have been observed in cultured human cells for arsenosugars in concentrations up to the micromolar range (Andrewes *et al*., 2004; Leffers *et al*., 2013a; Leffers *et al*., 2013b; Sakurai *et al*., 1997). In the studies with seaweed-eating sheep, no toxic effects were observed. However, the limited lifetime of the sheep (4-6 years) probably does not allow to assess long-term illness (Feldmann & Krupps, 2011). Recently, the effects of

arsenosugars on oxidative stress, DNA damage and behaviour were investigated in mice. Mice were treated orally for 40 days with 20, 30 or 50 mg/kg bw/day of the synthetic pentavalent arsenosugar, (R)-2',3'-dihydroxypropyl 5-deoxy-5-dimethyarsinyl-β-D-riboside. Dysfunction of cognitive and motor functions was observed together with increased levels of markers for oxidative stress and DNA damage (Bin Sayeed *et al*., 2013). Further research is thus required.

Remark: As^{V} compounds can *in vivo* be reduced to As^{III} compounds both with and without enzyme catalysis. Reduction of pentavalent arsenosugars to their trivalent counterparts via reaction with thiol compounds can therefore not be excluded. In a set of different *in vitro* toxicity studies, trivalent arsenosugars appeared to be at least 50-fold more active than its pentavalent counterpart in the plasmid DNA nicking assay and the cytotoxicity assay. Nevertheless, the trivalent arsenosugar was much less toxic than MMA^{III} and DMA^{III} (Andrewes *et al*., 2004).

DMA^{V} – As discussed for iAs, dimethylarsenic (DMA^{V}) is a tumor promotor and complete carcinogen in experimental animals (Kenyon & Hughes, 2001; Wei *et al*., 1999).

Thio-DMA^{V} – Thio-dimethylarsenic (thio-DMA^{V}) was more cytotoxic to cultured human cells than DMA^{V} (Naranmandura *et al*., 2013; Bartel *et al*., 2011; Naranmandura *et al*., 2007; Ochi *et al*., 2008). Furthermore, thio-DMA^{V} induced cell-cycle arrest, aneuploidy, chromosome structural aberrations, apoptotic mode of cell death, and abnormalities of spindle organization and centrosome integrity in cultured human cells (Ochi *et al*., 2008). A recent *in vitro* study indicated that although thio-DMA^{V} exerts its toxicity in a similar or even lower concentration range than As^{III}, the mechanisms involved are likely to be different (Ebert *et al*., 2013). To date, thio-DMA^{V} is considered to be the most toxic human metabolite identified after arsenosugar intake. Consequently, more research – and in particular *in vivo* studies – to characterize the toxicological profile of this compound is needed (Ebert *et al*., 2013).

Epidemiology

Epidemiological studies do not provide evidence for an association between chronic toxicity and seaweed consumption. In contrast, seaweed consumption has been associated with a reduced risk for certain types of cancer including breast cancer (Funahashi *et al*., 2001) and colorectal cancer (Hoshiyama *et al*., 1993). This inverse correlation may be associated with the high fiber content and vitamin content of seaweed (Andrewes *et al*., 2004).

C. Arsenolipids

Arsenolipids are lipid-soluble compounds. Although the occurrence of arsenolipids in fish and other types of seafood was first reported in the 1960s (Lunde, 1968), more information on the structures of these compounds has only recently become available due to the improvements of the analytical techniques (Meyer *et al*., 2014). In the meantime, several types of arsenolipids have been identified including (i) arsenosugars bound to a phospholipid (Garcia-Salgado *et al*., 2014; Morita & Shibata, 1988), (ii) As-containing hydrocarbons (Taleshi *et al*., 2008) and (iii) As-containing long-chain fatty acids (Rumpler *et al*., 2008).

Toxicokinetic data

One study reported DMA and DMA-containing fatty acids as the major urinary metabolites in humans following ingestion of cod liver oil. The chemical structures of the arsenolipids present in cod liver oil were not identified experimentally but assumed to be DMA-containing long chain fatty acids (Schmeisser *et al*., 2008). In a recent study, the toxicokinetic profile of phosphatidylarsenocholine, one of the major arsenolipids identified so far in marine organisms, has been investigated in mice. After oral administration, phosphatidylarsenocholine was mostly absorbed from the gastrointestinal tract, metabolized to AsB and almost completely (> 90 %) excreted mainly in urine. Although the majority of the administered As is excreted within

144 hours, the excretion rate was considerably slower compared to the arsenolipids present in cod liver oil as well as water-soluble As compounds (Fukuda *et al*., 2011).

Toxicodynamic data

At present, toxicodynamic data on arsenolipids are extremely limited. However, arsenolipids are - like inorganic arsenocompounds and arsenosugars - biotransformed into DMA possibly leading to the production of toxic intermediates. Furthermore, as discussed above, DMA itself demonstrates a unique toxicity and has been identified as a potential carcinogen. In addition, one recent *in vitro* study with three As-containing hydrocarbons showed that all three compounds were strongly cytotoxic (comparable to As^{III}). However, the toxic modes of action appeared to be different from those of As^{III} (Meyer *et al*., 2014). More studies to unravel the toxicological profile of As-containing hydrocarbons and other arsenolipids are thus urgently needed.

D. Establishment of health based guidance values for organic arsenic compounds

In their Scientific Opinion on As in Food (2009), the CONTAM Panel concluded that AsB is probably of no toxicological concern as it is not metabolized and rapidly excreted in its unchanged form. In contrast, a risk assessment for arsenosugars and arsenolipids – compounds that are metabolized to a multitude of As metabolites - is currently not possible, largely because of the lack of relevant toxicological data (EFSA, 2009a). Consequently, more toxicological data on these As species are urgently needed. Data in humans are preferred as experimental animals differ considerably from humans with regards to As metabolism and other aspects of toxicokinetics.

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3.2.3.1.3 Cd, Pb and Hg

Cd is a non-essential naturally present heavy metal that is also introduced in the food chain by industrial activities and agriculture. Cd accumulates primarily in the kidneys and may induce kidney dysfunction, skeletal changes and reproductive deficiencies. According to IARC, Cd and Cd compounds are classified as human carcinogens (Group I).

Pb is a non-essential naturally present heavy metal but mainly introduced in the environment by anthropogenic activities such as smelters and battery production plants. Pb has been used in pesticides, gasoline, paints, pipes contributing by this way to the environmental and food chain contamination. Some chronic effects of Pb poisoning are colic, constipation and anaemia. It may also induce increased blood pressure and cardiovascular disease in adults. Foetal neuro-developmental effects and reduced learning capacity in children are among the most serious effects.

Hg is an element present in trace amounts in air, water and soil. Anthropogenic emissions of Hg in the environment and in food chain include metallurgy, paper pulp transformation, waste combustion and fossil fuels. Hg is a toxic element found mostly in fish and fishery products. Methylmercury, the main form in which Hg is present in seafood is the most toxic among Hg species. It may induce alterations in the normal development of the brain of infants and may, at higher levels, induce neurological changes in adults.

3.2.3.1.4 Overview of existing health based guidance values for risk characterization

For some of the chemical species, not for all of them, health based guidance values (HBGV) for short- and (or) long term toxicity are proposed by several organisations. A selection of HBGV has been made as presented in Table 2.

It should be noted that, for chronic effects of As and Pb, no HBGV can be proposed as a consequence to the recent withdrawal of the former PTWI by the EFSA. The MoE approach can, then, be adopted as an alternative to carry out the risk characterization. The MoE is the ratio between the BMDL and the exposure. The BMDL is the lower one-sided confidence limit on the "benchmark dose", a dose of a substance associated with a specified low incidence of risk, generally in the range of 1–10 %, of a health effect. Thus, the BMDL can be used as the point of departure for derivation of an alternative option to HBGV for risk characterization (EFSA, 2009).

Table 2 *Proposed Health Based Guidance Values (HBGV) for the relevant species of the As, Cd, Hg and Pb contaminants*

Species	**Type of exposure considered and proposed HBGV**	**Comment**	**Reference**
Inorganic As	Short term : 5 µg/kg bw/d	"Minimal risk level" derived for acute effects (gastro-intestinal and facial oedema) after oral intake	ATSDR, 2007
	Long term : None (use of MoE approach)	$BMDL_{01}$ = 0.3 - 8 µg/kg bw/d Chronic toxicity (cancer of the lung, skin and bladder and skin lesions)	EFSA, 2009a
Cd	Short term : 4.3 µg/kg bw/d	Based on the NOAEL of 3 mg/person/day for short term effects (EFSA 2009; WHO 2011), assuming a body weight of 70 kg and applying an uncertainty factor of 10	FAVV-AFSCA, 2014
	Long term : 2.5 µg/kg bw/w	Chronic toxicity (renal tubular effects)	EFSA, 2009b
MeHg (expressed as Hg)	Short term : 1.3 µg/kg bw/d	Based on a NOAEL of 11.5 mg/kg maternal hair from Faeroe and Seychelles cohorts (neurodevelopmental effects on humans after prenatal exposure)	FAVV-AFSCA, 2014; EFSA, 2012c
	Long term : 1.3 µg/kg bw/w	Based on a NOEL of 11.5 mg/kg maternal hair from Faeroe and Seychelles cohorts (neurodevelopmental effects on humans after prenatal exposure)	EFSA, 2012c
Pb	Short term : none	No short term HBGV proposed by EFSA, ATSDR and US-EPA	FAVV-AFSCA, 2014
	Long term : None (use of MoE approach)	$BMDL_{10}$ = 0.63 µg/kg bw/d Chronic toxicity (nephrotoxic effects on humans) $BMDL_{01}$ = 1.5 µg/kg bw/d Chronic effects (cardiovascular effects) $BMDL_{01}$ = 0.5 µg/kg bw/d Chronic effects (neurodevelopmental toxicity)	EFSA, 2012b

3.2.3.2 *Exposure assessment*

Previous information on the consumers' exposure to As species in Belgium and Europe

In Belgium, the SPECAS study (SPECAS, 2010) demonstrated that, amongst all sampled food categories, the highest total As concentrations were found in those originating from the sea (marine fish, seafood, seaweed). Within these foods, As was almost exclusively present as organic compounds. Some samples of seaweed for human consumption contained up to 23000 µg/kg (in dry mass (DM)) and on average 135 µg/kg (DM) was inorganic. The seaweed "Hijiki" which is particularly known for its high iAs content was not included in this study.

In the EFSA study (EFSA, 2014a), the food group "Seaweed" was included in the food category "Vegetables and vegetable products". The maximum estimated mean values of iAs in this food category were found in seaweed, in particular in the dark greenish brown seaweed Kombu (middle bound, MB = 352.6 µg/kg). It should be noted, however, that results of all Hijiki samples – although they were all containing iAs levels > 1 mg/kg - were excluded from EFSA's exposure assessment, since only one eating occasion on Hijiki was reported in the EFSA Comprehensive Food Consumption Database.

FS were providing the highest number of results in the EFSA food category "Products for special nutritional use". The food group with the highest estimated mean concentration of iAs was "Algae formula (e.g. Spirulina, Chlorella)" with MB = 6133.8 µg/kg. With this food group a conservative approach was followed as the estimated iAs was calculated applying 70 % to the tAs reported as for the other food groups. This approach was based on the fact that Hijiki seaweed could also be found in food supplements and it is known that this seaweed accumulates high amounts of iAs in contrast to most of the other seaweed (EFSA, 2014).

In the SPECAS study the amount of iAs in FS varied between < LOD (limit of detection) and 142 µg/kg. It was concluded that FS prepared with seaweed need most attention. However the number of algae-based food supplements analysed was very limited (n = 3).

The SPECAS study made use of the national dietary survey (2004), as EFSA did for Belgium in its recent report (2014a), and showed that the average daily intake of total As by adults in Belgium is 1.04 µg/kg bw/d (using 70 kg bw). A large amount of this intake is due to AsB. The average daily intake of iAs by adults in Belgium is 0.11 µg/kg bw/d according to the SPECAS study but it must be noted that this study didn't take seaweed nor FS into account, as there were no consumption data available for Belgium. Biomonitoring of Flemish adolescents (14-15 years old) showed an average of 2.02 µg/l blood for tAs and 4.39 µg/l urine for the sum of As^{III}, As^{V}, MMA and DMA (Steunpunt Milieu & Gezondheid, 2015).

Dietary exposure to iAs was also assessed in the European vegetarian population but this assessment was based on a very limited number of subjects (no data from Belgium). These results indicate no remarkable differences between vegetarians and the general population (table 3) (EFSA, 2014a).

Table 3 *Dietary exposure to iAs in the European population (EFSA, 2014a)*

	mean dietary exposure to iAs (µg/kg bw/day) (min LB - max UB)	**95th percentile dietary exposure to iAs** (µg/kg bw/day) (min LB - max UB)
infants, toddlers and other children	0.20 - 1.37	0.36 - 2.09
adult population (adults, elderly and very elderly)	0.09 - 0.38	0.14 - 0.64
general population*	0.11 - 0.34	0.18 - 0.55
vegetarians*	0.10 - 0.42	0.28 - 0.60

LB = lower bound; UB = upper bound

* Comparing the five surveys with data on both vegetarians and general population

Estimated iAs levels were obtained from the reported occurrence data, and are those used to calculate the dietary exposure in the general and vegetarian population, except the iAs concentration for the alga Hijiki that were obtained from the UK FSA report published in 2004 (FSA, 2004). High consumption values were obtained from the EFSA Comprehensive Food Consumption Database using the maximum 95th percentile in the adult population for each food group, except for the alga Hijiki that was obtained from literature (Nakumara *et al*., 2008; Sawada *et al*., 2013). Although this alga is particularly consumed in the Asian market, it is also commercialized in Europe and can be found in restaurants, supermarkets and as part of FS of dietary fiber and/or minerals. The impact of the high consumption of these specific foods on the dietary exposure to iAs was especially significant for consumers of the alga Hijiki.

For the current assessment, dealing specifically with algae and FS, intake of As has been estimated, on the one hand, for consumers of FS containing algae (as such or as algal extracts) and, on the other hand, for consumers of algae products (seaweed salads, etc). In addition, a first evaluation of the potential intake of Cd, Hg and Pb by consumers of algae products has also been carried out. Intake of As and heavy metals through consumption of clay (as such or as ingredient of FS) has not been integrated in the scope of this assessment.

3.2.3.2.1 As intake via food supplements containing algae

Several scenarios were elaborated taking into account the contamination data available for total As and for iAs (data from the control programme of the Federal Agency for the Safety of the Food Chain - FASFC), as well as data on the consumption of the targeted FS (information available from the labels). Each scenario corresponds to one given FS that contains the algal ingredients. Total As and iAs intake for the selected scenarios are presented in Table 4.

Table 4 *Estimated intake of tAs and iAs via FS containing algae (products analysed as sold)*

FS #	Algae (species)	FS daily intake* (g/d)	tAs content (µg/g)	iAs content (µg/g)	tAs intake (µg/kg bw/d)	iAs intake (µg/kg bw/d)
22	Ascolphyllum nodosum	1.8	5.000	0.11	0.1286	0.00283
9	Chlorella	1	0.630	0.031	0.0090	0.00044
10	Chlorella	1	0.670	0.03	0.0096	0.00043
11	Chlorella	3	0.500	0.035	0.0214	0.00150
2	Fucus vesiculosus	12	0.092	0.042	0.0158	0.00720
3	Fucus vesiculosus	12	0.053	0.012	0.0091	0.00206
4	Fucus vesiculosus	10	0.226	0.006	0.0323	0.00086
12	Fucus vesiculosus	2.55	0.430	0.027	0.0157	0.00098
13	Fucus vesiculosus	1.5	5.300	0.044	0.1136	0.00094
14	Fucus vesiculosus	1.5	5.500	0.045	0.1179	0.00096
15	Fucus vesiculosus	10	0.240	0.006	0.0343	0.00086
16	Fucus vesiculosus	2.901	0.650	0.022	0.0269	0.00091
17	Fucus vesiculosus, Undaria pinnatifida	15	0.470	0.012	0.1007	0.00257
18	Kelp	0.35	23.900	0.43	0.1195	0.00215
19	Kelp	0.35	23.100	1.43	0.1155	0.00715
5	Lithothamnium calcareum	4	2.670	0.13	0.1526	0.00743
20	Lithothamnium Calcareum	0.47	2.260	0.006	0.0152	0.00004
21	Lithothamnium Calcareum	2.22	0.520	0.023	0.0165	0.00073
1	Spirulina	0.85	3.550	0.034	0.0431	0.00041
6	Spirulina	10	0.200	0.023	0.0286	0.00329
7	Spirulina	3	0.440	0.083	0.0189	0.00356
8	Spirulina	15	0.300	0.02	0.0643	0.00429

* According to the instructions on the FS label

3.2.3.2.2 As intake via algae consumption

In the absence of data on the consumption of edible algae by the Belgian population, some scenarios were built for long term exposure, based on information gathered by the Department of Public Health (Unit Nutrition and Food Safety) of the Ghent University. So, we estimated the algae consumption to 7 g/person/day for specific consumers (e.g. vegetarians), whilst the worst case estimate for heavy consumers corresponded to 21 g/person/day.

Contamination data for total and iAs in the edible algae were collected from international publications (cfr. Annexe 1). Because a sufficient number of studies provided consistent data on both total and iAs, studies that presented only data on total As where not retained for use in our study. In addition, only results from commercially available samples were selected. Samples from algae directly collected in the field were not included.

Table 5 *Estimated intakes of tAs and iAs via edible algae (Hijiki excluded) by several groups of consumers (products analysed as sold)*

Algae (species)	**Daily intake** (g/d)	**tAs content** (µg/g)	**Mean iAs content** (µg/g)	**iAs intake** (µg/person/d)	**iAs intake** (µg/kg bw/d)
All species except Hijiki	7*	32	0.35	2.45	0.035
All species except Hijiki	21**	32	0.35	7.35	0.105

* Vegetarian diet (long term exposure)
** "Worst case" vegetarian diet (long term exposure)

Table 6 *Estimated intakes of tAs and iAs via Hijiki algae by several groups of consumers (products analysed as sold)*

Algae (species)	**Daily intake** (g/d)	**tAs content** (µg/g)	**Mean iAs content** (µg/g)	**iAs intake** (µg/person/d)	**iAs intake** (µg/kg bw/d)
Hijiki	7*	105	64.8	453.6	6.48
Hijiki	21**	105	64.8	1360.8	19.44

* Vegetarian diet (long term exposure)
** "Worst case" vegetarian diet (long term exposure)

3.2.3.2.3 Cd, Hg and Pb intake via algae consumption

To estimate the intake of Cd, Hg and Pb via algae consumption, the same scenarios were taken as those elaborated for the estimation of As intake (see 3.2.3.2.2). As for the contamination data, the results of Cd, Hg and Pb determination of edible algae carried out by FASFC in 2012 were used. Those data are summarized in table 7.

Table 7 *Analysis results for edible algae (as sold) (n = 9) sampled by the FASFC in 2012. For the calculations of mean and median, the values < LOQ were set equal to LOQ)/2*

	Cd (mg/kg)	**Hg** (mg/kg)	**Pb** (mg/kg)
Minimum	< 0.01	< 0.01	< 0.02
Maximum	1.65	0.06	2.76
Median	0.01	0.005	0.11
Mean	0.42	0.015	0.524

Estimates of the intake of Cd, Hg and Pb via the consumption of edible algae are presented in table 8.

Table 8 *Estimated (long term) intake of Cd, Hg and Pb via edible algae (as sold) by specific consumers*

Daily intake (g/d)	**Mean Cd content** (mg/kg)	**Cd intake** (µg/kg bw/d)	**Mean Hg content** (mg/kg)	**Hg intake** (µg/kg bw/d)	**Mean Pb content** (mg/kg)	**Pb intake** (µg/kg bw/d)
7*	0.42	0.042	0.016	0.0016	0.524	0.052
21**	0.42	0.126	0.016	0.0048	0.524	0.157

* Vegetarian diet (long term exposure)
** "Worst case" vegetarian diet (long term exposure)

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3.2.3.3 Risk characterisation

3.2.3.3.1 As in food supplements containing algae

The risk characterization for As in FS has been carried out for different scenarios for which information on As intake has been obtained (see 3.2.3.2.1 exposure assessment). Intake of iAs could be compared to background mean dietary intake of the Belgian adult population as well as to some of the HBGV (Table 9).

Table 9 *Risk characterization for iAs intake following consumption of FS containing algae*

FS #	Algae (species)	Intake via FS consumption (µg/kg bw/d)	Number of times background intake of the Belgian adult population (0.11 µg iAs/kg bw/d)	% HBGV for acute toxicity (5 µg iAs/ kg bw/d)	MoE for chronic toxicity (BMDL = 0.3 - 8 µg iAs/kg bw/d)
22	Ascolphyllum nodosum	0.00283	0.02571	0.05657	106 – 2827
9	Chlorella	0.00044	0.00403	0.00886	677 – 18182
10	Chlorella	0.00043	0.00390	0.00857	700 – 18605
11	Chlorella	0.00150	0.01364	0.03000	200 – 5333
2	Fucus vesiculosus	0.00720	0.06545	0.14400	42 – 1111
3	Fucus vesiculosus	0.00206	0.01870	0.04114	146 – 3883
4	Fucus vesiculosus	< 0.00086	< 0.00779	< 0.01714	> 350 - > 9300
12	Fucus vesiculosus	0.00098	0.00894	0.01967	305 – 8163
13	Fucus vesiculosus	0.00094	0.00857	0.01886	318 – 8511
14	Fucus vesiculosus	0.00096	0.00877	0.01929	311 – 8333
15	Fucus vesiculosus	< 0.00086	< 0.00779	< 0.01714	> 350 - > 9300
16	Fucus vesiculosus	0.00091	0.00829	0.01823	329 – 8791
17	Fucus vesiculosus, Undaria pinnatifida	0.00257	0.02338	0.05143	117 – 3113
18	Kelp	0.00215	0.01955	0.04300	140 – 3721
19	Kelp	0.00715	0.06500	0.14300	42 – 1119
5	Lithothamnium calcareum	0.00743	0.06753	0.14857	40 – 1077
20	Lithothamnium Calcareum	< 0.00004	< 0.00037	< 0.00081	> 7447 - > 200000
21	Lithothamnium Calcareum	0.00073	0.00663	0.01459	411 – 10959
1	Spirulina	0.00041	0.00375	0.00826	727 – 19512
6	Spirulina	0.00329	0.02987	0.06571	91 – 2432
7	Spirulina	0.00356	0.03234	0.07114	84 – 2247
8	Spirulina	0.00429	0.03896	0.08571	70 - 1865

From the results obtained, it appears that the intake of iAs through the consumption of several FS (for which data on contamination and realistic consumption rate are available) is rather low (< 0.00004 – 0.00743 µg iAs/kg bw/d) compared to the background dietary intake by the Belgian adult population of 0.11 µg iAs/kg bw/d (SPECAS, 2010). Also, the intake is small compared to the HBGV for acute toxicity (5 µg iAs/kg bw/d). The MoE for chronic toxicity (BMDL of 0.3 µg iAs/kg bw/d) is rather high, ranging from 40.0 (FS#5) to > 7447 (FS#20). It must be noted, however, that this intake has to be added to the background intake and, thus, that the cumulated intake will be characterized by quite low MoE figures, as it has already been pointed out in other studies (SPECAS, 2010).

3.2.3.3.2 Inorganic As in edible algae

Table 10 *Risk characterization for iAs intake following consumption of edible algae (Hijiki species excluded) by specific consumers*

Algae (species)	**Daily intake** (g/d)	**iAs intake via edible algae consumption** (µg/kg bw/d)	**Number of times background intake** (0.11 µg/kg bw/d)	**% HBGV for acute toxicity**	**MoE** (BMDL range = 0.3 - 8 µg iAs/kg bw/d)
All species except Hijiki	7*	0.035	0.32	0.70	8.6 - 229
All species except Hijiki	21**	0.105	0.95	2.10	2.9 - 76.0

* Vegetarian diet (realistic case for long term exposure)
** Vegetarian diet (worst case for long term exposure)

From table 10 it becomes clear that the rather small consumption of edible algae (other than Hijiki) as recommended in the diet of some vegetarians could already contribute significantly to the exposure to iAs, ranging from 1/3 to 1 times the estimated background intake of Belgian consumers. In terms of acute toxicity, those levels of exposure are still well below the HBGV. For the chronic toxicity, however, it has to be noted that, when using the lowest BMDL of 0.3 µg/kg bw/d, the MoE is small (< 100) for average vegetarian consumers. For high algae consumers the MOE is also low (< 100), even when compared to the upper range of BMDL-values. Here, summing up the background intake and additional intakes such as those recommendable by some nutritionists to vegetarians, can lead to possible exposure levels close to the lowest BMDL, without any margin of exposure left anymore.

Table 11 *Risk characterization for iAs intake following consumption of Hijiki algae by specific consumers*

Algae (species)	**Daily intake** (g/d)	**iAs intake via edible algae consumption** (µg/kg bw/d)	**Number of times background intake** (0.11 µg/kg bw/d)	**% HBGV for acute toxicity**	**MoE** (BMDL range = 0.3 - 8 µg iAs/kg bw/d)
Hijiki	7*	6.48	58.91	129.6	0.046 – 1.235
Hijiki	21**	19.44	176.73	388.8	0.015 – 0.412

* Vegetarian diet (realistic case for long term exposure)
** Vegetarian diet (worst case for long term exposure)

The figures shown in Table 11 and corresponding to consumption of Hijiki algae either according to a low or to a high consumption rate, show that the intake of iAs is estimated to be very high whatever scenario selected. The estimated intakes are by far more important than the background intake and do exceed the HBGV for acute toxicity. In terms of chronic toxicity, the estimated intake associated to the selected scenarios are always much higher than the lowest dose associated to cancer induction. Hijiki algae consumption seems thus to be associated to severe health risks and must be avoided at whatever consumption rate.

3.2.3.3.3 Cd, Hg and Pb in edible algae

The results of the risk characterization exercise for As, Cd, Hg and Pb present in edible algae are presented in table 12.

Table 12 *Risk characterization following Cd, Hg and Pb intake via consumption of edible algae. Background intake is 0.14 µgCd/kg bw/day (Sci Com, 2009a), 0.047 µg MeHg/kg bw/day (Sioen, 2007) and 0.13 µg Pb/kg bw/day (Sci Com, 2009b)*

Element	**Daily intake** (g/d)	**Element intake via edible algae consumption** (µg/kg bw/d)	**Number of times background intake**	**% HBGV for acute toxicity**	**% HBGV for chronic toxicity**	**MoE**
Cd	7*	0.042	0.30	0.98	11.76	N.A.
	21**	0.126	0.90	2.93	35.28	N.A.
Hg (MeHg)	7*	0.0016	0.034	0.12	0.86	N.A.
	21**	0.0048	0.102	0.37	2.58	N.A.
Pb	7*	0.052	0.4	N.A.	N.A.	9.6 – 28.8
	21**	0.157	1.21	N.A.	N.A.	3.2 – 9.6

* Vegetarian diet (realistic case for long term exposure)
** Vegetarian diet (worst case for long term exposure)
N.A.: Not applicable; N.R.: not realistic (this scenario is considered as non plausible)

Overall it can be said that the potential intake of Cd and Pb linked to the consumption of edible algae is far from negligible, being within the range of the background dietary intake of the adult population in case of a (long term) daily consumption of 21 g algae (i.e. the worst case long term exposure scenario considered for vegetarians). As to the exposure to Hg, information is lacking about the chemical species present in edible algae (only total Hg has been measured). Table 12 presents, however, the results of the risk characterization considering that all the Hg is present in algae as MeHg. In this case, it appears that, on the long term, a daily consumption of 21 g algae will lead to an exposure reaching 10 % of the background intake.

For the other elements it was unfortunately not possible to compare the intake with the HBGV for acute toxicity since there is a lack of information about the actual metal concentrations in re-hydrated or cooked seaweed (i.e consumed like a fresh salad). However, by adopting a conservative approach and considering that the metal content remains unchanged in the fresh consumed product, it appears that, for Cd, the intake could be very high compared to the background intake or compared to the relevant HBGV for acute toxicity.

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3.2.4 Uncertainties

Aspects contributing to the uncertainty of the present risk assessment:

3.2.4.1 Analytical methods used and chemical species considered

No internationally standardised method currently exists for the determination of iAs in algae or FS based on algae. Most literature data on iAs in algae are the result of applying one particular analytical method, which is based on the solubilisation of iAs with strong HCl to form a covalent halide and subsequent extraction with a non-polar solvent, followed by back extraction of iAs and HG-AAS detection.

Other analytical options (e.g. HPLC-ICP-MS : high performance liquid chromatography for the separation of species, with inductively coupled plasma mass spectrometry, etc.), however, exist and have been applied frequently for the analysis of iAs in other matrices. HPLC-ICP-MS results from algae samples, as obtained in the SPECAS and BIOTRAS project seem to give rise to similar results, but the number of algae samples analysed in the latter studies was too limited to allow a well-funded comparison.

It should also be indicated that only iAs was considered in the risk assessment. Algae may however also contain high amounts of organic As compounds. These components could however not be taken up due to the lack of precise analytical methods and toxicological information (e.g. arsenolipids and arsenosugars).

Finally, it should be noted that the sampling procedure prior to preservation is also a point of attention. To preserve the speciation of arsenic compounds in a sample, it is advised to flash freeze the sample in liquid nitrogen and subsequently store it at -20°C. A slow freezing process may convert As species, leading to an inaccurate assessment of As speciation in a sample.

3.2.4.2 Lack of consumption data

Data on consumption of FS derived from algae are lacking. Hence, it was only possible to work out some scenarios based on the instructions written on the labels.

Additionally, it is not known how long consumers do take such FS (occasionally or for long periods). The same is true for the consumption of edible algae. Here also realistic scenarios for long term (vegetarians) exposure had to be built.

In addition, there is a lack of data on the consumption of specific species such as Hijiki algae for example.

Moreover, there is a need to have more information on profiles particularly at risk such as, for example, people cumulating iAs highly contaminated foodstuffs such as some specific FS, edible algae, rice and rice derived products.

On the other hand, there is also need for more data in specific population groups, such as pregnant women and children.

3.2.4.3 Uncertainties about the effect of food processing before consumption

Occurrence data were obtained from official national controls, analysing the products as sold (in case of FS). The potential leaching of As to the cooking liquid and/or hydratation water was not taken into account because only for Hijiki leaching data were available. Because cooking may

lead to a leaching of As from the matrix to the cooking liquid, this may lead to an overestimation of the exposure. In this respect recent BIOTRAs results indicate that it is advisable to cook As containing food matrices in plenty of water.

Some studies have investigated the effect of cooking on As concentration in seaweeds. In a study of Wondimu *et al*. (2007) and in the BIOTRAS project (2014) a reduction of respectively 40 % and 38 % of the tAs concentration in Hijiki was found after soaking and washing of the sample at room temperature. Both studies also confirmed that heating/cooking further reduced the As concentration. Wondimu *et al*. (2007) showed in natural Hijiki samples that the leaching effect was temperature dependent with the strongest reduction at 55 °C to 75 °C (60 % reduction), but again, the effect was smaller at boiling temperature (100 °C). In the BIOTRAS project the reduction caused by "washing+boiling" was 60 %, which corresponded to the largest decrease in Wondimu's study.

However, because of the limited availability of this kind of data for other types of algae, and to avoid wrong estimates of this reduction, we decided not to include the effect of leaching for the calculations in the present study. While some producers also claim that preparation steps can significantly reduce the fraction of iAs, exisiting studies show no (Rose *et al*., 2007) or only a very small effect (BIOTRAS, 2014).

Another point of attention for future research concerns the presystemic metabolism of arsenic. While As metabolism by human cell types has been described before, speciation changes of As by gut microorganisms is highly feasible. Studies with human gut microbiota indicate the potency of the gut microbiome to reduce, methylate and even thiolate arsenicals. Due to a difference in gut microbiome composition, this metabolic potency is characterized by a high interindividual variability. It is noteworthy that food matrix composition can also impact the efficiency at which As speciation changes take place. For example, As sorbed to dietary fiber in a food matrix may get released from that matrix under colon conditions due to fibre fermentation. That As fraction can then be metabolized by colon microbiota and subsequently be absorbed.

3.2.5 Recommendations

3.2.5.1 Recommendations for risk management

The current legislation is based on the determination of tAs whilst Health Based Guidance Values are only available for iAs. The current legislation needs to be adapted because there is no direct link between tAs and iAs in algae products.

Taken into account the above mentioned uncertainties related to the lack of toxicological information of many chemical species other than the iAs, the SHC recommends to set legal maximum levels based on "tAs, reduced by AsB" (cf. Feldman *et al*., 2011) instead of "tAs", as it is the case in current legislation.

Furthermore, the SHC recommends the competent bodies to take appropriate measures at national level (and by extension at European level) according to the findings of this report.

3.2.5.2 Recommendations for consumers

- Consumption of Hijiki has to be avoided;
- Restrain the consumption of other edible algae to 7 g (i.e. half a spoon of dried material) per day;
- Be aware that a normal diet does already provide a relatively high exposure to iAs;
- Since As is known to leach (partly) to the cooking liquid, it is advised not to consume the cooking liquid;
- Consumption of (food supplement based on) algae is not recommended for children and pregnant women;
- Consumers should avoid large cumulated intake of foodstuffs, substantially contributing to iAs exposure such as rice, algae and derived products.

3.2.5.3 Recommendations for research

- A comparison of iAs results in algae obtained by different analytical methods is necessary to confirm the trueness of the results;
- Toxicology of arsenosugars and arsenolipids, identification and quantitative analysis of all As species to be covered by the legislation;
- Risks related to As (and other heavy metals) due to the consumption of clay minerals (a.o. as "detoxification");
- Consumption data and/or total diet study with focus on the foodstuffs as consumed (cooked or rehydrated) in order to better assess the intake of As and other toxic elements.

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5. ANNEXES

ANNEXE 1: Table of data on FS selected from literature

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ALGAE TYPE	Species	common name or use	TOTAL As	INORGANIC As	RATIO		Reference	Origin
			total As (mg/kg DM)	iAs or As(v) (mg/kg DM)	% iAs of total			
GREEN ALGAE (CHLOROPHYTA	Enteromorpha sp,	green nori flakes (dried edible sea algae)	2,3	0,37	16,1	as sold	Almela et al., 2002	
	Enteromorpha sp,		2,15	0,346	16,1	as sold	Almela et al., 2006	unknown
	Ulva pertusa	AO nori (dried edible sea algae)	5,17	0,36	7,0	as sold	Almela et al., 2002	
	Ulva pertusa		3,24	0,268	8,3	as sold	Almela et al., 2006	unknown
	Ulva rigida	Sea lettuce	6,61-7,06	0,151-0,177		D.W.	Besada et al., 2009	
RED ALGAE (RHODOPHYTA)	Porphyra sp	NORI ?	33,6			as sold	almela et al., 2005	Spain
	Porphyra sp	NORI ?	27,19			as sold	almela et al., 2005	Spain
	Porphyra sp	NORI ?	32,7	0,189	0,6	as sold	almela et al., 2006	Japan
	Porphyra sp	NORI ?	24,3	0,383	1,6	as sold	almela et al., 2006	Spain
	Porphyra sp	NORI ?	20,8	0,176	0,8	as sold	almela et al., 2006	Korea
	Porphyra sp	NORI ?	18,4	0,131	0,7	as sold	almela et al., 2006	South Korea
	Porphyra sp	NORI ?	23,5	0,116	0,5	as sold	almela et al., 2006	South Korea
	Porphyra sp	NORI ?	41,7	0,402	1,0	as sold	almela et al., 2006	China
	Porphyra sp	NORI ?	58,3	0,223	0,4	as sold	almela et al., 2006	China
	Porphyra tenera		24,1	0,28	1,2	as sold	almela et al., 2006	Japan
	Porphyra tenera		23,2	0,167	0,7	as sold	almela et al., 2006	Japan
	Porphyra tenera	nori (dried edible sea algae)	23,7	0,57	2,4	as sold	Almela et al., 2002	
	Porphyra tenera	nori (dried edible sea algae)	28,3	0,19	0,7	as sold	Almela et al., 2002	
	Porphyra tenera	toasted nori (dried edible sea algae)	30	0,314	1,0	as sold	Almela et al., 2002	
	Porphyra umbilicalis	NORI	34,5	0,239	0,7	as sold	almela et al., 2006	Spain
	Porphyra umbilicalis	NORI	28,9-49,5	0,132-0,338		D.W.	Besada et al., 2009	
		NORI	23	<0,3	<1,3	as sold	Rose et al. 2007	China
		NORI	22	<0,3	<1,4	as sold	Rose et al. 2007	Japan
		NORI	18	<0,3	<1,6	as sold	Rose et al. 2007	Japan
		NORI	26	<0,3	<1,1	as sold	Rose et al. 2007	China
		NORI	32	<0,3	<0,9	as sold	Rose et al. 2007	unknown

		NORI	18	<0,3	<1,6	as sold	Rose et al. 2007	unknown
		NORI	29	<0,3	<1,0	as sold	Rose et al. 2007	USA
		NORI	35,5	0,15	0,43		BIOTRAS	
		NORI	34	0,08	0,22		BIOTRAS	
		NORI	29,5	0,06	0,19		BIOTRAS	
		NORI	21,9	0,14	0,64		SPECAS, 2010	
	Palmaria palmata	Atlantic dulse (dried tender Japanese sea algae)	7,56	0,44	0,1	as sold	Almela et al., 2002	
	Palmaria sp,		13	0,466	3,6	as sold	Almela et al., 2006	Spain
	Palmaria palmata		12,6	0,595	4,7	as sold	Almela et al., 2006	Japan
	Rhodymenia palmata		8,8	0,153	1,7	as sold	Almela et al., 2006	unknown
	Rhodymenia palmata		7,68	0,152	2,0	as sold	Almela et al., 2006	unknown
	Chondrus crispus	Irish moss	12,7	0,357	2,8	as sold	Almela et al., 2006	Spain
	Chondrus crispus	Irish moss	16,1	0,842	5,2	as sold	Almela et al., 2006	Spain
	Chondrus crispus	Irish moss	18,2	0,51	2,8	as sold	Rose et al. 2007	Spain (dried at 40°C)
	Chondrus crispus	Irish moss	23,2-25,5	0,217-0,225		D.W.	Besada et al., 2009	
	Gelidium spp.	Agar	<0,05-0,21	0,025-0,135		D.W.	Besada et al., 2009	
BROWN ALGAE (PHAECOPHYTA)	Undaria pinnatifida	WAKAME (dried edible sea algae)	32	0,15	0,5	as sold	Almela et al., 2002	
	Undaria pinnatifida	WAKAME (dried edible sea algae)	42	0,26	0,6	as sold	Almela et al., 2002	
	Undaria pinnatifida	Japanese wakame (dried tender Japanese sea algae)	34,6	0,18	0,5	as sold	Almela et al., 2002	Japan
	Undaria pinnatifida	WAKAME	41,4	<LD		as sold	almela et al., 2006	Japan
	Undaria pinnatifida	WAKAME	45,2	<LD		as sold	almela et al., 2006	Japan
	Undaria pinnatifida	WAKAME	46,2	1,12	2,4	as sold	almela et al., 2006	Spain
	Undaria pinnatifida	WAKAME	28	0,268	1,0	as sold	almela et al., 2006	Spain
	Undaria pinnatifida	WAKAME	32,3	0,371	1,1	as sold	almela et al., 2006	Spain
	Undaria pinnatifida	WAKAME	46	1,06	2,3	as sold	almela et al., 2006	Korea
	Undaria pinnatifida	WAKAME	41,5	0,61	1,5	as sold	almela et al., 2006	Japan
	Undaria pinnatifida	WAKAME	42,1-76,9	0,045-0,3446		D.W.	Besada et al., 2009	
		WAKAME	35	<0,3	<0,9	as sold	Rose et al. 2007	Korea
		WAKAME	42	<0,3	<0,7	as sold	Rose et al. 2007	Korea

		WAKAME	34	<0,3	<0,9	as sold	Rose et al. 2007	Korea
		WAKAME	29	<0,3	<1,0	as sold	Rose et al. 2007	unknown
		WAKAME	36	<0,3	<0,8	as sold	Rose et al. 2007	Japan
		WAKAME	29,8	0,128	0,4	as sold	SPECAS, 2010	
	Laminaria japonica	kombu (dried sea algae)	53	0,254	0,5	as sold	Almela et al., 2002	
	Laminaria japonica	Japanese kombu (dried tender Japanese sea algae)	47	0,297	0,6	as sold	Almela et al., 2002	Japan
	Laminaria japonica	KOMBU	116	1,44	1,2	as sold	almela et al., 2006	Japan
	Laminaria japonica	KOMBU	104	0,238	0,2	as sold	almela et al., 2006	Japan
	Laminaria sp		39,6	0,473	1,2	as sold	almela et al., 2006	Spain
	Laminaria sp		48,3	0,145	0,3	as sold	almela et al., 2006	Japan
	Laminaria digitata		65,7	0,251	0,4	as sold	almela et al., 2006	Japan
		KOMBU	51	<0,3	<0,6	as sold	Rose et al. 2007	unknown
		KOMBU	32	<0,3	<0,9	as sold	Rose et al. 2007	Japan
		KOMBU	69	<0,3	<0,4	as sold	Rose et al. 2007	Japan
		KOMBU	75	<0,3	<0,4	as sold	Rose et al. 2007	Korea
		KOMBU	75	<0,3	<0,4	as sold	Rose et al. 2007	USA
		KOMBU	19	[0,3]	[1,6]	as sold	Rose et al. 2007	Japan
		KOMBU	28	<0,3	<1,1	as sold	Rose et al. 2007	Japan
		KOMBU	51,7-68,3	0,052-0,443		D.W.	Besada et al., 2009	
		ARAME	32	<0,03	<0,9	as sold	Rose et al. 2007	Japan
		ARAME	31	<0,03	<1,0	as sold	Rose et al. 2007	Japan
		ARAME	28	<0,03	<1,1	as sold	Rose et al. 2007	Japan
	Eisenia bicyclis	ARAME	22,4	0,167	0,7	as sold	almela et al., 2006	Japan
	Eisenia bicyclis	ARAME	25,2	1,35	5,4	as sold	almela et al., 2006	Japan
	Eisenia bicyclis	ARAME	26,3	0,135	0,5	as sold	almela et al., 2006	Japan
	Eisenia bicyclis	ARAME	4,1	0,292	7,1	as sold	almela et al., 2006	Japan
	Eisenia bicyclis	ARAME	26,6	0,206	0,8	as sold	almela et al., 2006	Japan
	Eisenia bicyclis	ise wild arame (dried tender Japanese sea algae)	23,8	0,17	0,7	as sold	Almela et al., 2002	Japan
	Eisenia bicyclis	ise wild arame (dried edible sea algae)	29	0,185	0,6	as sold	Almela et al., 2002	

	Eisenia bicyclis	arame (dried edible sea algae)	30	0,15	0,5	as sold	Almela et al., 2002	
	Eisenia bicyclis	ARAME	27,9-34,1	0,041-0,170		as sold	Besada et al., 2009	
	Himanthalia elongata	SEA SPAGHETTI	23,6	<LD		as sold	almela et al., 2006	Spain
	Himanthalia elongata	SEA SPAGHETTI	31,2	0,202	0,6	as sold	almela et al., 2006	Spain
	Himanthalia elongata	SEA SPAGHETTI	21,3	<LD		as sold	almela et al., 2006	Spain
	Himanthalia elongata	SEA SPAGHETTI	32,9-36,7	0,166-0,245		D.W.	Besada et al., 2009	
	Fucus vesiculosus	FUCUS	40,4	0,291	0,7	as sold	almela et al., 2006	unknown
	Fucus vesiculosus	alga fucus	50	0,34	0,7	as sold	Almela et al., 2002	
	Durvillaea antartica		15,2	0,318	2,1	as sold	almela et al., 2006	Chile
		GEM	**32,0**	**0,35**				
		MIN	**2,15**	**0,056**				
		MAX	**116**	**1,440**				

BROWN ALGAE (PHAECOPHYTA)	Hisikia fusiforme	Hiziki	128	88	68,8	as sold	Almela et al., 2002	
	Hisikia fusiforme	Hijiki (dried edible sea alga)	141	85	60,3	as sold	Almela et al., 2002	
	Hisikia fusiforme	Japanese hijiki (dried tender Japanese sea algae)	115	83	72,2	as sold	Almela et al., 2002	Japan
	Hisikia fusiforme	HIJIKI	103,73	13,2	12,7	as sold	almela et al., 2005	Spain
	Hisikia fusiforme	HIJIKI	131,61	8,65	6,6	as sold	almela et al., 2005	Spain
	Hisikia fusiforme	HIJIKI	111	75,4	67,9	as sold	almela et al., 2006	Japan
	Hisikia fusiforme	HIJIKI	89,2	41,6	46,6	as sold	almela et al., 2006	Japan
	Hisikia fusiforme	HIJIKI	114	91,2	80,0	as sold	almela et al., 2006	Japan
	Hisikia fusiforme	HIJIKI	131	81,1	61,9	as sold	almela et al., 2006	Japan
	Hisikia fusiforme	HIJIKI	93,9	61,6	65,6	as sold	almela et al., 2006	Japan
	Hisikia fusiforme	HIJIKI	124	80,3	64,8	as sold	almela et al., 2006	Japan
	Hisikia fusiforme	HIJIKI	149	117	78,5	as sold	almela et al., 2006	Japan
	Hisikia fusiforme	HIJIKI	68,3	43,7	64,0	as sold	almela et al., 2006	Japan
	Hisikia fusiforme	HIJIKI	106	69,4	65,5	as sold	almela et al., 2006	Japan
	Hisikia fusiforme	HIJIKI	107	73	68,2	as sold	Rose et al. 2007	Japan
	Hisikia fusiforme	HIJIKI	112	80	71,4	as sold	Rose et al. 2007	Japan

	Hisikia fusiforme	HIJIKI	116	83	71,6	as sold	Rose et al. 2007	Japan
	Hisikia fusiforme	HIJIKI	100	69	69,0	as sold	Rose et al. 2007	Japan
	Hisikia fusiforme	HIJIKI	95	67	70,5	as sold	Rose et al. 2007	Japan
	Hisikia fusiforme	HIJIKI	110	81	73,6	as sold	Rose et al. 2007	Japan
	Hisikia fusiforme	HIJIKI	112	76	67,9	as sold	Rose et al. 2007	Japan
	Hisikia fusiforme	HIJIKI	102	72	70,6	as sold	Rose et al. 2007	Japan
	Hisikia fusiforme	HIJIKI	124	96	77,4	as sold	Rose et al. 2007	Japan
	Hisikia fusiforme	HIJIKI	80,9	27,7	34,2	as sold	Shimoda et al. 2010	
	Hisikia fusiforme	HIJIKI	112,6	55,8	49,6	as sold	Shimoda et al. 2010	
	Hisikia fusiforme	HIJIKI	86,7	51,2	59,1	as sold	Shimoda et al. 2010	
	Hisikia fusiforme	HIJIKI	86,3	51,1	59,2	as sold	Shimoda et al. 2010	
	Hisikia fusiforme	HIJIKI	118,6	71,3	60,1	as sold	Shimoda et al. 2010	
	Hisikia fusiforme	HIJIKI	37,1	9,2	24,8	as sold	Shimoda et al. 2010	
	Hisikia fusiforme	HIJIKI	48,7	13,6	27,9	as sold	Shimoda et al. 2010	
	Hisikia fusiforme	HIJIKI	103-147	32,1-69,5		D.W.	Besada et al., 2009	
	Hisikia fusiforme	HIJIKI	110	94	85,0	as sold	BIOTRAS, 2014	
		GEM	**105**	**64,8**	**59,9**			
		MIN	**37,1**	**8,7**				
		MAX	**149,0**	**117,0**				

*Data in grey were not taken into account for the calculations

AAS = atomic-absorption spectrometry
FI-HG-AAS = flow injection-hydride generation atomic-absorption spectrometry
HPLC-ICP-MS = high performance liquid - chromatography inductively coupled plasma mass spectrometry
ICP-MS = chromatography inductively coupled plasma mass spectrometry

ANNEXE 2: As speciation analysis

As speciation analyses consist of various steps involving on the one hand extraction and/or separation of As species, and on the other hand their detection and quantification. For each step different techniques are available that can be combined in various ways to obtain quantitative information on As species concentrations. A schematic overview of methods and combinations that have been described in literature is presented in Figure 1 (Chen *et al*., 2014).

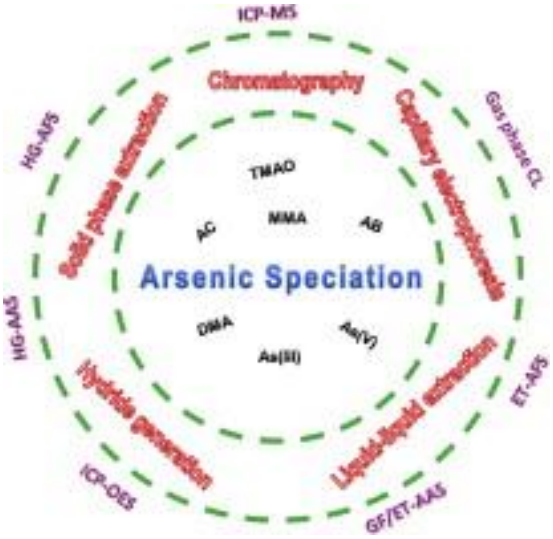

Figure 1 *Methods and method combinations that have been applied for extraction and/or separation of As species on the one hand (inner circle), and detection and quantification (outer circle) on the other hand. (Chen et al., 2014)*

In recent years the most commonly applied method for As speciation analysis has been the hyphenation of HPLC (high performance liquid chromatography) for the separation of species, with ICP-MS (inductively coupled plasma mass spectrometry) as element specific detector. Numerous studies that provide iAs results of large sample series have used this method, although the specific conditions of extraction and separation vary among studies. However, with upcoming regulations on the iAs concentration in the EU (actually only for rice and some rice products), considerable research efforts are directed towards the development of techniques that are faster and cheaper than HPLC-ICP-MS, but still offer sufficiently low detection limits (e.g. Musil *et al*., 2014).

It is beyond the scope of the present report to discuss all analytical possibilities that have been tested and reported in literature. For a recent review on As speciation methods we refer to Chen *et al.* (2014).

However, because the source data used in the current study originate from different studies that have applied different speciation methodologies, some comments and explanations to the methods used in these studies will be given below. These methods at the same time cover the ones that have been applied the most frequently in data-generating studies.

According to Francesconi and Edmunds (1998), methods for the identification and quantification of As species in marine samples may be divided into three categories depending on the level of information they provide:

- first level techniques, which only differentiate between iAs and organic As;
- second level techniques which are capable of analysing the two inorganic forms in addition to three of the simple methylated As species (MA, DMA, and TMAO: trimethylamine-N-oxide);

- third level techniques which extend the range to quaternary compounds such as AsB, and other more complex As species such as arsenosugars.

The following paragraphs extends for each of these levels on some of the relevant methods in relation to the source data on iAs used in the present report.

First level techniques include e.g. those based on the conversion of iAs to a covalent halide ($AsCl_3$ by treatment of the sample with strong HCl and e.g. KI (to reduce As^V to As^{III}). The $AsCl_3$ is then separated from the organoarsenic constituents by distillation or by extraction with a non-polar solvent. As in the two fractions is subsequently determined by standard techniques for the determination of tAs. These methods were employed widely in the 1970s and 1980s (e.g. Flanjack, 1982 in: Francesconi and Edmonds, 1998), but some variants – including a back extraction step - are still in use today (e.g. Munoz *et al*., 1999).

Although the first level techniques provide only limited information about As species, they remain relevant for human health studies which generally consider only the inorganic portion of total As. The method also has the advantage of being accessible to laboratories with only routine analytical instruments such as atomic absorption spectrometers. In addition, the method determines the inorganic/organic As quantities on the whole sample, not an extract thereof. Consequently, potential problems associated with extraction of biological tissue, needed for the solubilisation of As compounds, are avoided (Franscesconi and Edmonds, 1998).

Second level analyses for As species are provided by hydride generation (HG) techniques. The hydride generation technique, which makes use of a separation of the analyte element from the matrix by conversion to its volatile hydride, was first used to lower detection limits for total concentrations of elements like antimony, As, selenium (Se), on AAS (atomic absorption spectrometry) or ICP-OES (inductively coupled plasma atomic emission spectrometry). For the synthesis of these hydrides sodium tetrahydroborate ($NaBH_4$; sodium borohydride) is now almost universally applied. Because As^{III} and As^V react at different rates with sodium tetrahydroborate[II1] in acid solution it is normal practice to add e.g. iodide to the acidified sample solution to reduce As^V to As^{II1} prior to reaction with $NaBH_4$ when total As has to be measured.

The methyl derivatives (e.g. MA - monomethylarsonic acid, DMA) of many of the hydride forming elements can also be volatilised and determined following $NaBH_4$ reduction. For As speciation purposes the volatile arsines which may be flushed from the sample matrix, can be collected in a cold trap, and then separated from each other by e.g. fractional distillation or gas chromatography prior to As specific detection (Francesconi and Edmunds, 1998). Alternatively, some authors have exploited the difference in the rate of reduction of As species for the direct application of the HG technique for As speciation analysis, without a prior step of species separation (e.g. Anderson, Thompson and Culbard, 1986). In particular direct determination of iAs has been described based on the observation that iAs forms volatile arsine species with high efficiency when treated with $NaBH_4$ at acidic conditions, whereas most organoarsenic compounds do not form any or only less volatile arsines. High concentrations of HCl further reduce the production of the less volatile arsines (Petursdottir *et al*.,2014).

In the latter conditions, however, when only iAs quantification is targeted, the HG application obtains the characteristics of a first level technique. It can be used not only in combination with AAS, but also with e.g. ICP-MS (Petursdottir *et al*.,2014).

The third level methods combine chromatographic separation of the native As compounds with As-specific detection. HPLC with ion exchange or reversed phase columns is the most commonly used separation method. Although atomic absorption and atomic emission spectrometers can be directly connected to a HPLC system and used as As-specific detectors, they usually lack the low detection limits necessary to examine crude marine extracts. Mass spectrometry following decomposition of the As species and ionisation to the $^{75}As^+$ ion by ICP-MS provides the

necessary low detection limits. Such HPLC/ICP-MS systems allow the separation and determination of As species in e.g. crude marine extracts. When combined with appropriate standard compounds, HPLC/ICP-MS can provide comprehensive data on As species in marine samples (Francesconi and Edmunds, 1998). When routine analysis of only iAs is targeted, the HPLC-ICP-MS methods are rather time consuming and expensive compared to the level 1 or level 2 methods, (which therefore may be more attractive for routine purposes). It must be mentioned that also chromatographic methods, when working with reduced elution times by selection of specific elution conditions and when focussing at only 1 specific peak, tend to hold the characteristics of the first level methods, although the potential to serve as a third level methods remains present.

In the below table the source data of the present study (cf. annexe 1) are classified per technique and per level used.

First level techniques	**Second level techniques**	**Third level techniques**
Solubilization of iAs with strong HCl to form a covalent halide and subsequent extraction with a non-polar solvent, followed by back extraction of iAs and HG-AAS detection.	HG-AAS with/without separation step	HPLC-ICP-MS (anion exchange chromatography)
Almela *et al*., 2002 Almela *et al*., 2005 Almela *et al*., 2006 Besada *et al*., 2009 Rose *et al*., 2007 Shimoda *et al*., 2010	/	SPECAS, 2010 BIOTRAS, 2014

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6. COMPOSITION OF THE WORKING GROUP

All experts joined the working group ***in private capacity***. The names of the experts appointed by Royal Decree as well as members of the Committee and the Board are available on our website (web page: composition et fonctionnement). The general declarations of interests of the experts are available on our website (web page: Conflits d'intérêts).

The following experts were involved in drawing up the advisory report:

DE HENAUW Stefaan	food and public health	UGent
MAGHUIN-ROGISTER Guy	foodstuff analysis	ULg
MERTENS Birgit	toxicology, novel foods	WIV-ISP
PUSSEMIER Luc	residues and contaminants, chemical risks	CODA-CERVA
RUTTENS Ann	chemical analysis	CODA-CERVA
VLEMINCKX Christiane	toxicology	WIV-ISP

The administration was represented by:

DE BOOSERE Isabel	microbiological and chemical environmental process contaminants	FPS HFCSE, DG4

The working group was chaired by Mr. **Luc PUSSEMIER**, the scientific secretary was Ms. Anouck WITTERS.

The advisory report has been approved by the standing working group "Nutrition and Health, including Food Safety" (NHFS) on January, 28th 2015.

DESTAIN Jacqueline	industrial microbiology, technology	FUSAGx
FONDU Michel	chemistry, additives, contaminants	ULB
GOYENS Philippe	paediatrics, metabolism	ULB
KOLANOWSKI Jaroslaw	physiology and physiopathology of nutrition; physiopathology of obesity, metabolic syndrome and diabetes type 2	UCL
MAGHUIN-ROGISTER Guy	analysis of foodstuffs	ULg
MAINDIAUX Véronique	nutrition and dietetics	Institut Paul Lambin
MERTENS Birgit	toxicology, novel foods	WIV-ISP
PEETERS Marc	oncology	UA
PENNINCKX Michel	endocrinology, toxicology, biotechnology	ULB
PUSSEMIER Luc	residues and contaminants, chemical risks	CODA-CERVA
VAN DE WIELE Tom	microbiological technology, contaminants	UGent

The administration was represented by:

DE PAUW Katrien	food supplements	FPS HFCSE, DG4

The permanent working group NHFS was chaired by Mr. **Guy MAGHUIN-ROGISTER**, the scientific secretary was Ms. Anouck WITTERS.

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About the Superior Health Council (SHC)

The Superior Health Council is a federal advisory body. Its secretariat is provided by the Federal Public Service Health, Food Chain Safety and Environment. It was founded in 1849 and provides scientific advisory reports on public health issues to the Ministers of Public Health and the Environment, their administration, and a few agencies. These advisory reports are drawn up on request or on the SHC's own initiative. The SHC aims at giving guidance to political decision-makers on public health matters. It does this on the basis of the most recent scientific knowledge.

Apart from its 25-member internal secretariat, the Council draws upon a vast network of over 500 experts (university professors, staff members of scientific institutions, stakeholders in the field, etc.), 300 of whom are appointed experts of the Council by Royal Decree. These experts meet in multidisciplinary working groups in order to write the advisory reports.

As an official body, the Superior Health Council takes the view that it is of key importance to guarantee that the scientific advisory reports it issues are neutral and impartial. In order to do so, it has provided itself with a structure, rules and procedures with which these requirements can be met efficiently at each stage of the coming into being of the advisory reports. The key stages in the latter process are: 1) the preliminary analysis of the request, 2) the appointing of the experts within the working groups, 3) the implementation of the procedures for managing potential conflicts of interest (based on the declaration of interest, the analysis of possible conflicts of interest, and a Committee on Professional Conduct) as well as the final endorsement of the advisory reports by the Board (ultimate decision-making body of the SHC, which consists of 40 members from the pool of appointed experts). This coherent set of procedures aims at allowing the SHC to issue advisory reports that are based on the highest level of scientific expertise available whilst maintaining all possible impartiality.

Once they have been endorsed by the Board, the advisory reports are sent to those who requested them as well as to the Minister of Public Health and are subsequently published on the SHC website (www.shc-belgium.be). Some of them are also communicated to the press and to specific target groups (healthcare professionals, universities, politicians, consumer organisations, etc.).

In order to receive notification about the activities and publications of the SHC, please contact: info.hgr-css@health.belgium.be.

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