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Risk assessment of complex organoarsenic species in food

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

Abstract

The European Commission asked EFSA for a risk assessment on complex organoarsenic species in food. They are typically found in marine foods and comprise mainly arsenobetaine (AsB), arsenosugars and arsenolipids. For AsB, no reference point (RP) could be derived because of insufficient toxicity data. AsB did not show adverse effects in the two available repeat dose toxicity tests in rodents. It has not shown genotoxicity in *in vitro* assays. There is no indication of an association with adverse outcomes in human studies. The highest 95th percentile exposure for AsB was observed in 'Toddlers' with an estimate of 12.5 μg As/kg bw per day (AsB expressed as elemental arsenic). There is sufficient evidence to conclude that AsB at current dietary exposure levels does not raise a health concern. For glycerol arsenosugar (AsSugOH) a RP of 0.85 mg As/kg bw per day was derived based on the $BMDL_{10}$ values for cognitive and motor function in mice. A margin of exposure (MOE) of ≥ 1000 would not raise a health concern. The highest 95th percentile estimate of exposure for AsSugOH (for adult consumers of red seaweed Nori/Laver) was 0.71 μg As/kg bw per day (AsSugOH expressed as elemental arsenic), which results in an MOE > 1000, not raising a health concern. Based on qualitative consideration of all identified uncertainties, it is regarded likely that the dietary exposures to AsB and AsSugOH do not raise a health concern. No conclusions could be drawn regarding other arsenosugars. No risk characterisation could be conducted for arsenolipids, due to the lack of data.

KEYWORDS

Arsenobetaine, arsenolipids, arsenosugars, complex organoarsenic species, margin of exposure (MOE), risk assessment

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SUMMARY

In 2009, the EFSA Panel on Contaminants in the Food Chain (CONTAM) adopted a Scientific Opinion on the presence of arsenic in food. In that Opinion, complex organoarsenic species like arsenosugars and arsenolipids could not be considered in the risk characterisation, because of a lack of data. It was also concluded that the complex organoarsenic species arsenobetaine is not metabolised in humans and is excreted unchanged. While limited direct toxicity data were available, it was widely assumed not to be of toxicological concern. In 2021, the European Commission (EC) asked the European Food Safety Authority (EFSA) for an update of the 2009 risk assessment of inorganic arsenic in food, and for a consumer risk assessment for organic arsenic in food. An updated Opinion on inorganic arsenic, and an Opinion on small organoarsenic species were published in 2024. The risk assessment on complex organoarsenic species is presented here.

Complex organoarsenic species contain methyl groups and a larger organic group bound to arsenic. They are almost exclusively found in marine foods and generally only in trace amounts in terrestrial foods, and consist mainly of arsenobetaine (AsB), arsenosugars and arsenolipids. AsB is by far the major arsenic species occurring in fish, crustaceans and most molluscs. AsB is chemically stable and can be reliably quantified by most modern food analytical laboratories. Other species of the AsB group (e.g. arsenocholine (AsC)) are also sometimes reported but usually at low levels. Arsenosugars are the major arsenic species in marine seaweeds, in particular brown seaweeds. They are moderately chemically stable and, provided the analytical process is mild, they can be reliably quantified by experienced laboratories. Arsenolipids comprise several sub-groups of arsenic species (e.g. arsenic fatty acids (ASFAs) and arsenic hydrocarbons (AsHCs)), found usually at low levels (< 10%) in fish, crustaceans, molluscs and seaweeds. The various sub-groups have quite different chemical properties and stability, which complicates their measurement in food samples.

In rodents, AsB and AsC are well absorbed, not metabolised and rapidly eliminated in urine. In one study with mice, arsenosugars were partly metabolised to DMA(V) and excreted as arsenosugars or DMA(V) in the faeces (urine was not examined). No information on the toxicokinetics of arsenolipids in rodents was identified. In humans, AsB is absorbed but not metabolised and most of the AsB is excreted within days in the urine. While concentrations of AsB are usually low (only a few µg/L), they can be much higher in individuals who recently consumed fish or other seafood. Consumption of seafood is the primary reason for the high variability of total As in urine. Although AsB can also be detected in blood, quantification is challenging and therefore blood is rarely used for biomonitoring. Based on limited information there is low transfer (0.03%) to human milk.

Arsenosugars are metabolised to DMA(V) and several other minor As species, which are excreted in the urine within 4 days. There is inter-individual variability in the efficiency of arsenosugar metabolism and excretion via the urine.

Based on limited information, arsenolipids ingested from arsenolipid-containing food, are bioaccessible and bioavailable, and they are metabolised mainly to DMA(V) and some minor As species, which are excreted in the urine. Arsenolipids can also be transferred to human milk (transfer rate about 3%).

Because DMA(V) is the major urinary metabolite of both ingested iAs, and compounds as arsenosugars and arsenolipids, it cannot be used as a specific biomarker of exposure to arsenosugars or to arsenolipids.

Few toxicity studies are available on complex organoarsenic species. AsB, AsC and glycerol arsenosugar (AsSugOH) exhibited low acute toxicity with oral LD_{50} values exceeding 6000 mg/kg bw. There are indications that AsC, but not AsB, might have immunological effects. Exposing rats to AsB during pregnancy and lactation at doses up to 10 mg/kg bw per day did not result in reproductive toxicity. Mice dosed orally for 40 days with AsSugOH at 20, 30 and 50 mg/kg bw per day showed dose-related decreases in both passive avoidance and motor function.

In vitro genotoxicity studies on complex organoarsenic species were largely negative, with only a few reporting weak clastogenic effects for AsB, AsC and AsSugOH at highly cytotoxic concentrations. A single in vivo genotoxicity study was identified, which reported DNA damage in a Comet assay with lymphocytes and hippocampal tissue of mice exposed to AsSugOH.

The lack of cytotoxic and genotoxic effects in various mammalian cell systems suggests that AsB does not interact with biologically relevant macromolecules. In a study with mice, AsSugOH was found to increased oxidative stress. The arsenolipids, arsenic hydrocarbons AsHCs and AsFAs affected neurite outgrowth and mitochondrial membrane potential in vitro, AsHCs being more potent.

There are a few, mainly cross-sectional, studies examining associations between urinary excretion of various As species, in some of which AsB was quantified and health outcomes. There are no studies including quantification of arsenosugars or arsenolipids. The studies on AsB and health outcomes indicate some tendencies of inverse associations (reduced odds ratios), potentially explained by a beneficial effect of fish consumption. Therefore, the CONTAM Panel concluded that epidemiological studies provide no evidence for an association with adverse health outcomes.

The toxicity data for AsB and arsenolipids are insufficient to identify reference points (RPs) for these groups and therefore no health based guidance values (HBGVs) or margins of exposures (MOEs) could be derived. Dose–response modelling of the effects of AsSugOH on passive avoidance and motor function in a 40-day study with male mice data resulted in similar $BMDL_{10}$ values (3.7 mg/kg bw per day, expressed as AsSugOH). Overall, a value of 0.85 mg As/kg bw per day (expressed as elemental arsenic), was identified as an RP for AsSugOH. Given the limited toxicity data, it is not appropriate to establish a HBGV for AsSugOH and therefore an MOE approach was applied. The interpretation of the MOE should take into account the default uncertainty factor of 100 for inter- and intra-species differences, and an additional factor of 10 to account for major deficiencies in the database, indicating that a MOE of ≥ 1000 would not raise a health concern.

A total of 650 analytical results on AsB were submitted to EFSA. The highest numbers of samples were reported for 'Grains and grain-based products' ($n = 268$), followed by 'Fish and seafood' ($n = 131$) and 'Products for non-standard diets, food imitates and food supplements' ($n = 63$). The highest levels of AsB (all expressed as elemental arsenic on whole weight basis) among the samples of 'Fish and seafood' were reported for 'Molluscs', in particular for 'Oysters' with mean values of 1878 µg As/kg (LB = UB, $n = 27$); relatively high mean levels were also reported for 'Mussels' (853 µg As/kg, LB = UB, $n = 41$). No data on crustaceans were reported to EFSA. Among fish samples, 'Marine fish' had the highest mean levels (1106 µg As/kg, LB = UB, $n = 7$), followed by 'Diadromous fish' (276 µg As/kg, LB = UB, $n = 12$) and 'Freshwater fish' (LB-UB, 48.5–49.9 µg As/kg, $n = 6$). Considering the uncertainties associated with the AsB levels reported for seaweed and alga-based supplements, only occurrence data from fish, seafood and fish-based processed commodities were used to estimate dietary exposure to AsB.

As regards to arsenosugars, no occurrence data were reported to EFSA. To conduct the dietary exposure assessment, occurrence data on AsSugOH were extracted from the scientific literature with a focus on seaweeds, known to contain the highest levels of arsenosugars in food. In particular, the red seaweed known as Laver/Nori, and the brown seaweeds Wakame and Kombu were prioritised, since they seem to be the most important seaweeds used as food. Among the data retrieved, the highest mean levels are for Kombu (7.5 mg As/kg d.m., $n = 11$), followed by Nori/Laver (2.0 mg As/kg d.m., $n = 18$) and then Wakame (1.7 mg As/kg d.m., $n = 20$). Additionally, occurrence data were derived for two foods (sushi and miso soup) containing seaweed as ingredient, which are probably among the most popular seaweed-containing foods consumed by the European population that developed a taste for Asian cuisine. All occurrence values for AsSugOH are expressed as elemental arsenic.

No occurrence data on arsenolipids were reported to EFSA. Arsenolipid levels have been reported in the literature for seaweeds, fish, molluscs and crustaceans, but not in terrestrial foods. Among the more than 200 different arsenolipids in food, the dominant subgroups appear to be AsHCs, arsenosugar phospholipids (AsSugPLs), arsenic phosphatidylcholines (AsPCs) and arsenic triglycerides (AsTAGs). Quantification of arsenolipids is challenging due to the large number of species present in food, their instability during the analysis and the lack of analytical standards. Arsenolipids are particularly abundant in seaweeds, where they primarily occur as AsSugPLs, while AsHCs are the predominant species in seafoods.

Chronic dietary exposure assessment to AsB was conducted for consumers of fish, seafood and fish-based processed commodities ('consumers only'). The highest mean dietary exposure to AsB was 4.6 µg As/kg bw per day, in both 'Infants' and 'Toddlers'. The highest 95th percentile exposure was found for 'Toddlers' with an estimate of 12.5 µg As/kg bw per day. Dietary exposure estimates for special population groups such as 'Pregnant women' and 'Lactating women' were similar to those for the adult population. 'Fish meat', and particularly 'Marine fish', was overall the main contributor to the exposure to AsB across the different age classes.

Chronic dietary exposure to AsSugOH was estimated considering the individual consumption of red seaweed Laver/Nori and brown seaweeds Wakame and Kombu ('consumers only'). The highest mean exposure to AsSugOH was estimated in the 'Elderly' population (0.19 µg As/kg bw day) whereas the highest 95th percentile (high consumers) was estimated in 'Adults' (0.71 µg As/kg bw day), both due to the consumption of Laver/Nori. Chronic exposure to AsSugOH from the consumption of alga-based food supplements seems to be low as compared to that from the consumption of seaweeds as part of the diet.

Considering that no occurrence data on arsenolipids were submitted to EFSA, and that the available toxicological data did not allow the identification of RPs, or MOEs that do not raise a health concern for arsenolipids, no dietary exposure was conducted.

AsB is not metabolised and is excreted unchanged, and therefore is not likely to exhibit the toxicity of inorganic As. It is possible that intact AsB could exhibit some toxicity, but this has not been comprehensively assessed. Limited data from acute, immunotoxicity and reproductive toxicity tests are available for AsB, none of which showed effects up to the highest doses tested. When comparing these dose levels with the highest 95th percentile exposure to AsB, the MOEs based on the highest dose tested NOAELs from the two repeat dose studies are > 340 (based on the NOAEL of 4.2 mg As/kg bw per day from the reproductive study) and > 31,000 (based on the NOAEL of 387 mg/kg As bw per day in the short term immunotoxicity study). AsB has not shown genotoxicity in in vitro assays. In addition, there is no indication of an association with adverse outcomes in human studies. Overall, there is sufficient evidence to conclude that AsB at current dietary exposure levels does not raise a health concern. Comparing the highest 95th percentile exposures to AsSugOH in Laver/Nori consumers with the RP for neurobehavioural effects results in an MOE > 1000, not raising a health concern. Risk characterisation for arsenobetaine compounds other than AsB, arsenosugars other than AsSugOH and arsenolipids was not possible due to a lack of toxicity and exposure data.

Based on a qualitative assessment of all identified uncertainties, it is regarded likely that dietary exposures to AsB and AsSugOH do not raise a health concern. It is not known whether the assessment for AsSugOH applies to other arsenosugars.

There is a need for occurrence data on complex organoarsenic species and for improved understanding of the ADME of complex organoarsenic species, particularly in relation to inter-individual differences in humans. There is a general need for toxicity data on complex organoarsenic species which is currently very limited.

1 | INTRODUCTION

1.1 | Background and Terms of Reference

1.1.1 | Background

In 2009, the EFSA Panel on Contaminants in the Food Chain (CONTAM) adopted a Scientific Opinion on the presence of arsenic in food. Arsenic occurs naturally in soil and ground water. Inorganic arsenic may cause cancer of the skin, urinary bladder and lungs and EFSA calculated $BMDL_{01}$ values for these effects between 0.3 and 8 μg/kg bw per day, highlighting a possible risk to consumers on the basis of the estimated exposure. However, for the organic arsenic forms EFSA indicated that arsenobetaine, which is the major form of arsenic in fish and most seafood, is widely assumed to be of no toxicological concern. Arsenosugars and arsenolipids are mainly metabolised in humans to dimethylarsinate (DMA(V)), but sparse information is available regarding their toxicity. For other organoarsenic species no human toxicity data are available. Because of the lack of data, arsenosugars, arsenolipids, methylarsonate (MMA(V)) and dimethylarsinate (DMA(V)) could not be considered in the risk characterisation.

In its 2014 scientific report on dietary exposure to inorganic arsenic in the European population,[1] EFSA identified grain-based products as the main contributor to the exposure and also rice, milk and dairy products as important contributors. The heterogeneity of the food consumption data, the conversion of total arsenic to inorganic arsenic and the treatment of left censored data represented important uncertainties in the exposure assessment.

Commission Recommendation (EU) 2015/1381[2] recommended to Member States to monitor during 2016, 2017 and 2018 the presence of arsenic, preferably by determining the content of inorganic and total arsenic and, if possible, other relevant arsenic species, in a wide variety of food, and to provide these data to EFSA on a regular basis at the latest by October 2018.

The newly available occurrence data have been used for an updated consumer exposure assessment for inorganic arsenic, which was endorsed by the CONTAM Panel of 24–25 November 2020.

Since the publication of the 2009 opinion on arsenic in food new studies have become available on the toxic effects of inorganic arsenic and it is therefore appropriate to update the hazard characterisation. Taking into account the new exposure assessment and new information on the adverse health effects, a new risk assessment should be performed for the risks for human health related to inorganic arsenic in food. Since the publication of the 2009 opinion on arsenic in food new studies have become available on the toxic effects of organic arsenic. Furthermore, it has become clear that dimethylmonothioarsenate (DMMTA, thio-DMA(V)) is formed from methylarsonate (MMA(V)) due to the application of sulfate fertilisers, and that this substance could be more toxic than methylarsonate (MMA(V)). It is therefore appropriate to update the hazard identification and characterisation of organic and inorganic arsenic. On the basis of the available occurrence data for organic arsenic or literature information on organic arsenic in food, the exposure could be estimated in order to carry out an assessment for the risks for human health related to organic arsenic in food. In view of different properties of the organoarsenic species, arsenobetaine, arsenosugars and arsenolipids, it is appropriate to assess the organic arsenic species in 2 further separate opinions: an opinion on small organoarsenic species and an opinion on arsenobetaine, arsenosugars and arsenolipids which should also include other relevant complex organoarsenic species. After finalising the risk assessments on inorganic and organic arsenic, also the combined exposure to inorganic and organic arsenic should be assessed.

1.1.2 | Terms of Reference

In accordance with Art. 29 (1) of Regulation (EC) No 178/2002 the Commission asks EFSA for

- an updated consumer risk assessment for inorganic arsenic in food, taking into account
  - the updated exposure assessment, endorsed by the Panel in November 2020
  - newly available scientific information
- a consumer risk assessment for organic arsenic in food, taking into account
  - newly available scientific information
  - occurrence data for organic arsenic in food
  - literature data on the occurrence of organic arsenic in food
- a consumer risk assessment on the combined exposure to inorganic and organic arsenic, taking into account
  - the risk assessment on inorganic arsenic in food
  - the risk assessments on organic arsenic in food.

[1]EFSA CONTAM Panel, 2014. Scientific report of on the dietary exposure to inorganic arsenic in the European population. EFSA Journal, 12(3), 3597.
[2]Commission Recommendation (EU) 2015/1381 of 10 August 2015 on the monitoring of arsenic in food. OJ L213, 12.8.2015, p. 213.

1.2 | Additional information

1.2.1 | Properties of complex organoarsenic arsenic species relevant to their presence in food

1.2.1.1 | *Description and categories of complex organic arsenic species*

The second opinion delivered within the current mandate on arsenic in food (EFSA CONTAM Panel, 2024b) dealt with small, methylated arsenic species which were defined as those organoarsenic species that contain methyl groups, but no other organic groups, bound to arsenic. The present Opinion (Opinion 3) will deal with the more complex organoarsenic species; these species also contain methyl groups bound to arsenic but, in addition, they contain a larger organic group bound to arsenic. This additional organic group can vary from 2 carbons to >40 carbons, leading to many complex organic arsenic species occurring in nature (>200 reported at the time of writing) and their properties can differ markedly. To simplify the following discussion, the complex organic arsenic species are divided into three main sub-groups, namely, (i) the arsenobetaine group – alkyl chain contains 2 to 4 carbons; (ii) arsenosugars – the alkyl group contains a ribose sugar and (iii) arsenolipids – the alkyl group contains many, >10 carbons, which renders these species lipid-soluble.

For this report, the term arsenolipid is used to describe any arsenic species that is lipid-soluble, or at least more readily soluble in organic solvents (e.g. ethanol, diethyl ether, chloroform) than in water. Accordingly, the arsenic containing carboxylic acids with a small hydrocarbon chain (e.g. TMAP, trimethylarsoniopropionic acid, arsenobetaine), are grouped with the water-soluble species rather than with the arsenolipids.

1.2.1.2 | *The arsenobetaine group: Organoarsenic species with alkyl chain of 2 to 4 carbons*

Arsenobetaine (AsB) is the major naturally occurring organoarsenic species, particularly dominant in marine animals where it often constitutes >90% of the total arsenic content. It was first identified in lobster in 1977 (Edmonds et al., 1977), and since then there have been many hundreds of reports of AsB's presence in fish and other seafood (Taylor, Goodale, et al., 2017). AsB is also found in some terrestrial foods such as mushrooms (Braeuer & Goessler, 2019), but the concentrations are much lower than in seafoods. Over the years, additional organoarsenic species with alkyl chains of 2 to 4 carbons have been detected along with AsB, but they occur at much lower levels (usually <2% of AsB). These species are grouped together because they are all closely related structurally to AsB, and share similar properties in that they are small, polar species, usually cationic or amphoteric, and very water-soluble. Thus, they are amenable to similar methods of analysis. Common examples include dimethylarsinoylacetate (DMAA) and arsenocholine (AsC); properties of the species assigned to this group are recorded in Table 1.

TABLE 1 Properties of the arsenobetaine group – methylated organoarsenic species containing an additional alkyl group with 2–4 carbons.*

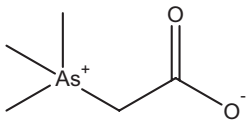
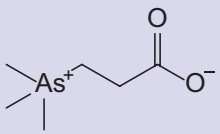
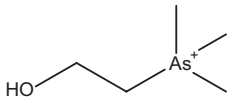
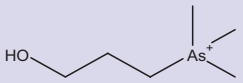
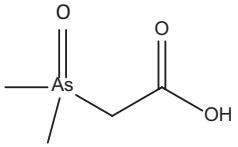
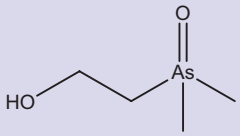
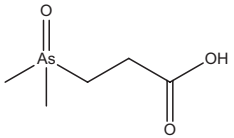
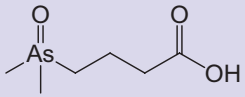
Acronym	Common name and synonyms	Chemical name (CAS number)	Formula and molecular mass	Structure	Properties	Comment
AsB AB	**Arsenobetaine**	2-Trimethylarsonium acetate (Carboxymethyl) trimethylarsonium hydroxide inner salt IUPAC: 2-(trimetharsaniumyl) acetate (CAS: 64436–13-1)	$C_5H_{11}AsO_2$ **178.06 Da** **179.07 Da (protonated mass)**		Stable solid, water-soluble, hygroscopic, cationic; pKa 2.2	The predominant arsenic species in seafood. Considered to be the stable end-product of a process for detoxifying inorganic arsenic. AsB is the arsenic analogue of glycine betaine, a common osmolyte. AsB standard is commercially available. Measured as a cation by LC at low pH.
TMAP AB2	Trimethylarsonio propionate Arsenopropiobetaine	Trimetharsoniopropionate	$C_6H_{14}AsO_2$ 193.10 Da		Stable solid, water-soluble	Fairly common arsenical, usually minor species but can reach 10% of total arsenic. Measured as a cation by LC at low pH.
AsC AC	Arsenocholine	(2-Hydroxyethyl) (trimethyl) arsonium IUPAC: (2-Hydroxyethyl) trimethylarsanium (CAS: 39895–81-3)	$C_5H_{14}AsO$ 165.09 Da		Stable solid, water-soluble hygroscopic, cationic; usually prepared as the bromide salt, which is non-hygroscopic	Common in seafood but usually present only at trace levels. AsC standard is commercially available. Measured as a strong cation by LC.
Homo-AsC AC2	Homo-arsenocholine Arsenohomo-choline	(3-Hydroxypropyl) (trimethyl) arsonium IUPAC: (3-Hydroxypropyl) (trimethyl)arsanium	$C_6H_{16}AsO$ 179.11 Da		Stable solid, water-soluble, cationic; has been prepared as the bromide salt	A trace arsenical reported so far only in mushrooms. Measured as a strong cation by LC.
DMAA	Dimethylarsinoyl acetic acid; Dimethylarsonio acetate	2-Dimethylarsanylacetic acid (CAS: 117929–06-3)	$C_4H_9AsO_3$ (as the acid) 180.03 Da			pK_a 4.0; Trace constituent of some foods; Usually measured as an anion by LC; can protonate at low pH.
DMAE	Dimethylarsinoyl ethanol; Dimethylarsonio ethanol	2-(Dimethylarsinoyl)-ethanol IUPAC: 2-(Dimethylarsoryl)-ethanol (CAS: 82563–93-7)	$C_4H_{11}AsO_2$ 166.05 Da		Weakly basic	Trace constituent of food; a breakdown product from arsenosugars, and a possible precursor to AsB. Measured as a weak cation by LC.
DMAP	Dimethylarsinoyl propionic acid Dimethylarsinoyl propionate	3-Dimethylarsinoyl-propionic acid IUPAC: 3-(Dimethylarsoryl) propanoic acid (CAS: 117929–07-4)	$C_5H_{11}AsO_3$ 194.06 Da		Weakly acidic	A urinary metabolite formed from arsenolipids; Measured as an anion by LC.

TABLE 1 (Continued)

Acronym	Common name and synonyms	Chemical name (CAS number)	Formula and molecular mass	Structure	Properties	Comment
DMAB	**Dimethylarsinoyl butanoic acid** **Dimethylarsinoyl butanoate**	**Dimethylarsinoyl butanoic acid**	$C_6H_{13}AsO_3$ 208.08 Da		Weakly acidic	A urinary metabolite formed from arsenolipids; Measured as an anion by LC.

Abbreviations: CAS, Chemical Abstracts Service; Da, Dalton; IUPAC, International Union of Pure and Applied Chemistry; LC, liquid chromatography; pKa, acid dissociation constant; pH, logarithm of the concentration of the H+ species in water.

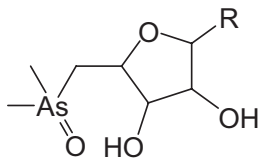
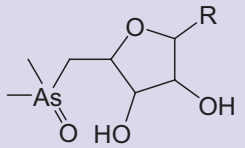
*The oxo-species (e.g. DMAE) could also be present in foods to some extent as the thio-analogue; in solution (e.g. an aqueous extract prepared for analysis) the thio-species will revert within hours to its more stable oxo-analogue. Given that the thio-analogues are rarely reported in foods, they are not presented in this table.

1.2.1.3 | *Arsenosugars*

These species are the major arsenic species found in marine seaweed[3] where they usually constitute > 70% of the total arsenic. About 20 arsenosugars have been reported, but usually only the four major species are present in significant amounts (Table 2). Arsenosugars are only occasionally found in terrestrial foods, and then only at low levels. The species are most commonly found in the oxo dimethylated form, $(CH_3)_2As(O)$-R, where R contains a ribose moiety, but depending on conditions the O can be substituted by S or CH_3; these thio and trimethyl analogs are much less common. The arsenosugars are water-soluble and can be cationic or anionic depending on the aglycone portion of the ribose group.

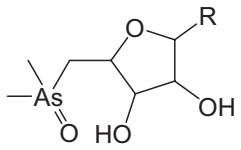
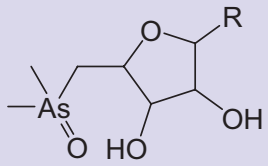
[3]Seaweeds, also known as macroalgae are multicellular plant-like organisms that generally live attached to rock or other hard substrata in coastal areas. They are classified into three groups on the basis of their thallus colour, corresponding to phylum Chlorophyta (green algae), Ochrophyta (brown algae) and Rhodophyta (red algae). The other type of algae are microalgae, which are unicellular algae that comprise several thousands of species, with *Chlorella* and *Arthrospira* (*Spirulina*, a gram-negative cyanobacterium) being probably the most used in food applications.

TABLE 2 The major arsenosugars found in foods, predominantly in marine seaweeds.[a]

Acronym	Common name and synonyms	Chemical name (CAS number)	Formula and molecular mass	Structure	Properties	Comment
AsSug-OH	Glycerol arsenosugar Glycerol arsenoriboside	2,3-Dihydroxy propyl 5-deoxy-5-(dimethylarsinoyl) riboside 2,3-Dihydroxy propyl 5-deoxy-5-(dimethylarsoryl) pentofuranoside (2R,3R,4S,5S)-2-(2,3-dihydroxy propoxy)-5-(dimethylarsoryl methyl) oxolane-3,4-diol CAS: 103476-61-5	$C_{10}H_{21}AsO_7$ 328.19 Da	 R = $OCH_2CHOHCH_2OH$	Water-soluble; cationic; pKa 3.6; stable at neutral pH but can degrade to DMA(V) at low and high pH.	One of the four major arsenosugars found in marine seaweeds. Rarely reported in terrestrial food. The thio analogue (where S replaces O bound to As) can also be present but usually at much lower concentrations. The trimethylated analogue (where CH_3 replaces O bound to As) can also occur but is rare. Pure standard has been prepared and is available in some laboratories, but there is no current commercial source. Algal extracts are often used as a standard for matching LC retention times. Measured as a cation by LC at low pH.
AsSug-PO4	Phosphate arsenosugar	3-'Glycerophosphoryl'-2-hydroxy-1-[5-deoxy-5-(dimethylarsinoyl)-β-ribofuranosyloxy] propane	$C_{13}H_{28}AsPO_{12}$ 482.25 Da	 R = $OCH_2CHOHCH_2OPO_3CH_2CHOHCH_2OH$	Moderately stable at neutral pH, but slowly degrades to the glycerol arsenosugar. Moderately acidic. Degrades to DMA(V) at low and high pH.	One of the four major arsenosugars found in marine seaweeds. Rarely reported in terrestrial food. The thio analog (where S replaces O bound to As) can also be present but usually at much lower concentrations. The trimethylated analog (where CH_3 replaces O bound to As) can also occur but is rare. No synthesised standard available. Algal extracts often used as a standard for matching LC retention times. Measured as an anion by LC.

(Continues)

TABLE 2 (Continued)

Acronym	Common name and synonyms	Chemical name (CAS number)	Formula and molecular mass	Structure	Properties	Comment
AsSug-SO3	Sulfonate arsenosugar	3-[5′-Deoxy-5′-(dimethylarsinoyl)-β-D-ribosyloxy]-2-hydroxypropane-1-sulfonic acid 2-Hydroxy-3-[[5-(dimethylarsinyl)-5-deoxy-β -D-ribofuranosyl]oxy] propane-1-peroxysulfonic acid	$C_{10}H_{21}AsSO_9$ 392.25 Da	 R = $OCH_2CHOHCH_2SO_3H$	Stable at neutral pH; Degrades to DMA(V) at low and high pH. Acidic.	One of the four major arsenosugars found in marine seaweeds; particularly high in brown seaweeds. Rarely reported in terrestrial food. The thio analog (where S replaces O bound to As) can also be present but usually at much lower concentrations. The trimethylated analogue (where CH_3 replaces O bound to As) can also occur but is rare. Pure standard has been prepared, but only scarce amounts are available; there is no current commercial source. Algal extracts are often used as a source of standard for matching LC retention times. Measured as an anion by LC.
AsSug-SO4	Sulfate arsenosugar	(2S)-3-[5-Deoxy-5-(dimethylarsinoyl)-β-D-ribofuranosyloxy]-2-hydroxypropyl hydrogen sulphate	$C_{10}H_{21}AsSO_{10}$ 408.25 Da	 R = $OCH_2CHOHCH_2OSO_3H$	Stable at neutral pH, but will very slowly degrade to AsSugOH when stored in aqueous solution Degrades to DMA(V) at low and high pH Strongly acidic.	One of the four major arsenosugars found in marine seaweeds; particularly high in brown seaweeds. Rarely reported in terrestrial food. The thio analog (where S replaces O bound to As) can also be present but usually at much lower concentrations. The trimethylated analogue (where CH_3 replaces O bound to As) can also occur but is rare. Pure standard has been prepared, but only scarce amounts are available; there is no current commercial source. Algal extracts are often used as a source of standard for matching LC retention times. Measured as an anion by LC.

Abbreviations: As, arsenic; CAS, Chemical Abstracts Service; CH_3, methyl group; Da, Dalton; LC, liquid chromatography; O, oxygen; P, phosphorus; pKa, acid dissociation constant; pH, logarithm of the concentration of the H+ species in water; S, sulfur.

[a]In addition to these four major arsenosugars, all dimethyloxo arsenic species, their thio- and trimethyl analogs are also occasionally found; if present at all, they are usually trace constituents.

1.2.1.4 | *Arsenolipids*

Although the presence of lipid-soluble arsenic in seafoods was first reported in the 1920s (Sadolin, 1928), it was not until 1988 that some of these lipid arsenic species were first identified as derivatives of the phosphate arsenosugar in which the end glycerol group was acylated with various long chain fatty acids (Morita & Shibata, 1988). Accordingly, these species were termed arsenosugar phospholipids (AsSugPL) and collectively they formed the first identified sub-group among the arsenolipids. Subsequent research on lipid-soluble arsenic, particularly in the last 15 years, has revealed the presence of several additional sub-groups including: arsenic hydrocarbons (AsHC), arsenic fatty acids (AsFA), arsenic fatty alcohols (AsFOH), arsenic phosphatidylcholines (AsPC), arsenosugar phytols (AsSUGPhytol) and arsenic triglycerides (AsTAG).

The major source of arsenolipids is marine food. Collectively, the arsenolipids usually constitute < 25% of the total arsenic in a sample, but for some seafoods, in sashimi-grade tuna for example, this value can be 50% or more (Taleshi et al., 2010). In fish oils, essentially all of the arsenic is present as arsenolipids (Schmeisser, Goessler, et al., 2005).

There have been > 200 arsenolipids identified (up to the time of drafting). The simpler arsenolipids such as the arsenic hydrocarbons (AsHC) and the arsenic fatty acids (AsFA), were the first to be identified; they were subsequently found in many seafoods and were initially thought to be major arsenolipids. This remains the case for the AsHCs. For the AsFAs, however, later studies indicated that they can be produced from larger arsenolipids (e.g. AsTAG) during the early analytical preparation stages prior to measurement (Pereira et al., 2016; Řezanka et al., 2019; Taleshi et al., 2014), and thus the natural abundance of the AsFAs has been probably overestimated. More data are required to ascertain the relative abundances of the various arsenolipid groups, and these relative amounts will certainly vary widely between food types. At this stage, however, the dominant arsenolipid sub-groups in foods appear to be: AsHC, AsSugPL, AsPC and AsTAG.

It is not practical nor of much value to list all the arsenolipids identified so far. Rather, Table 3 lists the major sub-groups, giving some examples and briefly describes their general properties.

TABLE 3 Properties of arsenolipids found in food: the major sub-groups with some examples are shown.

Acronym*	Common name and synonyms	Chemical name (CAS number)	Formula and molecular mass[1]	Structure example	Properties	Comment
AsFA **e.g. AsFA 362**	Arsenic fatty acid Arsenic-containing fatty acid	15-Dimethylarsinoyl-pentadecanoic acid	$C_{17}H_{35}AsO_3$ 363.19 Da	AsFA 362[2]	The most polar of the arsenolipid sub-groups; the smaller chain AsFA are sparingly water-soluble	Common in fish oil; might represent breakdown products of AsTAG.
AsHC **e.g. AsHC 332**	Arsenic hydrocarbon Arsenic-containing hydrocarbon	1-(Dimethylarsinoyl)-pentadecane	$C_{17}H_{37}AsO$ 333.21 Da	AsHC 332[3]	Moderately polar arsenolipid	Very common in in fish.
AsFOH **e.g. AsFOH 375**	Arsenic fatty alcohol	–	$C_{20}H_{44}AsO$ 375.26 Da	AsFOH 375[4]		Usually only minor amounts are found.
AsPC **e.g. AsPC 985**	Arsenic phosphatidylcholine	–	$C_{53}H_{85}O_9NAsP$ 986.52 Da	AsPC 985[5]		First identified in caviar.
AsPE **e.g. AsPE 1035**	Arsenic phosphatidylethanol-amine	–	$C_{57}H_{87}O_9NAsP$ 1036.54 Da	AsPE 1035[6]		Trace amounts relative to AsPC.
AsSugPhytol **e.g. AsSugPhytol 546**	Arsenosugar phytol	phytyl 5-Dimethylarsinoyl-2-O-methyl-ribofuranoside	$C_{28}H_{55}AsO_5$ 547.33 Da	AsSugPhytol 546[7]		
AsSugPL **AsPL** **e.g.** **AsSugPL 958**	Arsenosugar phospholipid Arsenic phospholipid	–	$C_{45}H_{89}O_{14}PAs$ 959.52 Da	AsSugPL 985[8]	Moderately polar arsenolipid	AsPL 958 is the most common arsenosugar phospholipid found in seaweeds. It can degrade to AsSug PO_4 and AsSugOH. Pure (synthesised) standard is not available. Source for analytical purposes is an algal extract.
AsTAG **e.g. AsTAG 930**	Arsenic triglycerides	–	$C_{53}H_{107}AsO_7$ 931.73 Da	AsTAG 930[9]	The most non-polar of the arsenolipids	
AsDAG	Arsenic diglycerides (not shown)	–				

Abbreviations: CAS, Chemical Abstracts Service; Da, Dalton; PO_4, phosphate group.

*The number in the acronym represents the nominal molecular mass of the example species.

[1]Monoisotopic mass of M + H species (except for AsOH 375).

[2]AsFA 362.

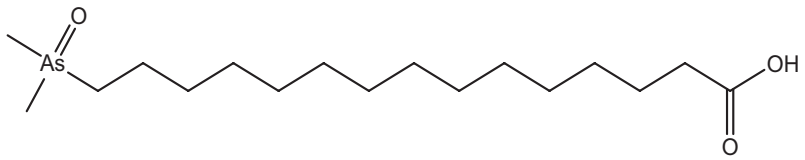
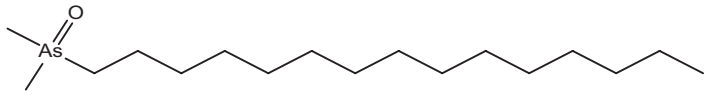
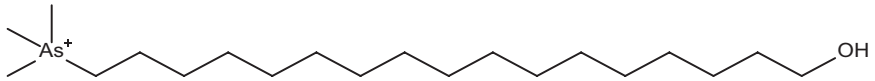


TABLE 3 (Continued)

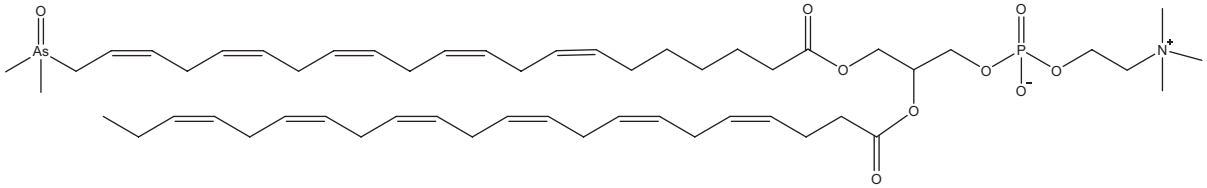
[3]AsHC 332.



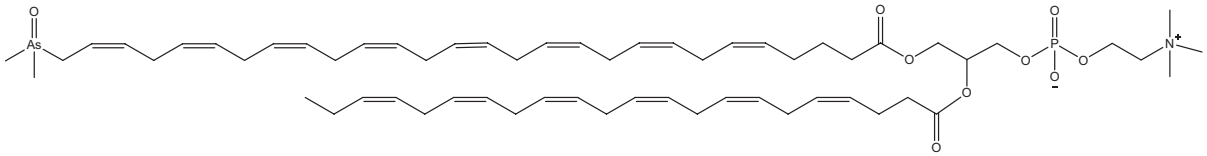
[4]AsFOH 375.



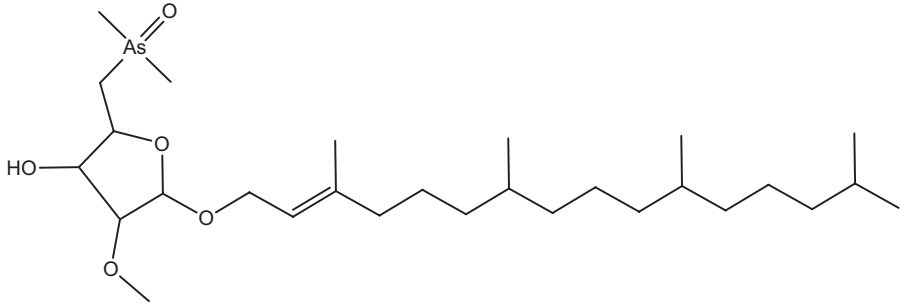
[5]AsPC 985.



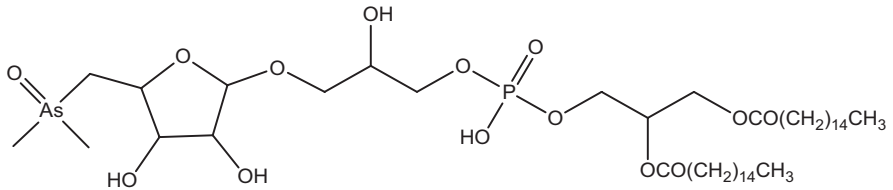
[6]AsPE 1035.



[7]AsSugPhytol 546.



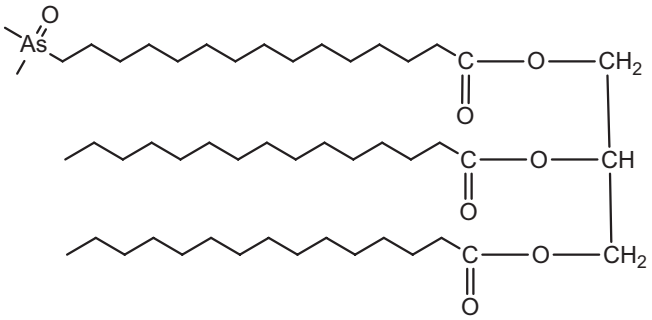
[8]AsSugPL 958.



(Continues)

TABLE 3 (Continued)

[9]AsTAG 930.



1.2.2 | Analytical methods for complex organoarsenic species in food

As discussed in the Opinion on small organoarsenic species (EFSA CONTAM Panel, 2024b), arsenic speciation analysis comprises three main stages, namely extraction, separation and detection. The large difference in properties displayed by the water-soluble arsenic species (Tables 1 and 2) compared with the arsenolipids (Table 3) translates into their requiring distinct methods for their measurement. It is thus prudent to deal separately with these two broad groups of species.

Before analysts embark on specific methods to measure individual organoarsenic species, they can obtain useful information by performing a solvent partitioning procedure to separate the water-soluble arsenic species from the lipid-soluble arsenic species. There are many combinations of solvents and steps that can be used, depending on sample type and the types of arsenic species therein, but the basic procedure will follow the steps outlined in the flow chart below (Figure 1). The data obtained from such a preliminary investigation will guide the future analyses: for example, when the total amount of lipid-soluble arsenic is low, a complete speciation analysis of the arsenolipids may not be justified.

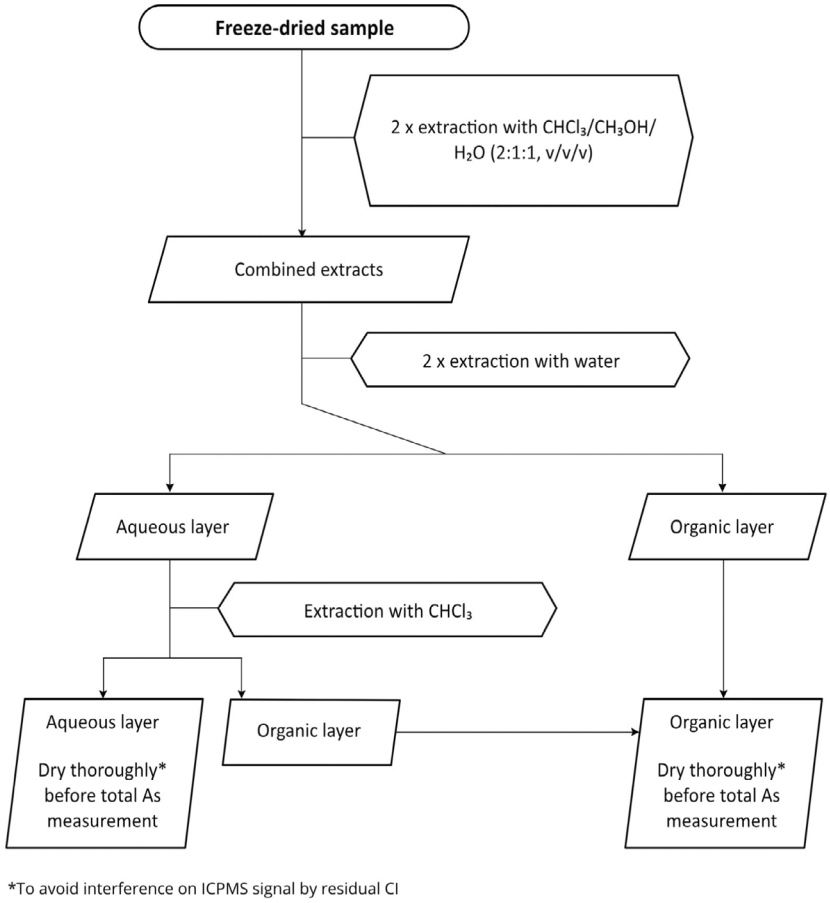


FIGURE 1 A generalised scheme for extraction and separation of total water- and lipid-soluble organoarsenic species from food.

1.2.2.1 | *Water-soluble complex organoarsenic species: the arsenobetaine group and the arsenosugars*

The extraction stage aims to transfer the arsenic species originally in the sample efficiently and unchanged to the solution phase. The species in the arsenobetaine group generally are stable in dilute acid or alkali, and thus can be extracted using various water-based extractants. The thio analogues, however, will slowly convert in water solutions to the oxo form. Additional care must be taken when extracting the arsenosugars because they are less robust and will degrade, eventually forming DMA(V), in solutions of low or high pH, especially when heated.

The separation of the complex organic arsenic species is performed almost exclusively with liquid chromatography (LC), and the conditions used are fundamentally unchanged from the first methods reported in the 1980s. The most common method uses ion-exchange columns; usually both cation- and anion-exchange columns are employed to provide a good coverage of the arsenic species. As a rule, cation-exchange LC will separate most of the arsenic species present in water extracts of foods from a marine animal source, whereas anion-exchange LC is favoured for foods based on marine seaweeds where the three acidic arsenosugars are often major species. Anion-exchange LC has the advantage of being able to also capture inorganic arsenic and the simple methylated arsenic species MMA(V) and DMA(V). Reversed-phase LC, in combination with ion-pairing agents, is also occasionally used to separate the water-soluble arsenic species. Reversed-phase LC can also be used directly (without ion-pairing agents) for some of the arsenic species, and it is particularly suitable for determining thio-arsenic species where the exchange of the O with S on the arsenic atom renders the arsenic species less polar.

Practical methods for detecting the complex organic arsenic species in food first became available with the introduction in the late 1980s of ICPMS (inductively coupled plasma mass spectrometry) as an arsenic detector coupled to LC. In time, LC–ICPMS enabled the routine determination of arsenobetaine and several other arsenic species for which standards were available. An arsenic species cannot be reliably identified in a sample without a standard to match LC retention times. Some organoarsenic standards, such as AsB and AsC, are available commercially, while others can be obtained by extracting a natural source; for example, an extract of a brown seaweed (Fucus) provides quantified standards for the four major arsenosugars (Madsen et al., 2000). In some commercially available certified reference materials (CRMs), arsenic species have been reported and these too can be used to confirm LC peaks.

When no appropriate standard is available, an arsenic species in a sample can still be identified by applying molecular mass spectrometry, usually with electrospray (ES) ionisation. The technique, ESMS (electrospray mass spectrometry) can provide information about the molecular mass and the structure of the organoarsenic species. For quantification, however, ESMS requires a standard, and the signal intensity is often strongly negatively influenced by the matrix.

The two types of mass spectrometry – elemental with ICPMS and molecular with ESMS – are often used together to provide a complementary and comprehensive picture of the complex organoarsenic species in a sample. Currently, however, the quantitative ability and robustness of ICPMS ensures that it remains the major detection method for measuring water soluble organic arsenic species in food.

In summary, a suitable analytical method for complex water-soluble organoarsenic species in food involves extraction with water, separation of the arsenic species by LC under both anion- and cation-exchange conditions, and detection and quantification of the arsenic species by ICPMS. Using this procedure, many of the water-soluble arsenic species in food can be routinely measured. When no standard is available, or an unknown arsenic species is detected, structural information can be obtained by coupling LC with ESMS.

1.2.2.2 | *Lipid-soluble complex organoarsenic species: the arsenolipids*

The arsenolipids are hydrophobic, but the several sub-groups cover a wide polarity range, and thus can require different organic solvents or mixtures thereof to extract them from the food sample and bring them into solution (Glabonjat et al., 2014). The major arsenolipid groups follow this general pattern of decreasing polarity: AsFA>AsFOH>AsHC>AsPC≈AsSUGPhytol≈AsPE>AsSugPL>AsTAG. Within each group, the polarity is also strongly influenced by the length of the carbon chain (longer chain = less polar) and to a lesser extent by the presence of double bonds, which increase polarity. Acceptable extraction efficiency across the arsenolipid polarity range has been reported for a mixture of dichloromethane (DCM) and methanol (MeOH) (2 + 1 v/v) (Glabonjat et al., 2014), and this mixture is a commonly used extractant for arsenolipids.

Because of the difficulties in coupling ICPMS with LC systems employing mobile phases with high organic solvent content, the technique is currently performed only in specialist laboratories. In contrast to ICPMS, ESMS is totally compatible with the LC organic mobile phases, and this is one reason for the increasing use of ESMS for arsenolipid measurements. The major reason, however, is that ESMS can provide structural information about the arsenolipids, many of which are very large molecules.

When dealing with arsenolipids, the combination of LC and ESMS is simpler to operate than LC–ICPMS, and LC–ESMS instruments are available in most food chemistry laboratories. Matrix effects can severely compromise ESMS detection, thereby requiring exacting standard addition procedures to obtain reliable quantitative data. Quantitative data with ICPMS for some of the arsenolipids is currently possible, but the methods are challenging and still not accessible to routine laboratories. The detection limits (LODs) achievable with LC–ICPMS when dealing with arsenolipids are higher than those obtainable with water-soluble arsenic species (ca 0.01–0.05 µg As/L) but can be estimated to be as low as 0.5 µg As/L, corresponding to about 5 µg As/kg in the food sample, depending on the food type. It is not straightforward to compare LODs for LC–ICPMS and LC–ESMS because the sensitivity of ESMS, unlike ICPMS, is dramatically affected by matrix. Estimations from the study of Khan et al. (2016) for pure AsHC standards in solution suggest that an LOD of < 0.001 µg As/L is achievable for LC–ESMS. There are no LOD data available for LC–ESMS analysis of arsenolipids in food samples, and a general LOD is difficult to state owing to the large and variable influence of the sample matrix on the ESMS signal. One might estimate that, under favourable conditions, an LOD of 5 µg As/kg could be achievable by LC–ESMS for some arsenolipids in food.

In summary, a routine analytical method for the quantification of arsenolipids in food is not currently available. It is not feasible to develop a method to cover the plethora of arsenolipids in food. A better approach, and with an eye to the future, might be to select the few arsenolipids of most toxicological concern, produce pure standards of these species, and distribute them to selected analytical laboratories to facilitate their developing the necessary quantitative methods. Additionally, reference materials should be prepared and certified for the most relevant arsenolipids.

1.2.3 | Previous assessments

In 2009, the EFSA CONTAM Panel assessed the risks to human health related to the presence of arsenic in food (EFSA CONTAM Panel, 2009) and evaluated both food-relevant inorganic and organic arsenic species. It was concluded that AsB is not of toxicological concern and that arsenosugars and arsenolipids could not be considered in the risk characterisation, because of the lack of data.

In 2011, the JECFA evaluated arsenic (FAO/WHO, 2011). Because of a general lack of data on both exposure to and toxicity on organic arsenic species, the JECFA considered only inorganic arsenic in their risk assessment.

In 2012, the International Agency for Research on Cancer (IARC) classified arsenic and inorganic arsenic species as carcinogenic to humans (Group 1), DMA(V) and MMA(V) as possibly carcinogenic to humans (Group 2B), and AsB and other organic arsenic species not metabolised in humans, as not classifiable as to their carcinogenicity to humans (Group 3).

1.2.4 | Legislation

Council Regulation (EEC) No 315/93[4] stipulates that food containing a contaminant in an amount unacceptable for public health shall not be placed on the market, that contaminant levels should be kept as low as can reasonably be achieved and that, if necessary, the European Commission may establish maximum levels (MLs) for specific contaminants in specific foods. These MLs are laid down in the Annex I of Commission Regulation (EU) 2023/915.[5] While MLs have been established for inorganic arsenic (sum of As(III) and As(V)) and for total arsenic in salt, so far no specific MLs have been established for organoarsenic species. Under Regulation (EC) No 333/2007[6] requirements for the sampling and analysis of inorganic and total arsenic in food are established.

2 | DATA AND METHODOLOGIES

The present risk assessment was developed applying a structured methodological approach, which implied developing a priori the protocol or strategy of the full risk assessment. The protocol in Annex A of this Opinion contains the method that was proposed for covering all the steps of the risk assessment process. The CONTAM Panel used its previous opinion on arsenic (EFSA CONTAM Panel, 2009) as a starting point for drafting the current Opinion.

2.1 | Collection and appraisal of data collected from the public literature

A call for a literature search and review was launched in January 2022 (NP/EFSA/BIOCONTAM/2021/01) with the aim to identify and review relevant literature related to small methylated arsenic species and arsenic species other than small methylated species including any other organic arsenic species. A final project report was delivered in August 2022 and was published by EFSA (Licht et al., 2022).

Briefly, search strings were designed to identify potentially relevant studies published from 1 January 2009 (the year in which the previous opinion was published) in three databases (PubMed, Web of Science, Scopus) until 29 April 2022 (the day the literature search was carried out). After removal of duplicates and applying inclusion criteria for relevance, the number of publications considered as relevant for the different fields for other organoarsenic species were as follows: analytical methods (222), human biomonitoring (46), occurrence/concentration and formation (117), exposure of humans (8), toxicokinetics (21), in vitro and in vivo toxicity (24), observations in humans (2) and toxic mode of action (15).

The report from the literature search contains a detailed explanation of the search terms and methodology applied as annexes summary tables containing key information about all individual studies (authors, abstracts, species tested, study details).

The summary tables were then screened by the Working Group (WG) members and used for the assessment when considered relevant by applying expert judgement.

In addition to the previously mentioned systematic literature search, a first supplementary search was conducted to complement the literature search that was commissioned by EFSA. The updated search was carried out on 3 March 2023. It was designed to capture also literature published after the outsourced literature search (timespan 1 May 2022 to 2 March 2023) and was confined to studies on toxicokinetics and in vitro as well as in vivo toxicity as it was agreed that for these fields also the very recently published literature needs to be considered. This search yielded 17 potentially relevant studies on TK, one study on in vitro toxicity and one on in vivo toxicity.

A second supplementary search was carried out on 5 November 2023 to capture also literature on the genotoxicity and toxicity of AsB and AsC published between 1 Jan 1970 and 31 Dec 2009, as it was agreed that for these compounds and endpoints also the literature published before the previous EFSA Opinion on arsenic (EFSA CONTAM Panel, 2009) needs to be re-evaluated. This search yielded 11 potentially relevant studies on genotoxicity of AsB and AsC, and 28 potentially relevant studies on toxicity of AsB and AsC.

Additional searches were conducted on August 4 and 27, 2024, respectively to retrieve studies on food processing relative to arsenobetaine, arsenosugars, arsenolipids (search August 5) and relative to arsenic species in seaweed and algae

[4]Council Regulation (EEC) No 315/93 of 13 February 1993 laying down Community procedures for contaminants in food. OJ L 37, 13.2.1993, p. 1–5.

[5]Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. OJ L 119/103, 5.5.2023, p. 103–107.

[6]Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the control of trace elements and processing contaminants in food. OJ L88, 28.2.2007, p.1–19.

(search August 27). The searches were limited to publication from 2009 to 2024 and yielded 22 (search 5 August) and 2 (search August 27) potentially relevant studies.

More detailed information (search terms, databases used, detailed results) regarding these supplementary searches can be found in Annex B.

In addition to the described literature searches, a 'forward snowballing approach' was applied during the process of drafting the Opinion by all WG members (see Jalali & Wohlin, 2012) in order to obtain further relevant information published until adoption of the Opinion.

2.2 | Inclusion criteria for human evidence

Table 4 depicts the criteria for criteria for consideration of epidemiological studies in the assessment.

TABLE 4 Criteria for epidemiological studies of long-term effects in humans.

Study design	In	Cross-sectional studies Cohort studies Case–control studies
Study characteristics	In	Duration of exposure > 6 months
Population	In	Any country. Any age groups
Exposure estimate	In	AsB in urine
Outcomes	In	Any long-term adverse outcomes
Covariates	Out	Lack of information on age or sex Lack of information on smoking or BMI for T2D outcome Lack of information on covariates (child characteristics/parents' education or maternal intelligence/ socioeconomic status) for neurodevelopment
Language	In	English
	Out	non-English
Time period	In	From 1 Jan 2009 to 29 April 2022
Publication type	In	Peer-reviewed original research, systematic reviews, meta-analyses
	Out	Expert opinions. Editorials. Letters. PhD theses. Conference proceedings. Conference abstracts.

Abbreviations: AsB, arsenobetaine; BMI, body mass index; T2D, type 2 diabetes.

2.3 | Occurrence data submitted to EFSA

2.3.1 | Data collection and validation

Occurrence data on AsB were collected as part of the EFSA call for continuous collection of chemical contaminants occurrence data in food and feed.[7] European national authorities and similar bodies, research institutions, academia, food business operators and other stakeholders were invited to submit analytical data on AsB in food and feed.

The data submission to EFSA followed the requirements of the EFSA Guidance on Standard Sample Description (SSD) for Food and Feed (EFSA, 2010a) and the EFSA Guidance on Standard Sample Description version 2.0 (EFSA, 2013). Occurrence data were managed following the EFSA standard operational procedures (SOPs) on 'Data collection and validation' and 'Analysis of data from the S-DWH for the assessment of dietary exposure'.[8]

Occurrence data were extracted in March 2024; data received after that date were not included in the assessment. The current assessment covered samples collected from 2012 onwards.

Occurrence data on neither arsenosugars nor arsenolipids in food were submitted to EFSA.

2.3.2 | Data analysis

Following EFSA's Technical report on handling of occurrence data for dietary exposure assessment to guarantee an appropriate quality of the data used in the exposure assessment (EFSA, 2021), the initial data set was evaluated by applying several data cleaning and validation steps. Special attention was paid to the identification of duplicates and to the accuracy of different parameters, e.g. information provided on analytical methods and their sensitivity, sampling strategy, expression of the results, etc., as well as to the codification of analytical results under the FoodEx classification (EFSA, 2011a, 2011b, 2015). The outcome of the data analysis is presented in Section 3.2 on Occurrence.

[7] https://www.efsa.europa.eu/en/call/call-continuous-collection-chemical-contaminants-occurrence-data-food-and-feed-2023.

[8] https://www.efsa.europa.eu/en/corporate/pub/sops.

The left-censored data (results below limit of detection (LOD) or below limit of quantification (LOQ)) were treated by the substitution method as recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' (WHO/IPCS, 2009). The same method is indicated in the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010b) as an option in the treatment of left-censored data. The guidance suggests that the lower bound (LB) and upper bound (UB) approach should be used for chemicals likely to be present in the food (e.g. naturally occurring contaminants, nutrients and mycotoxins). The LB is obtained by assigning a value of zero (minimum possible value) to all samples reported as lower than the LOD (< LOD) or the LOQ (< LOQ). The UB is obtained by assigning the numerical value of the LOD to values reported as < LOD, and the LOQ to values reported as < LOQ (maximum possible value), depending on whether LOD or LOQ is reported by the laboratory.

The variability of concentrations observed within the occurrence data set is evidenced by reporting for each food category/food group the mean concentration and a high concentration, the latter typically represented by the highest reliable percentile (HRP) limited to the 95th percentile of the distribution.[9]

2.4 | Occurrence data from the literature

Data from public literature collected as described in Section 2.1 above were used to comparatively assess the occurrence data submitted to EFSA on AsB. For one arsenosugar, glycerol arsenosugar (AsSugOH), occurrence data retrieved from the literature were used to estimate dietary exposure since no data were available in the EFSA database.

2.5 | Food consumption data

The EFSA Comprehensive European Food Consumption Database (EFSA Comprehensive Database) provides a compilation of existing national information on food consumption at individual level and was first built in 2010 (EFSA, 2011b; Huybrechts et al., 2011; Merten et al., 2011). Details on how the Comprehensive Database is used are published in the Guidance of EFSA (EFSA, 2011b). The latest version of the Comprehensive Database, updated in December 2022, contains results from a total of 83 different dietary surveys carried out in 29 different Member States covering 154,388 individuals.

Within the dietary studies, subjects are classified in different age classes as follows:

Infants:[10]	> 12 weeks to < 12 months old
Toddlers:	≥ 12 months to < 36 months old
Other children:	≥ 36 months to < 10 years old
Adolescents:	≥ 10 years to < 18 years old
Adults:	≥ 18 years to < 65 years old
Elderly:	≥ 65 years to < 75 years old
Very elderly:	≥ 75 years old

For the purpose of this Scientific Opinion and given that food consumption data on very young infants (0 to up to 3 months old) were scarce, separate exposure scenarios were not conducted for them.

Nine surveys provided information on specific population groups: 'Pregnant women',[11] 'Lactating women'[12] and 'Vegetarians'.[13] These surveys were used to identify specific concerns for these sub-population groups.

The food consumption data gathered by EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. Consumption data were collected using single or repeated 24- or 48-h dietary recalls or dietary records covering from 3 to 7 days per subject. Because of the differences in the methods used for data collection, direct country-to-country comparisons can be misleading.

When for one country and age class two different dietary surveys were available, only the most recent one was used. Not all countries provided consumption information for all age groups, and in some cases the same country provided more than one dietary survey.

[9]The highest percentile, lower than or equal to the 95th percentile, that can be reliably estimated when applying the following minimum sample size for each percentile: 5 samples for the P50, 11 samples for the P75, 29 samples for the P90 and 59 samples for the P95.

[10]According to the EFSA Scientific Committee Guidance on the risk assessment of substances present in food intended for infants under 16 weeks of age, the exposure assessment for these infants should be carried out separately from that for older infants, following the procedure described in the guidance (EFSA Scientific Committee, 2017). Based on this guidance, infants under 16 weeks of age should be excluded from the dietary exposure estimation of the infants age group. However, for the exposure assessment of AsB due to uncertainty in the reported individual ages of infants in the Comprehensive Database, the cut off age was based on a validated existing age group in this database corresponding to 12 weeks of age. Thus, food consumption data of infants between 12 and 16 weeks of age were also included in the exposure assessment. As the number of children within this age range in the database is limited, it is not expected that this will have affected the exposure estimate of AsB for infants of 16 weeks up to 12 months of age.

[11]Austria: ≥ 19 years to ≤ 48 years old, Cyprus: ≥ 17 years to ≤ 43 years old; Latvia: ≥ 15 years to ≤ 45 years old, Romania: ≥ 19 years to ≤ 49 years old, Spain: ≥ 21 years to ≤ 46 years old, Portugal: 17 years old to 46 years old.

[12]Greece: ≥ 28 years to ≤ 39 years old, Estonia: 18 years old to 45 years old.

[13]Romania: ≥ 12 years to ≤ 74 years old.

As suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011c), dietary surveys with only one day per subject were not considered for chronic exposure as they are not adequate to assess repeated exposure (EFSA, 2011c). Similarly, subjects who participated only 1 day in the dietary studies, when the protocol prescribed more reporting days per individual, were also excluded for the chronic exposure assessment. This resulted in a total of 49 different dietary surveys carried out in 22 different European countries used for the chronic dietary exposure assessment (84,676 subjects). These dietary surveys and the number of subjects available for the chronic exposure assessment are described in Annex C.

2.6 | Food classification

Consumption and occurrence data were codified according to the FoodEx2 classification system (EFSA, 2011a, 2011d). Since 2018, all consumption records in the Comprehensive Database as well as all occurrence data submitted to EFSA have been codified according to the FoodEx2 classification system (EFSA, 2015). The FoodEx2 classification system consists of standardised basic food items aggregated into broader food categories in a hierarchical parent–child relationship. Additional descriptors, called facets, are used to provide additional information about the codified foods (e.g. information on food processing and packaging material).

2.7 | Dietary exposure assessment

The CONTAM Panel considered it appropriate to estimate only chronic dietary exposure to AsB and to AsSugOH. As mentioned in Section 2.5. above, only the dietary surveys fulfilling the criteria for being reliable for calculation of the chronic dietary exposure were selected (see Annex C).

A. Preparation of the occurrence and consumption data for the AsB dietary exposure assessment

The occurrence data on AsB were carefully examined and grouped at the appropriate FoodEx2 levels before being linked to the consumption data.

Food categories represented by < 6 samples were considered as not suitable for the exposure estimations. Exceptions were made for four samples of marinated/pickled fish since literature data supported the presence of the reported levels of AsB. Additionally, when all data within a particular food category were below the LOD or LOQ and there was no information available that might indicate the presence of AsB, that food category was not considered in the dietary exposure estimations.

Different processed commodities (e.g. 'Fish pâté', 'Fish soup', etc.) were identified as potential contributors to the dietary exposure to AsB for which no occurrence data were available. AsB values for these processed commodities were derived using the reported occurrence for raw primary commodities (fish meat). Further details are provided in Section 3.3 on Exposure assessment.

Before estimating dietary exposure, occurrence data and consumption events for solid forms of certain foods (e.g. dehydrated soups) were adjusted by an appropriate dilution factor and these consumption events were reclassified to the liquid forms (EFSA, 2018a).

The different food commodities were grouped within each food category to better explain their contribution to the total dietary exposure to AsB.

B. Estimation of the dietary exposure to AsB

Chronic dietary exposure to AsB was estimated for consumers of fish and seafood ('consumers only') considering only the occurrence data submitted to EFSA, including those derived for few fish-based processed commodities. Food consumption and body weight data at the individual level were retrieved from the Comprehensive Database. Occurrence data and consumption data were linked at the relevant FoodEx2 level.

Chronic dietary exposure was estimated by combining mean AsB occurrence values for food samples collected in different countries (pooled European occurrence data) with the average daily consumption for each food at individual level in each dietary survey and population group. Consequently, individual average exposures per day and body weight were obtained for all individuals and then for 'consumers only'.

$$\bar{e}_i = \frac{\sum_{d \in D_i} \sum_{f \in F} \bar{x}_f \cdot c_{f,d,i}}{|D_i| \cdot \mathrm{bw}_i},$$

where:

$\bar{e}_i$ is the average exposure of individual *i*

$\bar{x}_f$ is the mean AsB concentration in each food or food group f (belonging to set of foods F_i for individual i)
$C_{f,d,i}$ is the consumed amount of food f by individual i on day d
bw_i is individual body weight of individual i
d is the survey day (belonging to the set of survey days D_i for individual i)
$|D_i|$ represents the number of survey days of individual i

The distributions of individual exposures were then used to calculate the mean and high (95th percentile) exposures per survey and per population group. These exposure estimates were obtained for two scenarios, a first scenario using the LB mean concentration of AsB and a second one using the UB mean concentration of AsB.

No exposure scenarios were conducted for breastfed and formula-fed infants as AsB occurrence data were not available.

C. Preparation of the occurrence and consumption data for the AsSugOH dietary exposure assessment

As mentioned in Section 3.2 on Occurrence data, the occurrence data used to estimate dietary exposure to AsSugOH were extracted from the literature as no data on this compound were submitted to EFSA. The extraction of the data was focused on seaweeds, in particular on AsSugOH levels in the brown seaweeds Wakame (*Undaria pinnatifida*) and Kombu (some *Laminaria* and *Saccharina* spp.), and in the red seaweed known as either Laver or Nori[14] (some *Porphyra* and *Pyropia* spp.). After a thorough assessment of the available literature, average AsSugOH values were derived for the seaweeds Wakame, Kombu and Nori/Laver.

Consumption data for the selected seaweeds were extracted from the EFSA Comprehensive Database. Additionally, eating occasions reported as 'Sushi' and 'Miso soup' were also identified. The average amount of seaweeds present in these two foods were assumed to be ~ 1% for Miso soup (Wakame seaweed) and ~ 5% for the different types of sushi (Nori seaweed) (Alves et al., 2017; Ficheux et al., 2022; González et al., 2021). Seaweed consumption reported as 'Algae and prokaryote organisms', without further information or with no information on whether the amounts refer to dry or fresh weight, was excluded from the exposure estimations.

D. Estimation of the dietary exposure to AsSugOH

Chronic dietary exposure to AsSugOH was estimated for consumers of seaweeds ('consumers only') considering only the occurrence data extracted from the literature. For each seaweed, chronic dietary exposure was calculated by combining mean AsSugOH occurrence values with the average daily consumption of each seaweed at individual level, as reported in the dietary surveys for each population group. The occurrence and consumption data were linked at the relevant FoodE2x level.

An exposure assessment was also conducted to account for the increased consumption of alga-based food supplements as a source of iodine. These supplements are mainly made of brown seaweeds, well known to contain high iodine concentrations but with high variations depending on the species, the parts used and the growth conditions (Blikra et al., 2022). To estimate chronic exposure, a mean AsSugOH level derived from selected brown seaweeds was combined with different doses as indicated by the manufacturers (one capsule/three capsules), also considering the amounts of seaweeds described per capsule.

Dietary exposure in the European population was complemented by exposure estimations in the Asian population using consumption data reported in the literature for Japan, China and Korea, and the average AsSugOH values derived for the seaweeds Wakame, Kombu and Nori/Laver.

In the absence of data on the occurrence of AsSugOH in human milk and infant formula, no exposure scenarios were conducted for breastfed and formula fed infants.

All analyses were run using the SAS Statistical Software (SAS enterprise guide 8.3, update 5).

3 | ASSESSMENT

3.1 | Hazard identification and characterisation

3.1.1 | Toxicokinetics

3.1.1.1 | *Toxicokinetics in rodents*

It had long been accepted that 'fish As', later shown to be AsB, was not metabolised in mammals including humans, and animal studies in the 1980s provided clear evidence for this view. The most comprehensive and focused study was reported

[14]The terms nori, laver and purple laver are often used interchangeably between North Atlantic and Pacific *Porphyra* and *Pyropia* species. In the North Atlantic coasts (both American and European), *Porphyra umbilicalis* is probably the most widely distributed *and the one with the highest commercial value*. It is known as 'Purple laver', 'Laver' or wild Atlantic Nori, and it has been used as food in Great Britain and Ireland for centuries (e.g. laverbread). In Asia, the most relevant 'Nori' seaweeds are *Pyropia yezoensis, Pyropia tenera* and *Pyropia haitanensis*.

by Vahter et al. (1983) who investigated the metabolism of AsB in mice, rats and rabbits after intravenous administration of ^{73}As-labelled arsenobetaine at a dose of 4 mg As/kg body weight (bw). They showed that within 3 days >98% (mice and rats) or 75% (rabbits) of the administered AsB was excreted unchanged in the urine. No other labeled arsenic species was detected in the urine, liver, kidney or lungs, of the mice, rat and rabbit demonstrating that AsB was not metabolised. A further study with mice injected interperitoneally with AsB also showed rapid elimination of unchanged AsB, with no evidence for any metabolism (Cannon et al., 1983).

In contrast to the conclusions from the studies of Vahter et al. (1983) and Cannon et al. (1983), two recent studies, from one research group, have reported experiments that according to authors show that AsB is significantly biotransformed in mice (Ye et al., 2022; Zhang et al., 2023). In both studies, mice were fed fish diets containing AsB for up to 60 days, after which the major tissues were removed and analysed for AsB, As(III), As(V), MMA(V) and DMA(V). The data suggest small increases in As(V) concentrations in some tissues, which the authors assume resulted from AsB metabolism. This interpretation seems questionable, however. The control group was not appropriate, and the fish diets fed to the mice already contained considerable amounts of iAs in addition to AsB. No quantitative data were provided to show that any of the AsB in the fish diets was changed to iAs. For these reasons, this Opinion notes the two studies by Zhang et al. (2023), Ye et al. (2022), but does not consider them further.

Kobayashi & Hirano (2016) orally administered AsB and AsC at a dose of 1.0 mg As/kg bw to male Sprague–Dawley rats. The authors found that the liver contained 3.2% and 11% of the administered dose for AsB and AsC, whereas the spleen contained 0.1%, respectively. The authors assessed that 72% and 60% of the dose were excreted in urine within 2 days after exposure to AsB and AsC, respectively. For faeces, 0.2 and 0.5% of the dose were excreted within 2 days for AsB and AsC, respectively.

Overall, the CONTAM Panel concludes, as in 2009, that AsB and AsC are well absorbed by rodents, not metabolised and are readily excreted as such.

For arsenosugars one toxicokinetic study has been identified in rodents. Wu et al. (2021) exposed mice to arsenosugars via an alga-containing feed and examined the arsenic species in faeces and various organs and muscle tissue. The authors concluded that the arsenosugars were not retained in mice, but were partly metabolised to DMA(V) which was then excreted via the faeces together with the arsenosugars. Unfortunately, urine was not collected in this study; it is also unclear whether or not the collected faeces were free of urine.

For arsenolipids no toxicokinetic studies have been identified in rodents.

3.1.1.2 | *Toxicokinetics in humans*

Arsenobetaine (and related species)

The early study of Cox (1925) on humans ingesting 'seafood arsenic', indicated that AsB is readily absorbed by humans, and is rapidly excreted unchanged in urine; the many qualitative and semi-quantitative studies (e.g. Cannon et al., 1981; Le et al., 1994) subsequently performed have clearly supported the early work. Although it is generally accepted that AsB is not metabolised at all in humans, and is quantitatively excreted within days following ingestion, there have been few studies specifically designed to detect if AsB is metabolised or retained in the body to some small extent. In a study where six volunteers ingested ^{74}As-labelled AsB, Brown et al. (1990) showed that < 1% of the arsenic remained in the body after 24 days. Newcombe et al. (2010), however, measured AsB in the urine of five volunteers who had been on an AsB-exclusion diet and showed that five days after their last AsB meal, AsB was still being excreted at a constant level in three of the volunteers.

There are also some recent preliminary results showing that the milk of nursing mothers can contain traces of AsB. For example, two samples of human milk from Norway contained AsB at levels estimated at 0.15 and 0.8 µg As/kg, the origin of which was thought to be the high content of fish in the mothers' diet (Stiboller et al., 2017). In the study of Xiong et al., 2020, a nursing mother consumed a salmon fillet with a known amount of AsB (1.72 µg As/g), and then the mother's milk was monitored for arsenic species over the next 3 days. AsB was first detected in the milk at $t = 4$ h after consumption of a salmon meal; the levels peaked at 0.21 µg As/kg in about $t = 8$ h before steadily decreasing to background at $t = 2$ days. The transfer rate of ingested AsB to human milk was calculated at just 0.03%.

There have been no reports of human toxicokinetic studies with trimethylarsoniopropionate (TMAP), AsC or DMAE.

In summary, arsenobetaine is absorbed in humans but is not metabolised. Most of the arsenobetaine ingested is excreted unchanged within days in the urine. There appears to be minimal transfer to human milk. This is in accordance with the conclusions in the 2009 Opinion.

Arsenosugars

Studies with nine (Le et al., 1994) and four (Ma & Le, 1998) volunteers showed that when seaweed were eaten, the several arsenosugars originally present were extensively metabolised and excreted in the urine mainly as DMA(V); other unidentified metabolites were also detected. Similar results were reported by Van Hulle et al. (2004) when five volunteers ingested brown seaweed. A study where nine volunteers ingested blue mussels, which contain arsenosugars in addition to AsB, showed that urine contained DMA(V) as the major metabolite of the arsenosugars, together with unchanged AsB (Lai et al., 2004). Some individual variability among the volunteers in the patterns of arsenic excretion has been noted by Le

et al. (1994), Ma and Le (1998) and by Lai et al. (2004), although quantitative data were not provided. On the other hand, Van Hulle et al. (2004) reported that their five volunteers had similar arsenic excretion patterns.

The above studies all used food items (seaweeds or mussels) that naturally contain other arsenic species in addition to the target species, arsenosugars. The study by Francesconi et al. (2002) used a single synthetic arsenosugar (AsSugOH) ingested by one volunteer, and showed that (i) arsenic excretion in urine first became significant at $t = 9–13$ h (after ingestion), with a maximum excretion between 22 and 30 h and had returned to low levels by $t = 60$ h; (ii) about 80% of the ingested arsenic was accounted for in the urine; (iii) DMA(V) accounted for 67% of the urinary arsenic, DMAE 5% and several unidentified arsenic metabolites collectively accounted for 25% of urinary arsenic; and (iv) unchanged AsSugOH was present as only a trace constituent in the urine. These data were consistent with a biphasic disposition of AsSugOH – slow absorption and elimination during the first 13 h, followed by rapid elimination. A follow-up study (Raml et al., 2005) confirmed the results of Francesconi et al. (2002) and identified four additional metabolites, namely DMAA, thio-DMAA, thio-DMAE and thio-AsSugOH.

The individual variability in arsenosugar metabolism mentioned above has been supported by a quantitative study involving six volunteers and synthetic AsSugOH (Raml et al., 2009). Whereas four of the volunteers excreted the bulk of their ingested arsenic (> 85%) during the four days of the experiment, two of the volunteers excreted only small amounts (totalling only 4% or 15% of the ingested dose). The missing arsenic was not accounted for, but may have been contained in the faeces, which was not collected in that study. The arsenic urinary metabolites also showed high variability: urine from the high arsenic excretors contained DMA(V) and > 10 other metabolites, whereas the urine from the two low excretors contained only DMA(V), unchanged AsSugOH and its thio-analogue. Furthermore, the excretion patterns appeared to be consistent within an individual; repeat experiments performed six months later with one low excreter and one high excreter producing results matching those from the first experiment.

The new arsenic metabolites (DMAA, thio-DMAA, thio-DMAE and thio-AsSugOH) identified in the studies using pure arsenosugar elucidated findings in a later study with edible seaweeds (Taylor, Goodale, et al., 2017). That study measured the arsenic species in three types of seaweeds, and in the urine of 11 volunteers who consumed small portions of the seaweeds. The results were consistent with the studies of Raml et al. (2005, 2009) by identifying the main urinary arsenic species as DMA(V), thio-DMAE, thio-DMAA and unchanged arsenosugars together with their thio analogues. Thio-DMA(V) was also detected but only at trace levels. The recovery of the main arsenic species in the urine over 3 days, as a percentage of total arsenic consumed was only 10%–28%, depending on algal type. Marked individual variability was also noted by Taylor, Goodale, et al. (2017) with 2 of the 11 volunteers recording almost no increase in urinary arsenic after ingestion of seaweed. The data also suggested that arsenosugar accessibility depended to some extent on the type of seaweed.

In summary, in most individuals tested so far, arsenosugars are bioavailable. The arsenosugars show biphasic excretion with > 85% of ingested arsenic excreted in the urine within 4 days. The major metabolite by far is DMA(V); minor metabolites include DMAE and DMAA, and their thio analogues. In some individuals, however, urinary excretion of arsenic following arsenosugar ingestion can be as low as 4%, although DMA(V) remains the major metabolite. The type of seaweed ingested (sample matrix) may influence the bioaccessibility and bioavailability of the arsenosugars contained within.

Arsenolipids

Toxicokinetic studies with arsenolipids have been performed with food rich in these compounds but not with synthesised compounds. Thus, Schmeisser, Rumpler, et al. (2005), Schmeisser et al., (2006) reported a study where two volunteers ingested cod liver, which contains arsenolipids in addition to arsenobetaine, and the arsenic species in the cod liver and in the volunteers' urine during the following 66 h were measured. The arsenolipids were efficiently metabolised to water-soluble compounds, which were excreted in the urine. For both volunteers, peak arsenic excretion was between 7 and 15 h, and by the end of the experiment about 90% of the ingested arsenic had been accounted for. The major arsenolipid metabolite was DMA(V), constituting > 70% and most of the remaining arsenolipid-derived arsenic comprised four small arsenic-containing fatty acids, namely DMAP, DMAB and their thio analogues. Arsenobetaine, also present in the urine samples, was presumed to come from what had been ingested, but the possibility remained that some of the arsenobetaine may have come from the arsenolipids. In a second experiment, one volunteer ingested cod liver oil, which contains only arsenolipids: arsenobetaine was not detected in the urine and the pattern of arsenolipid urinary metabolites matched that from the first experiment.

It should be noted that the above studies with arsenolipids were performed before the structures were known and are valid only for those species present in cod liver. There are now > 200 known arsenolipids; all of them, however, contain the dimethylarseno group ($Me_2As(O)$-), and it is likely that DMA(V) will be the major urinary metabolite in most cases. Definitive studies with pure compounds, however, will be difficult to perform; several of the synthesised arsenolipids have been shown to be cytotoxic, and thus ethics approval for human toxicokinetic studies is unlikely to be readily given.

Arsenolipids have also been detected in human milk. In five samples from Norway, Stiboller et al. (2017) reported AsHCs and AsFAs with a combined total of about 0.5 μg As/kg. The arsenolipids in the milk were likely to be a result of the high fish content of the typical Norwegian diet. The AsHCs probably came directly from fish; the AsFAs, however, were probably degradation products of larger arsenolipids (e.g. AsTAGs) present in fish. In the same study mentioned above reporting AsB in salmon and human milk, Xiong et al. (2020) also determined the transfer rate of AsHCs contained in the salmon to the mother's milk. At $t = 4$ h after the salmon meal, the AsHCs were detected in the milk and the levels peaked after 8 h at

about 0.33 µg As/kg. From the total AsHCs in the salmon meal, and the total collected in milk over 3 days, the transfer rate of AsHCs to human milk was estimated at about 3%. This value is 100-fold the value estimated for transfer rate of AsB to human milk.

Because DMA(V) is a major metabolite of arsenolipids, in urine at least, it seems possible that arsenolipids could result in increased DMA(V) in human milk. Neither of the two relevant studies (Stiboller et al., 2017; Xiong et al., 2020) conducted so far, has focused on DMA(V); although the data indicate that traces (0.04 µg As/kg) of DMA(V) occur in human milk, yet whether it results directly from traces in the ingested fish or from metabolism of arsenolipids present in the fish cannot be determined.

In summary, for the few individuals tested so far, arsenolipids ingested from arsenolipid-containing food, are bioaccessible, bioavailable and metabolised among others to DMA(V) and excreted.

3.1.2 | Biomarkers of exposure

3.1.2.1 | *Arsenobetaine*

As mentioned in Section 3.1.1 on Toxicokinetics, AsB is not metabolised, but is excreted in urine and the half-life seems in humans to be less than 20 h (Fowler, 2023; Popowich et al., 2016). As mentioned in Section 1.2.2.1, AsB can be analyzed in human urine using LC for separation and ICP–MS for detection. Many studies have demonstrated the presence of AsB in human urine, some of them in combination with assessment of potential health effects, examples of which will be reviewed in Section 3.1.5. on Observations in humans. Concentrations of AsB in urine are often only a few µg/L (see Table 6 of Section 3.1.5) but can be much higher in individuals who recently consumed fish or other seafood. The impact of AsB from seafood on total arsenic concentration in urine is the reason for the high variability of total As in urine in humans which typically varies more than hundred-fold in general population samples (Ellingsen et al., 2023). AsB can also be detected in blood, but quantification is complicated, and AsB in blood is rarely used for biomonitoring (Mandal et al., 2004; Shibata et al., 1994).

3.1.2.2 | *Arsenosugars and arsenolipids*

Arsenosugars and arsenolipids are metabolised to DMA(V) and other dimethylated As species and excreted in urine. It is therefore currently not possible to use arsenosugars and arsenolipids in urine for biological monitoring (Davydiuk et al., 2023; Taylor, Goodale, et al., 2017). Taylor, Goodale, et al. (2017) have suggested that thio-DMAE and thio-DMAA are unique urinary products of arsenosugars, and hence measurement of these metabolites in urine may allow an assessment of arsenosugar intake.

3.1.3 | Toxicity

The CONTAM Panel noted that the toxicity studies with complex organoarsenic species were summarised very briefly in the 2009 Opinion and decided to review all of the studies published since 1970 in order to provide a more comprehensive overview of the data, encompassing both cell-based and animal model studies.

3.1.3.1 | *Acute and repeated dose toxicity*

Acute toxicity

The 2009 Opinion noted that the oral LD_{50} value of AsB in weanling male mice was > 10,000 mg/kg bw, but did not describe other studies on toxicity of the complex organoarsenic species. The 2009 Opinion referred to the reviews of WHO (2001) and ATSDR (2007) and did not cite the primary source for the oral LD_{50} value of AsB, however it appears to be derived from the study of Kaise et al. (1985). In another acute study, intraperitoneal injection of AsB into mice at the dose of 360 mg/kg bw resulted in no mortality and no difference in behaviour compared to control mice (Cannon et al., 1983). Kaise and colleagues have also reported on the acute toxicity of AsC, with an oral LD_{50} of 6500 mg/kg bw in mice (Kaise et al., 1992). Sakurai (2002) reported that the oral LD_{50} of an arsenosugar (AsSugOH) was > 6000 mg/kg bw in mice.

Repeated dose toxicity

Kim et al. (2008) orally dosed groups of three male cynomolgus monkeys twice with AsB (3.56 mg/kg bw on day one, followed by 71.3 mg/kg bw on day 19) or AsC bromide (4.9 mg/kg bw on day one, followed by 98.1 mg/kg bw on day 19). Clinical observations, haematological parameters and serum biochemical parameters were recorded. Soft faeces and vomiting, and also an increase in reticulocyte percentage were reported following both doses of AsC but not AsB. Aspartate aminotransferase (AST) was reduced, and total cholesterol was increased following both doses of AsB but not

AsC. However, there was no control group of monkeys, and therefore these observations cannot be attributed to the administered substance.

Administration of AsB to male CDF1 mice at 1625 mg/kg bw on four occasions over one week (averaging 387 mg As/kg bw per day over the week) resulted in no changes in body weight, weight of spleen or thymus, or in numbers of Peyer's patches and functions of immune effector cells (Sakurai et al., 2004). In contrast, these authors found that administration of AsC to male CDF1 mice at 2500 mg/kg bw on four occasions over one week decreased the absolute and relative weight of the thymus, and survival of thymocytes. Survival of splenocytes was suppressed, but there were no other effects on the spleen or on body weight (Sakurai et al., 2005).

In an investigation of the proatherogenic potential of different arsenic species in male apo $E^{-/-}$ and DKO (double knockout apo $E^{-/-}$ *As3mt*$^{-/-}$) mice, administration of AsB (200 µg/L in drinking water for 13 weeks, equivalent to an AsB dose of 0.03 mg/kg bw per day) had no effect in this model system (Negro Silva et al., 2017).

The toxicity of three arsenic hydrocarbons (AsHC 332, AsHC 346 and AsHC 374) has been investigated in C57BL/6 mice dosed at the level of '3 mg/kg' by gavage for 4 weeks (Chen et al., 2023). The paper does not make clear whether this dose is expressed as As or as the tested AsHC, or if it is a daily dose or cumulative. There were no significant differences in body weight or liver weight. The authors reported that histological examination of the livers of mice showed mild changes, including steatosis and spherical microvesicles, for all three of the AsHC treatments. However, the CONTAM Panel noted that the figures presented in the publication were unclear and did not support conclusions on the histopathological changes. The concentrations of As and Fe in the livers of AsHC treated mice were increased and concentrations of Cu, Co, Mn, Cr, Ni, Se and Zn were decreased compared to control animals. Activities of ALT and AST were increased, and T-SOD activity and GSH content were decreased, as was the expression of the genes Soda, Sod2, Mt1 and Mt2. The effects of AsHC 332 were greater than those of the other 2 arsenic hydrocarbons. Because of the lack of clarity on the dose levels and histopathology, these data could not be considered further in the evaluation.

3.1.3.2 | *Reproductive and developmental toxicity*

Taylor et al. (2013) investigated the effects of oral exposure to AsB in rats during pregnancy and lactation. Groups of pregnant Sprague–Dawley rats received AsB via gavage at doses of 0, 0.1, 1 or 10 mg/kg bw per day (corresponding to 0, 0.042, 0.42 and 4.2 mg/kg bw per day expressed as elemental arsenic) from GD8 to GD20, PND12 or PND20, and the offspring were examined at GD20 or PND21, respectively, for body and organ weights, clinical chemistry and haematology. The offspring of further groups of dosed dams were followed up to PND90, with measurements of body weights, clinical chemistry and haematology and developmental milestones (eye opening, vaginal opening and estrus cycle, preputial separation). AsB did not result in any treatment-related alterations in dam general health, length of gestation, litter size, sex ratio or pup survival. For those offspring followed up until PND90, there was a small but significant reduction in body weight gain in both male and female pups, with no dose response relationship.

3.1.3.3 | *Neurotoxicity*

Neurobehavioural effects of a pentavalent arsenosugar, AsSugOH, have been investigated by Bin Sayeed et al. (2013). Groups of 10 male Swiss albino mice were dosed with 0, 20, 30 or 50 mg/kg bw per day AsSugOH orally for 40 days. The actual route of administration is not specified but is assumed to be gavage because the control group received 0.9% NaCl. Passive avoidance tests in a box with dark (delivering electric shocks) and illuminated (without shocks) chambers were performed on days 36–38. Motor function tests to evaluate muscle strength and coordination on a rotating rod were performed on days 39–40. The mice exhibited a dose-related decrease in both passive avoidance and motor function at all dose levels. There was no significant difference in bodyweight compared to controls. Biochemical parameters related to oxidative stress were also measured and are reported in Section 3.1.4 on Mode of action.

3.1.3.4 | *Genotoxicity*

<u>In vitro genotoxicity</u>

Arsenobetaine

Out of the various complex organoarsenic species, AsB has received the most attention in genotoxicity studies. Overall, AsB demonstrated either no genotoxic potential or, at most, a minimal genotoxic potential across different experimental systems. Lack of mutagenic activity was reported both in bacteria in the Ames test in the presence and absence of liver microsomal fraction (S9) (Cannon et al., 1983; Jongen et al., 1985) and in mammalian cells in the HGPRT assay in V79 hamster cells (Jongen et al., 1985) and in the TK assay in mouse lymphoma cells (Soriano et al., 2007). AsB had neither synergistic nor antagonistic effects on the mutagenic activity of benzo[a]pyrene or on the inhibition of metabolic cooperation by tetradecanoylphorbol-13-acetate (TPA) in V79 cells (Jongen et al., 1985). Negative results were also obtained when the genotoxic potential was analysed by the sister chromatid exchange (SCE) assay in V79 cells (Jongen et al., 1985; Kaise et al., 1998) and in primary cultures of human umbilical cord fibroblasts (Kaise et al., 1998) as well as by the chromosomal aberration assay in primary cultures of human umbilical cord fibroblasts (Kaise et al., 1998; Oya-Ohta et al., 1996). The

incidence of aberrations in AsB treated cells was not influenced by depletion of GSH, suggesting that AsB does not interact with cellular nucleophiles (Oya-Ohta et al., 1996).

The analysis of mitotic arrest and tetraploid formation gave also negative results (Eguchi et al., 1997). Consistent with the absence of significant genotoxic effects, no DNA damage was observed using the Comet assay in the TK6 human lymphoblastoid cell line (Guillamet et al., 2004).

Arsenocholine

Two studies were identified regarding the genotoxicity of AsC. Oya-Ohta et al. (1996) and Kaise et al. (1998) reported low genotoxic activity (MN and SCE assays) as compared to inorganic arsenic species on a molar basis and only at a highly cytotoxic concentration, in human umbilical cord fibroblasts and V79 cells. The analysis of mitotic arrest and tetraploid formation gave also negative results (Eguchi et al., 1997).

Arsenosugars

In one study, an arsenosugar (AsSugOH) induced chromosomal aberrations in primary cultures of human umbilical cord fibroblasts but only at very high concentrations (Oya-Ohta et al., 1996) and was negative in the SCE assay. On the basis of the differential toxicity of the trivalent versus pentavalent arsenic species, Andrewes et al. (2004) compared the in vitro toxicity of the pentavalent arsenosugar (AsSugOH), to that of its trivalent counterpart (where $Me_2As(O)^-$ is converted to Me_2As^-). The trivalent arsenosugar was positive in the in vitro nicking plasmid DNA assay, whereas the pentavalent arsenosugar was not. The trivalent arsenosugar was more cytotoxic than its pentavalent counterpart in normal human epidermal keratinocytes in vitro, however, both the trivalent and the pentavalent arsenosugars were significantly less cytotoxic than MMA(III) or DMA(III). Neither the pentavalent arsenosugar nor the trivalent arsenosugar was mutagenic in Salmonella TA104. Leffers et al. (2013) confirmed the lack of clastogenic potential of arsenosugars by analysing micronuclei frequency induced by AsSugOH and AsSugSO4 in human urothelial cells (UROtsa). Besides the negative results in the MN assay, no increase of bi- or multinucleated cells was observed.

Arsenolipids

Arsenic hydrocarbons (AsHCs) have been shown to be toxic in various in vitro systems including human cell lines in the same micromolar concentration range as compared to arsenite. Ebert et al. (2020) characterised the genotoxicity of thio-AsHC 348 (the thio analogue of AsHC 332) and directly compared it to AsHC 332. No induction of DNA damage by the Comet assay nor increase of micronuclei frequency was detected in HepG2 liver cells up to concentrations of 15 µM for 48 h exposure. This concentration caused a 40% decrease in both lysosomal integrity and dehydrogenase activity and a 50% decrease in cell number. Within this concentration range no induction of oxidative stress was reported. No induction of apoptosis nor necrosis was observed. Thio-AsHC 348, in contrast to AsHC 332, did not disturb the mitochondrial membrane potential, and, similar to AsHC 332, it was negative in inducing cellular oxidative stress.

Meyer et al. (2014) confirmed the lack of genotoxicity of AsHCs by analysing DNA damage and micronuclei frequency induced by AsHC 332, AsHC 360 and AsHC 444. In both cultured human urothelial (UROtsa) and liver (HepG2) cells these arsenolipids did not increase the number of micronuclei, nor the amount of bi- or multinucleated cells (data not shown). Additionally, they did not induce DNA strand breaks and Fpg-sensitive sites as measured by the alkaline unwinding assay in HepG2 cells. The lack of oxidative damage to DNA supports a mechanism of toxicity that is independent of oxidative stress.

Similar to the AsHCs, the arsenic fatty acids (AsFAs), as represented by a saturated (AsFA 362) and an unsaturated (AsFA 388) AsFA, were shown to lack clastogenic potential as detected by the micronucleus assay (Meyer et al., 2015). No increase of bi- or multinucleated cells was also reported.

In vivo genotoxicity

Only one in vivo study was identified where the pentavalent arsenosugar AsSugOH was tested for its potential to damage DNA (Bin Sayeed et al., 2013). The authors reported increased DNA damage, as measured by the alkaline comet assay, in both blood lymphocytes and hippocampus tissues from mice receiving daily oral doses of 20, 30 and 50 mg/kg bw of AsSugOH. Several comet assay parameters were measured, including the percentage of DNA in the head, the percentage of DNA in the tail and the tail moment (the product of tail DNA percentage and tail length). All measured comet parameters indicated statistically significant higher DNA damage in samples from the treated groups compared to the control group, and these changes were dose-dependent.

Summary of in vitro and in vivo genotoxicity

In general, these complex organoarsenic species, while exhibiting cytotoxic effects in cultured cells from various sources, including human cells, showed minimal if any in vitro genotoxic activity as evaluated by mutation assays in bacterial and mammalian cells, the comet assay, the sister chromatid exchange test and clastogenicity tests, such as micronuclei formation and chromosomal aberrations (see Table 5). When assessed alongside inorganic arsenic and small organoarsenic

species, the hierarchy of clastogenic potency was observed as follows: arsenite > arsenate > DMA(V) > MMA(V) > arsenosugar = AsB = AsC (Oya-Ohta et al., 1996; Kaise et al., 1998;). Also, in the case of the arsenolipids tested, a lack of clastogenic potential was observed in comparative studies with known clastogenic compounds (Meyer et al., 2014, 2015). Although no genotoxic effects were observed in in vitro cell systems, one in vivo study reported that arsenosugar (AsSugOH) induced DNA damage in a Comet assay in blood lymphocytes and hippocampus tissues of treated mice.

TABLE 5 Complex organoarsenic species in vitro genotoxicity.

Reference	Test system	Cells	Concentration/treatment time	Results	Comments
ARSENOBETAINE/ARSENOCHOLINE					
Cannon et al. (1983)	Ames test	Salmonella typhimurium (strains TA98, TA100, TA1535 and TA1537) +/−S9	AsB (1 mg, 100 µg and 10 µg)	**Negative:** both in the presence and absence of liver S9 fraction	
Jongen et al. (1985)	Ames test HGPRT mutation assay SCE assay	Salmonella typhimurium (TA97, TA98 and TA100) +/−S9 V79 Chinese hamster cells +/−S9	AsB Ames test: 1–5000 µg/plate HGPRT and SCE assays: 1–10 mg/mL	**Negative:** no mutagenic effects in any strain in the presence or absence of metabolising systems. **Negative**: negative in the HGPRT mutation assay and SCE test.	No effects on the activity of the positive controls benzo[a]pyrene and tetradecanoylphorbol-13-acetate in V79 cells.
Oya-Ohta et al. (1996)	CA assay	Primary cultures of human umbilical cord fibroblast cells for CA and SCE assays	AsC (1.2 to 12.1 ×10-2M) AsB (0.6–5.6 ×10-2M) arsenite, arsenate, tetramethylarsonium iodide for comparison	**Positive/weak:** AsC, AsB low activity as compared to inorganic arsenicals on a molar basis. The tested concentration corresponds to the amount that inhibits cell growth by 50% in BALB/c 3T3 cells.	The incidence of aberrations caused by AsB was not influenced by depletion of GSH, probably ASB does not interact with cellular nucleophiles.
Eguchi et al. (1997)	Analysis of mitotic arrest and tetraploid formation	Chinese hamster V79 cells	AsB (5.6 mM) AsC (6.1 mM)	**Negative** AsB and AsC did not induce mitotic arrest nor tetraploidy	
Kaise et al. (1998)	CA assay SCE assay	Primary cultures of human umbilical cord fibroblast cells for CA and SCE assays V79 Chinese hamster cells for the SCE assay BALB/c 3T3 cells: for cell growth inhibition	AsB, AsC Arsenite, arsenate, MMA, DMA(V), trimethylarsine oxide and TMAI for comparison	**Positive/weak**: AsB caused CA at a concentration of 10 mg/cm^3, mainly chromatid gaps and chromatid breaks. At this concentration osmotic pressure is likely to cause chromosomal breakage. **Negative**: AsB no effect in the SCE assay at a concentration of 1 mg/cm^3. Similar results for AsC	Arsenite induced CA after 24 h treatment at concentrations of 0.0001–0.001 mg/cm^3 The ranking order of these arsenic compounds in terms of clastogenic potency was arsenite > arsenate > DMA(V) > MMA(V) > trimethylarsine oxide > tetramethylarsonium iodide > AsB = AsC Cell growth inhibition in BALB/c 3T3 cells: no effect of AsB, AsC and trimethylarsine oxide at a concentration of 10 mg/cm^3.
Guillamet et al. (2004)	Comet assay	TK6 human lymphoblastoid cell line assay.	AsB; highest concentration 10 mM, for 30 min or 3 h. MMA(V), DMA(V), TMAI, TMACl for comparison	**Negative**: no induction of DNA damage	
Soriano et al. (2007)	TK mutation assay	L5178Y/*Tk*+/− mouse lymphoma cells	AsB (5–10,000 µM) MMA(V), DMA(V), tetraphenylarsenium for comparison	**Negative:** is not mutagenic after 4 and 24 h treatment	Inorganic arsenic compounds produce significant effects in the micromolar range, while the mutagenic organic arsenic compounds induce similar effects but in the millimolar range.

TABLE 5 (Continued)

Reference	Test system	Cells	Concentration/treatment time	Results	Comments
Leffers et al. (2013)	MN assay	human urothelial cells (UROtsa)	dimethylarsenoacetic acid (DMAA), dimethylarsenoethanol (DMAE), thio- DMAA and thio-DMAE	**Negative:** no increase of MN number nor of multinucleated cells at a concentration of 500 μM for 48 h	In the same study iAs(III) showed significant increase in the MN assay starting from 0.5 μM and DMA(V) in the frequency of bi- and multi-nucleated cells starting from 100 μM for 48 h
ARSENOSUGARS					
Oya-Ohta et al. (1996)	CA assay	Primary cultures of human umbilical cord fibroblast cells for CA and SCE assays	AsSugOH (0.3, 1.5 × 10-2M) Arsenite, arsenate, tetramethylarsonium iodide for comparison	**Positive/weak:** low activity as compared to inorganic arsenicals on a molar basis. The tested concentration corresponds to the amount that inhibits cell growth by 50% in BALB/c 3T3 cells.	The rank order of compounds in terms of clastogenic potency was arsenite > arsenate > DMAA > MAA > trimethylarsine oxide > arsenosugar = AsB = AsC.
Andrewes et al. (2004)	DNA nicking assay Ames test	pBR 322 plasmid DNA Salmonella typhimurium strain TA104	AsSugOH Trivalent counterpart of AsSugOH	**Positive:** DNA damage was observed in the nicking assay for trivalent arsenosugar at concentrations as low as 0.6 mM. **Negative**: no effect in the case of the pentavalent counterpart **Negative**: both arsenosugars showed no effect in the Ames test at doses as high as 1 mg/plate (3500 nmol/plate).	The trivalent arsenosugar exhibited at least equal potency at nicking pBR322 plasmid DNA when compared to DMA(III)
Leffers et al. (2013)	MN assay	human urothelial cells (UROtsa)	AsSugOH and AsSugSO4	**Negative:** no increase of MN number nor of multinucleated cells at a concentration of 500 μM for 48 h	In the same study iAs(III) showed significant increase in the MN assay starting from 0.5 μM and DMA(V) in the frequency of bi- and multi-nucleated cells starting from 100 μM for 48 h
ARSENOLIPIDS					
Meyer et al. (2014)	Alkaline unwinding (DNA breaks and Fpg-sensitive sites) MN assay	Human bladder (UROtsa) and liver (HepG2) cells.	AsHC 332, AsHC 360 and AsHC 444	**Negative:** no induction of DNA strand breaks and Fpg-sensitive sites in HepG2 cells at concentrations up to 50 μM for 48 h **Negative:** no increase of the MN frequency in Urotsa cells at concentrations up to 20 μM for 48 h	The arsenolipids are at least 600-fold more toxic than a glycerol arsenosugar and about 20 to 25-fold more toxic than their major metabolite DMAV.
Meyer et al. (2015)	MN assay	HepG2 cells	A saturated (AsFA 362) and an unsaturated (AsFA 388) arsenic-containing fatty acid (1–100 μM, 48 h incubation)	**Negative:** no significant micronuclei induction at concentrations up to 100 μM for 48 h	No increase of bi- or multinucleated cells occurred IC70 after 48 hrs: AsFA362 96 μM; AsFA388 83 μM

(Continues)

TABLE 5 (Continued)

Reference	Test system	Cells	Concentration/treatment time	Results	Comments
Ebert et al. (2020)	MN assay Comet assay	HepG2 liver cell line	thio-AsHC 348 up to 15 μM (48 h incubation) AsHC 332	**Negative**: thio-AsHC 348 did not induce micronuclei nor increase of DNA breaks up to 15 μM. No increase in binucleated cells with/ without micronuclei and polynucleated cell (data not shown).	Thio-AsHC 348 is a metabolite of AsHC 332 Similar cytotoxic concentration range for thioxo-AsHC348 and AsHC 332 but about 10 times lower As bioavailability for thio-AsHC 348 as compared to AsHC 332 No induction of apoptosis nor necrosis

Abbreviations: AsFA, Arsenic containing fatty acid; AsHC, Arsenic containing hydrocarbon; CA, chromosomal aberration; DMAA, dimethylarsinoylacetate; DMA(V), dimethylarsinic acid; DMAA(V), dimethylarsenoacetic acid; DMAE(V), dimethylarsenoethanol; DN, double negative; DNA, deoxyribonucleic acid; DP, double positive; ELISA, Enzyme-Linked Immunosorbent Assay; FLARE, Fluorescent Advanced Oxidation Protein Products; FPG, Formamidopyrimidine-DNA Glycosylase; GSH, glutathione; HGPRT, hypoxanthine guanine phosphoribosyl transferase; hOGG1, Human 8-Oxoguanine DNA Glycosylase 1; MDA, Malondialdehyde; MN, micronucleus; MMA(V), monomethylarsonic acid; $NaAsO_2$, sodium arsenite; uM, micromolar; ROS, Reactive Oxygen Species; SCE, Sister Chromatid Exchange; SOD, superoxide dismutase; SSB, Single-Strand Break; TK, thymidine kinase; UROtsa, human urinary tract epithelial cell line.

3.1.4 | Mode of action

The 2009 Opinion did not describe studies on modes of action of the complex organoarsenic species.

3.1.4.1 | *Arsenobetaine*

AsB and AsC were tested for in vitro immunotoxicity in a variety of murine and human cells. Kojima et al. (2002) investigated the effect of AsB on the viability and functions (NO_2- production, release of IL-1a and cellular lysosomal enzyme activity) of mouse macrophage RAW264.7 cells. The authors reported only minor effects on NO_2- production at concentrations up to 20 mM but not significant effects on the immune functions. AsB at the concentrations of 10^{-4} and 10^{-7} M had no effect on proliferation or cytokine content of human peripheral blood mononuclear cells (PBMC) (Di Giampaolo et al., 2004). AsB did not show any cytotoxicity in human monocyte-derived macrophages or dendritic cells at concentrations up to 5 mM, nor any effect on the differentiation of monocytes into macrophages or dendritic cells (Ohta et al., 2004). AsC was not cytotoxic in vitro at concentrations up to 10 mM in murine immune effector cells such as peritoneal macrophages, alveolar macrophages splenocytes, thymocytes or Peyer's patch lymphocytes and enhanced the viability of bone marrow cells (Sakurai, 2002). Overall, neither AsB nor AsC demonstrated in vitro immunotoxicity in human and murine cells.

AsB was not cytotoxic in vitro at concentrations up to 25 mM in mouse macrophage RAW264.7 cells, rat liver TRL1215 cells and human skin TIG-112 cells, in the presence or absence of the GSH synthase inhibitor BSO (25 μM) (Kojima et al., 2002). Therefore, AsB is non-toxic to these types of cells, regardless of the presence of a key protective antioxidant such as GSH.

Concentrations of AsB as high as 500 μM failed to induce either cytotoxic effects or neoplastic transformations in BALB/3T3 Cl A 31-1-1 cells (Sabbioni et al., 1991). When retention and intracellular distribution were investigated, the flow of AsB towards the intracellular compartment appeared to follow a simple diffusion mechanism, suggesting no binding with cellular macromolecules.

These findings align with the observed lack of genotoxic effects by both AsB and AsC in various mammalian cell systems, suggesting that these compounds do not interact with biologically relevant macromolecules.

3.1.4.2 | *Arsenosugars*

The study by Bin Sayeed et al. (2013) reported dysfunction of cognitive and motor functions in mice receiving daily oral doses of 20, 30 and 50 mg/kg bw of the pentavalent arsenosugar AsSugOH. The treated animals exhibited increased malondialdehyde (MDA) levels, decreased superoxide dismutase (SOD) activity and increased DNA damage, as detected by the Comet assay in both blood lymphocytes and hippocampus tissues. The authors suggest that these biochemical changes may account for the observed impairment in cognitive and motor functions in the exposed mice.

3.1.4.3 | *Arsenolipids*

Arsenolipids do not appear to induce oxidative stress or genotoxic effects in cultured cells (Ebert et al., 2020; Meyer et al., 2014). Meyer et al. (2014) investigated the cytotoxicity of three arsenolipids (AsHC 332, AsHC 360 and AsHC 444) in cell lines derived from human urothelium (UROtsa) and a human hepatoma (HepG2). Cytotoxicity was reported at low μM concentrations, associated with decreased ATP content of the cells, with AsHC 360 being the most and AsHC 332 the least cytotoxic, leading the authors to conclude that the cytotoxicity was due to a decline in energy levels. A subsequent study reported that a saturated (AsFA 362) and unsaturated (AsFA 388) arsenic-containing fatty acid were 10–20-fold less cytotoxic (Meyer et al., 2015).

Emerging evidence supports an association between exposure to arsenolipids, especially AsHCs, and neurotoxic effects. Witt, Ebert, et al. (2017), Witt, Meyer, et al. (2017) demonstrated that AsHC 332, AsHC 360 and AsHC 444 and the arsenic fatty acids, AsFA 362 and AsFA 388 interact with neuronal cells inducing significant cytotoxicity in cultures of pre-differentiated human neurons and astrocytes. AsHCs exhibited high cell accessibility in pre-differentiated neurons, affected neurite outgrowth and mitochondrial membrane potential. In contrast, arsenite did not substantially affect these two endpoints. However, both AsHCs and arsenite caused neurite toxicity in fully differentiated neurons. Interestingly, AsHCs did not exhibit substantial toxic effects in co-cultures of human astrocytes and neurons, suggesting a protective role of astrocytes against arsenic accumulation in neurons, highlighting the importance of glial-neuron interactions. AsHC 332, AsHC 360 and AsHC 444, and the arsenic fatty acids, AsFA 362 and AsFA 388 have been shown to penetrate and disrupt the blood–brain barrier in an in vitro model of the blood-cerebrospinal fluid barrier composed of porcine choroid plexus epithelial cells, which could also contribute to neurotoxic potential (Müller, Ebert, Bornhorst, et al., 2018; Müller, Ebert, Raber, et al., 2018).

AsHCs have been shown to accumulate in the brain tissue of *Drosophila melanogaster* (Niehoff et al., 2016) and tuna fish (Stiboller et al., 2019). Moreover, AsHCs, while exhibiting toxic effects in the fruit fly within a concentration range similar to that of arsenite, differ from arsenite by uniquely inducing developmental toxicity during the late developmental stages of the fruit fly (Meyer et al., 2014).

Zheng et al. (2021) investigated the potential neurotoxicity of AsHC 360 on long-term potentiation (LTP) in hippocampal slices isolated from male SD rats, aged 14–17 days. The field excitatory postsynaptic potential was recorded before and after treatment, and then the contents of Na, K, Ca, Mg, Mn, Cu, Zn and As were measured. The authors found no effect of

AsHC 360 at 1.5 µg As/L, enhancement of the LTP at 3.75–15 µg As/L, possibly associated with Ca^{2+} influx, and inhibition of the LTP at 45–150 µg As/L, possibly due to damage to the integrity of the synaptic cell membrane. They suggested that the results indicated that AsHCs may have a negative effect on development, learning and memory.

Three types of arsenolipids were tested in the worm *Caenorhabditis elegans*, namely two AsHCs, a saturated AsFA, and an arsenic-containing triacylglyceride (AsTAG) by Bornhorst et al. (2020). Although all arsenolipids were highly bioavailable, only the AsHCs were substantially metabolised to thiolated or shortened metabolic products, and induced significant toxicity, affecting development.

Several reports indicate that arsenolipids exhibit neurotoxic properties in vitro and were found in the brain of Drosophila. Arsenolipids have been measured also in brain of tuna (Stiboller et al., 2019). While flies have a blood brain barrier, that of fish is structurally and functionally more similar to the human system (Limmer et al., 2014; O'Brown et al., 2018). Although the mechanism is unclear, the potential for neurotoxicity in humans would require further investigation.

3.1.5 | Observations in humans

The CONTAM Panel identified no epidemiological studies that provided results from measurements or estimates of concentrations of complex organic As species in food. There are, however, many studies that examined associations between urinary excretion of various As species and health outcomes. Some of these studies quantified AsB in urine. The CONTAM Panel identified no such reports on AsC or other complex organoarsenic species including arsenolipids and arsenosugars. Therefore, the sections below refer to studies on AsB in urine. It should be noted that for many of the studies assessing As species in urine, the a priori hypothesis was that the inorganic As species (including their metabolites) might be associated with adverse health outcomes. However, some of the studies also reported results for AsB.

3.1.5.1 | *Selection of studies*

Cohort, case–control and cross-sectional studies characterised by estimates on the individual or group level of exposure to AsB were considered.

The reliability of all epidemiological studies was assessed in terms of design, exposure assessment, assessment of outcomes, confounding and risk of other sources of bias.

For some outcomes, studies exclusion criteria were applied from the start: Studies of type 2 diabetes were excluded if data were not available on smoking and BMI. Studies on neurodevelopment were excluded if data were not available for key covariates that are important for neurodevelopment. The covariates were based on the findings of Lanphear et al. (2005) who evaluated low-level exposure to lead and neurodevelopment: (1) sex and age, anthropometry or nutrition, (2) socioeconomic status (SES) or HOME (Home Observation Measurement of the Environment) score and (3) parental education/intelligence quotient (IQ). Because of some overlap of these factors (e.g. between SES and parental education, and between nutrition and SES), the selected studies were required to have adjusted for at least two of these groups of covariates. See table 4 in Section 2 on Data and Methodologies for further details on inclusion/exclusion criteria for human studies.

The CONTAM Panel notes that seafood is a major source of exposure to AsB. Fish consumption is often associated with decreased risk of cardiovascular disease and may be beneficial for neurodevelopment, unless the fish is contaminated with high levels of contaminants which may compromise neurodevelopment (e.g. MeHg, dioxins or PCBs). This should be taken into account when assessing possible associations between AsB in urine and health outcomes.

3.1.5.2 | *Studies on associations between AsB in urine and health outcomes*

The CONTAM Panel identified 13 studies in humans on associations between u-AsB and health outcomes as listed in Table 6. Most of them were cross-sectional, several of which used data from the United States National Health and Nutrition Examination Survey (US NHANES). Three studies examined associations with diabetes (type 2 or gestational diabetes) or related conditions (insulin resistance and beta cell function). For each of the other outcomes only one study was identified.

In summary, apart from diabetes, only one study per outcome was identified and for many of the studies no significant associations were found. Some tendencies towards reduced ORs could be due to protective effects of fish consumption. Therefore, epidemiological studies provide no evidence for an association between exposure to AsB and adverse health outcomes.

TABLE 6 Epidemiological studies on associations between AsB in urine and various health outcomes.

Reference Study population Design	Outcome definition	Population size (n) Case/control	Arsenic in urine (μg/L)	Results OR (95% CI) or β (95% CI)	Comment/confounders
Navas-Acien et al. (2008) US NHANES Cross-sectional	Type 2 Diabetes	788 adults	AsB 32% > LOD (0.4) Median 0.9 ≤ 0.4 > 0.4–2.0 > 2.0	POR for p80 versus p20 = 0.53 (0.22–1.26) 1.0 0.54 (0.28–1.05) 0.44 (0.16–1.21)	Adjusted for u-creatinine, sex, age, race, ethnicity, education, BMI, s-cotinine, hypertensive medication. Test for trend: $p = 0.14$. A model also including B-Hg showed similar results.
Shiue (2015) US NHANES Cross-sectional	Eczema (self-reported physician-diagnosed)	4979 adults, 310 (6.2%) with eczema	Means: AsB 13.6 μg/L.	POR per μg/L AsB: 0.81 (0.64–1.03)	Adjusted for u-creatinine, age, sex, birthplace. LOD and numbers < LOD not reported.
Thomas et al. (2015) Canada (MIREC study) Cohort	Small for gestational age (SGA)	1835 pregnant women	AsB (first trimester) 49% > LOD (0.75 μg/L as As) AsB (cases/women) < 0.75 (46/958) 0.75–2.25 (9/177) > 2.25 (47/656)	RR (OR) 1.0 1.20 (0.60–2.41) 1.65 (1.10–2.47)	Adjusted for smoking, parity, specific gravity. Basis for cut-offs unclear (reported to be tertiles).
Jain (2016) US NHANES Cross-sectional	Thyroid hormones (TSH, TT4, FT4, TT3, FT3) and antibodies (TGN)	4126 adults and adolescents	AsB (μg/L). 62% > LOD. Tertiles: < 0.40 0.40–2.73 > 2.73	Iodine-replete females: Sign inverse association with TSH and TGN, but not with thyroid hormones.	Linear regression estimates were performed separately for iodine-deficient and -replete men and women. In total 96 regression estimates. Adjustments not clearly presented.
Nong et al. (2016) US NHANES Cross-sectional	10-year Arteriosclerotic CVD risk calculated based on prediction equations	1570 hypertensive adults	AsB LOD 0.4 μg/L or 1.14 μg/L (two periods). 4% < LOD Quartiles men: ≤ 0.60 0.61–1.92 1.93–8.39 > 8.40 Women: ≤ 0.30 0.31–1.16 1.17–5.70 > 5.71	Predicted ASCVD risk 1.0 0.96 (0.88–1.06) 0.94 (0.85–1.03) 0.98 (0.89–1.07) 1.0 1.02 (0.92–1.12) 1.10 (0.99–1.21) 1.16 (1.03–1.30)	Prediction equations (from AHA) for ASCVD risk based on age, sex, race, BP, total serum cholesterol, HDL, smoking and diabetes. Model adjusted for age, race, u-creatinine, education, BMI, serum cotinine, total cholesterol, HDL. Test for trend: $p = 0.03$. Additional adjustment for self-reported seafood intake had minor impact on results. 'Sensitivity analyses' when also 2067 non-hypertensives in NHANES were included showed no significant associations in men or women.
Wang et al. (2016) China Case–control	Male infertility	101 infertile and 61 fertile men	AsB LOD (0.2 μg/L). Medians in μg/g creatinine: controls 7.80 cases 12.03. Quartile limits NR.	OR by quartiles (n cases) Q1 1.0 (23) Q2 0.4 (0.14–1.15) (19) Q3 0.83 (0.30–2.32) (26) Q4 2.23 (0.70–7.12) (33)	Adjusted for age, BMI, alcohol, smoking. *P* for trend 0.01. Highly significant trend for u-As(V). No correlations with sperm parameters.

(Continues)

TABLE 6 (Continued)

Reference Study population Design	Outcome definition	Population size (n) Case/control	Arsenic in urine (μg/L)	Results OR (95% CI) or β (95% CI)	Comment/confounders
Baek et al. (2017) Cross-sectional	Beta cell function (HOMA-IR and HOMA- β)	369 adults from KNHANES	AsB, Median 72 μg/g creatinine < 16.3 16.9–70.1 71.6–322 324–2621 < 16.3 16.9–70.1 71.6–322 324–2621	HOMA-IR β 1 −0.21(−0.45–0.03) −0.22 (−0.47–0.03) −0.04 (−0.31–0.23) HOMA-β (%) 1 −9.6 (−24.5–5.3) −17.8 (−33.3 – −2.3) −8.4 (−24.9–8.1)	Adjusted for age, BMI, smoking, alcohol, physical activity and B-Hg.
Ashley-Martin et al. (2018), Canada (MIREC study) Cohort	Gestational diabetes	1243 women, 42 cases with GD.	AsB (first trimester) 49% > LOD (0.75μg/L as As) AsB (cases/women) < LOD (14/539) ≥ LOD (28/552)	 1.0 1.96 (0.97–3.96)	Adjusted for age, specific gravity, education, pre-pregnancy BMI, parity, race, ln Cd. Also non-significant if u-DMA was added to model.
Jain (2021) US NHANES Cross-sectional	Renal function	10,590 adults	AsB 55% > LOD GM vary btw 1.13 and 2.09 μg/L in the five strata of eGFR.	Adjusted geometric mean AsB increased with decreasing eGFR category up to eGFR 45–60 mL/min/1.73 m^2 BSA, and then decreased at eGFR < 45 mL/min.	The dependent variable(s) in the study is AsB (and other As species), not eGFR. However, the authors state that the result is compatible with (but not proof of) AsB causing declining GFR, while more severe kidney disease causing decreased excretion of AsB (and other As species).
Rahman, Niemann, and Munson-McGee (2022) US NHANES Cross-sectional	Albumin in urine	4094 adults, 12.7% with albuminuria defined as albumin/creatinine ratio ≥ 30 mg/g	AsB. 60% < LOD (NR). Median 1.1 μg/L for ids with AsB > LOD?	POR of albuminuria (ref AsB < LOD, and quartiles for ids > LOD) < LOD 1.0 Q1 0.65 (0.36–1.14) Q2 1.51 (0.65–3.50) Q3 0.93 (0.44–1.95) Q4 1.11 (0.43–2.87)	Adjusted for age, gender, ethnicity, education, marital status, poverty index, BMI, alcohol, cotinine. Methods and results hard to follow.
Rahman, Niemann, and Yusuf (2022) US NHANES Cross-sectional	Sleep disturbance (self-reported)	1611 adults, 30% with sleep disorders	AsB. 55% < LOD (1.16 μg/L).	POR < LOD 1.0 ≥ LOD 1.18 (0.83–1.69)	Adjusted for age, race/ethnicity, serum cotinine, self-reported depression.
Soler-Blasco et al. (2022) Spain Cohort	Neurodevelopment in children (McCarthy scales; MSCA)	807 Mother–Child pairs in INMA	AsB LOD 0.02 μg/L. Maternal (first trimester) GM 20.7 μg/g creat	Positive but non-significant regression coefficients btw u-AsB and MSCA, apart from verbal 0.59 (0.11–1.07) per μg/L.	Adjusted for creatinine, study area, season, maternal age and place of birth, maternal and paternal education, maternal verbal intelligence, parity, seafood, child's sex, attendance at nursery. Some additional confounder adjustments for some of the subscales. Significant *inverse* associations for MMA and general and several subscales.

TABLE 6 (Continued)

Reference Study population Design	Outcome definition	Population size (n) Case/control	Arsenic in urine (μg/L)	Results OR (95% CI) or β (95% CI)	Comment/confounders
Lee et al. (2022) Korea Cross-sectional	MDA and C-peptide in urine	2044 Korean adults	AsB 0.5% < LOD (0.06 μg/L). GM 41 μg/L	MDA by AsB and MDA (quartiles) Means (SD) MDA C-peptide 1.37 (1.32) 33.5 (30.2) 1.57 (1.44) 36.4 (31.7) 1.79 (1.55) 41.1 (35.4) 1.91 (1.57) 46.0 (36.2)	Group comparisons by ANOVA, *F*-tests: $p < 0.01$. The focus of the paper is levels and predictors of As species (not MDA or C-peptide).

Abbreviations: AHA, American Heart Association; ANOVA, analysis of variance; As, arsenic; AsB, arsenobetaine; ASCVD, Atherosclerotic Cardiovascular Disease; B-Hg, blood mercury; BMI, body mass index; BP, blood pressure; BSA, body surface area; Cd, cadmium; CI, confidence interval; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; FT3, free triiodothyronine; FT4, free thyroxine; GD, gestational diabetes; GM, geometric mean; HDL, high-density lipoprotein; HOMA- β, Homeostatic Model Assessment of Beta-cell Function; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; INMA, INfancia y Medio Ambiente; KNHANES, Korea National Health and Nutrition Examination Survey; LOD, limit of detection; MDA, malondialdehyde; MIREC, Maternal–Infant Research on Environmental Chemicals; MMA, monomethylated arsenic; MSCA, Mullen Scales of Early Learning; n, number; NR, not reported; OR, odds ratio; p20/p80, 20th/80th percentile; POR, prevalence odds ratio; ref., reference; RR, risk ratio; s-cotinine, serum cotinine; SD, standard deviation; SGA, small for gestational age; TGN, thyroglobulin antibodies; TSH, thyroid-stimulating hormone; TT3, total triiodothyronine; TT4, total thyroxine; u-creatinine, urinary creatinine; u-As(V); urinary arsenate; u-DMA, urinary dimethylated arsenic; US NHANES, United States National Health and Nutrition Examination Survey.

3.1.6 | Consideration of critical effects

There are very few experimental animal toxicity studies available for the complex organic arsenic species. Epidemiological studies provide insufficient evidence for associations between exposure to these species and adverse health outcomes.

AsB has shown no evidence of toxicity in acute oral studies in mice with doses greater than 1500 mg/kg bw administered once (Kaise et al., 1985) or four times over 1 week (Sakurai et al., 2004). It is not genotoxic in *in vitro* assays (see Section 3.1.3.4). One reproductive and developmental study (Taylor et al., 2013) found no treatment-related findings; however, the CONTAM Panel noted that it was performed at relatively low dose levels (up to 10 mg/kg bw per day) and therefore provides limited information for this assessment. It is widely assumed that AsB is excreted unmetabolised (see Section 3.1.1. on Toxicokinetics), and would not exhibit toxicity in humans at relevant dietary exposure levels. However, the limited available data are insufficient to identify a reference point.

AsC has shown some evidence of immunotoxicity in mice dosed orally at 2500 mg/kg bw on 4 occasions over 1 week (the only dose level tested) (Sakurai et al., 2005). It has shown no or weak effects in *in vitro* genotoxicity studies (see Section 3.1.3.4). Similarly to AsB, the available data are insufficient to identify a reference point.

Arsenosugars showed no genotoxic effects in in vitro assays, with only one in vivo study reporting DNA damage in animal tissues (see Section 3.1.3.4). The only toxicity study available for arsenosugars (Bin Sayeed et al., 2013) reported dose-related neurobehavioural effects in mice orally dosed with AsSugOH for 40 days. The CONTAM Panel decided to conduct dose–response modelling on the data from this study (see Section 3.1.7).

Among the tested arsenolipid classes, the AsHC exerted highest toxicity in vitro. AsHC and AsFA have not shown evidence of genotoxicity in vitro (see Section 3.1.3.4). Three types of AsHC have been tested in a study where mice were dosed by gavage at '3 mg/kg' for 4 weeks (Chen et al., 2023). Due to the lack of detail regarding whether this dose was expressed as As, or as the tested AsHC, and whether it was administered daily or as a cumulative dose, as well as the unclear information regarding histopathological findings, the study is not informative and no reference point can be identified. Several reports indicate that AsHC exhibits neurotoxic properties in vitro (see Section 3.1.4.3), but no in vivo data are available.

3.1.7 | Dose response assessment for arsenosugars

The CONTAM Panel identified one study for dose–response modelling (Bin Sayeed et al., 2013) which investigated the effects of AsSugOH on motor function and contextual memory in male mice (see Section 3.1.3.3 on Neurotoxicity). The benchmark dose (BMD) modelling was carried out according to the 2022 EFSA BMD guidance (EFSA Scientific Committee, 2022) and the results were obtained using the EFSA web-tool for Bayesian BMD analysis, which uses the R-package [BMABMDR] version of 0.0.0.9087 for the underlying calculations.

The BMD analysis was performed on two endpoints: (1) time of entrance in the dark chambers (avoidance test) and (2) retention time on a rotating rod of male mice exposed to AsSugOH via gavage at doses of 0, 20, 30 and 50 mg/kg bw per day for 40 days (Bin Sayeed et al., 2013).

A BMR of 10% was chosen for the above-mentioned effects, as it is believed to reflect the natural variability of neurobehavioral endpoints. This selection was made due to the lack of biological considerations of severity that would justify a different BMR. This is also in line with the approach taken in the update of the risk assessment on polybrominated diphenyl ethers (PBDEs) where a BMR of 10% has been selected for a similar endpoint (EFSA CONTAM Panel, 2024).

Using model-averaging, the $BMDL_{10}$ of 3.75 mg AsSugOH /kg bw per day (0.86 mg/kg bw per day expressed as As) was estimated for time of entrance in the dark chamber. The corresponding figure for the retention time on the rotating rod is 3.71 mg AsSugOH /kg bw per day (0.85 mg/kg bw per day expressed as As).

Table 7 below provides an overview of the BMD results obtained from the study of Bin Sayeed et al. (2013). A more detailed description of the BMD analyses can be found in Annex G.

TABLE 7 Benchmark dose analysis, expressed as AsSugOH, for the effects of AsSugOH in Bin Sayeed et al. (2013).

Study	Endpoint measured	BMR (%)	BMDL (mg/kg bw per day)	BMD (mg/kg bw per day)	BMDU (mg/kg bw per day)
Bin Sayeed et al. (2013)	Time of entrance in the dark chambers (avoidance test)	10	3.75	7.87	13.96
	Retention time on a rotating rod[a]	10	3.71	5.91	10.77

Abbreviations: BMD, benchmark dose; BMDL, benchmark dose lower confidence limit; BMDU, benchmark dose upper confidence limit; BMR, benchmark response.
[a]The data were presented as a box plot (median, min-max) and estimated as mean ± SD using the formula suggested by Wan et al. (2013).

3.1.8 | Derivation of reference points and approach for risk assessment

As noted above, the available data are not sufficient to identify reference points for AsB, AsC or arsenolipids.

In the field of the arsenosugars there is one study for AsSugOH (Bin Sayeed et al., 2013) that allows for the identification of a Reference Point. The CONTAM Panel calculated a $BMDL_{10}$ for retention time on a rotating rod of 3.71 mg AsSugOH/kg bw per day (equivalent to 0.85 mg As/kg bw per day), and a $BMDL_{10}$ of 3.75 mg AsSugOH/kg bw per day for the time of

entrance in the dark chambers (equivalent to 0.86 mg As/kg bw per day). Overall, a value of 0.85 mg As/kg bw per day can be used as a reference point for AsSugOH.

Given the overall lack of toxicity data, the CONTAM Panel concluded that it is not appropriate to establish a health-based guidance value for any of the complex organoarsenic species and decided that a margin of exposure (MOE) approach would be appropriate for AsSugOH.

The CONTAM Panel concluded that the approach to evaluate the MOE for AsSugOH should take into account the default uncertainty factor of 100 for inter- and intra-species differences and an additional factor to allow for deficiencies in the database. According to the EFSA Scientific Committee Guidance on selected default values, an additional factor can be considered in case of deficiencies in the database on a case-by-case basis. Furthermore, a default value has not been proposed, as it will be directly dependent on the data set available (EFSA Scientific Committee, 2012). The WHO (1994, 1999) has recommended a factor of 3 or 5 if there are minor deficiencies in the database and a factor of 10 if there are major deficiencies in the database. In the case of AsSugOH, data on neurobehavioural effects are available, whereas studies on chronic toxicity or other effects such as reproductive toxicity are lacking. The CONTAM Panel applied an additional factor of 10 to account for major deficiencies in the database indicating that a MOE ≥ 1000 would not raise a health concern.

The available data do not allow identification of RPs or MOEs not raising a health concern for any of the other complex organoarsenic species (AsB, AsC, arsenolipids or arsenosugars other than AsugOH).

3.2 | Occurrence data

3.2.1 | Occurrence data in food considered for dietary exposure assessment

3.2.1.1 | *Occurrence data on AsB*

An initial number of 650 analytical results on AsB on food were extracted from the EFSA Data Warehouse (see Annex D for the raw data).

The convention in arsenic speciation analysis is to report the individual arsenic species expressed as elemental arsenic concentrations (e.g. µg As/kg food). At the moment of the data submission, data providers did not indicate whether the concentrations were expressed as elemental arsenic or as arsenic species/kg (e.g. µg AsB/kg food). Following clarification requests from EFSA and based on the feedback received, all data were converted into elemental arsenic concentrations (µg As/kg) before being used for dietary exposure assessment.

A thorough analysis of the occurrence data set was carried out to prepare the data for the dietary exposure assessment; data providers were contacted as needed to clarify any doubt about the data submitted. The following modifications were made based on the feedback received and/or expert judgement:

- 30 analytical results on AsB, four from samples codified as ´Marine fish´ and 26 as ´Rice grains', were excluded as they refer to Total Diet Studies (TDS) samples that were prepared as consumed before being analysed.

Few other samples, also submitted as TDS samples to EFSA, were kept in the final dataset. This refers to four samples of 'Marinated/pickled fish´ and 48 of 'Rice-based dishes, cooked'. These are samples that were not processed before being analysed, and with a level of aggregation matching the level of classification of the individual samples. In the case of 'Marinated/pickled fish´, each sample was made of three individual samples from different sampling points that were pooled before the analysis. To ensure a proportionate representation of the individual samples and, therefore, an accurate use of the occurrence data in assessing the dietary exposure, the mean concentrations per food category were calculated by weighting the reported analytical results for the number of samples pooled.

The reported LODs/LOQs were assessed to identify high values that might have a significant influence on the UB scenario (EFSA, 2018). Following the assessment, it was concluded that there was no need to apply any cut-off values on the dataset, and all samples were kept.

After excluding the 30 analytical results mentioned above, the final data set comprised 620 analytical results, all expressed in whole weight (w.w.) and as µg As/kg. A total of 577 analytical results were reported directly as µg As/kg, while 73 analytical results confirmed as reported as µg AsB/kg food were converted to µg As/kg by dividing the reported value by a factor of 2.4.

Recovery was not reported for any of the samples analysed for AsB; for 42 of these samples the analytical data were sent to EFSA as corrected for recovery, 108 samples were reported as not corrected and for 470 samples no information was available on whether corrected or not for recovery. The analytical data were not modified; the uncertainty associated with the lack of information on recovery is discussed in the corresponding section.

As regards the sampling strategy (EFSA, 2013), 56% of the samples were reported as 'Selective sampling', 35% as 'Objective sampling' and for 9% no information was provided. All samples were retained in the final dataset regardless of the sampling strategy.

The main detection method was mass spectrometry (93%), reported as ICP–MS, either coupled to liquid chromatography (80%) or just as ICP–MS. For some samples (7%), the analytical method was reported as just 'HPLC with standard detection methods' without further details. For most of the samples only the LOQ was provided although on a few occasions

the LOD was also reported. The lowest reported LOD and LOQ were 0.06 µg As/kg and 0.24 µg As/kg, respectively, for the analysis of samples of 'Rice-based dishes, cooked'. Some relatively high LOQs (160–170 µg As/kg) were reported for the analyses of specific matrices, codified as 'Food supplements and similar preparations' and 'Algae-based formulations (e.g. Spirulina, chlorella)'.

As shown in Figure 2, the samples were collected in seven European countries, 62% of them in Belgium. All food samples were taken from the EU market regardless of their country of origin. Information on the country of origin was not reported in ~66% of the samples, and 24% were originated from non-EU countries. The number of samples per sampling year is presented in Figure 3; they were collected between 2016 and 2022.

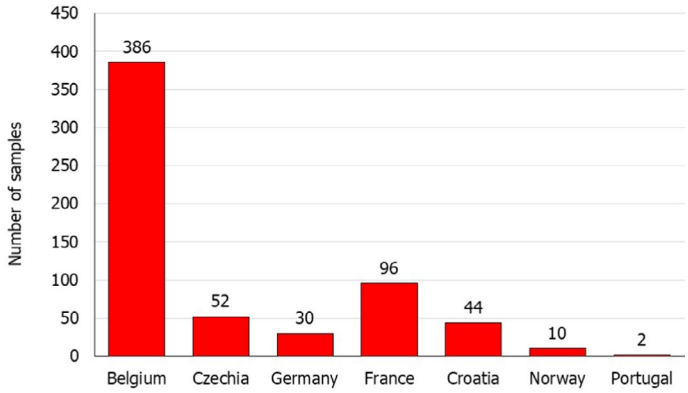


FIGURE 2 Distribution of analytical results reported for AsB across different EU countries (country of sampling; final cleaned dataset).

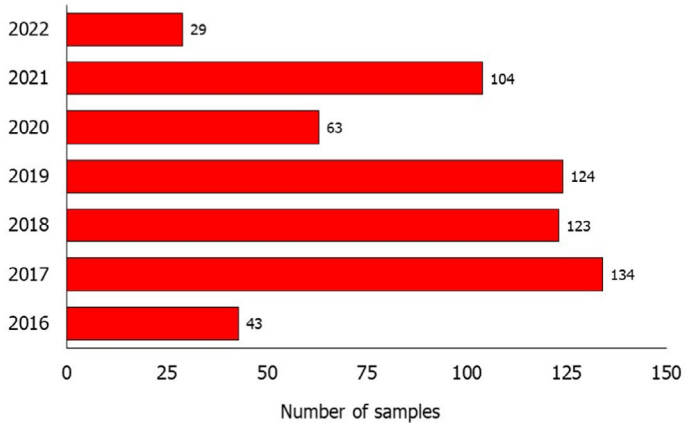


FIGURE 3 Distribution of analytical results reported for AsB by sampling year (final cleaned dataset).

The left-censored data accounted for 75% of the analytical results on AsB. Proportions of non-detected, non-quantified and quantified analytical results as well as the number of analytical results by FoodEx2 Level 1 food category are presented in Figure 4.

As can be seen in Figure 4, the vast majority of the quantified data were reported for samples codified as 'Fish and seafood' (128 out of 154 samples). The other food groups with quantified data were 'Composite dishes', 'Products for non-standard diets, food imitates and food supplements', and 'Vegetables and vegetable products'. The last two food groups include either seaweed samples or samples with seaweed as the main constituent (e.g. food supplements). The samples with quantified levels of AsB in the group 'Composite dishes' corresponded to a few samples codified as 'Rice-based dishes, cooked'.

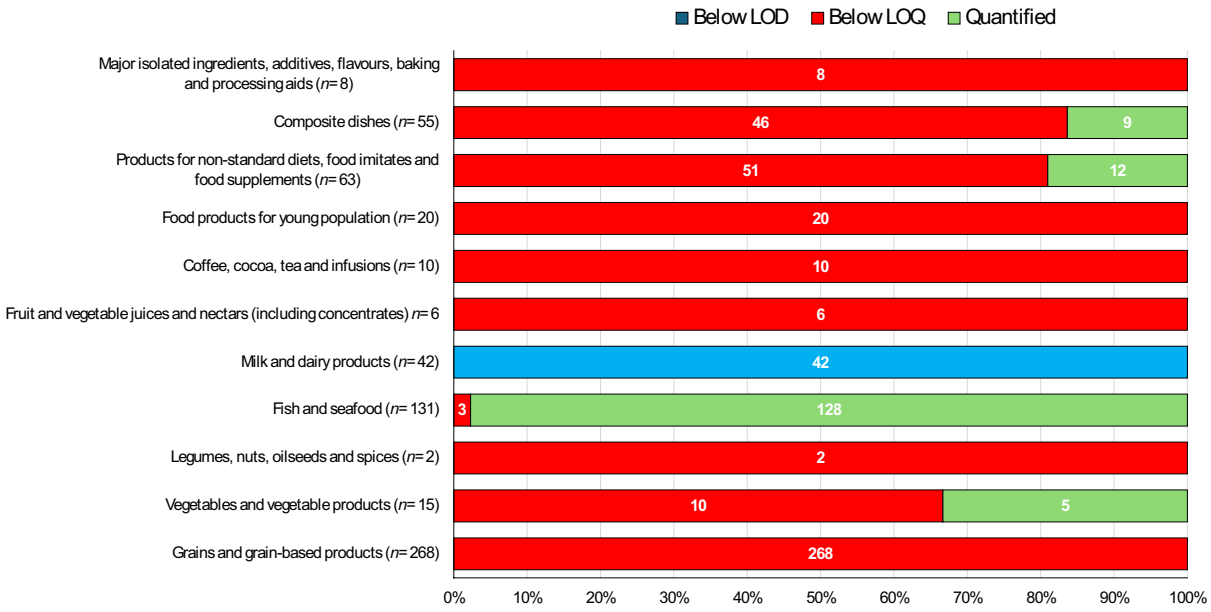


FIGURE 4 Overview of analytical results below LOD, below LOQ and quantified values in the final dataset of AsB across the different food categories (FoodEx2 Level 1).

In the final data set of 620 analytical results on AsB, 11 food categories at FoodEx2 Level 1 were represented, 5 of them with quantified data. The highest numbers of samples were reported for 'Grains and grain-based products' ($n = 268$), followed by 'Fish and seafood' ($n = 131$) and 'Products for non-standard diets, food imitates and food supplements' ($n = 63$). An overview of the number of analytical results, the proportion of left-censored data, and the mean and 95th percentile (P95) concentrations at FoodEx2 Level 1 is presented in Table 8. Summary statistics on the occurrence data on AsB codified at the different FoodEx2 levels are reported in Annex E.

TABLE 8 Summary of AsB occurrence data by food categories at FoodEx2 Level 1 (expressed as µg As/kg, w.w., weighted mean and P95).

			Mean		P95[a]	
	N	%LC	LB	UB	LB	UB
Grains and grain-based products	268	100	0.0	19.0	0.0	20.0
Vegetables and vegetable products[b]	15	67	4605	4618	–	–
Legumes, nuts, oilseeds and spices	2	100	0.0	4.2	–	–
Fish and seafood	131	2	877	877	–	–
Milk and dairy products	42	100	0.0	0.1	–	–
Fruit and vegetable juices and nectars (including concentrates)	6	100	0.0	1.0	–	–
Coffee, cocoa, tea and infusions	10	100	0.0	5.4	–	–
Food products for young population	20	80	25.0	38.8	–	–
Products for non-standard diets, food imitates and food supplements	63	81	326	355	132	170
Composite dishes	55	84	0.2	2.9	–	–
Major isolated ingredients, additives, flavours, baking and processing aids	8	100	0.0	20.0	–	–

Abbreviations: LB, lower bound; % LC, proportion of left-censored data; N, number of analytical results; P95, 95th percentile; UB, upper bound.

[a]The 95th percentiles obtained on occurrence data with fewer than 59 analytical results may not be statistically robust (EFSA, 2011b) and are, therefore, not reported in the table. The 95th percentile for 'Fish and seafood' was not calculated as this food category contains pooled samples.

[b]It includes both seaweed samples reported as dried and seaweed samples unprocessed.

Among the samples of 'Grains and grain-based products' ($n = 268$), most of them ($n = 153$) referred to different types of rice that were all reported as left-censored data. In the food category 'Composite dishes' ($n = 58$), nine samples codified as 'Rice-based dishes, cooked' ($n = 48$) reported quantified levels of AsB. Data providers communicated that these samples might contain small amounts of fish/seaweeds that could explain the reported levels (0.4–2.9 µg As/kg, mean = 1.1 µg As/kg). A similar situation occurred in four samples of 'Food products for young population', initially codified as 'Ready-to-eat cereal-based meal for children' due to the presence of rice as ingredient, and with mean levels of AsB of 125 µg As/kg (21.1–343 µg As/kg). Data providers confirmed that fish was also one of the ingredients; samples were re-codified as 'Ready-to-eat mixed meal for children'.

A total of 10 samples of seaweeds were available under the food group 'Vegetables and vegetable products', with 7 samples reported as dried seaweeds ($n = 7$), sand 3 as unprocessed. A large variation in the AsB levels was observed, with relatively high levels reported for three samples of dried Carrageen mosses, ranging from ~12,000 µg As/kg to 23,500 µg As/kg. AsB is usually reported in the literature as only a minor arsenic constituent in seaweeds. Although high AsB levels have been occasionally reported in seaweeds, they seem to be due to limitations of the analytical procedure (see Section 3.2.2).

Different types of supplements (vitamins, minerals, proteins) were reported under 'Food supplements and similar preparations' ($n = 55$) with no quantified levels of AsB. A total of 32 samples were submitted as 'Algae-based formulations (e.g. Spirulina, chlorella)', half of them made of the photosynthetic cyanobacterium Spirulina; relatively low levels of AsB were reported for these supplements (mean LB-UB, 12.5–34.5 µg As/kg, $n = 16$). Contrarily, for one supplement made of another cyanobacterium, *Aphanizomenon flos-aquae*, AsB levels were as high as 4600 µg As/kg. Relatively high levels were also reported for supplements made of brown seaweeds, as for tablets of *Ascophyllum nodosum* (Rockweed) (13,593 µg As/kg) or one sample made of Kelp (2367 µg As/kg). No AsB was quantified in other supplements also made of brown seaweeds, two made of *Undaria pinnatifida* (Wakame) and four reported as made of seaweeds from the genus *Fucus*. For few samples of dietary supplements made of the unicellular green microalgae Chlorella, mean LB-UB AsB levels were 22.0–62.8 µg As/kg ($n = 4$).

Because of the very high uncertainty associated with the reported occurrence values on AsB, the CONTAM Panel decided not to consider seaweeds and 'Algae-based formulations' (e.g. *Spirulina, Chlorella*)' when estimating dietary exposure to AsB.

As commented above, the highest number of quantified samples was reported for the food category 'Fish and seafood'. Three different groups of fish meat were represented, although the sample size was small (Table 9). The highest mean levels were reported for 'Marine fish' with mean values of 1106 µg As/kg (LB = UB, $n = 7$), followed by 'Diadromous fish' (276 µg As/kg, LB = UB, $n = 12$) and 'Freshwater fish' (LB–UB, 48.5–49.9 µg As/kg, $n = 6$). It is well described in the literature that AsB is the major arsenic species in marine fish, but its presence is much lower in freshwater fish (Amlund & Berntssen, 2004; Juncos et al., 2019). Most of the samples codified as 'Marine fish' were tuna fish, with mean AsB levels of 1023 µg As/kg (LB = UB, $n = 5$). The highest level was reported for one sample codified as 'Sardines and sardine-type fishes' (2600 µg As/kg). 'Diadromous fish' was mainly represented by ten samples of Atlantic salmon, all quantified, with mean AsB concentration of 317 µg As/kg and a maximum value of 600 µg As/kg. Overall, these levels are within the range of AsB levels reported in the literature on different fish species, for instance in sardines collected in Greek coastal areas (Kalantzi et al., 2017) or in salmon from local supermarkets in Barcelona (Spain) (Zmozinski et al., 2015).

The highest levels of AsB among the samples of 'Fish and seafood' were reported for 'Molluscs', in particular for 'Oysters' with mean values of 1878 µg As/kg (LB = UB, $n = 27$); relatively high mean levels were also reported for 'Mussels' (853 µg As/kg, LB = UB, $n = 41$). These values are also in line with those reported in the literature for samples of mussels and oysters collected in supermarkets across different European countries (Ciardullo et al., 2010; Moreda-Piñeiro et al., 2011; Serpe et al., 2013; Zmozinski et al., 2015). No data were reported to EFSA on crustaceans.

A few samples of processed/preserved fish were also available (Table 9). Most of them corresponded to samples codified as 'Canned tunas and similar' ($n = 25$) with reported mean values of 540 µg As/kg (LB = UB). These samples were merged with two samples of canned sardines under the food group 'Canned/jarred fish' before estimating dietary exposure. Six samples of smoked eels were grouped as 'Smoked fish' (mean LB-UB, 38.1–38.8 µg As/kg) and used as surrogates for all the eating occasions reported as 'Smoked fish'. Additionally, these mean values were used as surrogates for the eating occasions of 'Dried fish' and 'Salt-preserved fish'. The few samples of 'Marinated / pickled fish' ($n = 4$, LB = UB = 77.5 µg As/kg) were kept in the final dataset since literature data reported similar levels of AsB in this type of food commodities (Hackethal et al., 2021).

Table 9 also shows some samples of processed commodities identified by the CONTAM Panel as potentially relevant for the exposure to AsB for which the occurrence values were derived using the available data on raw primary commodities (fish meat). Accordingly, the available samples on 'Fish meat' ($n = 25$) were used to derive AsB levels in diverse fish-based commodities: 'Fish balls', 'Fish gratin', 'Fish pâté', 'Fish soup', 'Fish fingers, breaded' and 'Prepared fish salad'. To derive the AsB values, processing factors and recipes were used as needed from the EFSA's Raw Primary Commodity model (EFSA, 2019), assuming that no losses of AsB occur during the processing. Among the processed commodities, LB–UB mean values ranged between 213–214 µg As/kg and 272–273 µg As/kg for 'Fish soup' and 'Fish pâté', respectively.

Considering, (1) the very high uncertainty associated to the AsB occurrence values reported for seaweeds and 'Algae-based formulations (e.g. Spirulina, Chlorella)' and, (2) the relevance of fish and seafood in the context of this scientific Opinion due to their content of AsB, the CONTAM Panel decided to conduct the dietary exposure assessment for consumers of fish, seafood and fish-based processed commodities ('consumers only'). Table 9 shows the occurrence data on AsB as they were used for dietary exposure estimations.

TABLE 9 Summary statistics (expressed as µg As/kg, w.w., weighted mean) different food commodities as used to estimate dietary exposure to AsB.

			Mean		HRP[a]	
	N	%LC	LB	UB	LB	UB
Fish meat						
Unspecified fish (meat)[b]	25	8	454	454	500	500
Diadromous fish	12	0	276	276	400	400
Freshwater fish	6	33	48	50	14.4	14.4
Marine fish[b]	7	0	1106	1106	1030	1030
Molluscs						
Mussels	41	0	853	853	1069	1069
Oysters	27	0	1878	1878	2345	2345
Processed or preserved fish						
Canned/jarred fish	27	0	536	536	650	650
Smoked fish	6	17	38.1	38.8	48.3	48.3
Fish fingers, breaded[c]	25	8	241	241	265	265
Marinated/pickled fish	4	0	77.5	77.5	–	–
Composite dishes[c]						
Fish balls	25	8	232	232	255	255
Fish gratin	25	8	236	236	260	260
Prepared fish salad	25	8	218	218	240	240
Fish pâté	25	8	272	273	300	300
Fish soup	25	8	213	214	235	235

Abbreviations: LB, lower bound; % LC, proportion of left-censored data; N, number of analytical results; P95, 95th percentile; UB, upper bound.

[a]Highest reliable percentile that can be estimated when applying the following minimum sample size for each percentile: 5 samples for the P50, 11 samples for the P75, 29 samples for the P90 and 59 samples for the P95.

[b]The food group 'Unspecified Fish (meat)' was created considering all samples of freshwater fish, diadromous fish and marine fish to cover the relatively high amount of eating occasions reported as just 'Fish meat' without further details in the Comprehensive database (> 3000).

[c]Occurrence value derived using the 25 analytical results reported for 'Fish meat' and processing factors and recipes from the EFSA's Raw Primary Commodity model (EFSA, 2019).

3.2.1.2 | *Occurrence data on AsSugOH*

As mentioned in Section 2.3, no occurrence data on AsSugOH were submitted to EFSA. The highest arsenosugars levels in the literature are reported for seaweeds, where they are the dominant arsenic species. Much lower levels are found in seafood, with trace levels sometimes reported in fish (Taylor, Goodale, et al., 2017). Based on this, only AsSugOH levels in seaweeds were retrieved from the literature. The occurrence data were selected for the red seaweed known as Laver/Nori, and for the brown seaweeds Wakame and Kombu. These seaweeds seem to be the most important used as food, not only in Europe but also in Asian countries where the consumption of seaweeds has a long tradition as part of their diet, especially in Japan, China and Korea.

Table 10 shows the mean values derived for the selected seaweeds, citing the scientific papers used to retrieve the AsSugOH levels. The highest mean reported in the literature was for Kombu (7.5 mg As/kg d.m., $n = 11$), then for Nori/Laver (2.0 mg As/kg d.m., $n = 18$) and for Wakame (1.7 mg As/kg d.m., $n = 20$). Additionally, occurrence values were derived for two foods containing seaweeds as ingredient that are probably among the most popular foods consumed by the European population that developed a taste for Asian cuisine (sushi and miso soup). AsSugOH levels in these foods were derived as explained in Section 2.7 and corresponded to 0.1 mg As/kg and 0.02 mg As/kg for sushi and miso soup, respectively, both expressed in w.w.

TABLE 10 Mean AsSugOH levels derived from literature data for selected seaweeds and some food products containing seaweeds (expressed as mg As/kg, d.m.).

	N	Min–Max (mg As/kg d.m.)	Mean levels (mg As/kg d.m.)	Reference
Nori/Laver (red seaweed)	18	0.04–10.5	2.0	Almela et al. (2005), Garcia-Sartal et al. (2012), Taylor and Jackson (2016), Wolle et al. (2021), Yu et al. (2024)
Kombu (brown seaweed)	11	1.0– 24.6	7.5	García-Salgado et al. (2012), Wolle et al. (2021), Yu et al. (2024)
Wakame (brown seaweed)	20	0.38–6.9	1.7	Almela et al. (2005), García-Salgado et al. (2012), Wolle et al. (2021), Yu et al. (2024)

Abbreviations: d.m., dry matter; N, number.

3.2.2 | Occurrence data reported in the public literature

3.2.2.1 | *Arsenobetaine and related As species*

AsB was identified for the first time in the western rock lobster by Edmonds et al. (1977). Afterwards, it has been profusely quantified in fish, seafood and seaweeds, as well as in mushrooms, although in the latter typically at low concentrations.

In marine fish and most other seafoods, AsB is the predominant arsenic species usually constituting more than 70%–90% of the total arsenic content. These high levels seem to be related with the potential function of AsB as a cellular osmolyte, implying higher AsB levels at higher salinity (Popowich et al., 2016). However, while this has been supported in certain studies with mussels and pelagic fishes (Larsen & Francesconi, 2003; Taylor, Goodale, et al., 2017), changes in salinity did not affect muscular retention of AsB in Atlantic salmon (Amlund & Berntssen, 2004).

Different types of marine fish have been analysed for their content in AsB. Kalantzi et al. (2017) analysed sardines and anchovies collected in six areas in the Greek coastline. AsB concentrations ranged between 10.8 and 58.8 mg As/kg d.m. for anchovy and between 8.6 and 41.4 mg As/ kg d.m. for sardine, accounting for 67%–95% of the total arsenic. Other studies covered not only marine fish but also different types of seafood. As an example, Zmozinski et al. (2015), found AsB levels that ranged between 0.90 mg As/kg d.m. in tuna and 33.5 mg As/kg d.m. in white fish. These authors also analysed crustaceans, with the highest AsB concentration reported in prawns (2.21 mg As/kg d.m.), and different bivalves (oysters, clams, mussels, cockles), with the highest value reported in oysters (15.9 mg As/kg d.m.). All samples were collected in Spain except for four fish samples with origin in Brazil. The levels quantified in mussels (8.79 mg As/kg d.m.) were within the range of AsB concentrations reported by Serpe et al. (2013) in farmed mussels from the Italian coast (0.72–10.36 mg As/kg d.m.). Wolle, Conklin, and Wittenberg (2019), Wolle, Stadig, and Conklin (2019) determined arsenic species in the top ten most consumed fish and seafoods in the United States; for marine fish the highest AsB concentration was reported in some samples of cod (3.8 mg As/kg d.m.) while the highest levels were found in crab samples (20.1 mg As/kg d.m.). Marine samples contained the highest levels as compared to freshwater ones, and the median contribution of AsB to tAs varied from 29% in clams to 82% in crab samples.

Overall, freshwater fish are characterised by lower levels of tAs, and correspondingly lower accumulation of AsB as compared to marine fish and seafood. For freshwater fish, AsB levels of 0.20–0.28 mg/kg and 0.06–0.08 mg/kg d.m. were reported in creole perch and rainbow trout, respectively, in samples captured in different locations of the Argentinian lake Nahuel Huapi (Juncos et al., 2019). Other authors showed that AsB levels can be also relatively high in freshwater fish from areas rich in arsenic (Lower Silesia, Poland); levels up to 4.2 and 5.2 mg/kg (d.m.) were reported in trout and sturgeon, respectively (Komorowicz et al., 2019). A recent publication by Hoy and coworkers provided an extensive and comprehensive overview of levels of AsB in different freshwater fish worldwide, also describing most common challenges linked to arsenic speciation in freshwater fish (Hoy et al., 2023).

AsB is sometimes reported in seaweeds but usually as only a minor arsenic constituent. For example, a study on 25 samples from 12 seaweed species purchased at local markets in Barcelona found AsB in only 9 samples with values ranging from 0.003 to 0.46 mg As/kg (d.m.) (Yu et al., 2024). Similarly, Taylor and Jackson (2016) investigated the As content in 25 samples from 20 seaweed species harvested in New England (U.S.), and detected AsB in only 8 samples with values ranging from 0.02 to 0.19 mg As/kg d.m. The CONTAM Panel did, however, identify two scientific papers reporting remarkably high levels of AsB (up to 13.6 mg As/kg d.m.) in various seaweeds (Da Silva Junior et al., 2023; Peng et al., 2023). The data from these studies indicated an absence of arsenosugars, which was an unusual result considering that arsenosugars are well known to be the dominant As species in seaweeds (see below). After careful assessment of the methodology used in those two papers, the CONTAM Panel questioned the identification of AsB. A study by Huang et al. (2022) also reported high levels of AsB in several seaweed samples; the authors, however, clearly described the limitations of their analytical procedure and stated that the AsB assignment was uncertain. Because of the methodological limitations of the above three studies, the CONTAM Panel has not considered these data on AsB in seaweed. AsB seems not to be present in microalgae or detected at very low levels (Caumette et al., 2012; Popowich et al., 2016).

AsB is not present in terrestrial foods with the exception of some types of mushrooms. Although typically present at low concentration, AsB levels seem to widely vary in mushrooms and, in several cases, it was identified as the predominant organic species (Braeuer & Goessler, 2019; Nearing et al., 2014). In different samples of edible mushrooms obtained from local markets in China, AsB levels vary between 1.5 μg As/kg d.m. in *Lentinus edodes* (Shiitake) and 183 μg As/kg d.m. in mushrooms reported as 'Yellow boletus' without further details (Chen et al., 2017). More recently, levels between 45 μg As/kg and 150 μg As/kg d.m. were reported for the commonly eaten *Boletus edulis* (Walenta et al., 2024).

The other species in the arsenobetaine group sometimes reported are AsC, TMAP, DMAE and DMAA. Because AsC is a readily available standard and is one of the easiest As species to measure, it is often included in studies of As species in foods, whereas TMAP, DMAE and DMAA are less commonly measured. With few exceptions, the AsC levels reported are usually < 0.1 mg As/kg d.m. in fish and seafood. For example, in a study of arsenic species in 58 samples of molluscs, crustaceans and fish from the western Arabian Gulf, AsC was detected in several of the samples, but the levels were always below the LOQ (0.03 mg As/kg d.m.) (Krishnakumar et al., 2016). In a basket survey of the ten most consumed seafoods in the USA, Wolle, Conklin, and Wittenberg (2019) reported the arsenic species in 54 samples of fish, crustaceans and clams: AsC was detected in only 11 of the samples and concentrations were always < 0.01 mg As/kg d.m. That same study detected TMAP in 28 of the 54 samples, usually at low levels (< 0.04 mg As/kg d.m.), but seven samples of crab from three species contained

TMAP at 0.13–0.80 mg As/kg d.m. Wolle, Conklin, and Wittenberg (2019) also reported traces (0.001–0.028 mg As/kg d.m.) of DMAE or DMAA in 11 and 19, respectively, of the 54 samples analysed.

3.2.2.2 | *Arsenosugars*

Arsenosugars were first reported in seaweeds in 1981 and are now known to be the major arsenic species in marine seaweeds. They are found in brown, red and green seaweeds, but are particularly abundant in brown seaweeds. They can also occur, but usually at low levels, in marine animals consuming seaweeds. Arsenosugars are not normally associated with terrestrial organisms although there have been occasional reports of their presence at trace levels (EFSA CONTAM Panel, 2009).

The arsenosugars occur predominantly as the dimethylarsinoyl form ($Me_2As(O)$-) although the thio ($Me_2As(S)$-) and trimethyl (Me_3As-) forms can also be present. No less than ten sugar groups have been reported attached to the arsenic group; four of these arsenosugars are dominant, namely AsSugOH, $AsSugPO_4$, $AsSugSO_3$ and $AsSugSO_4$, at least two of which occur in essentially all marine seaweeds. Data from the literature on arsenosugars in edible seaweeds are shown in Table 11. Many studies reported concentrations for each of the four major arsenosugars. The literature data are shown in Table 11 as the sum of these four arsenosugars, together with the individual concentration of AsSugOH, which is the most studied arsenosugar in terms of toxicokinetics and toxicology.

TABLE 11 Individual levels (mg As/kg d.m.) of total arsenic (tAs), water-soluble arsenic, sum of arsenosugars and AsSugOH reported in the literature for different types of edible seaweeds (brown, red and green).

Sample type	Species name (common name)	Total As (mg As/kg d.m.)	Water-soluble As (mg As/kg d.m.)	Sum of arsenosugars (mg As/kg d.m.)	AsSugOH (mg As/kg d.m.)	Reference
Brown seaweeds	*Alaria esculenta* (Bladderlocks)	34.5	24.0	22.7	4.5	Taylor and Jackson (2016)
	Ascophyllum nodosum (Knotted wrack)	42	30	27.4	1.9	Wolle et al. (2021)
	Fucus spiralis (Spiral wrack)	16.3	7.7	6.0	0.56	Taylor and Jackson (2016)
	Fucus vesiculosus (Bladder wrack)	28.9	12.9	10.6	0.50	
		32.8	20.0	21.1	2.2	
	Laminaria digitata (Oarweed)	107	63.0	58.8	3.4	
		50.4	32.4	25.9	5.1	
	Laminaria longicruris (Atlantic Kombu)	74.1	52.4	51.7	3.6	
	Laminaria ochroleuca (Kombu)	56.4	31.5	34.5	8.1	Garcia-Sartal et al. (2012)
	Laminaria ochroleuca (Kombu)	84.2	52.5	24.3	8.4	Yu et al. (2024)
		98.9	76.8	56.5	9.5	
		59.5	41.1	39.5	24.6	
		51.0	39.1	31.6	8.8	
		51.5	10.9	31.3	17.6	
	Saccharina gyrata (Kombu)	85	78	67	0.99	Wolle et al. (2021)
	Saccharina japonica (Kombu)	63	50	48	4.2	
		58	46	44	2.7	
		65	57	54	3.2	
		65	46	43	11	
	Saccharina sp. (Kombu)	77	62	63	1.8	
	Saccharina latissima (Sugar kelp)	56.3	25.3	18.5	0.28	Taylor and Jackson (2016)
	Sargassum fusiforme (Hijiki)	87	66	6.3	1.2	Wolle and Conklin (2018a)
	Sargassum fusiforme (Hijiki)	104	26.3	9.9	1.24	Almela et al. (2005)
		132	18.6	7.8	1.79	
	Not available (Hijiki)	88	70	7.7	1.0	Wolle et al. (2021)
		86	70	5.6	0.71	
		105	85	15	1.9	
	Undaria pinnatifida (Wakame)	42	6	2.5	2.0	
		47	6	1.2	0.49	
		40	6	1.4	0.82	
		45	7	2.0	1.61	
		46	6	1.7	1.0	
		47	5	1.0	0.41	
		37	4	0.80	0.37	
		48	6	1.2	0.53	
		44	6	1.8	1.0	
		12	6	3.4	2.3	
		33	5	2.1	1.0	
		12	3	1.5	0.79	

TABLE 11 (Continued)

Sample type	Species name (common name)	Total As (mg As/kg d.m.)	Water-soluble As (mg As/kg d.m.)	Sum of arsenosugars (mg As/kg d.m.)	AsSugOH (mg As/kg d.m.)	Reference
	Undaria pinnatifida (Wakame)	32.1 28.2 41.9 43.3 30.4	6.2 6.4 2.8 3.6 24.7	3.8 4.5 0.69 1.4 19.3	3.2 4.2 0.38 1.1 6.9	Yu et al. (2024)
	Undaria pinnatifida (Wakame)	38.3	16.6	14.2	3.2	Garcia-Sartal et al. (2012)
	Undaria pinnatifida (Wakame)	49.3 47.4	2.5 23.1	0.19 21.4	0.19 1.79	Almela et al. (2005)
Red seaweeds	*Chondrus crispus* (Irish moss)	12.1 6.1	5.8 4.2	5.8 3.6	1.2 3.1	Taylor and Jackson (2016)
	Gracilaria vermiculophylla	11.8	2.9	2.4	1.1	
	Palmaria palmata (Dulse)	9.0	7.4	7.5	3.0	
	Palmaria palmata (Dulse)	34 32 33	11.7 10.9 8.7	9.3 8.0 6.3	1.9 2.6 1.8	Wolle et al. (2021)
	Polysiphonia lanosa	14.0	7.6	6.1	2.20	Taylor and Jackson (2016)
	Porphyra sp. (Nori)	33.6 27.2	22.0 19.7	21.3 21.2	10.5 5.3	Almela et al. (2005)
	Porphyra umbilicalis (Nori)	33.8 20.8 49.4 32.5	25.5 11.6 40.3 26.6	18.0 5.9 40.2 20.5	0.23 0.96 4.03 0.84	Yu et al. (2024)
	Porphyra umbilicalis (Nori)	48.8	30.0	30.5	4.3	Garcia-Sartal et al. (2012)
	Porphyra umbilicalis (Laver, Nori)	21	24	27	1.9	Taylor and Jackson (2016)
	Porphyra umbilicalis (Nori)	30	26	23	5.9	Wolle et al. (2021)
	Pyropia yezoensis (Nori)	20 12 13 21 13 13 22 24	17 10 11 18 11 11 18 18	12 2.1 6.2 6.4 4.8 5.4 5.9 6.8	0.06 0.06 0.42 - [(a)] 0.04 0.04 0.09 0.12	
	Pyropia sp. (Laver)	30	24	20	0.5	

(Continues)

TABLE 11 (Continued)

Sample type	Species name (common name)	Total As (mg As/kg d.m.)	Water-soluble As (mg As/kg d.m.)	Sum of arsenosugars (mg As/kg d.m.)	AsSugOH (mg As/kg d.m.)	Reference
Green seaweeds	*Ulva lactuca* (Sea lettuce)	1.2 3.1	0.95 1.6	0.25 0.35	0.24 0.18	Yu et al. (2024)
	Ulva lactuca (Sea lettuce)	5.3 4.1	0.4 1.0	0.10 0.32	0.07 0.06	Taylor and Jackson (2016)
	Ulva lactuca (Sea lettuce)	4.8	1.9	0.85	0.56	Garcia-Sartal et al. (2012)
	Ulva prolifera (Branched string lettuce)	14.6	1.1	0.62	0.46	Taylor and Jackson (2016)

Abbreviations: AsSugOH, glycerol arsenosugar; d.m., dry matter.

In summary, Table 11 shows that not considering the special case of Hijiki, all 11 edible species of brown seaweeds contain arsenosugars as their major water-soluble As species, with concentrations ranging from 0.2 to 67 mg As/kg d.m. and a mean of 16.2 mg As/kg d.m. Hijiki or Hiziki is a brown seaweed (*Sargassum fusiforme*, syn. *Hizikia fusiformis*) well documented as having high levels of iAs as compared to other seaweeds, which led several authorities in the past to advise consumers to avoid its consumption (EFSA, 2014, 2021). All species of red seaweeds have arsenosugars as major or significant As species with concentrations ranging from 2.1 to 30.5 mg As/kg d.m. and a mean of 13.2 mg As/kg d.m. Six samples from two species of green seaweeds contained arsenosugars at concentrations ranging from 0.10 to 0.85 mg As/kg, with a mean of 0.42 mg As/kg d.m.

As commented above, arsenosugars are usually present at low levels in marine animals. Fish generally contained only traces as reported for different samples of salmon, tuna, tilapia, pollock and cod purchased across supermarkets in United States (Wolle, Conklin, & Wittenberg, 2019; Wolle, Stadig, & Conklin, 2019), but levels of AsSugOH up to 0.70 mg As/kg d.m. have been occasionally reported as for instance in seabream captured in the Arabian gulf (Krishnakumar et al., 2016). Crustaceans and particularly molluscs seem to have overall higher levels of arsenosugars than fish. Wolle, Conklin, and Wittenberg (2019), Wolle, Stadig, and Conklin (2019) reported arsenosugars in four out of seven species of crabs, with the highest levels reported in swimming crab (*Portunus pelagicus*), around 0.11 mg As/kg d.m. The same authors reported arsenosugar levels up to 0.26 mg As/kg d.m. in different types of clams. In a pooled sample of blue mussels ($n = 50$) from the 2017 Norwegian surveillance, arsenosugar levels of ~ 2 mg As/kg d.m. were found, with AsSugPO4 as the major arsenosugar (Tibon et al., 2021).

3.2.2.3 | *Arsenolipids*

Arsenolipids have been reported so far in seaweeds, fish, molluscs and crustaceans; they have not been reported in terrestrial foods.

There are difficulties reporting quantitative data for arsenolipids in foods resulting from: (1) the large number of arsenolipids (> 200) found in foods; (2) the instability of many of the arsenolipids and the resultant artefacts produced during the analytical procedures; (3) the lack of analytical standards.

Several studies report a collective value for lipid-soluble arsenic in seafood, and some of these report individual quantitative data for the major arsenolipids. The most reliable data are for the AsHCs (As-hydrocarbons) – relative to other arsenolipids, the AsHCs are abundant in seafoods, stable and amenable to simpler HPLC analysis.

For these reasons, Table A.1 in the Appendix A summarises literature data on lipid-soluble arsenic in foods, together with concentrations of total AsHCs and that of AsHC332, the commonly reported AsHC for which toxicological data are also available. That table shows that lipid-soluble arsenic, as a percentage of total arsenic content, is usually < 10%; exceptions do occur such as fish oils (100%), Wakame (*Undaria pinnatifida*, up to 59%) and sashimi-grade (toro) tuna (49%). The major arsenolipids in seaweeds are the AsSugPLs, in agreement with arsenosugars being the dominant water-soluble As species in seaweeds. AsHCs, on the other hand, appear to be present in most seafood samples.

3.2.2.4 | *Effects of cooking and food processing on complex organoarsenic species*

There have been several studies investigating possible changes in complex organoarsenic species during cooking of fish, seafood and seaweed. When the muscle tissue of shark or crayfish was roasted at very high temperatures, the AsB originally present was partly converted to tetramethylarsonium ion (Hanaoka et al., 2001). Those results have been supported by further studies. Devesa et al. (2005) reported that the levels of tetramethylarsonium ion increased when fish (four species) were cooked, presumably because of partial degradation of AsB present in the fresh samples. In a quantitative study, Dahl et al. (2010) showed that the levels of tetramethylarsonium ion increased when fresh samples of cod, salmon or blue mussels were cooked by frying. These increases, which ranged from 0.01 to 0.35 mg As/kg, were presumed to result from partial degradation of AsB present in the fresh samples at levels ranging from 1.6 to 14 mg As/kg. When the fresh samples were boiled rather than fried, no increase in tetramethylarsonium ion was observed (Dahl et al., 2010).

In a qualitative study, Garcia-Sartal et al. (2012) observed no significant changes in arsenosugars contained in seaweed when the seaweed was cooked by boiling.

Liu et al. (2022) investigated the arsenolipids present in fish (two species), molluscs (three species) and a crustacean both before and after the samples had been cooked in a microwave. No quantitative data were obtained, but the authors used the relative peak areas (LC/ESMS) of the compounds in the raw and cooked samples to assess possible changes to AsHCs and AsFAs (other arsenolipids were not mentioned and presumably not measured). Based on those data, the authors suggested that during the microwave cooking (i) two of the five AsFAs originally present decreased slightly; the AsHCs remained unchanged and (iii) thio-arsenolipids were not formed. Those results contrasted with the quantitative study of Xiong et al. (2022) who investigated changes to arsenolipids when fresh salmon fillet was cooked by baking or by steaming. The fresh salmon contained mainly AsHCs whereas AsFAs were not detected, and during the cooking processes, about 30% of the AsHCs were converted to their corresponding thio-analogue for both baking and steaming.

Relevant to the above studies is the observation from Wolle, Conklin, and Wittenberg (2019), Wolle, Stadig, and Conklin (2019) that thiolation occurred when various standard dimethyloxo-arsenic species (i.e. with the group $Me_2As(O)$-) were heated in aqueous extracts of fish or crustaceans. It is likely that possible changes to a specific arsenic species during cooking could be influenced both by the specific cooking conditions (e.g. high temperature frying compared with steaming) and the type of fish or seafood being cooked.

3.3 | Exposure assessment

3.3.1 | Dietary exposure to arsenobetaine

As described in Section 2.7, chronic dietary exposure to AsB was conducted in consumers of fish, seafood and fish-based processed commodities ('consumers only'). The food commodities used for the dietary exposure assessment are described in Table 9 and the dietary surveys in Annex C. Table 12 shows the mean and 95th percentile exposure to AsB following the consumption of fish, seafood and fish-based processed commodities ('consumers only'). As most of the food categories in the occurrence data showed small or no differences between the LB and UB scenario, there were no differences between LB and UB exposure estimations after rounding up the exposure estimations to one decimal (Table 12). For each population group, the range of dietary exposures across the different dietary surveys is shown (min-max). Mean and 95th percentile exposures were considered for each individual dietary survey when at least 5 and 59 consumers were available, respectively. The exposure estimates calculated for each dietary survey across population groups ('consumers only') together with the different food contributors to the exposure are presented in Annex F.

The highest mean dietary exposure to AsB was in both 'Infants' and 'Toddlers' with estimates of 4.6 μg As/kg bw per day, and the highest 95th percentile exposure in 'Toddlers' with an estimate of 12.5 μg As/kg bw per day. In the adult population,[15] the maximum mean dietary exposure was 1.4 μg As/kg bw per day in both 'Adults' and 'Elderly', while the highest 95th percentile exposure was estimated in the 'Elderly' population (4.2 μg As/kg bw per day). The exposure estimates for 'Pregnant women' and 'Lactating women' were similar to those for the adult population. Hardly any studies assessing the dietary exposure to AsB could be retrieved from the scientific literature, preventing a comparison with the current estimates. There is just one recent study where the dietary exposure to AsB (median) was estimated for consumers of individual foods across different age groups (Hackethal et al., 2023). This study used occurrence data from the first German TDS (BfR MEAL Study) (Hackethal et al., 2021). The median exposure to AsB in consumers of fish, fish products and seafood was 0.94 μg As/kg bw per day for children 6–11 years ($n = 328$), and 0.49 μg As/kg bw per day in individuals ≥ 14 years ($n = 2892$). The highest exposure was estimated following the consumption of plaice/sole with estimates of 7.88 μg As/kg bw per day in children of 6–11 years ($n = 10$), and 6.32 μg As/kg bw per day in subjects ≥ 14 years ($n = 65$).

TABLE 12 Summary statistics of the chronic dietary exposure to AsB (μg As/kg bw per day) across European dietary surveys, considering the consumption of fish, seafood and fish-based processed commodities ('consumers only').

	Mean dietary exposure (μg As/kg bw per day)						
		Lower bound (LB)			Upper bound (UB)		
	N	Min	Median	Max	Min	Median	Max
Infants (> 12 weeks to < 12 months)	12	0.5	1.3	4.6	0.5	1.3	4.6
Toddlers (≥ 12 months to < 36 months)	15	0.5	1.3	4.6	0.5	1.3	4.6
Other children (≥ 36 months to < 10 years)	19	0.5	0.9	2.3	0.5	0.9	2.3
Adolescents (≥ 10 years to < 18 years)	21	0.2	0.6	1.3	0.2	0.6	1.3
Adults (≥ 18 years to < 65 years)	22	0.2	0.5	1.4	0.2	0.5	1.4
Elderly (≥ 65 years to < 75 years)	19	0.3	0.5	1.4	0.3	0.5	1.4
Very elderly (≥ 75 years)	13	0.3	0.5	0.8	0.3	0.5	0.8
Pregnant women	6	0.2	0.6	0.7	0.2	0.6	0.7
Lactating women	2	0.3	–	0.7	0.3	–	0.7
	95th percentile dietary exposure (μg As/kg bw per day)[a]						
		Lower bound (LB)			Upper bound (UB)		
	N	Min	Median	Max	Min	Median	Max
Infants (> 12 weeks to < 12 months)	12	1.5	4.0	10.1	1.5	4.0	10.1
Toddlers (≥ 12 months to < 36 months)	15	1.4	4.7	12.5	1.4	4.7	12.5
Other children (≥ 36 months to < 10 years)	19	1.5	3.0	7.3	1.5	3.0	7.3
Adolescents (≥ 10 years to < 18 years)	21	0.6	2.0	3.6	0.6	2.0	3.6
Adults (≥ 18 years to < 65 years)	22	0.6	1.5	3.9	0.6	1.5	3.9
Elderly (≥ 65 years to < 75 years)	19	0.7	1.8	4.2	0.7	1.8	4.2
Very elderly (≥ 75 years)	13	0.8	1.6	2.3	0.8	1.6	2.3
Pregnant women	6	1.2	1.7	1.9	1.2	1.7	1.9
Lactating women	2	0.9	–	0.9	0.9	–	0.9

Abbreviations: bw, body weight; N, number of dietary surveys.

[a]The 95th percentile exposure estimates obtained on dietary surveys with fewer than 59 observations may not be statistically robust (EFSA, 2011b) and are therefore not included in this table.

[15]In this scientific opinion, adult population refers to the population groups 'Adults', 'Elderly' and 'Very elderly'.

Figure 5 shows a summary of the average contribution to the dietary exposure to AsB of different food groups in selected age classes. 'Fish meat' was the main contributor to the exposure to AsB across the different age classes, being the only contributor in many dietary surveys of 'Infants' and 'Toddlers'. In these two age classes, the average contribution of 'Fish meat' ranged between 69% to 100% (median 99%) and 60% to 100% (median 93%), respectively. Among the different types of 'Fish meat', the most relevant for the exposure was 'Marine fish'. Other food commodities with an important role in the exposure to AsB were those codified as 'Processed or preserved fish', in particular canned/jarred fish. The maximum average contribution of 'Processed or preserved fish' across age classes varied between 18% in the 'Very elderly' and 45% in the 'Elderly'.

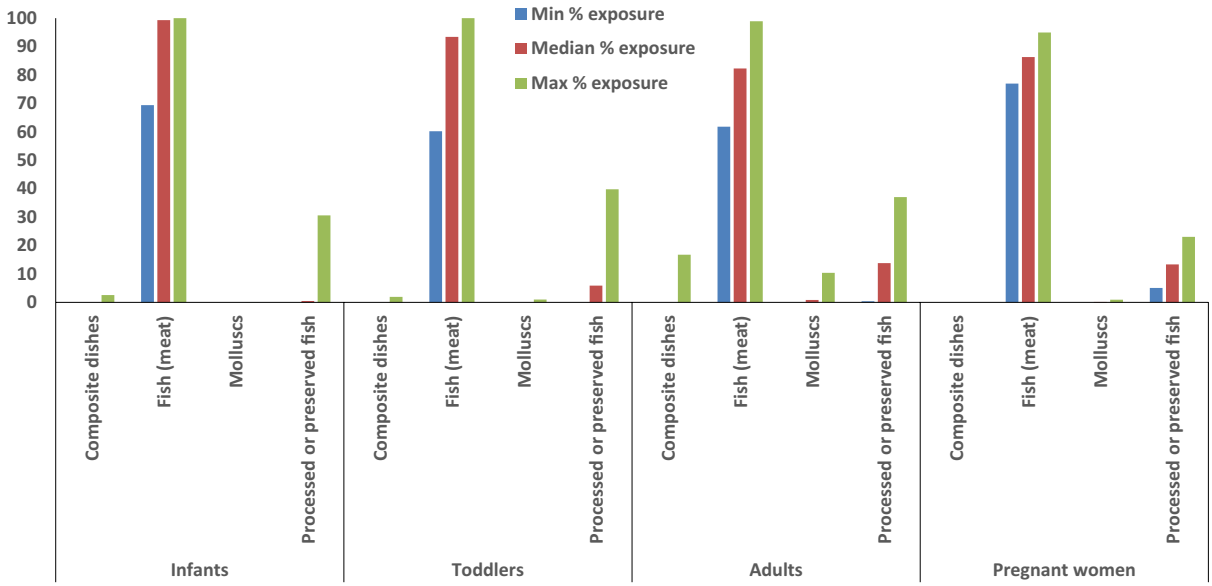


FIGURE 5 Average contribution of different food groups to the mean dietary exposure to AsB in selected population groups.

When interpreting the dietary exposure estimates, it is important to mention the limited data set available including the lack of data submitted to EFSA for several commodities that could be relevant for the exposure to AsB. For certain commodities (e.g. fish-based processed commodities), occurrence values were derived using AsB levels reported for raw commodities; the absence of data on different types of fish commonly consumed (e.g. cod, hakes, sole/plate) was also considered by grouping the available fish species at higher FoodEx2 level before estimating exposure. However, the lack of occurrence data in the EFSA database on specific seafoods such as crustaceans (shrimps) and molluscs (clams, squids or octopus) might have led to the underestimation of the dietary exposure to AsB for some individuals.

3.3.2 | Dietary exposure to glycerol arsenosugar (AsSugOH)

In Europe, there is evidence for the consumption of more than 150 edible species of algae, of which 14% are considered microalgae/cyanobacteria and 86% seaweeds (Mendes et al., 2022). In the last years, there has been an increasing consumption of seaweeds linked to the growing popularity of Asian cuisine in Europe. This consumption seems to be dominated by brown and red seaweeds, with red seaweed Laver/Nori, typically consumed as thin dried sheets in different types of sushi, representing 60% of the total consumption. Other seaweeds habitually consumed in Europe are the brown seaweeds Wakame and Kombu, as well as the red seaweed Dulse (*Palmaria palmata*). Among the microalgae/cyanobacteria, the most consumed is the cyanobacterium Spirulina, mainly as food supplements but also as an ingredient in different food products (e.g. pasta). Despite the increase in seaweed consumption, data on its consumption in Europe are limited, with the EFSA Comprehensive Database probably not fully capturing this tendency yet. Additionally, the complex taxonomy of seaweed species adds further uncertainty to the limited consumption data reported on seaweeds (EFSA, 2023).

A total of 466 eating occasions of seaweed (306 consumers) with adequate information to be used for the exposure estimations were retrieved from the EFSA Comprehensive Database (see Annex C for the dietary surveys considered). They corresponded to 401 eating occasions reported for Laver (156 as sushi and 245 as seaweed), 25 for Wakame (17 as miso soup +8 as seaweed) and 40 for Kombu, distributed across different age dietary surveys and age classes.

Chronic dietary exposure to AsSugOH was estimated considering the individual consumption of the red seaweed Laver/Nori and the brown seaweeds Wakame and Kombu ('consumers only'). Table 13 shows a selection of dietary exposure estimates for 'consumers only' of different types of seaweed grouped by age class. The highest mean exposure to AsSugOH was estimated in the 'Elderly' population (0.19 μg As/kg bw day) whereas the highest 95th percentile (high consumers) was estimated in 'Adults' (0.71 μg As/kg bw day), in both cases via the consumption of Laver/Nori. All the exposure estimates calculated for each age class ('consumers only') are presented in Annex F.

TABLE 13 Chronic dietary exposure to AsSugOH (μg As/kg bw per day) in the European population considering the consumption of selected seaweeds ('consumers only').

		Chronic dietary exposure (μg As/kg bw day)[a]			
	Seaweeds	**Number of consumers**	**Mean**	**75th percentile**	**95th percentile**
Other children	Laver/Nori	11	0.14	0.14	–
Adolescents	Laver/Nori	31	0.14	0.10	–
Adults	Kombu	27	0.13	0.05	–
Adults	Wakame	21	0.01	0.01	–
Adults	Laver/Nori	187	0.17	0.17	0.71
Elderly	Laver/Nori	7	0.19	–	–
Lactating women	Laver/Nori	7	0.04	–	–

[a]Only age classes with a minimum of six consumers for each seaweed are shown. Reliable percentiles for dietary exposure were estimated only if the following minimum number of consumers was available: 11 samples for the 75th percentile and 59 samples for the 95th percentile.

Chronic exposure to AsSugOH via the consumption of alga-based food supplements used as source of iodine was also estimated as described in Section 2.7. Although these food supplements are frequently marketed as 'Kelp', the description of the ingredients often reveals the use of other brown seaweeds such as Bladder wrack (*Fucus vesiculosis*) and Knotted wrack (*Ascophylum nodosum*) among others, instead of Sugar Kelp (*Saccharina latissima*). In the absence of data on food supplements, mean AsSugOH levels used for the exposure estimations were derived from the few samples of Bladder wrack, Knotted wrack and Sugar Kelp reported in Table 11 (1.2 mg As/kg d.m.; $n = 4$). The recommended dose on the product label is typically one capsule per day, although certain manufacturers recommend up to three capsules per day.

Assuming an average daily consumption of one capsule containing 300 mg powder from the three brown seaweeds mentioned above, the chronic dietary exposure to AsSugOH in an adult of 70 kg bw would be 0.005 μg As/kg bw per day. In a high consumer scenario, the consumption of three capsules per day containing 1000 mg powder could lead to an exposure to AsSugOH of 0.05 μg As/kg bw per day. These estimates, even though surrounded by high uncertainty, seem to indicate that the exposure to AsSugOH via consumption of alga-based food supplements (average and high consumer) is much smaller than that from the consumption of seaweeds itself.

3.4 | Risk characterisation

Arsenobetaine/Arsenocholine

It is widely assumed that AsB is non-toxic due to it being excreted unchanged. This means that arsenobetaine is not expected to exhibit toxicity because of the release of inorganic arsenic. However, this does not exclude the possibility that the intact AsB molecule could exhibit some toxicity, which has not been adequately investigated. AsB has not been found to exhibit acute toxicity following oral doses of up to 10,000 mg/kg bw in mice (corresponding to about 4000 mg/kg bw expressed as As). It did not exhibit immune effects following oral administration to mice at the single dose level of 1625 mg/kg bw (677 mg/kg bw expressed as elemental arsenic) on four occasions over one week (averaging 387 mg As/kg bw per day over the week). It did not exhibit reproductive toxicity when administered to rats during pregnancy and lactation at dose levels up to 10 mg/kg bw per day (4.2 mg/kg bw per day expressed as As), the highest dose tested. AsB has not shown genotoxicity in in vitro assays. In addition, there is no indication of an association with adverse outcomes in limited human studies.

The highest estimated 95th percentile human dietary exposure to AsB in the European population is 12.5 μg As/kg bw per day for toddler consumers of fish, seafood and fish-based processed commodities. Thus, although an RP cannot be derived for AsB, the MOEs based on the highest dose tested NOAELs from the repeat dose studies are > 340 (based on the NOAEL of 4.2 mg As/kg bw per day from the reproductive study) and > 31,000 (based on the NOAEL of 387 mg/kg As bw per day in the short term immunotoxicity study).

Overall, the CONTAM Panel is of the opinion that there is sufficient evidence to conclude that AsB at current dietary exposure levels does not raise a health concern.

No risk characterisation could be conducted for AsC, due to the lack of exposure and toxicity data.

Arsenosugars

The CONTAM Panel identified a reference point of 0.85 mg As/kg bw per day for AsSugOH, based on the $BMDL_{10}$ values for measures of passive avoidance and motor function. Taking into account an uncertainty factor of 100 for inter- and intra-species differences and an additional factor of 10 to account for major gaps in the database, an MOE ≥ 1000 would not raise a health concern. From literature data on the occurrence of AsSugOH and data for consumption of seaweed, the highest

95th percentile estimate of exposure was 0.71 μg As/kg bw per day for adult consumers of Nori/Laver. At this exposure level, the MOE would be 1197 and does not raise a health concern. However, it should be noted that there are only limited consumption data available. No risk characterisation can be conducted for arsenosugars other than AsSugOH due to the lack of toxicity and exposure data.

Arsenolipids

No risk characterisation could be conducted for arsenolipids, due to the lack of exposure and toxicity data.

3.5 | Uncertainty analysis

The evaluation of the uncertainties in the present assessment was performed following the principles laid down in the Guidance on uncertainty analysis in scientific assessments (EFSA Scientific Committee, 2018). The purpose of the uncertainty analysis is to identify and quantify the major uncertainties of the specific risk assessment and combine them to assess the overall certainty of the final conclusions. To harmonise uncertainty analyses, the CONTAM Panel applies an approach that structures the risk assessment process in broader groupings, as well as subgroupings. Sets of questions have also been defined to help the identification of uncertainties. There are four overarching elements of the approach: (i) chemical characterisation and analytical methods, (ii) exposure assessment, (iii) hazard identification and characterisation and (iv) risk characterisation. The uncertainties identified for (i), (ii), (iii) and (iv) are described in Tables 14–16.

A quantification of the uncertainty in estimated MOEs was also considered, consistent with assessments performed in the EFSA risk assessments on inorganic arsenic and on small organoarsenic species (EFSA CONTAM Panel, 2024a, 2024b). The MOE estimated for AsSugOH was above the MOE not raising a health concern (≥ 1000). Despite the uncertainty related to the incomplete information on toxicity of AsB, the CONTAM Panel concludes that at current dietary exposure levels AsB does not raise a health concern.

3.5.1 | Identification and impact assessment of uncertainties

3.5.1.1 | *Arsenobetaine*

For AsB, uncertainties related to chemical characterisation and analytical methods (Table 14), including issues related to the purity of commercially available species, were regarded to be of low impact for the overall conclusions.

With respect to hazard identification and characterisation (Table 14), uncertainty regarding toxicokinetics was also regarded to be of low impact since it was considered to have been sufficiently investigated. Regarding assessment of toxicity, there are only few human studies available, and they provided no evidence of an association between exposure to AsB and adverse health outcomes. Uncertainty related to available experimental studies was regarded to be of medium impact. These studies were not viewed as suitable for the establishment of a reference point. However, as a conservative approach, the NOAEL could be determined as the highest dose tested, i.e. since no effects were observed, the actual NOAEL might be higher. Also, it should be noted that there were only two studies available, where the dosing regimen differed and the NOAEL in one study was substantially lower than in the other. Overall, it is considered likely that AsB is nontoxic, but some uncertainty remains due to limited data.

Related to exposure assessment (Table 14b), the most relevant uncertainties associated with the EFSA occurrence data refer to the low number of samples available (lack of representativeness), with some identified missing food categories with no AsB data available (potential underestimation) and to the use of occurrence values for processed commodities derived from raw primary commodities (potential source of inaccuracy).

The uncertainties discussed above propagate to the risk characterisation, and while a NOAEL could be determined (as highest dose tested) it was not possible to formally establish an RP (Table 14c).

3.5.1.2 | *Arsenosugars*

Regarding chemical characterisation and analytical methods (Table 15a), uncertainties were identified, in particular, due to possible impurities related to the synthesis of arsenosugars species in the study used for RP derivation.

With respect to hazard identification and characterisation (Table 15a) no human studies are available, and only a single experimental study on AsSugOH relevant for quantitative dose–response analysis, was identified. Uncertainty related to toxicokinetics was regarded to be of medium impact for this study. Overall, the uncertainty related to using a single study was regarded as of high impact. Also, the results from this study may not be extrapolated to arsenosugars other than AsSugOH.

Related to the exposure assessment of AsSugOH (Table 15b), the most relevant uncertainties associated with the occurrence data retrieved from the literature are (1) the complex taxonomy of seaweeds that might lead to misreporting of seaweed species, (2) the low number of samples available per seaweed, and (3) the potential inaccuracy in deriving AsSugOH levels for certain composite foods (e.g. Sushi). Particularly important is the uncertainty surrounding the consumption

data on seaweeds, with a potential misclassification of the seaweeds consumed due to its complex taxonomy. Long-term (chronic) exposure assessment based on short dietary surveys (2–3 days duration) tends to overestimate the levels for high consumers, which can be particularly relevant for seaweeds due to their relatively low frequency of consumption in Europe.

Regarding risk characterisation (Table 15c), the main uncertainty identified relates to the limitation of the toxicity database discussed above, i.e. the MOEs derived cover AsSugOH from seaweed but it is not known whether this conclusion is applicable to other arsenosugars.

3.5.1.3 | *Arsenolipids*

For arsenolipids there are no occurrence data from EFSA, although data are available in the public literature. Also, there are no toxicity data (see Table 16). Therefore, no risk characterisation could be conducted for arsenolipids.

TABLE 14A **Arsenobetaine –** Elements of the CONTAM approach and relevance for the uncertainty analysis – chemical characterisation and analytical methods, hazard identification and characterisation.

Main group	Sub-group	Overarching questions	Description of uncertainty	Impact ranking[a]
Chemical composition and analytical methods	**Chemical composition**	Is there uncertainty associated with the dose in the critical studies used in the risk assessment?	Purity of commercially available As species.	**0**
			Purity of in-house synthesised As species, and possibility of wrong structure assignments.	**1** **2**
			Uncertainty related to exposure to natural mixtures of As species.	
	Analytical methods	Are the analytes being reliably identified and reported?	Yes, generally they are, but false assignments are still appearing in the literature.	**1**
		Are the target analytes being reliably quantified?	Yes, generally they are, but wrong quantitative data are still appearing in the literature.	**1**
Hazard identification and characterisation	**ADME**	Is there uncertainty in any aspect of ADME?	There is limited information about transfer to human milk and on on half-life in humans and rats.	**1**
	Studies in humans	Are there sources of uncertainties in the design of the human studies?	Most studies were cross-sectional, and apart from diabetes, only one study per outcome was identified.	**2**
	Toxicity studies in experimental animals: critical endpoints and critical study design	Are there sources of uncertainties in the design of the critical studies in the use of the animal model?	No critical studies were identified. Only two repeated dose studies are available with very different dose levels and neither showing any effects. It is assumed that AsB is not toxic but this assumption is based on limited evidence.	**2**
	Genotoxicity	Is there uncertainty on the genotoxicity of the species?	There is no evidence of genotoxic effects across different types of mammalian cells in in vitro assays. No in vivo studies were found.	**1**
	Mode of action	Are there uncertainties on the MoAs of the substance that could affect the conclusions of the risk assessment?	There is insufficient evidence on MOAs for AsB. But as there are no critical toxicity studies and it is assumed that AsB is not toxic (albeit not based on reliable scientific evidence) this is an uncertainty of low impact.	**1**

[a]0 – no or negligible uncertainty, 1 – uncertainty with low impact, 2 – uncertainty with medium impact, 3 – uncertainty with high impact.

TABLE 14B **Arsenobetaine –** Elements of the CONTAM approach and relevance for the uncertainty analysis – occurrence and exposure assessment.

Main group	Sub-group	Overarching questions	Description of uncertainty	Impact ranking[a]
Occurrence data	**Analytical measurements**	Is there uncertainty due to the performance of the analytical method? This may include identification, sensitivity and recovery.	Recovery was not reported for any of the samples analysed for AsB; for 42 of these samples the analytical data were sent to EFSA as corrected for recovery, 108 samples were reported as not corrected, and for 470 samples no information was available on whether corrected or not for recovery.	**1**
	Data reporting	Is there uncertainty on whether there are errors in the reported occurrence data or linked to missing information?	Potential errors in reporting the occurrence data (e.g. in the classification of the food category, of the compound, unit of measurement, parameter, fat vs. whole weight, etc.) – unidentified errors (not apparent from the data provided).	**1**
			There is uncertainty on the reported occurrence data for seaweeds and seaweed derived products (alga-based food supplements) mainly due to the complex taxonomy of seaweeds, but also to sample preparation and analysis.	**1**
		Is there uncertainty in the information on sampling strategy.	For around 10% of the samples no information was provided on the sampling strategy. It is possible that for some of these samples random sampling was not followed.	**1**
		Is there uncertainty in the occurrence data due to lack of data for potentially relevant major food categories?	Overall, the occurrence data set for AsB used for the exposure estimations is rather small (~150 samples). Although occurrence data were grouped to minimise the impact of missing fish species for which relatively high levels of AsB are reported in the literature (e.g. sole/plaice), there could be an underestimation of the exposure. Same applies to the lack of data on commodities that are well-known to possess relatively high levels of AsB (e.g. crustaceans (shrimps) and certain molluscs (clams, squids or octopus)).	**1–2**
		Is there uncertainty in the occurrence data due to left censorship and the substitution method.	The left-censored data accounted for 75% of the analytical results on AsB, although this proportion was much lower for the food categories eventually used to estimate dietary exposure (fish, seafood and fish-based processed commodities).	**1**
	Representativeness and completeness of the data	Is there uncertainty in the occurrence data due to limited data availability?	Extrapolation of data from one food category to others (e.g. deriving occurrence values for missing processed commodities using data reported in raw primary commodities).	**2**
			Low number of samples per food category.	**2**
			Low number of reporting sampling countries.	**1**
			Missing information on the detection method for few samples.	**1**

TABLE 14B (Continued)

Main group	Sub-group	Overarching questions	Description of uncertainty	Impact ranking[a]
Consumption data	**Data reporting**	Is there uncertainty in the consumption data due to errors e.g. in classification, body weight, age, memory errors etc.?	Unidentified errors in reporting consumption data (e.g. in the classification of the food, portion size, etc.).	**1**
			Body weight estimation (measured, self-reported or estimated).	**1**
			Memory errors and capacity to report details in dietary surveys, possible under and over reporting.	**1**
		Is there uncertainty in consumption data, e.g. due to methodology of the dietary survey, weekdays, national recipes?	Dietary survey methodology (dietary record vs. 24-h recall), dietary software, interview options (place, face to face vs. telephone and background of the interviewers) and use of portion-size measurement aids for the estimation of portion sizes).	**1**
			Long-term (chronic) exposure assessed based on few days of consumption per individual.	**1–2**
			Representativeness over different weekdays and seasons within dietary surveys.	**1**
			Sample size and response rate of the dietary surveys.	**1**
			Use of national standard recipes and ingredients factors for composite dishes (e.g. underestimation of minor ingredients, overestimation of standard ingredients, etc.) Sampling frame, method and design of the dietary surveys.	**1**
	Representativeness of the data	Is there uncertainty in the representativeness of the consumption data (e.g. of the countries, special population groups, sample size and response rates.	Lack of food consumption data for special population groups, including consumers only of specific foods of special interest, or following special diets, countries, etc.	**1**
Dietary Exposure estimates methodology		Is there uncertainty linked to the methodology used for calculating the exposure?	Long-term (chronic) exposure assessed based on short dietary surveys (2–3 days duration) tends to underestimate the proportion of individuals who consume particular food types, but at the same time, overestimates the levels for high consumers.	**1–2**

[a]0 – no or negligible uncertainty, 1 – uncertainty with low impact, 2 – uncertainty with medium impact, 3 – uncertainty with high impact.

TABLE 14C **Arsenobetaine –** Elements of the CONTAM approach and relevance for the uncertainty analysis – risk characterisation.

Main group	Sub-group	Overarching questions	Description of uncertainty	Impact ranking[a]
Risk characterisation	Uncertainty factors/Critical MOE/HBGV	Is there uncertainty in the selected (default or specific) UFs/critical MOE/HBGV?	Since no specific RP has been identified, it is not possible to identify UFs.	**3**

[a]0 – no or negligible uncertainty, 1 – uncertainty with low impact, 2 – uncertainty with medium impact, 3 – uncertainty with high impact.

TABLE 15A **Arsenosugars** – Elements of the CONTAM approach and relevance for the uncertainty analysis – chemical characterisation and analytical methods and hazard identification and characterisation.

Main group	Sub-group	Overarching questions	Description of uncertainty	Impact ranking[a]
Chemical composition and analytical methods	**Chemical composition**	Is there uncertainty associated with the dose in the critical studies used in the risk assessment?	Uncertainty for in-house synthesised arsenosugars species, arising from possible impurities (e.g. Bin Sayeed et al., 2013).	**3**
			Uncertainty when natural mixtures of arsenosugars are used.	**2**
	Analytical methods	Are the analytes being reliably identified and reported?	Arsenosugars (the four main species at least) are usually reliably identified.	**1**
		Are the target analytes being reliably quantified?	Reliable quantification of arsenosugars is possible but can have considerable uncertainty for some food matrices.	**1**
		Are different labs producing consistent and similar results?	Results are consistent in terms of the major identified arsenosugars, and generally show fair consistency for quantification. Possible changes during sample preparation and storage remain an issue.	**2**
Hazard identification and characterisation	**ADME**	Is there uncertainty in any aspect of ADME?	There is only one ADME study in rodents. There is limited information on transfer to milk in humans.	**2**
	Studies in humans	Are there sources of uncertainties in the design of the human studies?	Yes. There are no epidemiological studies.	**3**
	Toxicity studies in experimental animals: critical endpoints and critical study design	Are there sources of uncertainties in the design of the critical studies in the use of the animal model?	Relevance of the results of the single study on AsSugOH, which have not been replicated. Relevance of study on AsSugOH to other arsenosugars.	**3**
	Genotoxicity	Is there uncertainty on the genotoxicity of the species?	In vitro data indicate a lack of genotoxic potential for the two arsenosugars tested. A single in vivo study has shown evidence for DNA damage induction, as detected by the comet assay, in the blood and hippocampus tissue of AsSugOH-treated mice.	**3**
	Mode of action	Are there uncertainties on the MoA of the substance that could affect the conclusions of the risk assessment?	There is uncertainty related to the MoA for in vivo neurobehavioural effects of AsSugOH and the potential involvement of DNA damage.	**2**
	Dose–response analysis of critical endpoints	Is there uncertainty on the biological relevance of the selected BMR and (how) will this affect the results from the BMD analysis?	For AsSugOH a BMR of 10% for neurobehavioural effects was selected that is believed to reflect the natural variability of neurobehavioral endpoints. This is in line with previous EFSA opinions.	**1**
		Are there uncertainties in the selection of the RP that are not covered by the BMD confidence interval e.g. is model uncertainty covered.	As noted above, only a single study was available for BMD analysis which adds to the uncertainty in the RP selection.	**2**

[a]0 – no or negligible uncertainty, 1 – uncertainty with low impact, 2 – uncertainty with medium impact, 3 – uncertainty with high impact.

TABLE 15B **AsSugOH –** Elements of the CONTAM approach and relevance for the uncertainty analysis – occurrence and exposure assessment.

Main group	Sub-group	Overarching questions	Description of uncertainty	Impact ranking[a]
Occurrence data (all data on AsSugOH retrieved from the literature)	**Analytical measurements**	Is there uncertainty due to the performance of the analytical method? This may include identification, sensitivity and recovery	Occurrence data in the literature were thoroughly assessed to identify potential issues linked to sample preparation and/or the analytical method used (e.g. misidentification, interconversion).	**1**
	Data reporting	Is there uncertainty on whether there are errors in the reported occurrence data or linked to missing information?	There is some uncertainty on the occurrence data found in the literature mainly due to the complex taxonomy of seaweeds.	**1–2**
		Is there uncertainty in the occurrence data due to lack of data for potentially relevant major food categories?	Occurrence data for the most consumed seaweeds were retrieved from the literature.	**1**
	Representativeness and completeness of the data	Is there uncertainty in the occurrence data due to limited data availability?	Relatively low number of samples per type of seaweed. Generation of occurrence data for some foods (e.g. sushi, miso soup) using data available on seaweeds.	**1–2**
Consumption data	**Data reporting**	Is there uncertainty in the consumption data due to errors e.g. in classification, body weight, age, memory errors, etc.?	Missing information on some seaweed eating occasions (dried/fresh weight). Eating occasions classified at FoodEx Level 1 (Algae and prokaryotes organisms) without further information. Possible misclassification of seaweeds due to complex taxonomy.	**2**
			Unidentified errors in reporting consumption data (e.g. in the classification of the food, portion size, etc.).	**1**
			Body weight estimation (measured, self-reported or estimated). Memory errors and capacity to report details in dietary surveys, possible under and over reporting.	**1**
		Is there uncertainty in consumption data, e.g. due to methodology of the dietary survey, weekdays, national recipes?	Dietary survey methodology (dietary record vs. 24-h recall), dietary software, interview options (place, face to face vs. telephone and background of the interviewers) and use of portion-size measurement aids for the estimation of portion sizes).	**1**
			Long-term (chronic) exposure assessed based on few days of consumption per individual.	**2**
			Representativeness over different weekdays and seasons within dietary surveys.	**1**
			Sample size and response rate of the dietary surveys.	**1**
			Use of national standard recipes and ingredients factors for composite dishes (e.g. underestimation of minor ingredients, overestimation of standard ingredients, etc.) Sampling frame, method and design of the dietary surveys.	**1**
	Representativeness of the data	Is there uncertainty in the representativeness of the consumption data (e.g. of the countries, special population groups, sample size and response rates.	Lack of food consumption data for special population groups, including consumers only of specific foods of special interest, or following special diets, countries, etc.	**1**

(Continues)

TABLE 15B (Continued)

Main group	Sub-group	Overarching questions	Description of uncertainty	Impact ranking[a]
Dietary Exposure estimates methodology		Is there uncertainty linked to the methodology used for calculating the exposure?	Long-term (chronic) exposure assessed based on short dietary surveys (2–3 days duration) tends to underestimate the proportion of individuals who consume particular food types, but at the same time, overestimates the levels for high consumers. Exposure estimates based on only few consumers.	**2**

[a]0 – no or negligible uncertainty, 1 – uncertainty with low impact, 2 – uncertainty with medium impact, 3 – uncertainty with high impact.

TABLE 15C **Arsenosugars –** Elements of the CONTAM approach and relevance for the uncertainty analysis – risk characterisation.

Main group	Sub-group	Overarching questions	Description of uncertainty	Impact ranking[a]
Risk characterisation	Uncertainty factors	Is there uncertainty in the selected (default or specific) UFs?	The default UF for intra-species differences in kinetics and dynamics is considered sufficient as is the additional UF for limitations in the toxicological database. The additional factor of 10 for gaps in the database is considered conservative.	**0**
	Risk metric		The derived MOE specifically relates to AsSugOH and the result may not be extrapolated to other arsenosugars. Also, the MOE does not cover consumption of foods other than seaweed.	**3**

[a]0 – no or negligible uncertainty, 1 – uncertainty with low impact, 2 – uncertainty with medium impact, 3 – uncertainty with high impact.

TABLE 16 **Arsenolipids** – Elements of the CONTAM approach and relevance for the uncertainty analysis – chemical characterisation and analytical methods, hazard identification and characterisation.

Main group	Sub-group	Overarching questions	Description of uncertainty	Impact ranking[a]
Chemical composition and analytical methods	**Chemical composition**	Is there uncertainty associated with the dose in the critical studies used in the risk assessment?	Uncertainty for in-house synthesised As species, arising from possible impurities.	**2**
			Uncertainty related to exposure to natural mixtures of As species.	**2**
	Analytical methods	Are the analytes being reliably identified and reported?	Many, but not all of the arsenolipids can be reliably identified.	**2**
		Are the target analytes being reliably quantified?	The AsHCs can be reliably quantified in some cases; reliable quantification of most other arsenolipids is not currently being realised.	**2**
		Are different labs producing consistent and similar results?	There are only few data available to assess interlaboratory performance for arsenolipids. Different laboratories are generally consistent regarding identification (qualitative assessment) of arsenolipids, depending on the sample preparation. Consistent quantitative results between different laboratories are achievable for the AsHCs, but is not yet being shown for other arsenolipids.	**1**

TABLE 16 (Continued)

Main group	Sub-group	Overarching questions	Description of uncertainty	Impact ranking[a]
Hazard identification and characterisation	**ADME**	Is there uncertainty in any aspect of ADME?	There is no information on ADME in rodents. There is limited information on ADME in humans.	**2**
	Studies in humans	Are there sources of uncertainties in the design of the human studies?	Yes. There are no epidemiological studies.	**3**
	Toxicity studies in experimental animals: critical endpoints and critical study design	Are there sources of uncertainties in the design of the critical studies in the use of the animal model?	There are no relevant studies.	**3**
	Genotoxicity	Is there uncertainty on the genotoxicity of the species?	In vitro studies in different cell lines showed no clastogenic effects or oxidative damage to DNA. No in vivo studies were found.	**1**
	Mode of action	Are there uncertainties on the MoA of the substance that could affect the conclusions of the risk assessment?	There are only in vitro studies indicating neurotoxicity and no in vivo studies.	**2**
		Is there uncertainty on the human relevance of the MoA identified in experimental animals?	There are no in vivo studies.	**3**

[a]0 – no or negligible uncertainty, 1 – uncertainty with low impact, 2 – uncertainty with medium impact, 3 – uncertainty with high impact.

3.5.2 | Conclusions on Uncertainty

For AsB a dietary exposure assessment was conducted for consumers of fish, seafood and fish-based processed commodities ('consumers only').

An RP could not be derived for AsB. The MOEs based on the highest dose tested NOAELs from the two repeat dose studies are > 340 (based on the NOAEL of 4.2 mg As/kg bw per day from the reproductive study), and > 31,000 (based on the NOAEL of 387 mg/kg As bw per day in the short term immunotoxicity study).

The MOE of 340 is conservative since it is based on the highest estimated 95th percentile human dietary exposure, and because the NOAEL used was the highest dose tested in a single reproductive toxicity study. Also the much higher doses that have been tested in the short term immunotoxicity study, showed no effect. However, the toxicological database is incomplete. Based on qualitative evaluation of all identified uncertainties, it is regarded likely that they are covered by the MOE range.

For arsenosugars, an exposure assessment for AsSugOH related to the consumption of seaweeds ('consumers only') was conducted based on public literature, and an RP was established based on a single study. The estimated MOE (≈ 1200) is higher than the MOE considered not to raise a health concern (≥ 1000). This MOE is conservative in nature since it is based on a BMDL in combination with the highest P95 exposure. Also, based on quantitative considerations the MOE not raising a health concern is regarded to account for most of the identified uncertainties (e.g. limitations in the database). A health concern was therefore not raised despite the associated uncertainties. It is not known whether the assessment can be extrapolated to other arsenosugars.

4 | CONCLUSIONS

4.1 | General information

Arsenic species in the complex organoarsenic group (arsenobetaine (AsB), arsenosugars and arsenolipids) are almost exclusively found in marine food, although they have also been reported at lower levels in freshwater fish and crabs. AsB is by far the major arsenic species occurring in fish, crustaceans and most molluscs. It is chemically stable, and can be reliably quantified by most modern food analytical laboratories. Other species of the arsenobetaine group (e.g. arsenocholine (AsC)) are also sometimes reported but usually at low levels. Arsenosugars are the major arsenic species in seaweeds, in particular brown seaweeds; they are moderately chemically stable and, provided the analytical process is mild, they can be reliably quantified by experienced laboratories. Arsenolipids comprise several sub-groups of arsenic species (e.g. arsenic fatty acids (AsFAs), arsenic hydrocarbons (AsHCs)), found usually at low levels (< 10%) in fish, crustaceans, molluscs and seaweeds (marine food). The various sub-groups have quite different chemical properties and stability, which complicates their measurement in food samples.

4.2 | Toxicokinetics

- In rodents, AsB and AsC are well absorbed, not metabolised and rapidly eliminated in urine. In one study with mice, arsenosugars were partly metabolised to dimethylarsinic acid DMA(V) and excreted as arsenosugars or DMA(V) in the faeces; urine was not examined. No information on the toxicokinetics of arsenolipids in rodents was identified.
- In humans, AsB is absorbed but not metabolised and most of the arsenobetaine is excreted unchanged within days in the urine. Based on limited information there is low transfer (0.03%) to human milk. Arsenosugars are metabolised to DMA(V) and several other minor As species, which are excreted in the urine within 4 days. There is inter-individual variability in the efficiency of arsenosugar metabolism and excretion via the urine. Based on limited information, arsenolipids ingested from arsenolipid-containing food, are bioaccessible and bioavailable; they are metabolised mainly to DMA(V) and some minor As species, which are excreted in the urine. Arsenolipids can be transferred to human milk (transfer rate about 3%).

4.3 | Biomarkers of exposure

- AsB has been found in human urine in many studies. While concentrations are usually only a few µg/L, they can be much higher in individuals who recently consumed fish or other seafood. Consumption of seafood is the reason for the high variability of total As in urine in the general population. AsB can also be detected in blood, but quantification is complicated and therefore blood is rarely used for biomonitoring.
- Because DMA(V) is the major urinary metabolite of both ingested iAs, and compounds such as arsenosugars and arsenolipids, it cannot be used as a specific biomarker of exposure to arsenosugars or to arsenolipids.

4.4 | Toxicity in experimental animals

- Few toxicity studies are available.
- AsB, AsC and glycerol arsenosugar (AsSugOH) exhibited low acute toxicity with oral LD_{50} values exceeding 6000 mg/kg bw. There are indications that AsC, but not AsB, might have immunological effects.
- Exposing rats to AsB during pregnancy and lactation at doses up to 10 mg/kg bw per day (the highest dose) did not result in reproductive toxicity.
- Mice dosed orally with AsSugOH at 20, 30 and 50 mg/kg bw per day for 40 days showed dose-related decreases in both passive avoidance and motor function.

4.5 | Genotoxicity

- In vitro genotoxicity studies on complex organoarsenic species were largely negative, with only a few reporting weak clastogenic effects for AsB, AsC and AsSugOH at highly cytotoxic concentrations.
- A single in vivo genotoxicity study was identified which reported DNA damage in a Comet assay with lymphocytes and hippocampal tissue of mice exposed to AsSugOH.

4.6 | Mode of action

- The lack of cytotoxic and genotoxic effects in various mammalian cell systems suggests that AsB does not interact with biologically relevant macromolecules.
- In a study with mice, AsSugOH was found to cause increased oxidative stress.
- Arsenic hydrocarbons (AsHCs) and arsenic fatty acids (AsFAs) affected neurite outgrowth and mitochondrial membrane potential in vitro, AsHCs being more potent.

4.7 | Observation in humans

- There are a few, mainly cross-sectional, studies examining associations between urinary excretion of various As species, in some of which AsB was quantified, and health outcomes. There are no studies including quantification of arsenosugars or arsenolipids.
- The studies on AsB and health outcomes indicate some tendencies of inverse associations (reduced odds ratios), potentially explained by a beneficial effect of fish consumption.
- Therefore, the CONTAM Panel concluded that epidemiological studies provide no evidence for an association with adverse health outcomes.

4.8 | Derivation of reference points

- The toxicity data for AsB and arsenolipids are insufficient to identify RPs for these groups and therefore no health based guidance values (HBGVs) or margins of exposures (MOEs) could be derived.
- Dose–response modelling of the effects of AsSugOH on passive avoidance and motor function in a 40-day study with male mice data resulted in similar $BMDL_{10}$ values (3.7 mg/kg bw per day, expressed as the AsSugOH). Overall, a value of 0.85 mg As/kg bw per day (expressed as elemental arsenic) was identified as an RP for AsSugOH.
- Given the limited toxicity data, it is not appropriate to establish a HBGV for AsSugOH. A MOE approach should take into account the default uncertainty factor of 100 for inter- and intra-species differences and an additional factor of 10 to allow for major deficiencies in the data base, indicating that a MOE ≥ 1000 would not raise a health concern.

4.9 | Occurrence data

The concentrations of the different arsenic species are expressed as elemental arsenic.

Arsenobetaine

- The highest levels of AsB among the samples of 'Fish and seafood' were reported for 'Molluscs', in particular for 'Oysters' with mean values of 1878 µg As/kg (LB = UB, $n = 27$); relatively high mean levels were also reported for 'Mussels' (853 µg As/kg, LB = UB, $n = 41$). No data were reported to EFSA on crustaceans.
- Among fish samples, 'Marine fish' had the highest mean levels (1106 µg As/kg, LB = UB, $n = 7$), followed by 'Diadromous fish' (276 µg As/kg, LB = UB, $n = 12$) and 'Freshwater fish' (LB-UB, 48.5–49.9 µg As/kg, $n = 6$).

- Considering the uncertainties associated to the AsB levels reported for seaweed and alga-based supplements, only the occurrence data on fish, seafood and fish-based processed commodities were used to estimate dietary exposure to AsB.

AsSugOH

- No occurrence data on AsSugOH or any other arsenosugars were reported to EFSA. Scientific literature shows that seaweeds are the foods with the highest levels of arsenosugars, with these compounds being the dominant arsenic species in most seaweeds.
- To estimate dietary exposure to AsSugOH occurrence data from literature were retrieved for the most commonly consumed seaweeds in Europe: the red seaweed Laver/Nori and the brown seaweeds Wakame and Kombu.
- The highest mean AsSugOH levels in the scientific literature are reported for the seaweed Kombu ($n = 7.5$ μg As/kg, $n = 11$).

Arsenolipids

- No occurrence data on arsenolipids were reported to EFSA.
- Arsenolipids have been reported in the scientific literature in seaweeds, fish, molluscs and crustaceans but not in terrestrial foods.
- Among the more than 200 types of arsenolipids in food the dominant subgroups appear to be arsenic hydrocarbons (AsHCs), arsenosugar phospholipids (AsSugPLs), arsenic phosphatidylcholines (AsPCs) and arsenic triglycerides (AsTAGs).
- Quantification of arsenolipids in food is challenging due to their large number, their instability during the analysis and the lack of analytical standards.
- Arsenolipids are particularly abundant in seaweeds, mainly as AsSugPLs, while AsHCs are the dominant species in seafoods.

4.10 | Exposure assessment

Arsenobetaine

- Among consumers of fish, seafood and fish-based processed commodities ('consumers only'), the highest mean dietary exposure to AsB was 4.6 μg As/kg bw per day, in both 'Infants' and 'Toddlers'. The highest 95th percentile exposure was in 'Toddlers' with an estimate of 12.5 μg As/kg bw per day.
- Dietary exposure estimates for special population groups such as 'Pregnant women' and 'Lactating women' were similar to those for the adult population.
- 'Fish meat', and particularly 'Marine fish', was overall the main contributor to the exposure to AsB across the different age classes.

AsSugOH

- Among consumers of seaweeds ('consumers only'), the highest mean exposure to AsSugOH was estimated in the 'Elderly' population (0.19 μg As/kg bw day) whereas the highest 95th percentile (high consumers) was estimated in 'Adults' (0.71 μg As/kg bw day), in both cases via the consumption of Laver/Nori.
- The chronic exposure to AsSugOH from consumption of alga-based food supplements seems to be rather low as compared to that from the consumption of seaweeds as part of the diet.

Arsenolipids

- Considering that no occurrence data on arsenolipids were submitted to EFSA, and that the available toxicological data did not allow the identification of RPs, or MOEs that do not raise a health concern for arsenolipids, no dietary exposure was conducted.

4.11 | Risk characterisation

- Limited data from acute, immunotoxicity and reproductive toxicity tests are available for AsB, none of which showed effects up to the highest doses tested. When comparing these dose levels with the highest 95th percentile exposure to AsB, the MOEs based on the highest dose tested NOAELs from the two repeat dose studies are > 340 (based on the NOAEL of 4.2 mg As/kg bw per day from a reproductive study), and > 31,000 (based on the NOAEL of 387 mg/kg As bw per day in a short term immunotoxicity study).
- Overall, there is sufficient evidence to conclude that AsB at current dietary exposure levels does not raise a health concern.

- Comparing the highest 95th percentile exposure to AsSugOH in Laver/Nori consumers with the RP for neurobehavioural effects results in an MOE > 1000, not raising a health concern.
- Risk characterisation for arsenobetaine compounds other than AsB, arsenosugars other than AsSugOH, and arsenolipids was not possible due to a lack of toxicity and exposure data.

4.12 | Uncertainty analysis

- Based on a qualitative assessment of all identified uncertainties, it is regarded likely that dietary exposures to AsB and AsSugOH do not raise a health concern.
- It is not known whether the assessment for AsSugOH applies to other arsenosugars.

5 | RECOMMENDATIONS

- There is a need for occurrence data on complex organoarsenic species.
- There is a need for improved understanding of the ADME of complex organoarsenic species, particularly in relation to inter-individual differences in humans.
- There is a general need for toxicity data on complex organoarsenic species which is currently very limited.

ABBREVIATIONS

% LC	proportion of left-censored data
ADME	absorption, distribution, metabolism and excretion
AHA	American Heart Association
ALT	alanine aminotransferase
ANOVA	analysis of variance
Art.	article
As	arsenic
As(III)	arsenite
As(V)	arsenate
AsB	arsenobetaine
AsC	arsenocholine
ASCVD	atherosclerotic cardiovascular disease
AsDAG	arsenic diglyceride
AsFA	arsenic fatty acid
AsFOH	arsenic fatty alcohol
AsHC	arsenic hydrocarbon
AsOH	arsenic alcohol
AsPC	arsenic phosphatidylcholine
AsPE	arsenic phosphatidylethanolamine
AsSugOH	glycerol arsenosugar
AsSUGPhytol	arsenosugar phytol
AsSugPL	arsenosugar phospholipid
$AsSugPO_4$	phosphate arsenosugar
$AsSugSO_3$	sulfonate arsenosugar
$AsSugSO_4$	sulfate arsenosugar
AST	aspartate aminotransferase
AsTAG	arsenic triglyceride
ATSDR	Agency for Toxic Substances and Disease Registry
B-Hg	blood mercury
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
$BMDL_{01}$	benchmark dose lower confidence limit for a 1% extra risk
$BMDL_{10}$	benchmark dose lower confidence limit for a 10% extra risk
BMDU	benchmark dose upper confidence limit
BMI	body mass index
BMR	benchmark response
BP	blood pressure
BSA	body surface area
BSO	buthionine sulfoximine
bw	body weight
Ca	Calcium

CA	chromosomal aberration
ca	circa ('approximately')
CAS	Chemical Abstracts Service
Cd	cadmium
CH_3	methyl group
CI	confidence interval
Co	cobalt
CONTAM	Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA)
Cr	chromium
CRM	certified reference material
Cu	copper
CVD	cardiovascular disease
Da	Dalton
DCM	dichloromethane
DKO	Double Knockout (apo E–/– As3mt –/–)
DMA	dimethylated arsenic
DMA(III)	dimethylarsinous acid
DMA(V)	dimethylarsinic acid
DMAA	dimethylarsinoyl acetic acid
DMAB	dimethylarsinoyl butanoic acid
DMAE	dimethylarsinoyl ethanol
DMAP	dimethylarsinoyl propionic acid
DMMTA	dimethylmonothioarsenate
DN	double negative
DNA	deoxyribonucleic acid
DP	double positive
d.m.	dry matter
EEC	European Economic Community
eGFR	estimated glomerular filtration rate
ELISA	Enzyme-Linked Immunosorbent Assay
ES	electrospray
ESMS	electrospray mass spectrometry
FAO	Food and Agriculture Organization
Fe	iron
FLARE	Fluorescent Advanced Oxidation Protein Products
FPG	Formamidopyrimidine-DNA Glycosylase
FT3	free triiodothyronine
FT4	free thyroxine
fw	fresh weight
GD	gestational diabetes
GD	gestational day
GM	geometric mean
GSH	glutathione
HBGV	health based guidance value
HDL	high-density lipoprotein
HepG2	human hepatoma
HGPRT	hypoxanthine guanine phosphoribosyl transferase
hOGG1	Human 8-Oxoguanine DNA Glycosylase 1
HOMA-β	Homeostatic Model Assessment of Beta-cell Function
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HOME	Home Observation Measurement of the Environment
Homo-AsC	homo-arsenocholine
HPLC	high-performance liquid chromatography
HPLC-ESMS	high-performance liquid chromatography-electrospray mass spectrometry
HPLC-ICPMS	high-performance liquid chromatography-inductively coupled plasma mass spectrometry
HRP	highest reliable percentile
IARC	International Agency for Research on Cancer
iAs	inorganic arsenic
ICP	inductively coupled plasma
ICPMS	inductively coupled plasma mass spectrometry
INMA	Infancia y Medio Ambiente (translates to 'Childhood and Environment')
IPCS	International Programme on Chemical Safety

IQ	intelligence quotient
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
K	potassium
KNHANES	Korea National Health and Nutrition Examination Survey
LB	lower bound
LC	Liquid chromatography
LD_{50}	Lethal Dose 50%
LOD	limit of detection
LOQ	limit of quantification
LTP	long-term potentiation
MDA	malondialdehyde
MeHg	methylmercury
MeOH	methanol
Mg	magnesium
MIREC	Maternal–Infant Research on Environmental Chemicals
ML	Maximum Level
MMA(V)	monomethyarsonic acid
Mn	manganese
MN	micronucleus
MOA	mode of action
MOE	margin of exposure
MSCA	Mullen Scales of Early Learning
n	number of analytical results
Na	sodium
$NaAsO_2$	sodium arsenite
n.d.	not determined
NHANES	National Health and Nutrition Examination Survey (US: United States of America)
Ni	nickel
NOAEL	no observed adverse effect level
NR	not reported
OR	odds ratio
p20/p80	20th/80th percentile
P95	95th percentile
PBDE	polybrominated diphenyl ether
PBMC	human peripheral blood mononuclear cells
PCB	polychlorinated biphenyl
pH	logarithm of the concentration of the H+ species in water
pKa	acid dissociation constant
PND	postnatal day
PO_4	phosphate group
POR	prevalence odds ratio
ppb	parts per billion
Ref	reference
RF	renal function
ROS	Reactive Oxygen Species
RP	reference point
RR	risk ratio
SAS	Statistical Analysis System
SCE	Sister Chromatid Exchange
Se	selenium
SES	socioeconomic status
SGA	Small for Gestational Age
SO4	sulfate group
SOD	superoxide dismutase
sol	soluble
SOP	Standard operational procedure
SSB	Single-Strand Break
SSD	Standard Sample Description
t	time
T2D	type 2 diabetes
tAs	total arsenic

TDS	Total Diet Studies
TGN	thyroglobulin antibodies
TK	thymidine kinase
TMAP	trimethylarsoniopropionate
TSH	thyroid-stimulating hormone
T-SOD	Total Superoxide Dismutase
TT3	total triiodothyronine
TT4	total thyroxine
u-As(V)	urinary arsenate
u-AsB	urinary arsenobetaine
UB	upper bound
u-creatinine	urinary creatinine
u-DMA	urinary dimethylated arsenic
UF	uncertainty factor
UROtsa	human urinary tract epithelial cell line
U.S.	United States
US NHANES	United States National Health and Nutrition Examination Survey
V	volume
WG	Working Group
WHO	World Health Organization
w.w.	whole weight

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APPENDIX A

Occurrence of arsenolipids in seafoods

Table A.1 provides an overview about the occurrence of arsenolipids in seafoods.

TABLE A.1 Occurrence of arsenolipids in seafoods.

Sample	Total As (mg As/kg d.m.)	Lipid-soluble As (mg As/kg d.m.)	Sum of AsHCs (mg As/kg d.m.)	AsHC 332 (mg As/kg d.m.)	Reference/comment
Cod	1.4	0.05	n.d.	n.d.	Wolle and Conklin (2018b)
Haddock	5.9	0.07	n.d.	n.d.	
Mackerel	1.2	0.38	n.d.	n.d.	
Crab	22.1	0.22	n.d.	n.d.	
Shrimp	0.3	0.07	n.d.	n.d.	
Geoduck (clam)	2.9	0.38	n.d.	n.d.	
Oyster	2.9	0.84	n.d.	n.d.	
Kombu	26.5	2.92	n.d.	n.d.	
Salmon 3 species, $n=8$	0.45–1.75	0.1–0.7	n.d.	n.d.	Wolle, Conklin, and Wittenberg (2019), Wolle, Stadig, and Conklin (2019)
Tuna 2 species, $n=7$	3.8–12.3	0.25–1.15	n.d.	n.d.	
Tilapia 3 species, $n=4$	0.2–1.9	0.01–0.015	n.d.	n.d.	
Pollack 1 species, $n=3$	4.15–8.6	0.025–0.11	n.d.	n.d.	
Swai 1 species, $n=3$	0.04–0.05	<0.02	n.d.	n.d.	
Cod 3 species, $n=3$	2.9–22.1	0.035–0.43	n.d.	n.d.	
Catfish 1 species, $n=3$	0.1–0.35	0.02–0.15	n.d.	n.d.	
Crab 7 species, $n=10$	2.8–111	0.55–1.94	n.d.	n.d.	
Shrimp 10 species, $n=8$	1.7–38	0.05–0.85	n.d.	n.d.	
Clam 5 species, $n=5$	3.75–13.3	0.075–1.55	n.d.	n.d.	

TABLE A.1 (Continued)

Sample	Total As (mg As/kg d.m.)	Lipid-soluble As (mg As/kg d.m.)	Sum of AsHCs (mg As/kg d.m.)	AsHC 332 (mg As/kg d.m.)	Reference/comment
Asame *n* = 2	24–26	5–6	n.d.	n.d.	Wolle et al. (2021) 46 samples of seaweeds purchased at markets, comprising brown (8 species), red (5 species) and green (1 species) seaweeds. Values have been estimated from visual inspection of a bar graph (Figure 1) in the paper.
Hijiki *n* = 3	86–104	7–8	n.d.	n.d.	
Kombu (*Saccharina* spp.) 3 species, *n* = 6	55–85	3–10	n.d.	n.d.	
Knotted wrack (*Ascophyllum nodosum*) *n* = 2	28–42	3–6	n.d.	n.d.	
Oarweed (*Laminaria digitata*) *n* = 2	50–55	4–6	n.d.	n.d.	
Wakame (*Undaria pinnatifida*) *n* = 12	34–48	6–30	n.d.	n.d.	
Dulse (*Palmaria palmata*) *n* = 4	13–17	2–3	n.d.	n.d.	
Irish moss (*Chondrus crispus*) *n* = 3	10–12	0.5–0.8	n.d.	n.d.	
Red seaweed (*Pyropia* sp.) *n* = 1	32	3	n.d.	n.d.	
Purple laver (*Porphyra umbilicalis*) *n* = 1	30	3	n.d.	n.d.	
Susabi nori (*Pyropia yezoensis*) *n* = 8	13–26	1–4	n.d.	n.d.	
Sea lettuce (*Ulva sp.*) *n* = 2	6–7	2–3	n.d.	n.d.	

TABLE A.1 (Continued)

Sample	Total As (mg As/kg d.m.)	Lipid-soluble As (mg As/kg d.m.)	Sum of AsHCs (mg As/kg d.m.)	AsHC 332 (mg As/kg d.m.)	Reference/comment
Tuna	n.d.	n.d.	0.035	n.d.	Al Amin et al. (2020) Data, original values, based on wet weight, have been reported here as dry weight based on 80% moisture content (i.e. dw As value = 5 × ww As value)
Sea bream	n.d.	n.d.	0.40	n.d.	
Pacific saury	n.d.	n.d.	0.55	n.d.	
Skipjack	n.d.	n.d.	1.05	n.d.	
Sardine	n.d.	n.d.	1.0	n.d.	
Horse mackerel	n.d.	n.d.	0.40	n.d.	
Flounder	n.d.	n.d.	0.25	n.d.	
Flounder eggs	n.d.	n.d.	0.85	n.d.	
Whitebait	n.d.	n.d.	1.2	n.d.	
Salmon	n.d.	n.d.	0.25	n.d.	
Mackerel	n.d.	n.d.	0.05	n.d.	
Yellowtail	n.d.	n.d.	0.15	n.d.	
Shrimp	n.d.	n.d.	0.15	n.d.	
Squid	n.d.	n.d.	0.10	n.d.	
Octopus	n.d.	n.d.	0.10	n.d.	
Scallop	n.d.	n.d.	0.25	n.d.	
Clam	n.d.	n.d.	0.40	n.d.	
Algae	n.d.	n.d.	0.73	0.18	
Fish & Shellfish	n.d.	n.d.	0.18	0.18	Al Amin et al. (2018) Market basket survey. Mixture of fish, molluscs and crustaceans.
Wakame *(Undaria pinnatifida)*	40	2.7	0.51	0.02	García-Salgado et al. (2012) Major arsenolipids were AsSugPLs
Hijiki *(Hizikia fusiformis)*	113	1.8	0.37	0.31	García-Salgado et al. (2012) Major arsenolipids were AsSugPLs
Capelin (*Mallotus villosus)*	n.d.	n.d.	Identified as the major arsenolipid but not quantified		Amayo et al. (2011)
Brown seaweed (*Saccharina latissima)*	116	9	0.3	< 0.1	Petursdottir et al. (2019) Composite sample – approx. concentrations. Mean of old and young fronds. Major arsenolipids by far were the AsSugPLs

TABLE A.1 (Continued)

Sample	Total As (mg As/kg d.m.)	Lipid-soluble As (mg As/kg d.m.)	Sum of AsHCs (mg As/kg d.m.)	AsHC 332 (mg As/kg d.m.)	Reference/comment
B rown seaweed (*Alaria esculenta)*	93	5	0.2	< 0.1	Petursdottir et al. (2019) Composite sample – approximate concentrations in fronds. Major arsenolipids by far were the AsSugPLs.
Salmon (*Salmo salar)*	2.74	0.16	0.09	0.02	Xiong et al. (2022). With cooking, 26% of AsHCs were converted to their thio analogues
Kombu (processed product) *(Saccharina japonica)*	55	4.4	0.6 (estimated)	n.d.	Yu et al. (2018)
Kombu (fresh) *(Saccharina japonica)*	45	5.9	0.8 (estimated)	n.d.	Yu et al. (2018). Major arsenolipids were the AsSugPLs
Sugar kelp *(Saccharina latissima*)	78	3.2	0.9	0.02	Raab et al. (2013)
Sashimi-grade tuna	5.9	2.9	1.2	-	Taleshi et al. (2010)
Oil from Capelin *(Mallotus villosus)*	11.7	11.7	0.34	n.d.	Taleshi et al. (2008)
Skipjack tuna ($n = 5$) *(Katsuwonus pelamis)*	4.7	0.4	0.09	0.07	Stiboller et al. (2019)
Cod liver	2.6 1.3	2.0 0.33	n.d.	n.d.	Schmeisser et al. (2006)
Cod liver ($n = 26$)	2.1–240	1.8–16.4	ca 0.1 to 0.2	ca 0.08 to 0.15	Sele et al. (2015) Quantities estimated

Abbreviations: AsHC, arsenic hydrocarbon; d.m, dry matter; *n*, number of analytical results; n.d., not determined.

ANNEXES

ANNEX A

Protocol for the risk assessment

Annex A contains the risk assessment protocol selected by the CONTAM Panel to draft the opinion and is available under the Supporting Information section on the online version of the scientific output.

ANNEX B

Additional literature searches

Annex B contains the details of the additional literature searches complementing the literature search outsourced in preparation of the present risk assessment and is available under the Supporting Information section on the online version of the scientific output.

ANNEX C

Dietary surveys used for chronic exposure assessment AsB and ASugOH

The annex is provided as a separate Excel file containing the information on the dietary surveys used for the chronic dietary exposure assessment and is available on the EFSA Knowledge Junction community on Zenodo at: https://10.5281/zenodo.14186922.

ANNEX D

Raw occurrence data for AsB

The annex is provided as a separate Excel file containing the raw occurrence data set for AsB in food, and is available on the EFSA Knowledge Junction community on Zenodo at: https://10.5281/zenodo.14187244.

ANNEX E

Occurrence data set for AsB

The annex is provided in a separate Excel file containing summary statistics for the occurrence data on AsB at different FoodEx2 levels, and is available on the EFSA Knowledge Junction community on Zenodo at: https://10.5281/zenodo.14187259.

ANNEX F

Chronic dietary exposure assessment for AsB and AsSugOH

The annex is provided as a separate Excel file containing chronic dietary exposure estimations for AsB and AsSugOH, and is available on the EFSA Knowledge Junction community on Zenodo at: https://10.5281/zenodo.14187277.

ANNEX G

BMD modelling reports

Annex G contains the details of the BMD calculations carried out and is available under the Supporting Information section on the online version of the scientific output.