

Risk assessment of small organoarsenic species in food

EFSA Panel on Contaminants in the Food Chain (CONTAM) |

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Abstract

The European Commission asked EFSA for a risk assessment on small organoarsenic species in food. For monomethylarsonic acid MMA(V), decreased body weight resulting from diarrhoea in rats was identified as the critical endpoint and a BMDL₁₀ of 18.2 mg MMA(V)/kg body weight (bw) per day (equivalent to 9.7 mg As/kg bw per day) was calculated as a reference point (RP). For dimethylarsinic acid DMA(V), increased incidence in urinary bladder tumours in rats was identified as the critical endpoint. A BMDL₁₀ of 1.1 mg DMA(V)/kg bw per day (equivalent to 0.6 mg As/kg bw per day) was calculated as an RP. For other small organoarsenic species, the toxicological data are insufficient to identify critical effects and RPs, and they could not be included in the risk assessment. For both MMA(V) and DMA(V), the toxicological database is incomplete and a margin of exposure (MOE) approach was applied for risk characterisation. The highest chronic dietary exposure to DMA(V) was estimated in 'Toddlers', with rice and fish meat as the main contributors across population groups. For MMA(V), the highest chronic dietary exposures were estimated for high consumers of fish meat and processed/preserved fish in 'Infants' and 'Elderly' age class, respectively. For MMA(V), an MOE of ≥ 500 was identified not to raise a health concern. For MMA(V), all MOEs were well above 500 for average and high consumers and thus do not raise a health concern. For DMA(V), an MOE of 10,000 was identified as of low health concern as it is genotoxic and carcinogenic, although the mechanisms of genotoxicity and its role in carcinogenicity of DMA(V) are not fully elucidated. For DMA(V), MOEs were below 10,000 in many cases across dietary surveys and age groups, in particular for some 95th percentile exposures. The Panel considers that this would raise a health concern.

KEYWORDS

dimethylarsinic acid (DMA(V)), margin of exposure (MOE), monomethylarsonic acid (MMA(V)), risk assessment, small organoarsenic species

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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 this Opinion, it was concluded that small organoarsenic species like MMA and DMA and the 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 was not of toxicological concern. The European Commission has asked the European Food Safety Authority (EFSA) for an update of the risk assessment of inorganic arsenic of 2009. This Opinion was published in 2024. In addition to the risk assessment on small organoarsenic species that is presented here, the European Commission has also asked for a risk assessment on complex organoarsenic species.

Small organoarsenic species contain methyl groups, but no other organic groups, bound to arsenic. DMA(V) is by far the most abundant of these arsenic species in food, with highest concentrations found in rice, algae and other seafoods. The pentavalent oxo-analogues of small organoarsenic species are stable in food matrices while trivalent and thio-containing small organoarsenic species are less stable in solution converting readily to their pentavalent forms and slowly to their oxo-analogues, respectively, thereby complicating their analysis.

In experimental animals, MMA(V) and DMA(V) are well absorbed, distributed to various tissues and largely excreted unchanged in urine. To a limited extent, they can undergo further methylation, while there is no evidence for demethylation. There are no data allowing estimation of absorption rates for other small organoarsenic species. Pentavalent species can be reduced to their corresponding trivalent forms and there is *in vitro* evidence for presystemic thiolation and reduction of small organoarsenic species. The higher cellular uptake of the thio-compounds in comparison to their oxo-analogues can be explained by their lower polarity.

Only limited studies are available to assess toxicokinetics of ingested MMA(V) and DMA(V) in humans. When MMA(V) and DMA(V) are ingested by humans, they seem to be well absorbed (> 75%), essentially being excreted unchanged in urine but also may undergo limited further metabolism. In one human study, arsenic administered as a single oral dose of MMA(V) was efficiently excreted in urine, mainly as unmodified MMA(V) with only 13% (of total arsenic species) present as DMA(V). In the same study, when humans ingested arsenic as a single DMA(V) dose, there was no evidence for further metabolism and only unchanged DMA(V) was found in urine. Furthermore, MMA(V) and DMA(V) have been shown to pass through the placenta, and traces of DMA(V) have been detected in breast milk.

There is, so far, no arsenic species pattern in urine or blood that has been identified as a biomarker for exposure to small inorganic arsenic species.

MMA(V), DMA(V) and trimethylarsine oxide (TMAO) are of low acute toxicity in laboratory animals. There are no data on acute toxicity for other small organoarsenic species. The limited data available on repeated dose toxicity in laboratory animals indicate that MMA(V) has adverse effects on the gastrointestinal tract, kidney, thyroid and liver and that DMA(III), DMA(V) and TMAO have adverse effects on the urinary bladder and possibly on the kidney. There are no data for other small organoarsenic species. In developmental toxicity studies with MMA(V) and DMA(V) in rats and rabbits, developmental effects were only observed in the presence of maternal toxicity, while teratogenicity has not been observed with either compound. When pregnant mice were exposed to MMA(III), tumours were seen in the liver, lung and adrenals in male offspring and in the uterus and ovaries in female offspring. With MMA(V), no increases of tumour incidence were observed in a wide range of tissues investigated. While DMA(V) induces urinary bladder tumours in male and female rats, the results of studies with DMA(V) in mice are inconsistent, and do not provide convincing evidence of carcinogenicity. With TMAO, in a single study in male rats, increased hepatocellular adenomas were seen, but no hepatocellular carcinomas and no tumours in other examined tissues were reported. No carcinogenicity data are available for other small organoarsenic species.

MMA(III) and DMA(III) induce genotoxic effects *in vitro* including DNA single and double strand breaks, clastogenic and aneugenic effects. They inhibit DNA repair mechanisms, promote the generation of ROS, generate oxidative damage to DNA and have the potential to induce cell transformation. However, no *in vivo* genotoxicity studies on these compounds are available. MMA(V) and DMA(V) exert similar *in vitro* effects to those of MMA(III) and DMA(III) although the evidence is less robust. In the case of DMA(V), there are *in vivo* data showing the induction of oxidative DNA damage in various organs and DNA breaks in circulating lymphocytes of orally exposed rodents.

The induction of chromosomal damage *in vitro* by MMA(III), DMA(III), MMA(V) and DMA(V) in the absence of mutagenic effects suggests that DNA breaks induced by oxidative damage to DNA, along with the inhibition of DNA repair, may be pivotal for the clastogenic effects.

The *in vitro* genotoxic data on DMA(V) together with the *in vivo* data on induction of oxidative DNA damage and DNA breaks indicate involvement of DNA damage in the carcinogenic effects.

There are a number of plausible modes of action for the carcinogenicity of DMA(V), including genotoxicity, as well as cytotoxicity followed by regenerative cell proliferation. There is uncertainty regarding the relative importance of the different MOAs, and whether thresholded mechanisms can be assumed.

There are no studies on adverse health effects in humans related to concentrations of MMA and DMA in food only studies related to concentrations in urine. However, it is not possible to distinguish between ingested DMA and DMA formed by methylation of inorganic As or by catabolism of arsenosugars and arsenolipids. Therefore, studies on MMA(V) and/or DMA(V) in urine cannot be used for risk assessment for these compounds.

Only for MMA(V) and DMA(V) were the toxicological data sufficient to identify critical effects, and respective reference points. No other small organic arsenic species were included in the risk assessment.

For MMA(V) decreased body weight as an indicator of observed diarrhoea following dietary exposure in male and female rats was identified as the critical endpoint. Using model averaging and applying a BMR of 10%, a BMDL₁₀ of 18.2 mg MMA(V)/kg bw per day (equivalent to 9.7 mg As/kg bw per day) was calculated as a reference point. For DMA(V), increased total urinary bladder tumour incidence in male rats following exposure via drinking water was identified as the critical endpoint. Using model averaging and applying a BMR of 10%, a BMDL₁₀ of 1.1 mg DMA (V)/kg bw per day (equivalent to 0.6 mg As/kg bw per day) was calculated as a Reference Point.

For both MMA(V) and DMA(V), important data are missing, and therefore, the CONTAM Panel concluded that it is not appropriate to establish health-based guidance values but that a margin of exposure (MOE) approach needs to be applied. For MMA(V), there is inadequate evidence for genotoxicity in vivo and evidence that it is not carcinogenic. Therefore, the CONTAM Panel concluded that an MOE of ≥ 500 does not raise a health concern taking into account the default uncertainty factor of 100 for inter- and intra-species differences and an additional uncertainty factor of 5 to account for the deficiencies in the database.

For DMA(V), there is convincing evidence that it is a rodent carcinogen. The mechanisms of genotoxicity and its role in carcinogenicity of DMA(V) are however not fully elucidated. In the absence of established MoAs for the genotoxicity/carcinogenicity of DMA(V), the Panel applied an MOE of $\geq 10,000$ to the RP of 0.6 mg As/kg bw per day.

Occurrence data on small organoarsenic species were received only for MMA and DMA, all reported for the pentavalent species (and assumed if this information was not confirmed by the data providers). No data were received on the thio- analogues thio-DMA(V), dithio-DMA(V) and thio-MMA(V).

Following data cleaning, the final data set available for dietary exposure estimates comprised 1260 analytical results for DMA and 988 for MMA, all expressed in whole weight and as $\mu\text{g As/kg}$. For a total of 654 analytical results on DMA and 653 on MMA, no information was available on whether they were submitted as $\mu\text{g As/kg}$ or $\mu\text{g DMA(MMA)/kg}$; these samples were assumed to be reported as $\mu\text{g As/kg}$. Samples were collected in eight EU countries for DMA and in four countries for MMA between 2012 and 2021; most of the samples were collected in Italy (67% for DMA, 85% for MMA). For both DMA and MMA, occurrence values for various processed commodities were derived using occurrence data on raw primary commodities, and processing factors and recipes from the EFSA's Raw Primary Commodity model.

For DMA, the left-censored data accounted for 44% of the analytical results. Overall, the highest levels of DMA were reported for seaweeds and seaweed-based foods. Rice samples were reported mainly as 'Rice grain, polished' ($n=221$, mean LB=UB=40.0 $\mu\text{g As/kg}$) and 'Rice grain, brown' ($n=110$, mean LB=UB=27.8 $\mu\text{g As/kg}$). Different levels of DMA were reported depending on the type of fish; the highest average levels were for the few samples of 'Marine fish' ($n=34$, LB=UB=36.5–45.0 $\mu\text{g As/kg}$).

For MMA, the left-censored data accounted for 94% of the analytical results, included all the reported 411 samples of rice and the 35 of fish. The few quantified data were reported almost exclusively on molluscs (mainly mussels and oysters), with 27 samples of oysters all quantified (mean LB=UB=227 $\mu\text{g As/kg}$). Occurrence data submitted to EFSA on MMA were complemented with data from the literature (mainly on fish meat and processed/conserved fish) before estimating dietary exposure.

The highest DMA dietary exposure was estimated for the young population, in particular in one dietary survey for 'Toddlers', with LB–UB mean exposures of 0.130–0.157 $\mu\text{g As/kg bw per day}$ and LB–UB 95th percentile exposures of 0.397–0.477 $\mu\text{g As/kg bw per day}$. In the adult population (adults, elderly and very elderly), dietary exposure was similar across the different population groups with maximum LB–UB estimates of 0.038–0.044 $\mu\text{g As/kg bw per day}$ and 0.133–0.158 $\mu\text{g As/kg bw per day}$ for mean and 95th percentile exposures, respectively. Overall, the main contributors to the dietary exposure of DMA across population groups were 'Fish, seafood, amphibians, reptiles and invertebrates' and 'Grains and grain-based products', and additionally for part of the young population ('Infants, 'Toddlers'), 'Food products for young population' (all at FoodEx2 level 1).

Dietary exposure to DMA was also estimated for consumers of rice and for consumers of fish meat across European countries ('consumers only'). When considering consumers of rice, the highest mean and highest 95th percentile exposure (high consumers) was identified in one dietary survey in the age class 'Other children' with estimates (LB-UB) of 0.076–0.078 $\mu\text{g As/kg bw per day}$ and 0.186–0.190 $\mu\text{g As/kg bw per day}$, respectively. Similarly, for consumers of fish meat, the highest mean and highest 95th percentile exposure (high consumers) was identified in one dietary survey in the age class 'Toddlers' with estimates (LB-UB) of 0.165–0.205 $\mu\text{g per As/kg bw day}$ and 0.415–0.511 $\mu\text{g As/kg bw per day}$.

For MMA, highest exposures were estimated for high consumers of fish meat in infants and high consumers of processed/preserved fish in the elderly age class, in both cases with MMA estimates of 0.342 $\mu\text{g As/kg bw per day}$. Among the consumers of molluscs, the highest mean and 95th exposures were estimated in the adult population, with 0.090 $\mu\text{g As/kg bw per day}$ and 0.185 $\mu\text{g As/kg bw per day}$, respectively.

For risk characterisation of DMA(V), the RP of 0.6 mg As/kg bw per day was compared to the estimated exposures resulting in MOE values that range from 150,000 (lowest minimum LB exposure across national consumption surveys) to 3800 (highest maximum UB exposure across national consumption surveys) for the mean exposure estimates, and from 35,300 (lowest minimum LB) to 1300 (highest maximum UB exposure) for the 95th percentile exposure estimates across dietary surveys and age groups. Calculated MOEs are below 10,000 in most of the dietary surveys, in particular for 95th percentile exposures.

The uncertainty analysis indicated that it is probable that the MOE is higher than 10,000 with respect to the mean exposure while the opposite applies for the 95th percentile of exposure, at least for the younger age classes.

The MOEs resulting from a comparison of the RP for DMA(V) with exposure estimates from the different scenarios on rice-based infant formula ranged from 3200 to 970 and as these were lower than 10,000.

The MOEs derived for DMA(V) in the specific exposure scenario for rice consumers were lower than 10,000 at maximum average exposures for 'Infants', 'Toddlers' and 'Other children' and at maximum P95 exposures for all groups except 'Lactating women' and 'Vegetarians'. The corresponding MOEs derived in the specific exposure scenario for fish consumers were lower than 10,000 at maximum average and P95 exposures for 'Infants', 'Toddlers', 'Other children', 'adolescents' and 'adults' and at maximum P95 exposures for almost all age groups.

The CONTAM Panel noted that these MOEs below 10,000 raise a health concern but also noted the uncertainties associated with interpretation of the mode of action for carcinogenicity with regard to the extent to which DNA-reactive mechanisms contribute to DMA(V) genotoxicity as well as the role of genotoxicity in its carcinogenicity.

For risk characterisation of MMA(V), the RP of 9.7 mg As/kg bw per day was compared with exposure estimates for the different age groups focussing on 'consumers only' via the main food commodities for which quantified MMA(V) data were available. The selected food commodities were 'Fish meat', 'Molluscs' and 'Processed/preserved fish'. The MOEs ranged from 3,233,000 (average exposure, minimum) to 28,000 (P95 exposure, maximum) for fish meat consumers, from 9,670,000 (average exposure, minimum) to 52,000 (P95 exposure, maximum) for molluscs consumers and from 9700,000 (average exposure, minimum) to 28,000 (P95 exposure, maximum) for processed/conserved fish consumers. All MOEs were well above 500 for average and high consumers and thus do not raise a health concern.

The CONTAM Panel concluded that there is a need for robust validated analytical methods for determining all small organoarsenic species in food and that certified reference materials especially for trivalent and thio-containing small organoarsenic species in products such as rice and seafood are required. There is also a need for improved understanding of the human metabolism of small organoarsenic species following ingestion and the human health implications. In order to better characterise these compounds toxicological studies on developmental effects and neurotoxicity for MMA(V) and DMA(V) and more toxicity data on small organoarsenic species other than MMA(V) and DMA(V) are needed. The mechanisms of induction of DNA damage by small organoarsenic species should be investigated to clarify its mode of interaction with DNA. Further studies on genotoxic properties are needed for all small organoarsenic species. Occurrence data on small organoarsenic species in breast milk are needed, together with additional occurrence data on foods (e.g. rice, fish and seafood, rice-based infant formulas) to confirm the present exposure estimations and food contributors.

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 BMDL₀₁ 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 compounds no human toxicity data are available. Because of the lack of data, arsenosugars, arsenolipids, methylarsenate (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) 2015/1381² 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

¹EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2014. Scientific report of on the dietary exposure to inorganic arsenic in the European population. EFSA Journal, 12(3):3597.

²Commission 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 | Chemical properties of small organoarsenic species relevant to their presence in food

Arsenic occurs in group 15 in the periodic table under nitrogen and phosphorus. Although arsenic shows similarities to phosphorus in terms of its inorganic chemistry (e.g. the oxyanions arsenate/phosphate), in terms of its organic chemistry, it more closely resembles nitrogen by forming a wide range of stable compounds with carbon. These organoarsenic compounds almost always contain one or more methyl groups, and often additionally contain a more complex organic group. This Opinion deals with those organoarsenic species that contain methyl groups, but no other organic groups, bound to arsenic. These will be dealt with in a separate CONTAM Panel Opinion on complex organoarsenic species to be developed after the present one.

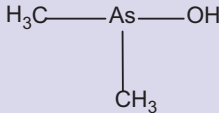
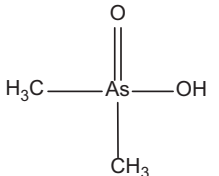
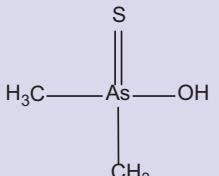
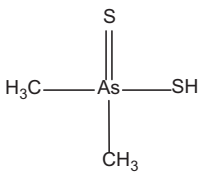
The small organoarsenic species found in food almost always contain arsenic in the +5 oxidation state, and usually also contain oxygen (the oxo-analogue) or sulfur (the thio-analogue). Dimethylarsinic acid (DMA(V)) is by far the most abundant of these arsenic species in food, being reported in a range of samples with highest concentrations found in rice, and in algae and other seafoods. Relevant species and their properties are presented in [Table 1](#).

The oxo-analogues of methylated species with arsenic in the +5 oxidation state are stable, both in food and in extracts of food, which makes their analysis relatively straightforward. The species with arsenic in the +3 oxidation state, however, are labile in solution where they readily convert to the As(V) form. This lability greatly complicates their analysis. The thio-arsenic species can also convert, albeit slowly, to their oxo-analogues (i.e. the As-S bond is replaced by an As-O bond) in solution, particularly under mild acid conditions. Such conversions could compromise the relative amounts of oxo/thio arsenic species reported in foods. For example, there is recent evidence that thio-dimethylarsinic acid (thio-DMA(V)) is being underestimated in foods owing to its conversion to DMA(V) during the chemical analysis (Dai et al., [2022](#)).

TABLE 1 Small organoarsenic species relevant to food.

Acronym ^a	Common name and synonyms	Chemical name (CAS number)	Formula and molecular mass	Structure ^b	Properties	Comment
MMA(III)	Monomethylarsonous acid; methylarsonous acid; methylarsonite	Methylarsonous acid (CAS 25400–23-1)	CH ₃ AsO ₂ 123.971 Da		Unstable in water readily converting to MMA(V)	A proposed intermediate in the biomethylation of inorganic arsenic. Available commercially; also chemically produced in situ by hydrolysis of methylarsine oxide or diiodomethylarsine
MMA(V) MMA MA MMAA	Monomethylarsonic acid; methylarsonic acid; methylarsonate	Methylarsonic acid (CAS 124–58-3)	CH ₃ AsO ₃ 139.970 Da		Stable water-soluble solid; pKa values 3.4 and 8.2	An intermediate in biomethylation of inorganic arsenic. A common albeit usually minor species in food. Commercially available
thio-MMA(V) MMMTA	Monomethylthioarsonic acid; thio methylarsonic acid; thio monomethylarsonic acid	Methylarsonothioarsonic acid	CH ₃ AsO ₂ S 156.036 Da			Occasional occurrence in rice at low concentrations
dithio-MMA(V) MMDTA	Monomethyldithioarsonic acid; methyldithioarsonic acid	Methylarsono-dithioic acid	CH ₃ AsOS ₂ 172.101 Da			Occasional occurrence in rice at low concentrations
MMA MA MMAA	Monomethylated arsenic			See footnote ^c		The term MMA is often used to signify all monomethylated arsenic species comprising MMA(III), MMA(V) and the two monomethylated thio arsenicals. In solution, MMA(V) is the most stable form and the other monomethylated forms readily convert to MMA(V)

TABLE 1 (Continued)

Acronym ^a	Common name and synonyms	Chemical name (CAS number)	Formula and molecular mass	Structure ^b	Properties	Comment
DMA(III)	Dimethylarsinous acid; hydroxydimethylarsane; dimethylarsonite; dimethylarsinite	Dimethylarsinous acid (CAS 55094-22-9)	C ₂ H ₇ AsO 121.998 Da		Unstable in water readily converting to DMA(V)	A transient intermediate in the biomethylation of inorganic arsenic. Chemically produced by hydrolysis of dimethyliodoarsine. Only rarely reported in food
DMA(V) DMA DMAA	Dimethylarsinic acid; dimethylarsinate; cacodylic acid, oxo-DMA(V)	Dimethylarsinic acid (CAS 75-60-5)	C ₂ H ₇ AsO ₂ 137.997 Da		Stable water-soluble solid; pKa 6.3	A major product of inorganic arsenic biomethylation reactions and metabolite of several organic arsenic species. A significant species in many foods of both marine and terrestrial origin
thio-DMA(V) DMMTA	Dimethylthioarsinic acid; thio-dimethylarsinic acid; dimethylated monothioarsenate	Dimethylarsino-thioic acid (CAS 754217-65-7)	C ₂ H ₇ AsOS 154.063 Da		Slowly converts to DMA(V); conversion can be rapid in dilute acid	Increasingly reported in foods, particularly in rice. Current analytical methods employing dilute acid might not be suitable for this species. Commercially available
dithio-DMA(V) DMDTA	Dimethyldithioarsinic acid	Dimethylarsino-dithioic acid (CAS 65165-11-9)	C ₂ H ₇ AsS ₂ 170.129 Da		Slowly converts to DMA(V) via thio-DMA; conversion can be rapid in dilute acid	Detected in rice and rice products
DMA	Dimethylated arsenic			See footnote ^c		The term DMA is often used to signify all dimethylated arsenic species comprising DMA(III), DMA(V) and the two dimethylated thio arsenicals. In solution, DMA(V) is the most stable form and the other dimethylated forms readily convert to DMA(V)

(Continues)

TABLE 1 (Continued)

Acronym ^a	Common name and synonyms	Chemical name (CAS number)	Formula and molecular mass	Structure ^b	Properties	Comment
TMAO	Trimethylarsine oxide	(CAS 4964-14-1)	C ₃ H ₉ AsO (136.025 Da)		Stable water-soluble species; pKa 3.6	A common but usually minor species in food. Commercially available
thio-TMA	Thio-trimethylarsine	Trimethylarsine sulfide (CAS 26386-93-6)	C ₃ H ₉ AsS (152.090 Da)			No studies found confirming occurrence in food
TETRA	Tetramethylarsonium	Tetramethyl-arsonium ion (CAS 27742-38-7)	C ₄ H ₁₂ As (135.059 Da)		Stable water-soluble species	A common but usually minor species in food

Abbreviations: CAS, Chemical Abstracts Service; pKa, acid dissociation constant.

^aThe acronym in bold in the first column represents the specific nomenclature used throughout this Opinion. Synonyms are also listed acknowledging that nomenclature varies in the scientific literature.

^bArsenic species are drawn in their fully protonated form.

^cIn this Opinion, the terms ‘ΣMMA,’ ‘ΣDMA,’ ‘MMA’ and ‘DMA’ collectively encompass specific groups of arsenic compounds. ‘ΣMMA’ is an abbreviation representing the sum of MMA(III), MMA(V) and two monomethylated thio arsenicals while ‘ΣDMA’ refers to the sum of DMA(III), DMA(V) and two dimethylated thio arsenicals. Furthermore, ‘MMA’ is used as an abbreviation for the sum of MMA(III) and MMA(V), and ‘DMA’ stands for the sum of DMA(III) and DMA(V).

^dThe names for the fully protonated forms (monomethylarsonic acid and dimethylarsinic acid) are often used in the literature although the species are usually measured as the anion, namely monomethylarsonate or dimethylarsinate.

1.2.2 | Analytical methods for small organoarsenic species in food

During the 1980s, vapour generation techniques that rely on the chemical conversion of the original arsenic species to its hydride were increasingly combined with liquid chromatography (LC) whereby the native arsenic species were first separated by LC and then converted to their volatile derivatives before transport to an arsenic-selective detector. Various modifications of this method were subsequently reported. Although methods based on vapour generation were widely used for measuring small organoarsenic species in food samples throughout the 1980s and 1990s, and are still reported today, they became less common following the introduction in the late 1980s of ICPMS (inductively coupled plasma mass spectrometry) combined with LC. The technique, LC-ICPMS, is robust and capable of very low detection limits (limits of detection (LODs) can be in the order of 10 ng As/L), thereby allowing the direct measurement of the arsenic species in extracts of food without the cumbersome vapour generation step. LC-ICPMS proved to be faster and much more reliable than the previously used techniques. Today, most food chemistry laboratories operate ICPMS instruments, and LC-ICPMS is the best and most common instrumental technique for measuring arsenic species in food. Accordingly, the following information on analytical methods focuses on ICPMS as the arsenic-selective detector.

The convention in the field of arsenic speciation analysis is to present the concentrations of the various species in terms of their arsenic content. Thus, occurrence data from the literature presented in this document refer to the concentrations of the various arsenic species in terms of $\mu\text{g As/g}$ and not as $\mu\text{g As species/g}$.

1.2.3 | The three main steps in arsenic speciation analysis

Analytical methods for quantifying individual arsenic species applied to solid samples such as food, usually comprise three stages: extraction, separation and measurement. Throughout the process, the analyst should be aware of possible chemical changes to the arsenic species during extraction, storage and separation.

1.2.3.1 | Extraction of arsenic species

The optimal extraction process will vary depending on sample type and the arsenic species present. The most common extractants are water, water/methanol mixtures, dilute aqueous acids or various enzyme solutions (Reid et al., 2020). Water is a mild extractant but might have low efficiency or produce a solution that is difficult to work with (e.g. forms an emulsion). Water/methanol mixtures are mild and give cleaner extracts, but they could compromise quantification of species at the measurement stage because of the 'carbon enhancement effect' (Larsen & Stürup, 1994). Aqueous acid mixtures are generally efficient giving clean extracts but could produce artefacts. Although common species such as MMA(V) and DMA(V) are stable in dilute acids, their thio-analogues might not be. As mentioned above in Section 1.2.1, a recent study has shown that thio-DMA(V) originally present in rice is converted to DMA(V) during aqueous acid extraction (Dai et al., 2022). If this conversion applies to other sample types as well, thio-DMA(V) might be underestimated and its occurrence in food might be more widespread than previously thought, as suggested by Dai et al. (2022). Thio-MMA(V) could also be similarly affected, converting to MMA(V) during extraction, leading to an underestimation of the thio-species in food. Another example of changes in speciation taking place during extraction/storage is provided by MMA(III) in mushrooms: This species was readily extracted into water, but quickly transformed to MMA(V) if left in solution (Braeuer et al., 2018).

The sample type could also affect the stability of the arsenic species in the extraction solution. For example, samples rich in natural thio-compounds are likely to produce extracts with reducing properties favouring the retention, or even the formation, of MMA(III)/DMA(III) and thio-analogues of MMA(V)/DMA(V). Because of the potential problems when using acidic extractants, enzyme solutions are sometimes preferred as a mild option to extract the arsenic species. In the case of rice extracted with an enzyme solution, the various arsenic species were efficiently extracted, the thio-DMA(V) remained intact and it could be reliably quantified (Dai et al., 2022). Enzyme extractions have, however, several disadvantages: They are time consuming and costly, and each sample type might require a different enzyme group (e.g. amylases or proteases).

Although arsenic extraction methods usually strive to retain the original form of the As species in the sample, there may, on occasion, be the need to have data on the sum of monomethyl (or dimethyl) As species. In such cases, and using monomethylated arsenic species as the example, the analytical precision could be improved by adding a reactant, such as peroxide, to the extract to ensure that any MMA(III) or thio-MMA(V) species are totally converted to MMA(V) before the separation and detection stages. Although the choice of extractant is the most critical component of the extraction process, other factors that need to be considered include agitation (e.g. shaking or sonication), temperature, time, centrifugation or filtration. Multistep extraction procedures can marginally increase extraction yields of arsenic, but such methods are time-consuming and introduce additional sources of error. Thus, the general application of multistep extraction procedures should not be encouraged. Rather, a simple, time-efficient procedure suitable for handling large sample numbers that gives adequate and consistent recovery of the intact arsenic species should be the priority.

In summary, the preferred extraction solutions for the oxo-As(V) species are water, aqueous methanol or aqueous acids, whereas aqueous enzyme solutions could have advantages for the thio-As(V) species. Water is an efficient extractant for MMA(III), but the solution should be quickly analysed to minimise transformation to MMA(V). Deoxygenating the extractant solutions is likely to minimise the transformation of the methylated As(III) and thio-arsenicals to the As(V) oxo-species.

1.2.3.2 | Separation of arsenic species

LC employing anion-exchange conditions is by far the most common method for effecting a separation of the small organoarsenic species, with a wide range of mobile phases being reported (Braeuer & Goessler, 2019; Reid et al., 2020). The Hamilton PRP-X100 column and a mobile phase based on phosphate buffers are often reported LC conditions for separating small organoarsenic species, including MMA(III). These conditions are also suitable for measuring inorganic arsenic species, thereby broadening their applicability. The two cationic species, TMAO and TETRA, can be separated by cation-exchange LC; because DMA(V) is amphoteric (at low pH it is protonated), it is also amenable to cation-exchange LC.

Reversed-phase LC, usually with ion-pairing, has also been used but the separations can be compromised by matrix effects. The less polar thio-organoselenicals are more amenable than their oxo-analogues to reversed-phase LC. This form of chromatography is also milder than anion-exchange LC, and thus might be better suited to labile arsenic species; for example, thio-DMA(V) could be detected, along with oxo-DMA(V), in extracts of rice when reversed-phase LC was used, but only oxo-DMA(V) was detected when anion-exchange HPLC was employed (Dai et al., 2022).

In summary, the preferred separation method for the methylated oxo-As(V) and As(III) species is anion-exchange LC, whereas reversed-phase LC is recommended for the thio-analogues.

1.2.3.3 | Detection of arsenic species

ICPMS is an excellent detector for the quantification of arsenic and is well suited to coupling with HPLC. Care should be taken if methanol, or some other organic solvent, is present in the extract; an arsenic species that co-elutes with methanol will give an enhanced and unpredictable signal response (Raber et al., 2010) thereby jeopardising reliable quantification. ICPMS is robust and provides low detection limits for individual species (limits of detection (LODs) can be in the order of 10 ng As/L of extract). This low detection limit capability translates into lower sample/extractant ratios thereby simplifying the extraction step and enhancing HPLC separation. Importantly, the ICPMS response is essentially independent of the type of arsenic species, which means that mass balance and column recovery can be reliably assessed. This useful characteristic of ICPMS also allows quantification of any unknown arsenic species in a sample.

1.2.3.4 | Summary

In view of the different chemistries displayed by the oxo- and thio-arsenicals, there is no single method covering all the small organoarsenic species. For the methylated oxo-arsenicals, a suitable method would entail extraction with water, aqueous methanol or dilute aqueous acid, followed by separation of the species by anion-exchange HPLC and then selective detection of arsenic by ICPMS. For the thio-arsenicals, which are inherently less stable than their oxo-analogues, a current preferred method is to use an enzyme extraction rather than using aqueous acids, and to perform the separation with reversed-phase HPLC prior to ICPMS detection. When values for the total MMA and total DMA content are deemed sufficient, and finer details such as oxo/thio and oxidation state are not required, the water-based extraction and anion-exchange LC-ICPMS is the preferred method. In such cases, the analytical precision could be improved by adding a reactant (e.g. peroxide) to the extract to ensure that the thio- or As(III) species are totally converted to oxo-As(V) species MMA(V) and DMA(V). The relevance of an analytical approach focusing on total MMA and DMA would be clearer if the fate of thio-arsenicals (and methylated As(III) species) in food following digestion were known.

The development of a reliable arsenic speciation method applicable to food samples requires reference materials covering a range of sample types and certified for small organoarsenicals including the thio-analogues. This need is currently not being met.

1.3 | 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 arsenobetaine is not of toxicological concern and that arsenosugars, arsenolipids, MMA and DMA 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 for their risk assessment.

In 2012, the International Agency for Research on Cancer (IARC) concluded that there was sufficient evidence in experimental animals for the carcinogenicity of dimethylarsinic acid (DMA(V)), calcium arsenate and sodium arsenite, and limited evidence in experimental animals for the carcinogenicity of sodium arsenate, gallium arsenide, arsenic trioxide and trimethylarsine oxide. There was inadequate evidence in experimental animals for the carcinogenicity of MMA(V) and arsenic trisulfide. Overall, there was sufficient evidence in experimental animals for the carcinogenicity of inorganic arsenic compounds. Taking into account all of the evidence, the IARC classified arsenic³ and inorganic arsenic compounds as

³Metallic/elemental arsenic.

carcinogenic to humans (Group 1), DMA(V) and MMA(V) as possibly carcinogenic to humans (Group 2B), and arsenobetaine and other organic arsenic compounds not metabolised in humans, as not classifiable as to their carcinogenicity to humans (Group 3).

In a recent Opinion of the CONTAM Panel on an update of the 2009 Opinion on inorganic arsenic (EFSA CONTAM Panel, 2024), organic species were only considered in cases where they are human inorganic arsenic metabolites. The evaluation of these organic arsenic metabolites was restricted to genotoxicity. The Panel concluded that the methylation of inorganic arsenic, particularly to trivalent methylated species but also to thiolated metabolites, should be regarded as an activation process that forms more reactive species, which exert stronger cyto- and genotoxic effects.

1.4 | Legislation

Council Regulation (EEC) No 315/93⁴ 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 333/2007⁶ 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 risk assessment on iAs in food (EFSA CONTAM Panel, 2009) as a starting point for drafting the current Opinion. The draft Opinion was posted for a public consultation from 26 March to 7 May 2024. The comments received were considered for finalising the Opinion. The outcome of the public consultation (comments received with responses to these) can be found in Annex H.

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 organoarsenic species and arsenic species other than small organoarsenic species including any other organic arsenic species. A final project report has been delivered in August 2022 and was published by EFSA (Licht et al., 2022).

Briefly, search strings were designed to identify potentially relevant studies published since 1 January 2009 (the year in which the previous opinion was published) in three databases (PubMed, Web of Science, Scopus). After removing duplicates and applying inclusion criteria for relevance the number of publications considered as relevant for the different fields for small organoarsenic species were as follows: analytical methods (303), human biomonitoring (101), occurrence/concentration and formation (153), exposure of humans (15), toxicokinetics (37), in vitro and in vivo toxicity (88), observations in humans (3) and toxic mode of action (84).

The report contains a detailed explanation of the search terms and methodology applied as annexes to summary tables containing key information about all individual studies (authors, abstracts, compounds 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, two supplementary literature searches were conducted to complement the literature searches that were commissioned by EFSA. The first search was carried out on 2 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 (TK) 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 five relevant studies on TK, seven studies on in vitro toxicity and 0 on in vivo toxicity.

In addition, it was found that, for the fields of in vivo genotoxicity and in vivo toxicity, the timelines for publications to be considered needed to be extended including also publications issued prior to 2009, because there was minimal reporting of the toxicity of small organoarsenic species in the 2009 opinion. Therefore, a complementary search (to the outsourced

⁴Council 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.

⁵Commission 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.

⁶Commission 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 L 88, 28.2.2007, p. 1–19.

literature search) on these specific fields was carried out on 3 August 2023 for publications on in vivo genotoxicity (time span 1 January 2000 to 3 August 2023) and in vivo toxicity (time span 1 January 1970 to 3 August 2023). This search yielded 29 relevant studies on in vivo genotoxicity and 49 studies on in vivo toxicity.

More detailed information 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 | Occurrence data

2.2.1 | Occurrence data submitted to EFSA

2.2.1.1 | Data collection and validation

Occurrence data on MMA and DMA 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 MMA and DMA in food and feed.

Analytical data on MMA and DMA can be reported to EFSA as Methylarsonite (MMA(III)), Methylarsonate (MMA(V)), Dimethylarsinite (DMA(III)) and Dimethylarsinate (DMA(V)).

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 ver.2.0 (EFSA, 2013). Occurrence data were managed following the EFSA standard operational procedures (SOPs)⁸ on 'Data collection and validation' and 'Analysis of data from the S-DWH for the assessment of dietary exposure'.

Occurrence data were extracted on 11 September 2023; the current assessment focused on samples collected between 2012 and 2022. Data received after that date were not included in the assessment.

2.2.1.2 | Data analysis

Following EFSA's Technical report on handling of occurrence data for dietary exposure assessment (EFSA, 2021) to guarantee an appropriate quality of the data used in the exposure assessment, 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 3.6.1 on Occurrence data.

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, 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

2.2.2 | Occurrence data from the literature

Data from public literature collected as described in Section 2.1 above were used to compare and support the reliability and accuracy of the occurrence data submitted to EFSA on MMA and DMA. Additionally, during the development of the scientific opinion, additional publications were collected by applying a 'snowballing approach'. Occurrence data published in the literature on different food commodities (e.g. rice-based infant formula, fish) were also used to carry out specific dietary exposure scenarios for MMA and/or DMA (see Section 3.7 on Exposure assessment).

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>.

⁹The 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.

2.3 | 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 conducted for them (see Section 3.7 on Exposure assessment). The food consumption data available in the Comprehensive Database for this subgroup were not considered in the exposure estimates for the general population.

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 subpopulation 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 three 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.

As suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011c), dietary surveys with only 1 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 D.

2.4 | 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 a large number 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).

¹⁰According 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 MMA/DMA 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 MMA/DMA for infants of 16 weeks up to 12 months of age.

¹¹Austria: ≥ 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: 17–46 years old.

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

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

2.5 | Dietary exposure assessment

2.5.1 | Human dietary exposure assessment

The CONTAM Panel considered it appropriate to estimate the chronic dietary exposure to MMA and DMA separately (see Section 3.5 on Derivation of reference points and approach for risk assessment). As mentioned in Section 2.3, only the selected dietary surveys fulfilling the criteria for being reliable for calculation of the chronic dietary exposure were selected (see Annex D).

A) Preparation of the occurrence and consumption data

The food categories represented by either a very low number of samples (< 6 samples) or for which all data were below the LOD or LOQ were considered as not suitable, and they were not used for the exposure estimations. Exceptions were made for five samples of marinated/pickled fish and three samples codified as salads, because literature data supported the presence of the reported levels of DMA.

Different processed commodities, for which no occurrence data were available, were identified as potential contributors to the dietary exposure to MMA and/or DMA. MMA and DMA values for these processed commodities were derived using the reported occurrence for raw primary commodities (e.g. rice, fish) and recipes and processing factors as described in the EFSA's Raw Primary Commodity model (EFSA, 2019), assuming that no contaminant losses occur during the processing. This refers for instance to fish-based commodities such as 'Fish balls', 'Fish gratin', 'Prepared fish salad', 'Fish pâté', 'Fish soup' and 'Fish fingers, breaded', rice-based commodities such as 'Rice flakes', 'Rice flour', 'Rice porridge', 'Rice popped' and 'Rice drink', and composite dishes such as 'Fish soup', 'Rice and meat meal', 'Rice and vegetables meal', 'Rice pudding' and 'Rice, meat and vegetables meal'.

For DMA, the data on seaweed with reported moisture content were also expressed in dry weight to allow an adequate linkage with the consumption of dry seaweeds. Still for DMA, the reported values for 'Simple cereals which have to be reconstituted with milk or other appropriate nutritious liquids' ($n=7$) were also used to cover eating occasions of 'Cereals with an added high protein food which have to be reconstituted with water or other protein-free liquid'. Additionally, the reported values for DMA in 'Smoked fish' ($n=11$) were also linked to the eating occasions of 'Dried fish' and 'Salt-preserved fish'.

Before linking the consumption data to the corresponding occurrence data, the following adjustments were made to reduce uncertainty and reach more accurate exposure estimates:

- Occurrence data and consumption events for solid forms of certain foods (e.g. dehydrated soups, porridge) were adjusted by an appropriate dilution factor and these consumption events were reclassified to the liquid forms (EFSA, 2018).
- Occurrence data and consumption events for 'Simple cereals which have to be reconstituted with milk or other appropriate nutritious liquids' and 'Cereals with an added high protein food which have to be reconstituted with water or other protein-free liquid' were expressed in liquid form using a factor of seven and four, respectively.

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

B) Estimation of the dietary exposure

For calculating the chronic dietary exposure to DMA and MMA, 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.

For DMA, chronic dietary exposure was calculated by combining mean DMA 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.

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

where:

- $\bar{e}_i$ $\bar{e}_i$ is the average exposure of individual i
- $\bar{x}_f$ is the mean DMA 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 DMA and a second one using the UB mean concentration of DMA.

Special attention was paid to consumers of certain commodities that are known to contribute to the exposure to DMA. Accordingly, ‘consumers only’ exposure scenarios were conducted for consumers of rice and consumers of fish using the Comprehensive Database and the occurrence data reported to EFSA.

Dedicated exposure scenarios were also conducted for infants below 16 weeks of age as this group of population is expected to be exclusively fed on breast milk and/or infant formula. To characterise the dietary exposure to DMA via infant formula, mean (200 mL/kg bw per day) and 95th percentile (P95) (260 mL/kg bw per day) default consumption values were used as indicated in the Guidance of the EFSA Scientific Committee on the risk assessment of substances present in food intended for infants below 16 weeks of age (EFSA Scientific Committee, 2017). Levels of DMA in rice-based infant formula were retrieved from the literature (Meyer et al., 2018). No exposure scenario was conducted for breastfeeding infants as insufficient DMA occurrence data were available.

For MMA, due to the high amount of left-censored data (94%, see Section 3.6, Occurrence data), individual exposure scenarios for ‘consumers only’ of selected food commodities were conducted. An additional exposure dietary exposure scenario (in ‘consumers only’) was conducted to cover the plausible situation where individuals might be exposed to MMA through the consumption of more than one food commodity containing MMA. Occurrence data (LB = UB) from the literature on different types of fish, shrimps/prawns and processed or preserved fish e.g. canned herrings, were combined with occurrence data reported to EFSA. Food consumption and body weight data at the individual level were retrieved from the Comprehensive Database. No exposure scenarios were conducted for breastfeeding infants and infants fed on infant formula as MMA occurrence data were not available.

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 | Animal studies

Table 2 summarises the relevant toxicokinetic studies on small organoarsenic species in experimental animals.

TABLE 2 Toxicokinetic studies on small organoarsenic species in experimental animals.

Arsenic species	Animals	Dose/concentration/treatment time	Results	Reference
MMA(III) MMA(V) [¹⁴ C]	Female B6C3F1 mice Female B6C3F1 mice	0.4 mg As/kg bw single oral gavage 0.4, 40 mg As/kg bw Single oral gavage	- Strong methylation to DMA 2 h post-exposure: > 75% of As in tissue (kidney, liver, lung) as DMA > 90 of excreted urinary dose DMA(III), DMA(V) - Oral absorption (MMA(V) derived radioactivity) 81/60% for 0.4/40 mg/kg bw 2-h post-exposure: < 25% of As in tissues (kidney, liver, lung) as DMA 8-h post-exposure 80% of MMA(V) excreted in urine and faeces < 10% of urinary excreted dose DMA	Hughes et al. (2005)
thio-MMA(V)	Wistar rats	0.5 mg As/kg bw i.v. single dose	- Distribution in liver and RBC (after metabolism to DMA(III)) 12 h post-injection, 35% of dose urinary excreted as thio-MMA(V)	Naranmandura, Bu, et al. (2010)
MMA(V) DMA(V)	Swine	80–100 µg As/kg bw Oral gavage	- Oral bioavailability 16.7% - Oral bioavailability 33.3%	Juhász et al. (2006)
DMA(V) [⁷⁴ As-DMA]	Male NMRI mice Male Sprague Dawley rats	0.4 mg As/kg bw Oral administration	- Mice, rats: 80% absorption - Mice: > 99% elimination, 3 days - Rats: ~ 50% elimination, 3 days; accumulation in blood - Fast distribution 0.5–6 h - No demethylation	Vahter et al. (1984)
DMA(V)	Female CD-1 mice	50 µg As/kg bw	- Absolute bioavailability 48% - Formation of DMA(III) - Levels of bound DMA highest in intestine (relative to liver, lung, muscle, brain)	Twaddle et al. (2019)
DMA(V)	Female Wistar rats	100/200 mg/L in drinking water, 10 weeks	- Accumulation in liver, kidney - Urinary DMA excretion, traces of urinary TMAO	Liu et al. (2015)
DMA(V) DMA(III) Thio-DMA(V) Dithio-DMA(V)	Hamsters	0.5 mg As/kg bw i.v. single dose	- DMA(V), dithio-DMA(V): quickly unmodified to organs; excretion unmodified (> 70% 1 h post-administration) - DMA(III), thio-DMA(V): 60 of dose accumulation in liver, kidney, lung; 8%–14% of dose excreted as DMA(V)	Naranmandura, Iwata, et al. (2010)
DMA(V)	Male Sprague Dawley rats	1.0 mg As/kg bw Oral single dose	- Distribution in whole blood, RBC, liver, spleen 2 days after administration 17% of administered dose excreted via urine within 2 days	Kobayashi and Hirano (2016)
DMA(V)	Mice, hamsters	40 mg As/kg bw Oral single dose	Urinary excretion as DMA and 3.5 (mice)/6.4 (hamsters) % as TMAO	Marafante et al. (1987)
DMA(V)	Female Fisher 344 rats	0, 4, 40, 100, 200 mg/L in drinking water, 14 days	- Blood: highest concentrations of dimethylated As - Lung, liver, kidney: dimethylated > trimethylated As - Bladder, urine: trimethylated > dimethylated As - Urine: DMA, thio-DMA(V), thio-TMA	Adair et al. (2007)

Abbreviations: DMA(III), Dimethylarsinite; DMA(V), Dimethylarsinate; dithio-DMA(V), Dimethyldithioarsinic acid; MMA(III), Methylarsonite; MMA(V), Methylarsonate; RBC, red blood cells; thio-DMA(V): Dimethylthioarsinic acid; thio-TMA, Thio-trimethylarsine; TMAO, Trimethylarsine oxide.

In experimental animals, the pentavalent MMA(V) and DMA(V) are well absorbed (>40%–50% of ingested dose), distributed to various tissues and largely excreted unchanged in urine. Notably they can to a limited extent undergo further methylation, while there is no evidence for demethylation.

There are no studies available that would allow absorption rates for other small organoarsenic species to be calculated. One i.v. study in hamsters and rats, however, showed efficient distribution of DMA(III), thio-DMA(V), dithio-DMA(V) (hamsters) and thio-MMA(V) (rats) to various organs, and urinary excretion of the pentavalent arsenic species following methylation of the monomethylated species and some interconversion of the pentavalent species. After a single oral dose in mice, MMA(III) was extensively methylated and more than 90% of the excreted dose in urine was DMA(V) and DMA(III).

Studies in cultured human cells indicate that the lower cellular uptake and transfer across *in vitro* intestinal barriers of pentavalent small organoarsenic species as opposed to their trivalent forms is largely based on the nature of the pentavalent species being negatively charged at physiological pH. The trivalent species are neutral and more membrane permeable. Inside the cell, pentavalent species can be reduced to their trivalent partners. Moreover, there is *in vitro* evidence for presystemic thiolation as well as reduction of small organoarsenic species (recently summarised in El-Ghiaty et al., 2023). The higher cellular uptake of the thio-compounds in comparison to their oxo-analogues could be explained by their lower polarity.

3.1.1.2 | Human studies

MMA and DMA commonly occur in food in their stable pentavalent states. However, only limited studies are available to assess toxicokinetics of ingested MMA(V) and DMA(V) in humans. When MMA(V) and DMA(V) are ingested by humans, they seem to be well absorbed (>75%), essentially being excreted unchanged in urine but also may undergo limited further metabolism. In one human study, arsenic administered as a single oral dose of MMA(V) was efficiently excreted in urine, mainly as unmodified MMA(V) with only 13% (of total arsenic species) present as DMA(V).

In the same study, when humans ingested arsenic as a single DMA(V) dose, there was no evidence for further metabolism and only unchanged DMA(V) was found in urine (Buchet et al., 1981). Furthermore, MMA(V) and DMA(V) have been shown to pass through the placenta (Concha et al., 1998; Devesa et al., 2006; Lindgren et al., 1984), and traces of DMA(V) have been detected in breast milk (Stiboller et al., 2017; Xiong et al., 2020).

No human toxicokinetic studies have been identified for other small organoarsenic species. There are also no human studies proving the presystemic thiolation of DMA or MMA.

3.1.2 | Biomarkers

Arsenic species in urine including MMA and DMA together with iAs and sometimes thio-DMA(V) are usually measured in urine as biomarkers for iAs exposure. The parallel measurement of urinary arsenobetaine, other complex organic arsenic species, DMA and sometimes thio-DMA(V) is used as marker for seafood consumption. Since small organoarsenic species are food contaminants but also metabolites of iAs and complex organoarsenic species, there is so far no arsenic species pattern in urine or blood that has been identified as a biomarker for exposure for small organoarsenic species (Taylor et al., 2017).

3.1.3 | Toxicity

The CONTAM Panel noted that the toxicity studies with small 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 | *In vitro* toxicity

Since the initial studies on the cytotoxic potential of methylated arsenicals in cells in culture, it appeared that trivalent arsenic species were more cytotoxic than their pentavalent counterparts and iAs (Hirano et al., 2004; Petrick et al., 2000; Styblo et al., 2000). More recent studies have confirmed these findings. Moe et al. (2016), by using real-time cell-electronic sensing analysis in two human cell lines, namely urinary bladder carcinoma T24 cells and human lung adenocarcinoma A549 cells, established the following decreasing order of cytotoxicity based on the 24 h IC₅₀ values: MMA(III) ~ DMA(III) > thio-DMA(V) > iAs(III) > DMA(V) > MMA(V). In such an analysis, toxicity is measured as overall cell number, adhesion quality and cell morphology, as a function of time (Stefanowicz-Hajduk & Ochocka, 2020). The trivalent forms of arsenic were the most cytotoxic in both cell lines tested. In the Caco-2 human cell line, the pentavalent forms, MMA(V) and DMA(V), did not produce toxic effects on the intestinal monolayer, but the trivalent species exerted toxicity by mechanisms related to the induction of oxidative stress (Calatayud et al., 2013). Toxicity was evaluated by measuring mitochondrial activity, induction of ROS, apoptosis and necrosis, levels of lipid peroxidation and intracellular GSH as well as enzymatic activity and gene expression of toxicity-associated proteins. The analysis of cytotoxicity of iAs(III), MMA(III) and DMA(III) by trypan blue staining of viable cells in three human cell lines, urothelial (1T1), keratinocyte (HEK001) and bronchial epithelial (HBE)

cells, corresponding to target organs for iAs-induced cancer, showed that MMA(III) and DMA(III) had 4- to 12-fold greater potency compared to iAs (Dodmane et al., 2013). It is of note that the toxicity of MMA(III) was also significantly higher than that of iAs(III) in several types of human brain cells including primary brain cells in vitro suggesting that MMA(III) is the physiological neurotoxin rather than iAs species (Yoshinaga-Sakurai et al., 2020).

Although less studied, thio-DMA(V) was identified as a potentially relevant toxic arsenical species in comparative in vitro studies with other arsenical species. Comparative cytotoxicity studies (Bartel et al., 2011; Ebert et al., 2011; Leffers, Unterberg, et al., 2013) conducted using colony forming ability (CFA) and automated cell counting in the human A549 lung cell line showed that thio-DMA(V) exerted approximately fivefold stronger cytotoxicity and showed a twofold higher cellular bioavailability as compared to arsenite. The cytotoxicity order corrected for intracellular arsenic was the following: thio-DMA(V) ~ iAs(III) ~ MMA(III) > DMA(III) $\gg$ MMA(V) ~ DMA(V). Leffers, Unterberg, et al. (2013) characterised further the cytotoxic effect of thio-DMA(V) in A549 cells. Although no increase in the level of reactive oxygen and nitrogen species (RONS) was detected, a strong disturbance of the oxidative defence system was observed after incubation with nanomolar concentrations of thio-DMA(V). Both GSH and GSSG (oxidised GSH) levels were significantly decreased and, accordingly, RONS levels were strongly increased in cells already under oxidative stress pre-treatment with H₂O₂. The high cytotoxicity of thio-DMA(V) was confirmed in human immortalised urothelial cells (UROtsa cells) (Ebert et al., 2014; Leffers, Ebert, et al., 2013). Thio-DMA(V) was slightly more cytotoxic than arsenite and significantly (100-fold) more cytotoxic than DMA(V) on the basis of IC₇₀ and CFA. The higher cellular bioavailability of thio-DMA(V) as compared to DMA(V) was confirmed, but this factor alone did not account for cytotoxicity ranking. In EJ-1 human bladder cancer cells thio-DMA(V) (Naranmandura et al., 2011) was confirmed to be highly toxic as measured by the MTS colorimetric assay based on the conversion of the tetrazolium salt by living cells. Its cytotoxicity was found to be comparable to that of trivalent DMA(III).

In summary, the CONTAM Panel concluded that in a variety of cells in culture, including cell lines corresponding to target organs for iAs (inorganic arsenic)-induced cancer, trivalent methylated arsenic species are more cytotoxic than both their pentavalent counterparts and iAs. MMA(III), a potent inducer of ROS that targets specifically mitochondria, appears to be the most cytotoxic in various cell systems. Several in vitro studies in human cells indicate that thio-DMA(V) exerts its cytotoxic effects at a concentration range similar to that of DMA(III) and iAs and is significantly more cytotoxic than DMA(V). When taking into account the cellular arsenic concentrations, thio-DMA(V) appears to exhibit greater potency than DMA(III) and iAs(III) although factors such as the cell type are important sources of variability. In addition, it is important to acknowledge that relying solely on total cellular arsenic concentration may not be ideal, as there is a lack of knowledge about the specific arsenic species present inside the cells and their intracellular distribution.

3.1.3.2 | Toxicity in experimental animals

3.1.3.2.1 | Acute toxicity

The 2009 EFSA opinion reported oral LD₅₀ values for MMA(V) (102–3184 mg/kg bw, depending on animal species) and DMA(V) (644–1800 mg/kg bw, depending on animal species) and > 10,000 mg/kg bw for TMAO. No new acute toxicity data were identified for the small organoarsenic compounds. Overall, MMA(V), DMA(V) and TMAO are of low acute toxicity in laboratory animals.

3.1.3.2.2 | Repeated dose toxicity

The 2009 EFSA opinion reported that MMA(V) has effects on the gastrointestinal tract, kidney and thyroid (ATSDR, 2007).

The study of Arnold et al. (2003) provided the NOAEL and LOAEL for **MMA(V)** (3.0 mg/kg bw per day and 25.7 mg/kg bw per day) noted in the previous Opinion. MMA(V) was administered at 0, 50, 400 or 1300 mg/kg in the diet of male and female Fischer F344 rats ($n=60$ per group and sex), and at 0, 10, 50, 200 or 400 mg/kg in the diet of male and female B6C3F1 mice ($n=52$ per group and sex) for 2 years in studies conducted according to US EPA Pesticide Assessment Guidelines (US EPA, 1982a and b, respectively). The highest dietary concentration for rats was reduced to 1000 mg/kg during week 53 and then to 800 mg/kg during week 60 due to high mortality in the male rats. At the end of the dosing period, the survival of male rats was 58, 50, 55 and 33% for the groups receiving 0, 50, 400 or 1300/1000/800 mg MMA(V)/kg diet, respectively. The corresponding survival of female rats was 82, 67, 78 and 65%, respectively. In rats, diarrhoea was observed at the mid and high doses, starting from week 4 and 3, respectively, with dose-related severity and persisting throughout the study. The primary target organ for non-neoplastic changes was the large intestine, with ulcerative and inflammatory lesions, which were more severe in males than in females. These were observed primarily in the high-dose groups, with some occurrence in the mid-dose groups. There was a dose-related increase in water consumption, possibly related to the diarrhoea.

In mice, there was no treatment-related mortality and survival rates at the end of the study were in the region of 83%–92% for males and 65%–82% for females. Loose and mucoid faeces was observed at the high dose starting from week 40 with occasional episodes of diarrhoea. In addition, an increased water consumption at the top two doses throughout most of the study was reported. Intestinal toxicity was observed as in rats but was less severe.

The CONTAM Panel previously identified the NOAEL for diarrhoea based on the dosage reported by the authors for male rats after 13 weeks of dosing, based upon the recorded feed intake and bodyweights. However, the current CONTAM Panel preferred to recalculate the doses based on the data for body weights and food consumption at different times of the study. Subsequently, the average dose was derived by considering the timely contribution of these individual doses.

The estimated doses averaged over the 2-year study period in rats were 2.6, 22.7 and 66.6 mg/kg bw per day in male rats, 3.2, 28.9 and 78.5 mg/kg bw per day in female rats, 1.5, 7.6, 30.4 and 72.4 mg/kg bw per day in male mice, and 1.8, 9.3, 38.67 and 95.1 mg/kg bw per day in female mice.

In addition to the gastrointestinal effects, there were a number of other treatment-related findings in the study of Arnold et al. (2003). The relative kidney weights were significantly increased in the mid- and high-dose male rats, and the relative weight of the heart was significantly increased in the mid- and high-dose female rats and in the high-dose male rats. Other changes in organ weights appeared related to decreased body weight. Hypertrophy of the thyroid follicular epithelium in high-dose male and female rats was considered to be possibly treatment-related, whereas histopathological findings in the kidney (basophilic tubules, hydronephrosis, pyelonephritis and cortical tubular cystic dilatation) were considered to be the result of urinary tract obstruction caused by inflammation of the ureter secondary to peritonitis, which was commonly observed in the rats. In the mice, progressive glomerulonephropathy and nephrocalcinosis were observed in the high-dose males and nephrocalcinosis also occurred in the female high-dose mice, and there was a significant positive trend in the combined occurrence of follicular cell hyperplasia in the thyroids. The lowest NOAEL was 2.6 mg/kg bw per day based on diarrhoea in male rats.

Nephrotoxicity of **DMA(V)** has been investigated in F344/DuCrj rats following gavage administration at 0, 57, 85 and 113 mg/kg bw per day for 4 weeks (Murai et al., 1993). There were dose-related decreases in body weight and survival at all dose levels, with mortality higher and occurring more quickly in female rats than in males. Histopathological observations of the kidneys of rats that died included proximal tubular degeneration and necrosis, which were not seen in the animals that survived, leading the authors to conclude that death could be attributed to nephrotoxicity. Hyperplasia of the epithelium covering the papillae was also prevalent. In addition, ulcerative colitis-like lesions were observed in the caecum of all surviving rats dosed with DMA(V).

Arnold et al. (1999) administered **DMA(V)** at 0, 2, 40 or 100 mg/L in drinking water (equivalent to 0, 0.18, 3.6 or 9 mg DMA(V)/kg bw per day¹⁴) to groups of 10 female F344 rats for 8, 10 or 20 weeks. Groups of 10 male rats received 0 or 100 mg/L DMA(V) in drinking water (equivalent to 0 or 9 mg DMA(V)/kg bw per day) for 10 weeks. In the females dosed at 40 and 100 mg/L, urothelial toxicity and hyperplasia were observed by light and scanning electron microscopy, and cell proliferation (BrdU labelling) was increased. The effects were less marked in the male rats, and resolved after a period of 10 weeks without DMA(V) administration. No effects were apparent at the low dose, equivalent to 0.18 mg DMA(V)/kg bw per day.

Shen et al. (2006) administered drinking water containing 187 mg/L **MMA(V)**, 184 mg/L **DMA(V)**, 182 mg/L **TMAO** or with no arsenic compounds to groups of three male or three female F344 rats for 13 weeks. These concentrations are equivalent to 16.8 mg MMA(V)/kg bw per day, 16.6 mg DMA(V)/kg bw per day and 16.4 mg TMAO/kg bw per day.¹⁵ There were no effects on body weight, food and water intake, absolute or relative liver or kidney weights in any of the treatment groups. Examination of the urinary bladder by scanning electron microscopy showed changes to the urothelium, which were minimal in male and female rats treated with MMA(V) or TMAO. More severe damage was observed in the rats treated with DMA(V), with the damage being more severe in females than in males.

Dodmane et al. (2013) administered 0 or 77.3 mg/L **DMA(III)** in drinking water (equivalent to 14 mg/kg bw per day¹⁶) ad libitum to groups of 10 12-week-old female C57BL/6 wild-type and arsenic 3+ methyltransferase (As3mt) knockout mice for 4 weeks. There was no significant difference in body weight or food/water intake in either of the treated groups compared to the relevant control. There was a small significant increase in the relative kidney weight in the treated knockout mice. Based on observations of hyperplasia, cell proliferation and ultrastructural changes in the urothelium of the urinary bladder, the authors concluded that DMA(III) induced urothelial cell cytotoxicity and regenerative cell proliferation, which were greater in As3mt knock-out mice than in wild-type mice.

Shahida et al. (2020) treated groups of seven Sprague Dawley rats (sex not reported) with monosodium methylarsonate (sodium salt of **MMA(V)**) at doses of 0 or 63.2 mg/kg bw per day for 6 months via gavage to investigate its effect on hepatocytes and sinusoidal endothelial cells. The authors reported that MMA(V) caused necrotic and apoptotic changes in the liver and loss of normal morphology of organelles in the hepatocytes together with defenestration of sinusoidal epithelial cells.

In summary, the limited data on repeated dose toxicity currently available for the small organoarsenic species indicate that MMA(V) has effects on the gastrointestinal tract, kidney, thyroid and liver, whereas DMA(III), DMA(V) and TMAO have effects on the urinary bladder and possibly the kidney. The differences in study design do not allow the determination of whether the toxic properties of these compounds differ. The data allow identification of N/LOAELs only for MMA(V). The lowest NOAEL for MMA(V) was 2.6 mg/kg bw per day, based on the occurrence of diarrhoea in rats dosed for 2 years. Carcinogenicity data are considered in Section 3.1.3.2.4 below. No repeated dose toxicity data are available for other small organoarsenic species.

3.1.3.2.3 | Developmental and reproductive toxicity

The most comprehensive study of developmental toxicity is that of Irvine et al. (2006), who published a review of earlier studies and also included previously unpublished data generated in developmental studies of MMA(V) and DMA(V) conducted according to regulatory guidelines in the late 1980s in the context of the toxicological assessment of these

¹⁴Applying the EFSA default conversion factor of 0.09 for subchronic drinking water rat studies.

¹⁵Applying the EFSA default conversion factor of 0.09 for subchronic drinking water rat studies.

¹⁶Applying the EFSA default conversion factor of 0.18 for subacute mouse studies.

substances as active ingredients in herbicides. They noted that the published studies (e.g. Chernoff et al., 1990; Kavlock et al., 1985; Rogers et al., 1981) had limitations such as effects only seen in the presence of maternal toxicity.

The regulatory studies reported by Irvine et al. (2006) involved administration of **MMA(V)** to pregnant rats or rabbits by gavage at doses of 0, 10, 100 and 500 mg/kg bw per day during gestation day (GD) 6–15 in the rat, and at doses of 0, 1, 3, 7 and 12 mg/kg bw per day during GD 7–19 in the rabbit. In the rat, maternal body weight gain was decreased at some time points in animals dosed at 100 mg/kg bw per day and more consistently at 500 mg/kg bw per day. At the highest dose, decreased fetal weight in rats and retardation of skeletal ossification in rabbits were observed, but there was no treatment-related developmental toxicity at lower doses. There was no evidence of teratogenicity with **MMA(V)**. **DMA(V)** was administered at doses of 0, 4, 12 and 36 mg/kg bw per day in the rat, and at doses of 0, 3, 12 and 48 mg/kg bw per day in the rabbit during the same periods of organogenesis. In the rat, there was a decrease in maternal body weight gain throughout most of the study, and in maternal body weight at termination, which was statistically significant at 36 mg/kg bw per day. Developmental toxicity was also observed at the top dose, with an increased incidence of foetuses with diaphragmatic hernia. In the rabbit, there was severe maternal toxicity at the top dose, resulting in no foetuses surviving for examination. There was no treatment-related maternal or developmental toxicity at 12 mg/kg bw per day in either rat or rabbit. The authors concluded that the NOAELs for **MMA(V)** were 100 and 7 mg/kg bw per day for rat and rabbit, respectively. For **DMA(V)**, the NOAEL was 12 mg/kg bw per day for both rat and rabbit.

No more recent data on developmental or reproductive toxicity were identified.

3.1.3.2.4 | Carcinogenicity

Tokar, Diwan, Thomas, and Waalkes (2012) treated groups of 10 pregnant CD1 mice with 0, 12.5 and 25 mg/L **MMA(III)** in drinking water from GD 8–18 (equivalent to 0, 2.3 and 4.5 mg/kg bw per day¹⁷). At weaning, groups of 25 pups were randomly grouped according to maternal exposure and they were followed up to 104 weeks of age. **MMA(III)** did not affect survival, body weight, birth weight or litter size of offspring and was well tolerated also by mothers. In male offspring, there was an increased incidence of hepatocellular carcinoma at the top dose, a non-dose-related increase in adrenal cortical adenoma at both **MMA(III)** doses, and an increase in lung carcinoma at the lower dose, but not at the top dose. In female offspring, increased uterine adenomas were seen at the high **MMA(III)** dose, and total uterine tumours were increased at both doses. Ovarian adenomas were increased at both doses but not in a dose-dependent manner and there was no increase in ovarian carcinomas. Oviduct hyperplasia and adrenal cortical adenomas were increased in a dose-dependent manner. Neither lung nor liver cancer incidences were increased in females.

As reported in Section 3.1.3.2.2, Arnold et al. (2003) administered **MMA(V)** at 0, 50, 400 or 1300 mg/kg in the diet of male and female Fischer F344 rats ($n=60$ per group and sex), and at 0, 10, 50, 200 or 400 mg/kg in the diet of male and female B6C3F1 mice ($n=52$ per group and sex) for 2 years in studies conducted according to US EPA Pesticide Assessment Guidelines, 1982a and b, respectively. The highest dietary concentration for rats was reduced to 1000 mg/kg during week 53 and then to 800 mg/kg during week 60 due to high mortality in the male rats. The CONTAM Panel estimated that the doses averaged over the 2-year study period in rats were 2.6, 22.7 and 66.6 mg/kg bw per day in male rats; 3.2, 28.9 and 78.5 mg/kg bw per day in female rats; 1.5, 7.6, 30.4 and 72.4 mg/kg bw per day in male mice; and 1.8, 9.3, 38.7 and 95.1 mg/kg bw per day in female mice. There were no treatment-related neoplastic effects in either rats or mice in the wide range of tissues examined.

Shen, Wanibuchi, Salim, Wei, Doi, et al. (2003) administered **MMA(V)** to groups of 42–45 male Fischer 344 rats in drinking water at 0, 50 and 200 mg/L for 104 weeks. These concentrations were equivalent to 0, 3 and 12 **MMA(V)**/kg bw per day.¹⁸ Liver sections were stained for glutathione S-transferase positive (GST-P) foci, urinary bladder sections were stained for proliferating cell nuclear antigen (PCNA) and histopathological examination was performed on a wide range of tissues. There were no treatment-related differences in tumour incidence. Statistically significant increases in the number and area of GST-P foci were observed in the liver of rats treated at 200 mg/L, indicating promotional activity. A significant increase of PCNA-positive cells was observed in the urinary bladder of rats treated at both doses.

Hayashi et al. (1998) administered **DMA(V)** to small groups of male A/J mice at 0, 50, 200 or 400 mg/L in drinking water for 25 weeks ($n=10$) or 50 weeks ($n=14$), equivalent to 0, 7.5, 30 and 60 mg **DMA(V)**/kg bw per day.¹⁹ The authors reported that, at 50 weeks, there was a statistically significant increase of the number of lung tumours (hyperplasia, alveolar adenoma, papillary adenoma and adenocarcinoma) per mouse, but not in the number of tumour-bearing mice. A further analysis of the tumours from the same experimental animals, investigated 'tumour progression effects' of **DMA(V)**, and found that there was a dose-related increase in mice with papillary adenoma and/or adenocarcinoma at 50 weeks but not at 25 weeks. No other endpoints were reported.

In a study reported in a short communication, groups of 36 male Fischer 344 rats received **DMA(V)** at 0, 12.5, 50 or 200 mg/L in drinking water for 104 weeks (Wei et al., 1999). These doses are equivalent to approximately 0, 0.63, 2.5 and 10 mg **DMA(V)**/kg bw per day.²⁰ There was a dose-related increase of the incidence of papillomas and carcinomas of the urinary bladder in the rats treated with 50 and 200 mg/L **DMA(V)** and none in the rats of the low-dose group. Other tissues were

¹⁷Applying the EFSA default conversion factor of 0.18 for subacute drinking water mouse studies.

¹⁸Calculated from the total intake of **MMA** per rat, assuming an average bw of 300 g.

¹⁹Calculated using the default conversion factor of 0.15 for subchronic drinking water mouse studies.

²⁰Calculated using the default conversion factor of 0.05 for chronic drinking water rat studies.

not examined. A subsequent publication from the same laboratory presented the same data for urinary bladder and included data for a wide range of additional tissues, none showing a statistically significant increase of tumours (Wei et al., 2002).

Salim et al. (2003) administered **DMA(V)** to groups ($n=30$) of p53 heterozygous knockout and wild-type C57BL/6J mice at 0, 50 or 200 mg/L in drinking water (equivalent to 0, 4.5 and 18 mg/kg bw per day) for 18 months. There was a significant increase in the number of tumour-bearing mice in both the wild-type and knockout mice, and an increase in the numbers of tumours per mouse in the wild type. There was no statistically significant increase in any tumour type in either strain.

DMA(V) was administered at 0, 2, 10, 40 or 100 mg/kg in the diet of male and female Fischer F344 rats ($n=60$ per group and sex) and at 0, 8, 40 or 500 mg/kg in the diet of male and female B6C3F1 mice ($n=56$ per group and sex) for 2 years (Arnold et al., 2006) in studies conforming to US EPA Pesticide Assessment Guidelines (US EPA a and b, respectively). These dietary concentrations are equivalent to approximately 0, 0.1, 0.5, 2.0 and 5 mg/kg **DMA(V)** bw per day in rats and to 0, 1.2, 6 and 75 mg/kg **DMA(V)** bw per day in mice.²¹ In rats of both sexes, papillomas and carcinomas with degeneration of the urothelium, necrosis and urothelial cell hyperplasia were observed in the urinary bladder. The carcinomas were found primarily in the rats of the top dose group, although there were single incidences of either papillomas or carcinoma also in the males of the lower dose groups, resulting in a statistically significant positive trend in females and when both sexes were combined. The number of lesions in the males was too small for statistical analysis. Non-neoplastic lesions (progressive glomerulonephropathy and nephrocalcinosis) were reported in the kidneys of the male rats of the 40 and 100 mg/kg groups and the female rats of the 100 mg/kg group, which were attributed to an increase in the aging nephropathy of the rat. Based on the kidney and bladder lesions, the authors reported that the NOAEL for rats was the dietary concentration of 10 mg/kg, which the CONTAM Panel calculated to be equivalent to approximately 0.5 mg/kg **DMA(V)** bw per day. In mice of both sexes, treatment-related non-neoplastic lesions were observed in the urinary bladder (increased vacuolisation of the superficial cells of the urothelium) and kidneys (progressive glomerulonephropathy and nephrocalcinosis), but there were no treatment-related neoplastic lesions in any of the multiple tissues examined (including lung). The authors reported the NOAEL to be the dietary concentrations of 40 mg/kg in male mice, and 8 mg/kg in female mice, which the CONTAM Panel calculated to be equivalent to 6 and 1.2 mg/kg **DMA(V)** bw per day, respectively.

In a study in OGG1 mutant and wild-type mice, groups of five or six mice of each sex were given 0 or 200 mg/L **DMA(V)** in drinking water for 72 weeks, equivalent to 0 and 18 mg/kg bw per day²² (Kinoshita et al., 2007). In the wild-type mice, there was no difference between the dose groups in the number of tumour-bearing mice, or in the numbers of tumours at specific sites, including the liver and lung. In the OGG1 mutant mice, there was a statistically significant increase in the number of tumour-bearing mice and also in the incidence of lung tumours (discussed further in Section 3.1.5).

Fujioka et al. (2020) treated groups of 10 pregnant CD-1 mice with 0 or 200 mg/L **DMA(V)** in drinking water from GD 8–18 (equivalent to 0 and 36 mg/kg bw per day²³ **DMA(V)**) and offspring were sacrificed at 84 weeks for histopathological examination of a range of tissues. It appears that all pups were followed up and there is no description of whether a potential litter effect was taken into account. **DMA(V)** did not affect survival, terminal body weight or kidney and liver weights. The authors reported a statistically significantly increased incidence and multiplicity of lung adenomas/adenocarcinomas, and of hepatocellular carcinomas in male offspring. There were no significant increases in tumours in the female offspring and no significant differences in the incidence of other tumours and no tumours in the urinary bladder in any of the groups. In a separate experiment, pregnant mice were dosed by the same regime and then the offspring were assessed for markers of cell proliferation in the lung epithelium (but not liver) at postnatal day (PND) 0 and 6 weeks of age. There were no histopathological effects in the lung of offspring except a significantly increased Ki67 labelling index.²⁴ There were no differences between the groups in the histologic findings in lung at 6 weeks of age. Overall, the authors concluded that transplacental exposure to **DMA(V)** increased lung and liver tumour incidence in male but not female mice and that further studies are needed to investigate the dose response relationship of these effects.

TMAO was administered to male Fischer 344 rats in drinking water at concentrations of 0, 50 or 200 mg/L ($n=42$, 42 and 45, respectively), equivalent to 0, 2.5 and 10 mg/kg **TMAO** bw per day²⁵ for 104 weeks (Shen, Wanibuchi, Salim, Wei, Kinoshita, et al., 2003). There was a statistically significant increase of hepatocellular adenoma at the higher dose level. No hepatocellular carcinomas were found and there were no increases of tumours in other tissues examined.

Table 3 summarises the key findings in the carcinogenicity studies described above.

²¹Calculated using the EFSA default conversion factors of 0.05 and 0.15 for chronic feed studies in rats and mice, respectively.

²²Calculated using the EFSA default conversion factor of 0.09 for chronic drinking water studies in mice.

²³Calculated using the EFSA default conversion factor of 0.18 for subacute drinking water studies in mice.

²⁴The Ki-67 protein (also known as MKI67) is a cellular marker for proliferation.

²⁵Calculated using the EFSA default conversion factor of 0.05 for chronic drinking water studies in rats.

TABLE 3 Summary of key findings in the carcinogenicity studies.

Organoarsenic species	Animals	Doses/treatment time	Results	Reference
MMA(III)	CD1 mice Dams pups (male/female) N=50 for control and 25 per group	0, 2.3 and 4.5 mg/kg bw per day Drinking water From GD 8–18 25 pups per dose group followed up for 104 weeks	<u>Males</u> Hepatocellular carcinoma: 0/24, 3/25, 5/23* Adrenal cortical adenoma: 0/24, 7/25*, 4/23*, lung carcinoma: 4/24, 11/25*, 9/23 <u>Females</u> Uterine adenomas+carcinomas: 0/23, 6/23*, 7/23* Ovarian adenomas: 0/23, 7/23*, 5/23* Adrenal cortical adenomas: 0/23, 2/23, 6/23*	Tokar, Diwan, and Waalkes (2012)
MMA(V)	Fischer F344 rats Adult male/female N=60 per group and sex B6C3F1 mice Adult male/female N=52 per group and sex US EPA (1982) guidelines;	Male rats: 2.6, 22.7 and 66.6 mg/kg bw per day Female rats: 3.2, 28.9 and 78.5 mg/kg bw per day Male mice: 1.5, 7.6, 30.4 and 72.4 mg/kg bw per day Female mice: 1.8, 9.3, 38.7 and 95.1 mg/kg bw per day Diet 2 years	No treatment-related neoplastic effects in either rats or mice in the wide range of tissues examined	Arnold et al. (2003)
MMA(V)	Fischer 344 rats Adult male N=42–45 per group	0, 3 and 12 mg/kg bw per day Drinking water 104 weeks	No increases in tumour incidences in a wide range of tissues	Shen, Wanibuchi, Salim, Wei, Doi, et al. (2003)
DMA(V)	A/J mice Adult male N=14 per group	0, 7.5, 30 and 60 mg /kg bw per day 50 weeks	Lung tumours (hyperplasia, alveolar adenoma, papillary adenoma and adenocarcinoma): 0.5 ± 0.52, 1.07 ± 1.00, 1.07 ± 1.07, 1.36 ± 1.01* tumours per mouse Number of mice with lung hyperplasia, alveolar adenoma, papillary adenoma and adenocarcinoma: 7/14, 10/14, 9/14, 11/14 Number of mice with papillary adenoma and adenocarcinoma: 2/14, 5/14, 7/14, 10/14*	Hayashi et al. (1998)
DMA(V)	Fischer 344 rats Male N=36 per group	0, 0.63, 2.5 and 10 mg/kg bw per day Drinking water 104 weeks	<u>Urinary bladder</u> Papillomas: 0/28, 0/33, 2/31, 2/31 Carcinomas: 0/28, 0/33, 6/31*, 12/31* No tumours at other sites	Wei et al. (1999) Wei et al. (2002)
DMA(V)	p53 heterozygous knockout and wild-type C57BL/6J mice Adult male N=30 per group	0, 4.5, 18 mg/kg bw per day Drinking water 18 months	Number of wild-type mice with tumours: 3/30, 9/30*, 9/30* Number of tumours per wild-type mouse: 6, 17*, 18* No significant increases in individual tumour type or site Number of p53 heterozygous mice with tumours: 14/29, 18/29, 19/30 Number of tumours per p53 heterozygous mouse: 23, 33, 36* No significant increases in individual tumour type or site	Salim et al. (2003)

TABLE 3 (Continued)

Organoarsenic species	Animals	Doses/treatment time	Results	Reference
DMA(V)	Fischer F344 rats Adult male/female N=60 per group and sex B6C3F1 mice Adult male/female N=56 per group and sex US EPA (1982) guidelines	Rats: 0, 0.1, 0.5, 2.0 and 5 mg/kg bw per day Mice: 0, 1.2, 6 and 75 mg/kg bw per day Diet 2 years	<u>Rats:</u> Urinary bladder papilloma + carcinoma: males 0/60, 1/59, 1/60, 1/58, 2/59 Females: 0/60, 0/59, 0/60, 0/40, 10/60 <u>Mice:</u> No treatment-related neoplastic lesions in any of a wide range of tissues	Arnold et al. (2006)
DMA(V)	OGG1 mutant and wild-type mice Adult male/female N=5 or 6 per group and sex	0 or 18 mg/kg bw per day Drinking water 72 weeks	Results in wild-type mice Number of tumour-bearing mice: 5/10, 6/12 No significant increase in any tumour type Results in mutant mice Number of tumour-bearing mice: 0/10, 10/10 Total lung tumours: 0/10, 5/10. No significant increase in any other tumour type	Kinoshita et al. (2007)
DMA(V)	CD-1 mice Dams/offspring N=10 dams	0 and 36 mg/kg bw per day Drinking water GD 8–18 All pups followed up for 84 weeks	<u>Male offspring</u> lung adenoma+adenocarcinoma incidence: 8/53, 20/60* Lung adenoma+adenocarcinoma multiplicity: 0.23 ± 0.66, 0.52 ± 0.85* Hepatocellular carcinoma incidence: 0/53, 6/60* Hepatocellular carcinoma multiplicity: 0, 0.12 ± 0.37* <u>Female offspring</u> No significant differences No significant differences in incidence of tumours at other sites, including urinary bladder	Fujioka et al. (2020)
TMAO	Fischer 344 rats Adult male N=42–45 per group	0, 2.5 and 10 mg/kg bw per day drinking water 104 weeks	Hepatocellular adenoma: 6/42, 10/42, 16/45* No hepatocellular carcinomas and no increases of tumours in other tissues	Shen, Wanibuchi, Salim, Wei, Kinoshita, et al. (2003)

Abbreviations: DMA(V), dimethylarsinic acid; MMA(III), monomethylarsonous acid; MMA(V), monomethylarsonic acid; OGG1, 8-Oxoguanine DNA Glycosylase 1; TMAO, Trimethylarsine oxide.

*Statistically significant at $p < 0.05$.

3.1.3.2.5 | Summary on carcinogenicity

One study was available for MMA(III), in which dosing of pregnant mice resulted in tumours of the liver, lung and adrenals in male offspring and of the uterus and ovaries in female offspring (Tokar, Diwan, & Waalkes, 2012).

Two studies with MMA(V) found no increases in tumour incidences in a wide range of tissues (Arnold et al., 2003; Shen, Wanibuchi, Salim, Wei, Doi, et al., 2003).

No studies with DMA(III) were available.

Two comprehensive carcinogenicity studies demonstrated that DMA(V) induced tumours in the urinary bladder of male and female rats (Arnold et al., 2006; Wei et al., 1999).

In mice, a small study of DMA(V) (Hayashi et al., 1998) reported a statistically significant increase of papillary adenoma and adenocarcinoma of the lung. A study with dosing of pregnant mice and only one dose level, reported tumours of the lung and liver, but not urinary bladder in the male offspring but not in females (Fujioka et al., 2020). In contrast, the guideline study by Arnold et al. (2006) found no tumours in mice (Arnold et al., 2006). Salim et al. (2003) reported a significant early induction of tumours in DMA(V)-treated both wild-type and p53+/- mice, with no evidence for organ-tumour specificity. In contrast, significant increases in total tumour incidence and multiplicity were reported in DMA(V)-treated OGG1-/- mice when compared to their control OGG1-/- group or DMA(V)-treated or untreated wild-type mice. The tumours induced in the DMA(V)-treated group of OGG1-/- mice were mainly in the lungs (50%) and malignant lymphomas/leukaemias (40%) (Kinoshita et al., 2007). The CONTAM Panel noted the inconsistencies in the data, and that greater weight should be attributed to the guideline study of Arnold et al. (2006), and concluded that these studies do not provide convincing evidence of carcinogenicity in mice. From the two comprehensive carcinogenicity studies in rats the CONTAM Panel identified a lowest NOAEL of 0.5 mg DMA(V)/kg bw per day.

TMAO has been tested in one study in male rats, which demonstrated formation of hepatocellular adenomas but no hepatocellular carcinomas.

No carcinogenicity data are available for other small organoarsenic species.

3.1.3.3 | Genotoxicity

3.1.3.3.1 | In vitro genotoxicity

Since the first genotoxicity studies on methylated arsenicals, it emerged that the genotoxic potential in cultured cells was influenced by the oxidation state of arsenic present in these methylated compounds. Methyloxoarsine and iododimethylarsine, which are the precursors of MMA(III) and DMA(III), respectively, were shown to directly nick isolated DNA in vitro. Conversely, iAs(III) and pentavalent methylated arsenicals did not exhibit nicking activity (Mass et al., 2001). Given that these effects are assessed on isolated DNA exposed to elevated concentrations of the arsenical species, their relevance for hazard identification remains uncertain. In the same study, MMA(III) and DMA(III) were shown to be more potent inducers of DNA single-strand breaks (SSBs) in human lymphocytes than iAs and their pentavalent counterparts. Chromosomal damage was analysed by Kligerman et al. (2003) who found that among the six arsenicals tested – MMA(III), DMA(III), MMA(V), DMA(V), iAs(III), iAs(V) – MMA(III) and DMA(III) were the most effective agents in causing chromosomal aberrations (CAs) in human blood lymphocytes. Chromatid and isochromatid deletions were the most prevalent type. Methylation was reported to reduce the clastogenic potency of iAs(V) but increased the clastogenic potency of iAs(III). The methylated arsenicals also exhibited characteristics of spindle poisons suggesting a potential involvement in inducing aneuploidy. In this study, cytotoxicity was not explicitly assessed; however, the mitotic index, a crucial parameter for measuring chromosomal damage and an indirect indicator of cytotoxicity, is appropriately reported. Additionally, clastogenic effects of MMA(III) and DMA(III) were observed at concentrations (starting from 0.6 μ M in the case of MMA(III) and 1.35 μ M for DMA(III)) in the same micromolar range as those reported in urine of humans and rodents exposed to arsenic. In this same study, all six arsenicals did not exhibit mutagenic effects in the Ames test. In addition, MMA(III) and DMA(III) increased the frequency of mutations at the thymidine kinase (TK) locus in LY5178 mouse lymphoma cells but only at highly cytotoxic levels. The positive results in the TK mutation assay can be ascribed either to the fact that this assay detects a wide range of genetic alterations, including chromosomal mutations (Wang et al., 2009), and/or to excessive levels of cytotoxicity (OECD, 2015). In line with these findings, MMA(III) induced significant increases in mutagenesis at the *gpt* locus of Chinese hamster G12 cells only at highly cytotoxic concentrations and most mutants exhibited transgene deletions. Some increase, but not significant, was also induced by DMA(III) at highly cytotoxic concentrations (Klein et al., 2007). DMA(III) was shown to induce mitotic abnormalities, such as centrosome abnormality, multipolar spindles, multipolar division and aneuploidy, in several cell lines at subtoxic concentrations (Ochi et al., 2003, 2004). Both MMA(III) and DMA(V) induced high levels of oxidative DNA damage at low physiologically relevant doses in HeLaS3 cells as detected by the formamidopyrimidine–DNA glycosylase (FPG)-modified comet assay (Schwerdtle, Walter, Mackiw, & Hartwig, 2003).

Subsequent studies conducted after 2009 largely corroborated these findings. Kitamura et al. (2009) showed that DMA(III) affects both the formation of the mitotic spindle and the final step of cell division by inhibiting cytokinesis and induces the formation of multinuclear cells. Kligerman et al. (2010) investigated the mechanism of MMA(III) induced chromosomal aberrations by treating lymphocyte cultures in different cell cycle phases. A significant increase was only detected when cells were treated in late G₁- or S-phase but not in G₀- or early G₁-phase. The authors concluded that MMA(III)-induced cytogenetic damage is short-lived. Suzuki et al. (2009) showed that DMA(III) induces abnormal localisation of the Aurora

B kinase in HepG2 cells and proposed that this is the mechanism underlying the induction of spindle abnormality and multinucleated cells. Since high levels of cytotoxicity may induce chromosomal damage as a secondary effect, it is relevant that MMA(III), MMA(V) and DMA(V) induced chromosomal damage at non-cytotoxic/slightly cytotoxic concentrations (Bartel et al., 2011; Meyer et al., 2015). These methylated arsenicals, including thio-DMA(V), were shown to be more effective inducers of chromosomal damage compared to iAs (Bartel et al., 2011). Leffers, Ebert, et al. (2013) observed an increased frequency of bi- and multinucleated cells but not of micronuclei in human bladder urothelial cells (UROtsa) after 24–48 h incubation with either thio-DMA(V) or DMA(V) at incipient cytotoxic concentrations (micromolar range), indicating cell-cycle arrest and disturbance in mitosis. No increase of micronuclei frequency or of bi- or multinucleated cells was reported by Unterberg et al. (2014) after long exposure times (from 7 to 21 days) of UROtsa cells to subtoxic doses of thio-DMA(V), while arsenite was confirmed to be clastogenic at subtoxic doses. Remarkably, both thio-DMA(V) and iAs(III) demonstrated the ability to cause global DNA hypomethylation at picomolar concentrations.

Several studies have reported that exposure to methylated arsenic species induces DNA damage, primarily DNA breaks, including both SSBs and double-strand breaks (DSBs), and oxidative DNA damage. In 2007, Wang and colleagues showed that MMA(III) and DMA(III) induced higher levels of cytotoxicity and greater levels of oxidative damage to DNA, as measured by the FPG/endolIII-modified comet assay, in human urothelial transformed cells when compared to their pentavalent counterparts. Additionally, these compounds led to a significant elevation in lipid peroxidation, protein carbonylation, nitric oxide, superoxide, hydrogen peroxide and cellular free iron when compared to the pentavalent arsenicals. In their study, Wnek et al. (2009) investigated the impact of MMA(III) on a URO-MSC cell line that had undergone malignant transformation upon MMA(III) exposure. Chronic and low-level exposure of UROtsa cells to 50 nM MMA(III) caused persistent elevation of DNA SSB as measured by the comet assay, increased levels of reactive oxygen species (ROS) and reduced poly-(ADP-ribose) polymerase (PARP) activity. Orihuela et al. (2013) showed that MMA(III) causes oxidative DNA damage (ODD) measured as DNA-nitron adducts by the immuno spin-trapping (IST) assay at non-toxic levels, independently of cellular biomethylation capacity and without requiring further methylation. Conversely, iAs(III) ODD formation after acute iAs exposure in biomethylation-deficient cells occurred only after exposure to concentrations of iAs that exceeded the LC_{50} . Notably, thio-DMA(V) did not induce DNA strand breaks (up to cytotoxic concentrations) as measured by the comet assay (Bartel et al., 2011) in human lung cells suggesting that its toxicity likely stems from distinct mechanisms. Rehman et al. (2014) showed that phosphorylation of the histone H2A variant (γ -H2AX), a biomarker of DNA DSB, increased significantly in cellular nuclei after exposure to MMA(III) and DMA(III) in the micromolar range and decreased in the presence of N-acetylcysteine (NAC). These data strongly suggest that the two metabolites were primarily responsible for inducing oxidative stress, which is what led to DNA damage. Induction of apoptosis was also observed at low concentrations. The detection of γ -H2AX foci can be influenced by apoptosis-induced DNA fragmentation. However, given the temporal distinction between detection of γ -H2AX (3-h exposure time) and apoptosis (12-h exposure time), it is unlikely that apoptosis significantly interfered with the analysis of γ -H2AX. Moreover, to prevent potential interference with the observed DNA damage response due to excessive cell death, experiments were performed using concentrations marginally lower than the IC_{50} , as estimated from the MTT assay. These concentrations were chosen to minimise cytotoxic effects while still inducing a measurable response in the γ -H2AX foci assay. The use of the human 8-oxoguanine DNA glycosylase 1 (hOGG1)-modified comet assay confirmed the induction of guanine base oxidation upon MMA(III) exposure of primary cultures of mouse bone marrow, spleen, thymus cells (Xu et al., 2016) and activation of the DNA damage response (DDR) by DSB formation, as detected by increased γ -H2AX fluorescence, in mouse thymus cells (Xu et al., 2017). As in the study by Rehman et al. (2014), γ -H2AX foci were detected at concentrations below the IC_{50} , as estimated from a cell proliferation assay. Overall, these two studies do not allow to conclude whether MMA(III) and DMA(III) induce double strand breaks at sub-cytotoxic or cytotoxic concentrations because of different incubation times for the assessment of cytotoxicity and γ -H2AX induction. No significant induction of DNA breaks was reported in HepG2 liver cells after exposure to DMA(V) (Benhusein et al., 2016) in line with a lower DNA-damaging capacity by pentavalent species. MMA(III) was shown to induce DSB (as measured by γ -H2AX foci), oxidative stress and apoptosis at lower doses than CD4 and CD8 double negative (DN) thymic T cells (Xu et al., 2017). The DN cells were much more sensitive to MMA(III)-induced DNA damage and apoptosis than the CD4 and CD8 double positive (DP) cells, probably due to the higher intracellular levels of MMA(III). Since apoptosis and γ -H2AX foci were measured at the same exposure time (13 h), it cannot be ruled out that the detection of γ -H2AX foci might have been influenced by the occurrence of apoptosis. Xu et al. (2018) investigated the effect of MMA(III), the major arsenical species found in immune system cells, on natural killer (NK) cells. MMA(III) inhibited cell growth and induced DNA damage and oxidative stress at low to moderate concentrations (20 and 50 nM). A strong correlation was found between DNA damage, oxidative stress and cytotoxicity.

Arsenic methylation proficient and deficient cell lines (methylating hepatocytes vs. non-methylating urothelial cells) were used to further explore the toxic effects of methylated arsenicals (Dopp et al., 2010). The non-methylating UROtsa cells were shown to accumulate higher amounts of MMA(III) in the cytosol and to be less prone to arsenic-induced cytotoxicity than the methylating hepatocytes where arsenic compounds are more distributed into the cell organelles. Induction of DNA breaks was detected in human hepatocytes at concentrations as low as 5 μ M MMA(III) (Dopp et al., 2008). The authors noted that this concentration lies within the concentration range of arsenic in urine in humans (5 μ M As(III) = 650 μ g/L). A correlation between DNA damage induction and free radical formation was reported although a significant increase of DNA breaks was already detected at relatively low ROS levels.

The well-established inhibitory effect of inorganic arsenic on DNA repair enzymes has led to investigations into the potential interference of methylated arsenical species with these enzymes and their subsequent impact on cellular responses.

Back in 2003, Schwerdtle and coworkers (Schwerdtle, Walter, & Hartwig, 2003) showed that both trivalent (MMA(III) and DMA(III)) and pentavalent (MMA(V) and DMA(V)) methylated metabolites of arsenic increased the formation and diminished the repair of BPDE-DNA adducts at non-cytotoxic concentrations in A549 human lung cells. Notably, MMA(III) exhibited the greatest potency. It is of note that MMA(III) was able to release zinc from human nucleotide excision repair XPA protein and inhibit the activity of isolated Fpg. In a study by Wnek et al. (2011), it was observed that UROtsa cells exposed to MMA(III) exhibited elevated levels of SSB in DNA and, upon removal of MMA(III), the activity of poly(ADP-ribose) polymerase-1 (PARP-1) increased while DNA damage decreased. Mass spectrometry analysis showed that MMA(III) can bind to a synthetic peptide representing the zinc-finger domain of PARP-1 and is able to remove zinc from it in a dose-dependent manner. Based on these findings, the authors hypothesised two interrelated potential mechanisms by which MMA(III) may make UROtsa cells more vulnerable to genotoxic insult: (i) increased levels of MMA(III)-induced DNA damage through the generation of ROS, and (ii) the direct inhibition of PARP-1 by MMA(III). Notably, Ebert et al. (2014) showed that also thio-DMA(V) specifically inhibits cellular poly(ADP-ribosyl)ation at 35,000-fold lower concentrations (i.e. ≥ 0.1 nM) than arsenite. A previous study conducted by Ebert et al. (2011) demonstrated that methylated arsenical species may affect base excision repair (BER) through various mechanisms. DMA(V) specifically affected the activity of 8-oxoguanine DNA glycosylase (OGG1), an enzyme involved in the repair of oxidative DNA damage. Arsenite, on the other hand, affected the protein level of DNA ligase III, while MMA(V) influenced the protein content of X-ray cross complementing protein 1 (XRCC1), both of which are essential proteins in the BER pathway. Notably, the trivalent methylated metabolites exhibited substantial effects on these BER proteins only at cytotoxic concentrations. It should be considered that measurements of DNA repair protein levels or cleavage activity using cell extracts provide valuable information, but they may not fully represent the entire DNA repair process as it occurs in living cells.

In summary, a substantial amount of evidence indicates that MMA(III) and DMA(III) exhibit genotoxic effects in mammalian cells when tested *in vitro*. These effects include the induction of DNA single and double-strand breaks, as well as clastogenic and aneugenic effects. Furthermore, they inhibit DNA repair mechanisms, promote the generation of ROS, generate oxidative damage to DNA and have the potential to induce cell transformation. The pentavalent analogues, MMA(V) and DMA(V), exhibit similar genotoxic characteristics, albeit with lower potency and some inconsistent results. Thio-DMA(V) presents distinct characteristics showing high cytotoxicity, potential interference with cell division, inhibition of DNA repair, yet without inducing DNA breaks or oxidative base lesions. It is important to note that the specific effects of methylated arsenical species can vary depending on the compound, its concentration, duration of exposure and the cell or tissue type under investigation (Tables 4 and 5).

TABLE 4 In vitro tests with small organoarsenic species.

Test system	Cells	Concentration treatment time	Results	Comments	Reference
Mutagenicity					
Ames test Prophage induction assay in <i>E. coli</i> TK mutation assay	Salmonella TA98, TA100 or TA104 LY5178 mouse lymphoma cells	MMA(III), DMA(III), As(V), As(III), MMA(V), DMA(V) Positive controls (2-Nitrofluorene, 2-aminoanthracene and methylglyoxal) MMA(III) from 0.09 to 0.57 μM ; DMA(III) 0.65–1.51 μM Positive controls (MMS and mitomycin C)	Negative , Ames test and prophage induction: negative all six arsenicals Positive , TK mutation assay: MMA(III) and DMA(III) increased the frequency of mutations at the TK locus in mouse cells but only at highly cytotoxic concentrations	Also induction of small colonies (chromosomal mutations)	Kligerman et al. (2003)
Mutation assay at the <i>gpt</i> locus	Chinese hamster G12 cells	MMA(III); DMA(III)	Positive , MMA(III): statistically significant increase of mutation frequency only at highly cytotoxic concentrations (0.6, 0.8 and 1.0 μM for 72 h at survival levels of 43%, 23% and 11%, respectively) Negative , DMA(III): increase but not significant of the mutagenic response at 0.3 μM and 0.4 μM (72 h) at highly cytotoxic doses (survival levels 5%–7%)	Most mutants exhibited transgene deletions. Some non-deletion mutants exhibited altered DNA methylation	Klein et al. (2007)
Clastogenicity and aneuploidy					
Chromosomal aberrations and SCE	Human lymphocytes	MMA(III), DMA(III), As(V), As(III), MMA(V), DMA(V) SCE and CA (24 h exposure): MMA(III) up to 2 μM , DMA(III) up to 3 μM , MMA(V) up to 3 mM, DMA(V) up to 10 mM, As(III) up to 10 μM , As(V) up to 100 μM	Negative/weakly positive , SCE induction: As(V), As(III), and MMA(III) were negative for SCE induction; MMA(V), DMA(V), and DMA(III) (in increasing order of potency), were only weak inducers of SCE. Positive , Chromosomal aberrations: MMA(III) and DMA(III) were the most effective agents in causing chromosomal damage (chromatid and isochromatid deletions the most prevalent)	Trivalent methylated arsenicals were approx. 3 orders of magnitude more potent than the methylated pentavalent forms. Concentrations of MMA(III) and DMA(III) that induced CA (starting from 0.6 μM for MMA(III) and 1.35 μM for DMA(III)) were in the same micromolar range of those reported in urines of exposed humans or rodents	Kligerman et al. (2003)
Chromosomal aberrations Aneuploidy	SHE cells	DMA(III) (Dimethylarsine iodide as a model) 24 h exposure from 0.1 to 5 μM	Positive , Chromosome structural aberrations (gaps, breaks and pulverisations) and numerical changes, such as aneuploidy, hyper- and hypo-tetraploidy induced at subtoxic concentrations		Ochi et al. (2003)
Aneuploidy	Syrian hamster embryo cells	DMA(III) (Dimethylarsine iodide(DMI) as a model)	Positive , Effective induction of aneuploidy, centrosome abnormality and multipolar spindles (0.5 μM DMI, 24 h incubation), low cytotoxicity	Morphological transformation of cells detected at concentrations from 0.1 to 1.0 μM	Ochi et al. (2004)
Effects on cytokinesis	V79 cells	DMA(III) (micromolar range, 2 h)	Positive : Enhancement of tetraploids and multinucleated cells (1.25 μM); mitotic arrest (2.5 μM)		Kitamura et al. (2009)

(Continues)

TABLE 4 (Continued)

Test system	Cells	Concentration treatment time	Results	Comments	Reference
Chromosome aberration assay	Freshly prepared splenic lymphocyte cultures (induction of mitogenesis by ConA)	MMA(III), 3–5 μ M (12–17 h)	Positive: MMA(III) (0.5 μ M) induced significant increase of chromosome aberration frequency when cells were treated in late G ₁ - or S-phase; MMA(III) was negative when treatment was confined to the G ₀ - or G ₁ -phase of the cell cycle	DNA lesions produced by MMA(III) that can lead to cytogenetic damage are short-lived	Kligerman et al. (2010)
Micronucleus assay	Human A549 lung cells	MMA(III), DMA(III), MMA(V), DMA(V), thio-DMA(V), arsenite (1, 24 h)	Positive: small but significant increase of MN frequency at non cytotoxic/slightly cytotoxic concentrations: MMA(III) (0.5, 1 μ M), thio-DMA(V) (5 μ M), MMA(V) (250 μ M), and DMA(V) (250 μ M). At highly cytotoxic concentrations, DMA(III) (5 μ M) and thio-DMAV (30 μ M) showed strongest effects. At cytotoxic concentrations significant increases also for arsenite ($\geq$ 50 μ M)	Based on the effective cellular arsenic concentrations, the cytotoxic order was: thio-DMA(V) $\sim$ arsenite $\sim$ MMA(III) > DMA(III) $\gg$ MMA(V) $\sim$ DMA(V) Thio-DMA(V) and especially DMA(III) increased the formation of multinucleated cells (significant effects at cytotoxic concentrations)	Bartel et al. (2011)
Micronucleus assay	UROtsa cells human bladder urothelial cells	thio-DMA(V), DMA(V)	Positive: increased frequency of bi- and multi-nucleated cells Negative: no micronuclei induction after 24–48 h incubation with either thio-DMA(V) or DMA(V) at incipient cytotoxic concentrations (micromolar range). iAsIII induced micronuclei formation even in the subcytotoxic concentration range		Leffers et al. (2013)
Micronucleus assay	UROtsa cells human bladder urothelial cells	thio-DMA(V), arsenite	Negative: Neither increase of micronuclei frequency or of bi- or multinucleated cells long exposure times (from 7 to 21 days) of UROtsa cells to subtoxic doses of thio-DMA(V). Arsenite was confirmed to be clastogenic at subtoxic doses	Both thio-DMAV and iAsIII caused global DNA hypomethylation at picomolar concentrations	Unterberg et al. (2014)
Micronucleus assay	Human liver HepG2 cells	DMA(V) 0-1-10-100-500 μ M (48 h)	Positive: significant increase of MN at 100 and 500 μ M, IC70 155 μ M		Meyer et al. (2015)
DNA breaks, oxidative damage to DNA, DNA repair enzymatic assays					
In vitro DNA nicking assay Comet assay (calculation of tail moment)	ϕ X174 DNA Human lymphocytes	Nicking assay (2 h, 37°C) various concentrations MMA(III) (as MAs(III)), DMA(III) (as DMAs(III)), iAs(III), sodium arsenate, MMA(V), DMA(V) Comet assay: (2 h, 37°C) range of concentrations used: MMA(III) up to 80 μ M; DMA(III) up to 90 μ M; iAs(III), iAs(V), MMA(V) and DMA(V) up to 1 mM	Positive: MMA(III) (30 mM) and DMA(III) (150 μ M) directly nick DNA in vitro. IAs(III) (up to 300 mM), sodium arsenate (up to 1 M), MMA(V) (up to 3 M) and DMA(V) (up to 300 mM) do not exhibit nicking activity Positive: MMA(III) and DMA(III) more potent inducers of DNA SSB than iAs and their pentavalent counterparts. MMA(III) linear increase from 10 to 80 μ M; DMA(III) linear increase up to 20 μ M; MMA(V), DMA(V), iAs(III), iAs(V) no effect up to 1000 μ M	Regarding induction of SSB, MMA(III) and DMA(III) were 77 and 386 times more potent than iAs(III), respectively No information on cytotoxicity	Mass et al. (2001)

TABLE 4 (Continued)

Test system	Cells	Concentration treatment time	Results	Comments	Reference
In vitro DNA nicking assay with FPG FPG-modified comet assay	PM2 DNA HeLa S3	MMA(III), DMA(III), MMA(V), DMA(V) Nanomolar to micromolar range, short-term (0.5 ± 3 h) and long-term (18 h) incubation	Positive , PM2 DNA: only DMA(III) (> 10 µM) generated SSB. Positive , HeLa S3 cells: trivalent arsenicals, MMA(III) and DMA(III), and pentavalent arsenicals, MMA(V) and DMA(V), induced Fpg-sensitive sites (nanomolar or micromolar concentration range, respectively; non cytotoxic doses) after both short-term and long-term incubations		Schwerdtle, Walter, Mackiw, and Hartwig (2003)
Comet assay (endo III and Fpg digestion before electrophoresis) (calculation of tail moment)	Human urothelial carcinoma (BFTC905) and transformed cells (NTUB1)	Cytotoxicity: In BFTC905 cells, the IC ₅₀ values for iAs(III), MMA(III), DMA(III), MMA(V), DMA(V), and iAs(V) were 0.13, 0.13, 0.52, 3.04, 0.38, 9.25, and 11.25 µM, respectively Lipid peroxidation, protein carbonylation, and oxidative DNA damage: 0.2 µM (IC50) arsenicals for 1 or 24 h. DMA(V) at the concentrations of 1 and 2 µM caused oxidative DNA damage in both cell lines	Positive : MMA(III), DMA(III), iAs(III) were more potent in inducing cytotoxicity, lipid peroxidation, protein carbonylation, oxidative DNA damage, nitric oxide, superoxide, hydrogen peroxide, and cellular free iron than their pentavalent counterparts in both cell lines.		Wang et al. (2007)
Alkaline comet assay	Human A549 lung cells	MMA(III), DMA(III), MMA(V), DMA(V), thio-DMA(V), arsenite (1, 24 h) Positive control: H ₂ O ₂	Negative : thio-DMA(V) did not induce DNA strand breaks (up to cytotoxic doses) Only thio-DMA(V) was tested for DNA breaks	Based on the effective cellular arsenic concentrations, the cytotoxic order was: thio-DMA(V) ~ arsenite ~ MMA(III) > DMA(III) ≫ MMA(V) ~ DMA(V)	Bartel et al. (2011)
Comet assay (Flow cytometry)	URO-MSC cell line that had undergone malignant transformation upon MMA(III) exposure.	MMA(III)	Positive : Chronic and low-level exposure to 50 nM MMA(III) caused persistent elevation of DNA SSB as measured by the comet assay, increased levels of ROS and reduced PARP activity.		Wnek et al. (2009)
Oxidative DNA damage (ODD) measured as DNA-nitron adducts by the immuno spin-trapping (IST) assay	Human cells	MMA(III)	Positive : MMA(III), ODD was detected at non-toxic levels, independently of cellular biomethylation capacity. iAsIII ODD formation after acute iAs exposure in biomethylation-deficient cells (concentrations > LC50).		Orihuela et al. (2013)
Comet assay (calculation of olive tail moment)	HepG2 liver cells	DMA(V) (10 µM for 24 h), cell viability: 85–90% Arsenate, sodium arsenite	Negative : no increase of SSB with DMA(V). Increase of SSB with arsenate and arsenite		Benhusein et al. (2016)

(Continues)

TABLE 4 (Continued)

Test system	Cells	Concentration treatment time	Results	Comments	Reference
Comet assay Free radicals measurement by the thiobarbituric acid test. (calculation of tail moment)	Non-methylating cells: UROtsa transformed human urothelial cells and CHO-9 Chinese hamster primary fibroblasts; methylating cells: primary human hepatocytes	MMA(III) iAs(III)	Positive: significant induction of DNA breaks: primary human hepatocytes at 5 μ M MMA(III) and 50 μ M iAs(III) (1 h); UROtsa cells at 5 μ M MMA(III) and 5 μ M iAs(III) (1 h); CHO cells at 10 μ M (1 h), iAs(III) at 500 μ M. Induction of free radicals with cell-type specific differences		Dopp et al. (2010)
BPDE-induced DNA adducts detection by LC/fluorescence assay	A549 human epithelial lung adenocarcinoma cells. XPA in vitro zinc release FPG activity	MMA(III) and DMA(III) from 2.5 to 7.5 μ M, MMA(V) and DMA(V) 250 and 500 μ M (16-hr incubation)	Positive: MMA(III), DMA(III), MMA(V) and DMA(V) inhibited the repair dose-dependent at non- cytotoxic concentrations (5, 2.5 and 250 μ M, respectively). Positive: MMA(III) and DMA(III) showed a concentration-dependent zinc release starting at low micromolar concentrations; MMA(V) and DMA(V) showed no or only slight effects up to 10 mM. Positive: MMA(III) and DMA(III) inhibited the activity of isolated Fpg at 1 mM; MMA(V) and DMA(V) showed no effects on Fpg activity up to 10 mM	MMA(III) and DMA(III) exerted higher cytotoxicity as compared to arsenite and to MMA(V) and DMA(V)	Schwerdtle et al. (2003)
DNA repair enzymes cleavage assay, gene expression and western blotting of cell extracts	A549 human epithelial lung adenocarcinoma cells	DMA(III), MMA(III), MMA(V), DMA(V), arsenite (24 h) followed by preparation of cell extracts	Positive: DNA repair protein specific inhibitory effects by DMA(V) (OGG1) and MMA(V) (XRCC1) (starting at ≥ 3.2 μ M cellular arsenic); the trivalent methylated metabolites effective only at cytotoxic concentrations.	Cytotoxic effects correlate with cellular uptake in the decreasing order DMA(III), MMA(III), arsenite, MMA(V), DMA(V)	Ebert et al. (2011)
Comet assay (calculation of tail length)	Human bladder cancer cells (EJ-1)	DMA(III) (12 μ M, 3, 6, 24 h exposure) 50% lethality iAs(III)	Positive DMA(III), increase of DNA breaks after 3, 6 and 24 h exposure; iAs(III), after 6 and 24 h exposure	DMA(III) increased ROS levels after 3, 6, 24 h exposure; iAs(III) only after 24 h exposure; DMA(III), 60% reduction of GSH (5 μ M for 24 h); iAs(III), increased GSH levels; DMA(III) (2 μ M), decreased expression of p21 and p53 (1–24 h exposure); iAs(III) increased expression of p21 and p53	Naranmandura et al. (2011)

TABLE 4 (Continued)

Test system	Cells	Concentration treatment time	Results	Comments	Reference
γ-H2AX foci Intracellular ROS generation by fluorescent probe (DCFH-DA) Apoptosis by annexin V-FITC and propidium iodide (PI) staining.	Human myeloid leukaemia HL-60 cells	MMA(III), DMA(III) and iAs(III) (1 μM for 12 h) γ-H2AX foci: 5 μM for 3 hrs MMA(III) (IC50 3 μM) DMA(III) (IC50 2 μM) IAAs(III) (IC50 10 μM) 24 h exposure	Positive: MMA(III) and DMA(III) increased oxidative stress, loss of mitochondrial membrane potential, increased γ-H2AX foci and apoptosis. IAAs(III) no effects	NAC prevented the generation of γ -H2AX foci and apoptosis	Rehman et al. (2014)
Alkaline and hOGG1-modified (FLARE) comet assay (measure of % DNA in tail)	Primary cultures of mouse bone marrow, spleen, thymus cells	MMA(III), sodium arsenite 5, 50 and 500 nM (4 h)	Positive: MMA(III), cells from all the three organs showed increased DNA breaks associated with oxidative base lesions starting at 5 nM Arsenite: bone marrow cells significant increase of DNA breaks from 5 to 500 nM, spleen and thymus cells only at 500 nM	MMA(III) is more genotoxic than As(III) in cell cultures	Xu et al. (2016)
Alkaline comet assay (measure of % DNA in tail) and γ-H2AX foci forming assay ROS detection by flow cytometry	Primary cultures of mouse thymus cells	MMA(III) 5, 50 and 500 nM (18 h)	Positive: MMA(III) significant increase of DNA breaks in DN cells in the whole dose range; DP cells at 50 and 500 nM. Significant increase of γ-H2AX fluorescence and reactive oxygen species level (DN cells, 5, 50 and 500 nM; DP cells, 500 nM) Note: DN, double negative, do not express CD4 or CD8; DP, double positive, express both cell markers	Significant increase of early apoptotic cells in DP cells in the whole dose range and in DN cells only at 500 nM MMA(III); of late apoptotic cells in DN cells in the whole range of doses and in DP cells only at 500 nM MMA(III)	Xu et al. (2017)
Comet assay (measure of % DNA in tail) ROS analysis by DHE	Mouse natural killer (NK) cells	MMA(III) (10–250 nM, 18 h), As(III)	Positive: MMA(III) induced significant increase (starting from 20 nM) of DNA SSB and oxidative stress at low to moderate concentrations	Strong correlations between DNA damage, oxidative stress and cytotoxicity	Xu et al. (2018)
Comet assay (calculation of tail moment) PARP1 activity by ELISA	UROtsa cells Human bladder urothelial cells	MMA(III), continuous exposure (50 nM, 12 weeks)	Positive: DNA SSB: increased levels; PARP1: upon removal of MMA(III), the activity increased while DNA damage decreased	Elevated DNA SSB coincide with increased levels of ROS. Mass spectrometry analysis showed that MMA(III) can bind to a synthetic peptide representing the zinc-finger domain of PARP-1 and is able to remove zinc from it in a dose-dependent manner	Wnek et al. (2011)

(Continues)

TABLE 4 (Continued)

Test system	Cells	Concentration treatment time	Results	Comments	Reference
Alkaline unwinding in combination with FPG PARP-1 activity by immune slot-blot technique	UROtsa cells Human bladder urothelial cells	thio-DMA(V), arsenite	Negative: thio-DMA(V) no significant induction of DNA SSB or FPG-sensitive sites after 1, 24 and 48 h incubation with subcytotoxic concentrations. Positive effect in case of 48 h incubation with already cytotoxic concentrations of 5 µM. The same effects with arsenite Positive: Thio-(DMA(V) inhibits H ₂ O ₂ -induced cellular poly(ADP-ribosyl)ation at 35,000-fold lower concentrations than arsenite		Ebert et al. (2014)

Abbreviations: As(III), arsenite; As(V), arsenate; CA, chromosomal aberration; CHO, Chinese Hamster Ovary; ConA, Concanavalin A; DCFH-DA, 2',7'-Dichlorofluorescein Diacetate; DHE, dihydroethidium; DMA(III), dimethylarsinous acid; DMA(V), dimethylarsinic acid; DMAs(III), iododimethylarsine; DMI, dimethylarsine iodide; DN, double negative; DNA, deoxyribonucleic acid; DP, double positive; ELISA, Enzyme-Linked Immunosorbent Assay; FLARE, Fluorescent Advanced Oxidation Protein Products; FPG, Formamidopyrimidine-DNA Glycosylase; hOGG1, Human 8-Oxoguanine DNA Glycosylase 1; iAs, inorganic arsenic; iAs(III), arsenite; IST, immuno spin-trapping; M, molar; MAs(III), methyloxoarsine; mM, millimolar; MMA(III), monomethylarsonous acid; MMA(V), monomethylarsonic acid; MN, micronucleus; NAC, N-Acetyl Cysteine; nM, nanomolar; NK, natural killer; ODD, oxidative DNA damage; PARP1, Poly(ADP-ribose) Polymerase 1; PI, propidium iodide; ROS, Reactive Oxygen Species; SCE, Sister Chromatid Exchange; SSB, Single-Strand Break; thio-DMA(V), dimethylthioarsinic acid; TK, thymidine kinase; UROtsa, human urinary tract epithelial cell line; V-FITC, V-Fluorescein Isothiocyanate.

3.1.3.3.2 | *In vivo genotoxicity*

The 2009 EFSA Opinion acknowledged the limited number of *in vivo* studies concerning the genotoxic effects of methylated arsenic metabolites. Most of the research in this area had concentrated on assessing the presence of DNA breaks and oxidative damage to DNA in *ex vivo* cells/tissues from mice or rats that were orally exposed to DMA(V) via drinking water. Some of these studies involved administration to rodents of very high DMA(V) doses (1500 mg/kg bw) (Yamanaka et al., 1989, 2001; Yamanaka & Okada, 1994). On the basis of their findings, Yamanaka and colleagues proposed that DMA(V), upon transforming into dimethylarsine, produces dimethylarsenic peroxy radicals (DMPR) and other free radicals by interacting with molecular oxygen and induces DNA damage. The presence of DMPR has been verified in cells exposed to dimethylarsine, demonstrating properties similar to OOH, potentially causing DNA damage such as strand breaks and crosslinks. However, the confirmation of this radical's induction lacks support from other studies. In studies performed in rodents at DMA(V) exposure levels in drinking water below 200 mg/kg bw (Vijayaraghavan et al., 2001; Wei et al., 2002), there was a consistent observation of heightened levels of 8-oxo-2'-deoxyguanosine (8-oxodG) in various cells and tissues from the exposed rodents including the target organs of arsenic carcinogenesis (lung, liver, and urinary bladder). Increased levels of 8-oxodG were also detected in urine and serum and DNA breaks in PBMCs' DNA of exposed mice at doses ranging from 188 to 375 mg/kg (Jia et al., 2004). Shen et al. (2003a, 2003b) found significantly higher levels of 8-oxodG in the liver of mice exposed to 200 mg/L of TMAO(V) in drinking water within a 2-year carcinogenicity study. These mice showed a significant increase of hepatocellular carcinomas (see section on Carcinogenicity). Similarly, increased formation of 8-OHdG was found in the liver DNA following TMAO(V) treatment of rats in drinking water (Kinoshita et al., 2007). In this same study, DMA(V) induced a significant increase of 8-oxodG in the bladder, while MMA(V) turned out to be negative. In addition, cell proliferation and apoptosis indices were significantly increased by TMAO(V) in the liver and by DMA(V) in the bladder of rats.

More recently, Xu et al. (2016) addressed the *in vivo* induction of DNA damage by MMA(III) using a mouse model exposed to iAs(III) in drinking water. By HG-CT-ICP-MS (hydride generation-cryotrapping ICP-MS) analysis they report that MMA(III) is the major arsenical species present in mouse bone marrow and thymus cells after a 30-day drinking water exposure to 100 and 500 ppb iAs(III). A dose-dependent increase of DNA damage as measured by the Comet assay was observed in NK cells from As(III) exposed mice and was attributed to MMA(III). Nevertheless, because of concerns about the rigour of the analytical procedure, in particular the method's suitability for specifically determining MMA(III), the CONTAM Panel does not agree that the observed effects can be attributed to MMA(III).

Regarding the mutagenic potential of methylated arsenicals in the previous opinion (EFSA CONTAM Panel, 2009), two studies were reported following *i.p.* injection of DMA(V) in mice. After one injection of DMA(V) in mice (300 mg/kg, bw), aneuploidy was observed in mouse bone marrow cells but no chromosome aberrations (Kashiwada et al., 1998). Additionally, when DMA(V) was *i.p.* injected into MutaMice (10.6 mg/kg per day, 5 consecutive days), it caused only a weak increase in the mutant frequency of the lacZ gene in the lung, but not in the bladder or bone marrow (Noda et al., 2002). Since 2009, only one additional study was identified related to *in vivo* clastogenicity of methylated arsenicals. Wang et al. (2009) conducted a study to explore whether a 1-week oral exposure of F344 female rats to 100 mg/L of DMA(V) or As(V) in drinking water, followed by a 24-h recovery period, could increase the MN frequency in the bone marrow cells. Neither DMA(V) nor As(V) exposure increased MN frequencies in the bone marrow, and DMA(V) did not increase cyclophosphamide (CP)-induced MN. In this same study, the repair of DNA damage in urinary bladder transitional cells, which are known to be susceptible to arsenic-induced cancer, was investigated. No alterations in the sensitivity to cyclophosphamide (CP)- or formaldehyde-induced DNA damage, or in the repair of H₂O₂-induced DNA damage, measured by the comet assay, was observed in urinary bladder transitional cells. The authors acknowledge that the negative results might be affected by several factors such as the repair time allowed before testing the cells and/or the requirement of longer than 1-week exposure time to arsenicals.

In summary, oral administration of DMA(V) or TMAO to rodents results in oxidative damage to DNA in specific organs. One study reports the occurrence of DNA breaks in circulating PBMC of mice orally exposed to DMA(V). Further genotoxicity assays are required to determine the genotoxic potential of these arsenicals. Additionally, the absence of genotoxicity studies on the other small methylated arsenical species necessitates further investigation.

TABLE 5 In vivo tests with organoarsenic species.

Test system	Animals	Concentration/treatment time	Results	Comments	Reference
Alkaline elution Filter binding assay Electron-spin-resonance	ICR mice Pulmonary mice cells isolated from mice orally administered with DMA(V)	DMA(V)-Na (1500 mg/kg) orally administered	Positive: increased DNA ssb in mouse lung cells at 12 h after administration (fully repaired within 24 h)		Yamanaka et al. (1989, 1993), Yamanaka and Okada (1994)
8-OHdG levels by EC-HPLC Comet assay (measure of length and fluorescence intensities of comet tails)	Eight-week-old female MT-I/II null (MT-/-) and wild-type mice (six mice for each treatment group)	DMA(V): 0–750 mg/kg by oral gavage, 24 h treatment	Positive: significant increase of 8-OHdG levels in serum and urine of both MT-I/II null and wild-type mice; increased levels in MT null mice compared to wild-type mice starting from 375 mg/kg in serum and from 188 mg/kg in urine Positive: dose-dependent increase of DNA breaks in PBMC (doses 188 and 375 mg/kg) in both MT-I/II null and wild-type mice. At the highest dose (750 mg/kg), the levels of DNA SSB in PBMC of MT-I/II null mice significantly higher than the wild-type mice	MT-I/II confers some protection against DNA SSB and oxidative damage presumably by acting as an antioxidant	Jia et al. (2004)
8-OHdG levels by ELISA	Kidney tissues of DMA(V)-exposed NCI-Black Reiter (NBR) rats	DMA(V): 5, 10 and 20 mg/kg body weight by gavage, once a day, 5 days a week, for a period of 4 weeks	Positive: increase of 8-OHdG levels in the kidney of the rats treated, with 10 mg/kg bw	Increased proliferation in the tissues of treated rats; no effects on apoptosis. Presence of vacuolated degeneration and dilation of the proximal tubule cells in two groups (10 and 20 mg/kg body weight)	Vijayaraghavan et al. (2001)
8-oxodG by HPLC-ECD	F344 rats/144 males Urinary bladder	DMA(V): 0, 200 mg/L in drinking water for 2 weeks	Positive: significant increase of 8-oxodG in urinary bladder at 200 mg/L	Molecular analysis of urinary bladder carcinomas: No MSI alterations; Low rates of Ha-ras mutations; no alteration of p53, K-ras or beta-catenin	Wei et al. (2002, 2003)
8-oxodG by HPLC-ECD	F344 rats/129 males Liver	TMAO(V) ad libitum at concentrations of 0, 50 or 200 mg/L in drinking water. 104 weeks	Positive: significant increase in liver of 8-OHdG at 200 mg/L. in drinking water	Induction of cell proliferation in the normally appearing parenchyma as determined by PCNA index	Shen, Wanibuchi, Salim, Wei, Kinoshita, et al. (2003)

TABLE 5 (Continued)

Test system	Animals	Concentration/treatment time	Results	Comments	Reference
8-oxodG by HPLC-ECD	F344 rats/100 males Liver and urinary bladder	MMA(V) (1.62 mM), DMA(V) (1.45 mM) and TMAO(V) (1.47 mM) in drinking water for 5, 10, 15 and 20 days	Positive: significant increase of 8-OHdG in the liver of rats after TMAO(V) treatment at days 15 and 20, and DMA(V) in the bladder after 20 days of treatment Negative: MMA(V) did not induce increased 8-OHdG levels in the liver of rats	Hydroxyl radical levels significantly increased after 15 and 20 days of TMAO(V) and DMA(V) treatment in the liver microsomal fraction Cell proliferation and apoptosis indices significantly increased by TMAO(V) in the liver and by DMA(V) in the bladder of rats	Kinoshita et al. (2007)
Comet assay MN assay	F344 rats/female/6 weeks old; groups of treated rats consisted of six rats with the exception of negative control groups with 3 or 4 rats	100 mg/L DMA(V) (0.72 µM) in drinking water for 1 week. 100 mg/L As(V) as control	Negative: No effects were induced by DMA(V) or As(V) either CP- H ₂ O ₂ - or formaldehyde-induced DNA damage, or on DNA repair in urinary bladder transitional cells. Negative: Neither DMA(V) nor As(V) increased the MN frequency, nor MN frequency induced by CP treatment in bone marrow	The negative results might be affected by several factors: 24 h recovery time without DMA(V) allowed to cells before testing, requirement of longer than 1 week exposure time to arsenicals	Wang et al. (2009)

Abbreviations: 8OHdG, 8-hydroxydeoxyguanosine; 8-oxodG, 8-oxo-2'-deoxyguanosine; ALS, amyotrophic lateral sclerosis; As, arsenic; iAs(III), arsenite; As(V), arsenate; CP, cyclophosphamide; DHE, dihydroethidium; DMAA, dimethylarsinate; DMA(V), dimethylarsinate; DNA, deoxyribonucleic acid; EC-HPLC, high-performance liquid chromatography-electrochemical detection; ED, electrochemical detection; ELISA, Enzyme-Linked Immunosorbent Assay; H₂O₂, hydrogen peroxide; HPLC, high-performance liquid chromatography; ICP-MS, inductively coupled plasma mass spectrometry; ICR, Institute of Cancer Research; MN, micronucleus; MSI, microsatellite instability; Na, sodium; NBR, NCI-Black Reiter; NK, natural killer; ppb, parts per billion; PBMC, peripheral blood mononuclear cell; PCNA, proliferating cell nuclear antigen; ROS, reactive oxygen species; SOD, superoxide dismutase; SSB, single-strand break; TMAO(V), trimethylarsine oxide; wt, wild type.

3.1.3.3.3 | Summary of *in vitro* and *in vivo* genotoxicity

Since assessing the genotoxic potential of small organoarsenic species is crucial in the decision process of the risk assessment approach, a summary of the scientific evidence including *in vitro* and *in vivo* studies is presented for each compound, along with its weighting. This assessment aims to draw conclusions about the likelihood or strength of a substance inducing genotoxic damage.

Most available data in the literature are on *in vitro* genotoxicity and primarily focus on the trivalent methylated arsenicals, specifically MMA(III) and DMA(III). There are fewer studies on the genotoxicity of pentavalent arsenicals such as MMA(V) and DMA(V) and even fewer on sulfur derivatives like thio-DMA(V). In terms of *in vivo* genotoxicity, there is a notable gap in research, except for studies that have measured DNA damage in various cells and organs of rodents exposed to DMA(V).

MMA(III)

MMA(III) is negative in *in vitro* bacterial assays for point mutations. Positive outcomes were observed in the TK locus mutation assay in LY5178 mouse lymphoma cells and at the *gpt* locus in Chinese hamster G12 cells but only at highly cytotoxic concentrations, often resulting in transgene deletions. It is an effective inducer of chromosomal aberrations in human lymphocytes (Kligerman et al., 2003, 2010) and micronuclei in human A549 epithelial lung adenocarcinoma cells (Bartel et al., 2011) at low toxicity levels. It causes DNA breaks (Dopp et al., 2010; Mass et al., 2001; Wnek et al., 2009, 2011; Xu et al., 2016, 2017, 2018) along with oxidative damage, at subtoxic concentrations in a variety of human cell lines (Orihuela et al., 2013; Schwerdtle, Walter, Mackiw, & Hartwig, 2003; Wang et al., 2007; Xu et al., 2016). Two studies (Rehman et al., 2014; Xu et al., 2017) report induction of γ -H2AX foci, an indirect marker of double-strand breaks, at concentrations marginally below the IC_{50} , as estimated from cell proliferation assays. Induction of apoptosis was also observed at low concentrations (Rehman et al., 2014; Xu et al., 2017). Additionally, MMA(III) induced inhibition of the repair of BPDE-DNA adducts at non-cytotoxic concentrations (Schwerdtle, Walter, & Hartwig, 2003) and inhibition of PARP1 activity under continuous exposure to nanomolar concentrations, likely via direct binding to the zinc finger domain of PARP1 (Wnek et al., 2011) in human cells. Induction of apoptosis via oxidative stress was also reported in the nanomolar concentration range (Rehman et al., 2014; Xu et al., 2017).

No studies were identified on the *in vivo* genotoxicity of MMA(III).

DMA(III)

DMA(III) induces chromosomal aberrations in human lymphocytes (Kligerman et al., 2003) and Chinese hamster V79 cells (Ochi et al., 2003), and is particularly effective in inducing aneuploidy at low doses in hamster cells (Kitamura et al., 2009; Ochi et al., 2004). It was shown to increase the formation of multi- and binucleated cells at low concentrations and to induce micronuclei in human lung cells only at highly cytotoxic concentrations (Bartel et al., 2011). It induces single and double DNA breaks (Mass et al., 2001; Naramandura et al., 2011; Rehman et al., 2014), along with oxidative damage to DNA (Schwerdtle, Walter, Mackiw, & Hartwig, 2003). One study (Rehman et al., 2014) reports induction of γ -H2AX foci, an indirect marker of double-strand breaks, in cultures of human cells at concentrations marginally below the IC_{50} , as estimated from a cell proliferation assay. It causes apoptosis through oxidative stress (Rehman et al., 2014).

No studies were identified on the *in vivo* genotoxicity of DMA(III).

MMA(V)

MMA(V) induces chromosomal aberrations in human lymphocytes (Kligerman et al., 2003), albeit it is significantly less potent compared to its trivalent analogue. It causes micronuclei formation (Bartel et al., 2011), and DNA damage (primarily oxidative damage to DNA) in human cell cultures (Schwerdtle, Walter, Mackiw, & Hartwig, 2003; Wang et al., 2007). These effects are reported in the micromolar range at non-cytotoxic or slightly cytotoxic concentrations. There is specific evidence of XRCC1 inhibition in extracts of human epithelial lung cells (Ebert et al., 2011).

MMA(V) has been shown to be ineffective in the induction of oxidative damage to DNA in the liver of orally exposed rats (Kinoshita et al., 2007).

DMA(V)

DMA(V) has been reported to be a weak inducer of chromosomal aberrations in human lymphocytes being significantly less potent than its trivalent analogue (Kligerman et al., 2003). It induces micronuclei formation (Bartel et al., 2011; Meyer et al., 2015) in cultures of human cells, with one conflicting study (Leffers et al., 2013) showing lack of micronuclei induction in human bladder cells but multinucleated cell formation, suggesting that it interferes with cell division. It induces oxidative damage to DNA (Schwerdtle, Walter, Mackiw, & Hartwig, 2003; Wang et al., 2007). These effects occur in the micromolar range at non-cytotoxic/incipient cytotoxic concentrations. No induction of DNA breaks was reported in two studies (Benhusein et al., 2016; Mass et al., 2001). DMA(V) exhibits specific inhibition of OGG1 in extracts of human epithelial lung cells (Ebert et al., 2011).

In vivo DMA(V) has been demonstrated to generate ROS, including superoxide anions and hydroxyl radicals, specifically in the bladder of rats orally exposed to this arsenical (Kinoshita et al., 2007). Several studies present evidence of DMA(V)-induced oxidative damage to DNA bases in kidney, liver and urinary bladder of rats exposed to DMA(V) in drinking water (Kinoshita et al., 2007; Vijayaraghavan et al., 2001; Wei et al., 2002). In a mouse study, elevated levels of 8-oxodG were observed in the serum and urine of exposed animals, alongside increased levels of DNA breaks in PBMC (Jia et al., 2004). In in vitro experiments, DMA(V) has been shown to inhibit specifically Ogg1 (Ebert et al., 2011). Notably, Kinoshita et al. (2007) (see also section on Carcinogenicity) showed a statistically significant increase in the occurrence and number of lung tumours in DMA(V)-treated homozygote Ogg1 deficient mice, suggesting that the persistent accumulation of 8-oxodG may contribute to the carcinogenicity of DMA(V).

THIO-DMA(V)

For thio-DMA(V), there are conflicting findings regarding micronuclei induction and multinucleated cell formation (Bartel et al., 2011; Leffers et al., 2013; Unterberg et al., 2014). No induction of DNA breaks or oxidative damage to DNA have been reported (Bartel et al., 2011), but there is evidence of inhibition of DNA damage-induced PARP1 at extremely low concentrations (≥ 0.1 nM) in human bladder urothelial cells (Ebert et al., 2014).

No studies were identified on the in vivo genotoxicity of thio-DMA(V).

TMAO

TMAO has been shown to induce hydroxyl radicals and oxidative damage to DNA specifically in the liver of exposed rats in two studies (Shen et al., 2003a, 2003b; Kinoshita et al., 2007).

In summary, there is evidence supporting the genotoxicity of MMA(III) and DMA(III) in vitro. No studies were conducted in in vivo animal models. In the case of the pentavalent species, MMA(V) and DMA(V), the evidence of a genotoxic potential in vitro is less robust due to their comparatively limited extent of investigation and some conflicting data. In the case of DMA(V), in vivo data show the induction of oxidative damage to DNA in various organs and one study reports the occurrence of DNA breaks in circulating PBMC.

Table 6 below presents assessment of the strength of evidence for in vitro and in vivo genotoxicity of small organoarsenic species is presented below.

TABLE 6 Strength of evidence assessment of genotoxicity.

	MMA(III)	DMA(III)	MMA(V)	DMA(V)	Thio-DMA(V)
In vitro	ADEQUATE EVIDENCE^a • Clastogenic • Induces DNA SSB and DSB, and oxidised DNA lesions • DNA repair disturbance	ADEQUATE EVIDENCE^a • Clastogenic, aneugenic • Induces DNA SSB and DSB, and oxidised DNA lesions • DNA repair disturbance	MODERATE EVIDENCE^b • Clastogenic • Induces DNA breaks and oxidised DNA lesions • DNA repair disturbance	MODERATE EVIDENCE^b • Clastogenic • Induces DNA breaks and oxidised DNA lesions • DNA repair disturbance	INADEQUATE EVIDENCE^c • DNA repair disturbance
In vivo	No studies	No studies	INADEQUATE EVIDENCE^c • No increase of 8-oxodG in liver of exposed rats (one study)	LIMITED EVIDENCE^d • Induces DNA breaks in PBMC (one study) and 8-oxodG in serum and urines of exposed mice • Induces 8-oxodG in kidney, liver and bladder of exposed rats	No studies

^a**Adequate evidence:** This level involves consistent findings from multiple studies supporting the substance's genotoxic effects.
^b**Moderate evidence:** Evidence that is fairly consistent but might have limitations in terms of number of studies.
^c**Inadequate evidence:** This level indicates a lack of sufficient or reliable data to decide about genotoxic effects.
^d**Limited evidence:** This level includes preliminary or conflicting evidence from a few studies that suggest genotoxic potential.

3.1.4 | Cell transformation

Previous work showed that MMA(III) and DMA(III) can transform cells in culture (Bredfeldt et al., 2006). Waalkes' laboratory confirmed the oncogenic potential of MMA(III) in a cell transformation assay in both methylation-deficient and proficient cells that acquired cancer cell characteristics concurrently with oxidative stress induction during chronic MMA(III) exposure (0.25–1.0 μ M, up to 30 weeks) (Tokar et al., 2013). In a previous work, the same laboratory (Kojima et al., 2009) showed that iAs can also induce a malignant phenotype in methylation-deficient cells but without generation of oxidative damage while MMA(III) does so more rapidly and with ROS induction, suggesting different underlying mechanisms.

3.1.5 | Mode of action

3.1.5.1 | *Mode of action for cytotoxicity and genotoxicity*

Various factors have been identified as potential contributors to the elevated cytotoxicity associated with trivalent methylated arsenicals. These factors include high chemical reactivity, which may result in biologically relevant interactions with essential proteins and DNA, enhanced cellular uptake, specific targeting of mitochondria, the generation of ROS and interference with cellular signalling pathways. MMA(III) and DMA(III) have been found to be more potent than iAs(III) as inhibitors of the activities of purified GSH reductase and of thioredoxin reductase in vitro (Lin et al., 1999, 2001; Styblo et al., 1997). The hypothesis was formulated that methylated arsenicals might compromise the antioxidant mechanisms by consuming GSH and inhibiting the enzymes responsible for its recycling. Naranmandura et al. (2011) showed that MMA(III) is a potent inducer of ROS primarily in mitochondria of rat liver cells while DMA(III), that is less cytotoxic, generates ROS in other organelles and iAs, that presents the lowest cytotoxicity, does not generate ROS. This observation suggests that the interference with the functioning of mitochondrial respiratory chain complexes could also account for the specificity of cytotoxic effects. Methylated arsenicals efficiently inhibit pyruvate dehydrogenase complex (PDC), which can disrupt the conversion of pyruvate to acetyl-CoA and impair aerobic energy production (Petrick et al., 2001). Bornhorst et al. (2012) showed that a disturbance of the cellular energy-related nucleotides occurs in lung cells with arsenite, DMA(V) and MMA(III), all inducing cellular ROS, but not with MMA(V). More recent studies indicate that a complex mechanism underlies cell death by inorganic and methylated arsenical forms. Dodmane et al. (2013) analysed cytotoxicity and gene expression changes for iAs(III), MMA(III) and DMA(III) in three human cell types, urothelial (1T1), keratinocyte (HEK001) and bronchial epithelial (HBE) cells, corresponding to target organs for iAs-induced cancer. Each of the arsenicals induced slightly different changes in signalling pathways within and across various cell types. However, when effects on the affected pathways from all three arsenicals were looked at in combination, they exhibited similarities across different cell types. The major signalling pathways altered included nuclear factor E2-related factor 2 (NRF2)-mediated stress response, interferon, p53, cell cycle regulation and lipid peroxidation. Induction of apoptosis has also been investigated for some methylated species. Rehman et al. (2014) found that MMA(III) and DMA(III) caused apoptosis in HL-60 cells via activation of caspase-3, -9 with PARP cleavage and these effects were significantly attenuated by pretreatment with an antioxidant (NAC). Apoptotic DNA fragmentation was also markedly observed in HL-60 cells exposed to either MMA(III) or DMA(III), while iAs(III) had no relevant effects.

It is important to note that certain pentavalent methylated arsenicals may demonstrate cytotoxicity levels that are comparable to those of trivalent methylated arsenicals. An example of this is thio-DMA(V), which exhibits cytotoxicity within a concentration range similar to iAs(III), DMA(III) and MMA(III) and is several fold more cytotoxic than DMA(V) (see Section 'In vitro toxicity'). Various factors can contribute to thio-DMA(V)'s increased cytotoxicity, including the presence of sulfur, which can give rise to unique chemical interactions, greater bioavailability and specific impacts on cellular pathways. Shimoda et al. (2018) showed that both DMA(III) and thio-DMA(V) induce apoptosis and increase caspase activity in HepaRG cells but through different signalling pathways. Exposure of human bladder cancer EJ-1 cells to thio-DMA(V) (Naranmandura et al., 2011) caused reduced protein expression of p53 and p21, increased DNA damage, increased intracellular ROS and a substantial 60% reduction in intracellular GSH, suggesting the involvement of oxidative stress in the cytotoxic effects. In contrast, exposure to iAs(III) led to a significant increase in the protein expression of p21 and p53, with no concurrent increase in ROS, which required extended exposure times, and to increased levels of GSH. However, it should be acknowledged that comparing the cytotoxicity of various methylated arsenicals is complex, as the precise ranking of their cytotoxicity may vary depending on the study and the parameters under consideration.

The available evidence in in vitro assays indicates that methylated arsenicals may possess genotoxic properties, following a gradient of decreasing genotoxicity from trivalent methylated arsenicals to their pentavalent analogues, and finally to iAs. Similar to iAs, these methylated arsenical compounds exhibit a lack of mutagenic activity, while concurrently causing chromosomal damage, such as chromatid breaks and gaps, as well as disturbances in cell division, ultimately resulting in aneuploidy. In addition, they trigger the production of ROS and lead to the occurrence of various types of DNA damage, including DNA breaks and oxidative type of damage to the DNA bases. Some small organoarsenic species have been found to inhibit distinct DNA repair enzymes, potentially contributing to their genotoxic effects. Failure to repair oxidative lesions (e.g. replication fork stalling and collapsing due to BER intermediates or unrepaired SSB during the S phase) can lead to the formation of DSB as observed upon exposure to trivalent methylated arsenicals. This mechanism has been proposed in the adverse outcome pathway (AOP) network that links oxidative DNA damage to chromosomal aberrations (Cho et al., 2022). It is of note that MMA(III) has been reported to inhibit the activity of PARP1 thus interfering with SSB repair. This interference would in turn increase the likelihood of DSB formation, ultimately increasing the risk of clastogenic events (Helleday, 2011). Regarding in vivo studies, data are currently limited to pentavalent small organoarsenic species. Organ-specific induction of oxidative damage to DNA has been reported after oral administration of moderate to high doses of DMA(V) to rodents. Notably, a study found the induction of DNA breaks, as detected by the alkaline comet assay, in PBMCs of mice orally exposed to DMA(V), suggesting that exposure to DMA(V) may lead to both types of damage, DNA breaks and oxidised base lesions. DNA breaks may arise either during the processing of oxidised base lesions or due to radical species.

Recent studies have also highlighted the induction of epigenetic modifications by some small organoarsenic species.

Thio-DMA(V) is notable for its distinctive characteristics, including the absence of induced DNA strand breaks, but inhibition of PARP at low concentrations, as well as its influence on cell cycle and mitosis without causing clastogenic effects.

In conclusion, small organoarsenic species induce cytotoxic and genotoxic effects in various cultured cell types. Overall, the trivalent oxo species show greater potency than the pentavalent analogues, except for the thio forms. The absence of mutagenic effects but the induction of chromosomal damage suggests that DNA breaks induced by oxidative damage to DNA, along with the inhibition of DNA repair, may be the pivotal events causing clastogenic effects. The *in vitro* genotoxic profile of DMA(V) together with the occurrence of oxidative damage to DNA in several organs and DNA breaks in PBMCs of orally exposed rodents suggest a contribution of DNA damage to DMA(V) animal carcinogenicity (see Section 3.1.5.3 on Mode of action for carcinogenicity). The intricate mechanisms influencing cell death and genomic stability are complex and require further investigation. The distinct genotoxic properties of some methylated arsenicals in contrast to iAs call for further exploration. Small methylated arsenicals such as DMA (III) have been shown to disrupt mitotic progression by altering the mitotic spindle apparatus. This mechanism may also lead to aneuploidic as well as clastogenic effects.

3.1.5.2 | Mode of action for carcinogenicity

DMA(V) has been found to induce tumours in the urinary bladder of male and female rats, but not of other tissues, and not in mice. Female rats appeared to be more sensitive than male rats. Cohen et al. (2007) proposed that the carcinogenic mode of action of DMA(V) involves cytotoxicity followed by regenerative cell proliferation in a thresholded process. In a 20-week study, reported above (see Section 3.1.3.2.2 Repeated dose toxicity), they found evidence for an increase in cell proliferation, together with cytotoxicity, rather than an increase in urinary solids or alteration in urinary composition (Arnold et al., 1999). In further short-term studies, cytotoxicity of the superficial cells of the urothelium was observed by SEM as 6 h after commencing feeding of DMA(V) at 100 mg/kg in the diet, whereas increased cell proliferation was not observed until 3 days after treatment started (Cohen et al., 2001). Based on their observations, Cohen et al. (2007) proposed that a reactive metabolite was responsible for the cytotoxicity rather than urinary solids or altered urinary composition. They postulated that the reactive metabolite would be DMA(III), which was supported by evidence that 2,3-dimercaptopropane-1-sulfonate (DMPS), which interacts with trivalent arsenicals, inhibited the cytotoxicity and hyperplasia (Cohen et al., 2002), and demonstration of DMA(III) in urine following administration of dietary DMA(V) to rats (Cohen et al., 2006).

The carcinogenicity studies of Wei et al. (1999, 2002) included mutation analysis of the urinary bladder tumours induced by **DMA(V)** in male rats (reported in Wei et al., 2003). The tumours had a low rate of *H-ras* mutations and no mutations in *p53*, *K-ras* or β -catenin genes. Two mutations were reported in exon 1 of *H-ras*, both resulting in an identical substitution of glycine for valine. Increased COX-2 expression was noted in the majority of tumours and in a further experiment formation of 8-OHdG was significantly increased after treatment of rats with 200 mg/L DMA(V) in drinking water for 2 weeks. The authors concluded that ROS is likely to play an important role in the early stages of DMA carcinogenesis.

Wei et al. (2005) provided further support for their hypothesis using *in vitro* and *in vivo* studies in which antioxidants were co-administered with DMA(V) or DMA(III). N-Acetylcysteine (NAC) and ascorbic acid (at mM concentrations), but not melatonin, Tiron²⁶ or Trolox²⁷ inhibited the cytotoxicity of MMA(III), DMA(III) in MYP3 rat urinary bladder cells. NAC also inhibited the cytotoxicity of DMA(V) and TMAO. In subsequent *in vivo* studies with DMA(V), sodium ascorbate but not melatonin or NAC, inhibited the proliferative effects of DMA(V) on the bladder epithelium. Microarray analysis revealed no consistent modifying effect on gene expression. The authors concluded that the results suggested that oxidative stress is at least in part involved in induction of bladder carcinogenesis by DMA(V), and that proteins and/or lipids may be the targets of the damage. The CONTAM Panel noted that the studies were performed with a high dietary concentration of 10,000 mg/kg sodium ascorbate, selected on the basis that it results in an approximate 10-fold increase in concentrations of urinary ascorbic acid compared to the control group, and the relevance was uncertain.

Salim et al. (2003) administered **DMA(V)** to groups ($n=30$) of *p53* heterozygous knockout and wild-type C57BL/6J mice at 0, 50 or 200 mg/L in drinking water (equivalent to 0, 4.5 and 18 mg/kg bw per day) for 18 months. There was a significant increase in numbers of tumours per mouse and in the number of tumour-bearing mice in the wild type. In the knockout mice, the incidence in the control group was higher than in the wild type and only the number of tumours per mouse was statistically increased. The predominant tumours were malignant lymphomas and sarcomas, but the incidence of specific tumour types did not differ significantly from control in either mouse strain. The authors concluded that DMA(V) exerted its carcinogenic effect on spontaneous development of tumours in both animal genotypes investigated and noted an earlier induction of tumours in the *p53* $-/-$ knockout versus wild-type mice.

Kinoshita et al. (2007) administered **DMA(V)** to small groups ($n=5$ or 6) of male and female *Ogg1* knock-out mice²⁸ and wild-type C57BL/6 at 0 or 200 mg/L in drinking water for up to 72 weeks, equivalent to 0 and 18 mg DMA(V)/kg bw per day.²⁹ There was a statistically significant increase in incidence and multiplicity of lung tumours in DMA(V)-treated homozygote *Ogg1* knockout mice, compared to the non-treated control groups. There was no increase in DMA(V)-treated wild-type mice, compared to the non-treated control groups. The authors suggested that the results indicated a role for persistent accumulation of DNA oxidative adducts in carcinogenicity of DMA(V) in homozygote *Ogg1* mice. An et al. (2005) reported that administration of 0 or 400 mg/L DMA(V) to mice for periods of 2–25 weeks led to lipid peroxidation products in the secretory granules of the terminal bronchiolar Clara cells, accompanied by ultrastructural morphological changes. The

²⁶Trade name, 4,5-Dihydroxy-1,3-benzenedisulfonic acid disodium salt.

²⁷Trade name, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

²⁸Mice carrying a mutant gene for the enzyme 8-hydroxyguanine DNA glycosylase 1 (OGG1).

²⁹Calculated using the default conversion factor of 0.09 for chronic drinking water mouse studies.

authors concluded that the Clara cells are a major target for DMA-induced oxidative stress and may play an important role in lung tumour progression in mice. However, the CONTAM Panel considered that the relevance of the studies was uncertain, in view of the conflicting evidence regarding whether DMA(V) induces lung tumours in mice.

In the study in which **TMAO** was administered to male Fischer 344 rats in drinking water at concentrations equivalent to 0, 2.5 and 10 mg/kg TMAO bw per day for 104 weeks (Shen, Wanibuchi, Salim, Wei, Kinoshita, et al., 2003), the levels of 8-OHdG and staining for proliferating cell nuclear antigen (PCNA) in liver were significantly higher in the top dose rats than in controls, leading the authors to suggest that oxidative DNA damage and enhanced cell proliferation have mechanistic roles in TMAO induced liver tumorigenicity.

A number of studies have reported that various small methylated organoarsenic species, particularly DMA(V) promote carcinogenicity, when administered to rats or mice after a genotoxic carcinogen.

Yamamoto et al. (1994, 1995, 1997) administered **DMA(V)** to groups of 20 male F344/DuCrJ rats in drinking water at 0, 50, 100, 200 or 400 mg/L (equivalent to 0, 4.5, 9, 18 and 36 mg/kg bw per day DMA(V)³⁰) for 24 weeks after pretreatment with a combination of 5 carcinogens [diethylnitrosamine, N-methyl-N-nitrosourea, 1,2-dimethylhydrazine, N-butyl-N-(4-hydroxybutyl)nitrosamine and N-bis(2-hydroxypropyl)nitrosamine, referred to as DMBDD]. A further two groups of 12 rats that did not receive DMBDD were treated with 100 or 400 mg/L DMA(V). There were no untreated controls. In the initiated groups, DMA(V) significantly increased the incidence of tumours in the urinary bladder (all doses), kidney (at 200 and 400 mg/L), liver (at 200 and 400 mg/L) and thyroid gland (at 400 mg/L). No treatment-related tumours or proliferative lesions were observed in the rats that did not receive DMBDD.

Yamanaka et al. (1996) initiated groups of 9–13 male ddY mice with 4-nitroquinoline 1-oxide (4NQO) and then administered **DMA(V)** at 0, 200 or 400 mg/L (equivalent to 0, 30 and 60 mg/kg bw per day DMA(V)³¹) in drinking water for 25 weeks. There was no untreated control group. A statistically significant increase in the multiplicity of lung tumours was reported at 400 mg/L DMA(V), but not in the numbers of tumour-bearing mice. There was no increase in lung tumours in mice that were not pretreated with 4NQO and no treatment-related tumours of the other organs examined. A subsequent publication from the same laboratory reported that co-treatment with epigallocatechin (EGCG) suppressed the effects, leading to the conclusion that oxidative stress was involved in the promotion (Mizoi et al., 2005).

Wanibuchi et al. (1996) administered N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) at 0 or 0.05% in drinking water to male Fischer 344 rats for 4 weeks, and the BBN-treated rats were then changed to administration of **DMA(V)** in drinking water at 0, 2, 10, 25, 50 and 100 mg/L for 32 weeks ($n = 20$ per group) (equivalent to 0, 0.2, 0.8, 2.1, 4.4 and 9.2 mg/kg bw per day DMA(V)³²). The rats that did not receive BBN were treated with 0 or 100 mg/L DMA(V) ($n = 12$ per group), equivalent to 0 and 8.6 mg/kg bw per day DMA(V)¹⁹. At the end of the dosing period, the animals were examined for tumours of the urinary bladder. There was a dose-related increase of incidence and multiplicity of papillomas and carcinomas in the rats treated with both BBN and DMA(V), commencing from the group receiving 25 mg/L DMA(V) (equivalent to approximately 2 mg DMA(V)/kg bw per day¹⁹). There were no tumours in the groups that did not receive BBN. In a further experiment, groups of five rats were treated with 0, 10, 25 or 100 mg/L DMA(V) in drinking water for 8 weeks without prior DDN treatment. At the top dose of DMA(V), all animals died after 4 weeks. The animals of the lower DMA(V) dose groups showed significantly increased cell proliferation and ultrastructural changes of the urinary bladder epithelium. The authors concluded that DMA(V) has the potential to promote rat urinary bladder carcinogenesis, and that one mechanism involved is stimulation of cell proliferation in the urinary bladder epithelium.

In a subsequent study, Wanibuchi et al. (1997) investigated the potential of DMA(V) at concentrations of 25, 50 and 100 mg/L in drinking water, to promote rat hepatocarcinogenesis following administration of diethylnitrosamine. The rats were also subjected to 2/3 partial hepatectomy to maximise the effects. DMA(V) resulted in a dose-dependent increase in number and areas of GST-P-positive foci, associated with an increase in ornithine decarboxylase activity and formation of 8-OHdG. The authors concluded that DMA(V) has the potential to promote rat liver carcinogenesis, possibly via a mechanism involving cell proliferation and DNA damage caused by oxygen radicals.

Chen et al. (1999) reported that administration of DMA(V) in drinking water for 6 weeks to male rats increased the incidence of urinary bladder tumours induced by BBN.

Vijayaraghavan et al. (2000) found that 200 mg/L **DMA(V)** in drinking water did not promote renal or liver carcinogenesis in rats pretreated with N-ethyl-N-hydroxyethylnitrosamine (EHEN).

Nishikawa et al. (2002) pretreated groups of 20 rats with diethylnitrosamine (DEN) and then administered **MMA(V)**, **DMA(V)** or **TMAO** at 100 mg/L (equivalent to 12 mg/kg bw per day MMA(V), DMA(V) or TMAO³³) or no arsenic compound, in drinking water, for 6 weeks. All animals were subjected to partial hepatectomy after 1 week. The number and size of GST-P foci were increased compared to controls with no differences between the effects of MMA(V), DMA(V) and TMAO.

Tokar, Diwan, and Waalkes (2012) treated groups of pregnant CD1 mice with 0 and 85 mg/L arsenite in drinking water from GD8-18. The male offspring were then exposed to 0 or 200 mg/L **DMA(V)** in drinking water from weaning until 104 weeks of age (equivalent to 0 and 18 mg/kg bw per day³⁴). DMA(V) alone increased the incidence of lung adenocarcinoma, adrenal

³⁰Calculated using the EFSA default conversion factor of 0.09 for subchronic drinking water studies in rats.

³¹Calculated using the EFSA default conversion factor of 0.15 for subchronic drinking water studies in mice.

³²Calculated from the total intake of DMA per rat, assuming an average bw of 300 g over the course of the dosing period.

³³Applying the EFSA default conversion factor of 0.12 for subacute drinking water studies in rats.

³⁴Applying the EFSA default conversion factor of 0.09 for chronic drinking water studies in mice.

adenoma and urinary bladder hyperplasia, but not renal or liver carcinomas. Exposure of dams to arsenite, followed by exposure of offspring to DMA(V) increased the incidence of renal cell and hepatocellular carcinoma compared to gestational arsenite exposure alone.

In contrast to the initiation–promotion studies described above, Anetor et al. (2009) administered DMA(V) at 0 or 200 mg/L in drinking water to groups of rats for 4 weeks prior administration of 2-acetylaminofluorene in the diet for 2 weeks. Based on observations of GST-P foci and the PCNA index in the liver, the authors concluded that DMA(V) exhibited weak initiation potential. Xie et al. (2004) administered MMA(V) or DMA(V) to *v-Ha-ras* transgenic (Tg.Ac) mice in drinking at concentrations of 1500 and 1000 mg/L expressed as arsenic followed by topical administration of TPA and studied changes in gene expression. The CONTAM Panel noted that these high doses of MMA(V) and DMA(V) resulted in high mortality, and therefore, the results could not be interpreted.

In summary, there are a number of plausible modes of action for the carcinogenicity of **DMA(V)**, including cytotoxicity followed by regenerative cell proliferation, which could also provide an explanation for the promotional activity observed in a number of studies. However, DMA(V) has also shown moderate evidence of genotoxicity *in vitro*, and limited evidence *in vivo*. There is also some evidence suggesting uncertainty regarding the importance of genotoxicity in the MOA for carcinogenicity, and whether thresholded mechanisms can be assumed.

MMA(III) has shown some evidence of carcinogenicity in mice. No mode of action studies was identified with the exception of the genotoxicity studies. Since there is adequate evidence that MMA(III) is genotoxic *in vitro*, and no studies *in vivo*, the CONTAM Panel concluded that the mechanism of carcinogenicity is likely to involve genotoxicity.

3.1.5.3 | Mode of action for other endpoints

Bae et al. (2009) reported that MMA(III) and DMA(III) at μ M concentrations induced procoagulant activity and apoptosis in freshly isolated human platelets, which they suggested could play a role in arsenic-associated cardiovascular disease.

Negro Silva et al. (2017) investigated whether methylated arsenic intermediates were proatherogenic and whether As3MT-mediated biotransformation was required for effects. Four-week-old male apo E^{-/-} and DKO (double knockout *apo E^{-/-} As3mt^{-/-}*) mice received various arsenic compounds at concentrations equivalent to 0.2 mg As/L (200 ppb As) in drinking water for 13 weeks. The arsenicals were 0.35 mg/L m-sodium arsenite, 0.78 mg/L MMA(V), 0.37 mg/L MMA(III) or 0.43 mg/L DMA(V) (equivalent to 0.05, 0.12, 0.06 and 0.06 mg/kg bw per day of the named compound).³⁵ All substances were atherogenic in Apo E^{-/-} mice (increased plaque size in aortic arch/sinus). With DKO mice lacking As3MT, sodium arsenite did not cause any effects suggesting that formation of methylated intermediates is required to induce atherogenic effects. The authors concluded that their findings indicate that As3MT acts to promote cardiovascular toxicity of arsenic. In a subsequent study, male and female apo E^{-/-} and double knockout apo E^{-/-} *As3mt^{-/-}* mice were exposed to 200 mg/L of m-sodium arsenite, **MMA(V)**, **MMA(III)** or **DMA(V)** in drinking water from conception to weaning, followed by assessment of atherogenic plaques in the aortic arch and sinus of the offspring at 18 weeks of age (Negro Silva et al., 2021). Early life exposure to the methylated arsenical compounds resulted in larger plaques than arsenite, with sex-specific differences in the composition of the plaques.

3.2 | Observations in humans

No human studies were identified on health outcomes related to concentrations of small organoarsenic species in food.

In contrast, a large number of studies have been published with results on MMA and DMA in urine. Many of these studies also investigated associations between MMA or DMA and various outcomes in humans, and they have been discussed in the EFSA opinion on inorganic As (EFSA CONTAM Panel, 2024). MMA and DMA are urinary metabolites of inorganic As. Because MMA is not an important As species in food, MMA in urine most likely results from methylation of inorganic As. The situation with DMA, however, is more complex: In addition to DMA being a urinary metabolite from ingested inorganic arsenic, it is the major metabolite from ingested arsenosugars and arsenolipids, and can also be a significant As species in food. It is thus not possible to distinguish between ingested DMA and DMA formed by methylation of inorganic As or by catabolism of arsenosugars and arsenolipids. Therefore, studies reporting associations between MMA or DMA in urine and various health outcomes in humans cannot be used for risk assessment for these compounds.

3.3 | Consideration of critical effects

MMA(III) has shown some evidence of carcinogenicity in one non-standard study (Tokar, Diwan, Thomas, & Waalkes, 2012) involving dosing of pregnant CD1 mice with MMA(III) in drinking water from GD 8–18 (equivalent to 0, 2.3 and 4.5 mg/kg bw per day³⁶), with follow-up of the pups to 104 weeks of age. A variety of different tumours were reported, with tumour sites differing between males and females and no clear dose dependency. Taking into account the *in vitro* evidence of

³⁵Applying the EFSA default conversion factor of 0.15 for sub-chronic mouse studies.

³⁶Applying the EFSA default conversion factor of 0.18 for sub-acute drinking water mouse studies.

genotoxicity, the CONTAM Panel concluded that carcinogenicity is likely to be a critical effect of MMA(III). MMA(III) has not been tested for other types of toxicity.

MMA(V) has effects on the gastrointestinal tract, kidney, thyroid and liver. Ulcerative and inflammatory lesions were reported in the large intestine, which were more severe in rats than in mice, and more severe in males than in females (Arnold et al., 2003). The effect seen at the lowest dose was diarrhoea, which was associated with decreased body weight. Arnold et al. (2003) also reported increased relative kidney weight and morphological alterations in the kidney of rats, which were possibly due to urinary tract obstruction caused by inflammation of the ureter secondary to peritonitis, and morphological alterations in the thyroid of rats. Effects on the kidney and thyroid were also observed in mice, at higher dose levels than in rats. Necrotic and apoptotic changes were reported in the liver of rats dosed with MMA(V) at 63.2 mg/kg bw per day for 6 months via gavage (Shahida et al., 2020), but since this was the only dose level tested, the study is not informative about the dose–response relationship. MMA(V) has also been tested for developmental toxicity in rats and rabbits (Irvine et al., 2006). In the rat, maternal body weight gain was decreased at some time points in animals dosed at 100 mg/kg bw per day and more consistently at 500 mg/kg bw per day. Maternal toxicity (decreased body weight gain) was reported at some time points in rats dosed at 100 mg/kg bw per day and more consistently at 500 mg/kg bw per day. In rabbits, maternal toxicity (decreased body weight gain) was reported in rabbits dosed at 12 mg/kg bw per day throughout the dosing period. Fetal toxicity (decreased fetal weight in rats and retardation of skeletal ossification in rabbits) was observed at the highest dose tested in each species (rat: 500 mg/kg bw per day, rabbit: 12 mg/kg bw per day), but there was no treatment-related developmental toxicity at lower doses. The NOAELs were 100 and 7 mg/kg bw per day for rat and rabbit, respectively. There was no evidence of teratogenicity with MMA(V). The CONTAM Panel considered that developmental effects were not a critical effect of MMA(V) because they were observed in the presence of maternal toxicity. In 2-year carcinogenicity studies in rats and mice, MMA(V) did not induce tumours at gavage doses of up to 70–100 mg/kg bw per day. Taking into account the inadequate evidence of genotoxicity and the absence of tumours in a well-conducted carcinogenicity study, the CONTAM Panel concluded that carcinogenicity is not a critical endpoint of MMA(V). Overall, the Panel concluded that the critical effect of MMA(V) is intestinal toxicity, resulting in diarrhoea and decreased body weight.

DMA(III) is genotoxic *in vitro* with a range of effects including clastogenicity, but no *in vivo* data are available. It has shown some evidence of effects on the urinary bladder epithelium (Dodmane et al., 2013) and the cardiovascular system (Negro Silva et al., 2017). However, the CONTAM Panel concluded that available data are insufficient to define the critical effects of DMA(III).

DMA(V) has shown adverse effects on the kidney (proximal tubular degeneration and necrosis) and urinary bladder epithelium (Arnold et al., 1999; Murai et al., 1993). Two comprehensive carcinogenicity studies (Wei et al., 1999 and 2002; Arnold et al., 2006) demonstrated that administration of DMA(V) via drinking water or diet induced tumours in the urinary bladder of male and female rats, and not in mice. The lowest NOAEL was 0.5 mg DMA(V)/kg bw per day. DMA(V) has also been tested for developmental toxicity in rats and rabbits (Irvine et al., 2006). Maternal and developmental toxicity (increased incidence of fetuses with diaphragmatic hernia) were observed at 36 mg/kg bw per day but not at lower doses in the rat. In the rabbit, there was severe maternal toxicity at 48 mg/kg bw per day, resulting in no fetuses surviving for examination. The NOAEL was 12 mg/kg bw per day in both rats and rabbits. The CONTAM Panel considered that developmental effects were not a critical effect of DMA(V) because they were observed in the presence of maternal toxicity. The CONTAM Panel concluded that carcinogenicity is the critical effect of DMA(V). There are a number of plausible modes of action for the carcinogenicity, including genotoxicity and also cytotoxicity followed by regenerative cell proliferation. There is uncertainty regarding the importance of the genotoxicity in the MOA for carcinogenicity, and whether thresholded mechanisms can be assumed.

The toxicological data available for other small organoarsenic species are insufficient to identify critical effects.

3.4 | Dose response assessment for MMA(V) and DMA(V)

The CONTAM Panel identified two studies to be dose–response modelled, namely Wei et al. (1999) and Arnold et al. (2003) for incidence of urinary bladder tumours and the decrease of body weight (due to diarrhoea), for DMA(V) and MMA(V), respectively. 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 0.0.0.9071/0.0.0.9073/0.0.0.9077 for the underlying calculations.

For DMA(V), the BMD analysis was performed based on total urinary bladder tumour incidence in male rats as well as on the incidence of papillomas and carcinomas of the urinary bladder in male rats exposed to DMA(V) through drinking water at concentrations providing doses equivalent to 0, 0.63, 2.5 and 10 mg DMA(V)/kg bw per day for 104 weeks (Wei et al., 1999) (see Section 4.1.4.2 on Carcinogenicity). Using model-averaging, the lowest BMDL₁₀ of 1.1 mg/kg bw per day was estimated for the total incidence of urinary bladder tumours. The default benchmark response (BMR) for quantal data was selected, i.e. an extra risk of 10%.

For MMA(V), the BMD analysis was based on decreased body weight as a continuous variable in male and female rats as an indicator of observed diarrhoea following dietary exposure to MMA(V) at doses of 0, 3.2, 28.9 and 78.5 mg MMA(V)/kg body weight per day for females and 0, 2.6, 22.7 and 66.6 mg MMA(V)/kg body weight per day for males for 104 weeks (Arnold et al., 2003) (see Section 3.1.3.2.2 on Repeated dose toxicity). The BMD analysis was conducted as a covariate analysis, utilising sex as covariates, as it was not possible to model the reduction in rat body weight gain with the available data.

In line with the WHO guidance relating to the magnitude of body weight change considered to represent adversity, a BMR of 10% was selected (FAO/WHO, 2015; WHO, 2020). Using model-averaging, the lowest $BMDL_{10}$ of 18.2 mg/kg bw per day was estimated for the decreased body weight of male rats at week 104.

Table 7 below provides an overview of the BMD results obtained from the studies of Arnold et al. (2003) and Wei et al. (1999). A more detailed description of the BMD analyses can be found in Annex C.

TABLE 7 Benchmark dose (BMD) analysis for the effects of MMA(V) in Arnold et al. (2003) and DMA(V) in Wei et al. (1999).

Study	Compound	Response	BMR (%)	BMDL (mg/kg bw per day)	BMD (mg/kg bw per day)	BMDU (mg/kg bw per day)	Lowest non-zero dose (mg/kg bw per day)
Arnold et al. (2003)*	MMA(V)	Decreased body weight of male rats, week 104	10	18.2	24.2	32.9	2.57
Wei et al. (1999)	DMA(V)	Carcinoma incidence in male rats	10	1.4	2.7	5.0	0.63
		Papilloma incidence in male rats	10	8.7	17.8	28.6	0.63
		Total urinary bladder tumour incidence in male rats*	10	1.1	2.2	4.0	0.63

Abbreviations: BMD, benchmark dose; BMDL, benchmark dose lower confidence limit; BMDU, benchmark dose upper confidence limit; BMR, benchmark response; bw, body weight.

*No. of rats bearing either one or two tumours.

#A sensitivity analysis has been carried out to explore the effect of the correlation between body weights measured at different time points (see Annex C).

3.5 | Derivation of reference points and approach for risk assessment

The toxicological data available for the small organoarsenic species are incomplete. Some data are available for MMA(V) and DMA(V) that allow identification of Reference Points, i.e.:

MMA(V) - BMDL₁₀ for decreased body weight of 18.2 mg MMA(V)/kg bw per day (equivalent to 9.7 mg As/kg bw per day);
DMA(V) - BMDL₁₀ for extra risk of urinary bladder tumours of 1.1 mg DMA(V)/kg bw per day (equivalent to 0.6 mg As/kg bw per day).

However, important data are missing, such as for example the potential for reproductive toxicity and neurotoxicity. Therefore, the CONTAM Panel concluded that it is not appropriate to establish health-based guidance values and decided to apply a margin of exposure (MOE) approach for both MMA(V) and DMA(V).

The CONTAM Panel considered whether the diarrhoea was a local concentration-related effect, which would indicate that there would be less interspecies and interindividual variation in toxicokinetics and smaller uncertainty factors would be appropriate (WHO, 2020). However, the mechanism by which MMA(V) causes diarrhoea is unknown. The diarrhoea in rats did not commence until week 3 or 4 of dosing (depending on the dose group), it is not an acute effect and involvement of systemic exposure is also possible.

The CONTAM Panel concluded that the approach to evaluate the MOE for MMA(V) 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 MMA(V), data on chronic toxicity and on developmental effects are available, whereas studies on other effects such as reproductive toxicity and neurotoxicity are lacking. The CONTAM Panel considered an additional factor of 5 to be appropriate for deficiencies in the database.

The EFSA Scientific Committee recommends that for substances that are genotoxic and carcinogenic and have the potential to directly interact with the genetic material (DNA) of the cells of the body and to cause cancer an MOE of 10,000 or higher, if it is based on the BMDL₁₀ from an animal study, would be of low concern from a public health point of view (EFSA Scientific Committee, 2005).

For DMA(V), there is convincing evidence of carcinogenicity in rodents, but it is not clear to what extent genotoxicity is a contributing factor in carcinogenesis. The mechanisms underlying the genotoxicity associated with DMA(V) exposure are unclear, but the Panel concluded that it is likely that DMA(V) causes DNA damage by either thresholded or non-thresholded mechanisms, or both.

Recognising these uncertainties, the Panel concluded that the MOE of 10,000 or higher, as specified for substances that are genotoxic and carcinogenic, is appropriate for identification of a level of low concern. The MOE is calculated based on the BMDL₁₀ of 1.1 mg/kg bw per day for urinary bladder tumours in the rat. The Panel points out that this approach may be too conservative depending on the mechanism of genotoxicity and its role in the mechanism of carcinogenicity.

The available data do not allow identification of reference points or MOEs of low health concern for any of the other small organoarsenic species.

Similarly, the available data do not support grouping of any of the small organoarsenic species for the purposes of an assessment of the risks associated with combined exposure that would be compatible with the EFSA guidance (EFSA Scientific Committee, 2021).

3.6 | Occurrence data

3.6.1 | Occurrence data in food considered for dietary exposure assessment

An initial number of 2766 analytical results on DMA ($n = 1524$) and MMA ($n = 1242$) on food were extracted from the EFSA Data Warehouse covering the sampling years 2012–2022 (see Annex E for the raw data).³⁷

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 DMA/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; when information was not available, the data were assumed to be submitted as elemental arsenic.

³⁷For DMA, analytical results were initially submitted as dimethylarsinate (1522) and as dimethylarsinite ($n = 2$). For MMA, analytical results were submitted as methylarsonate ($n = 786$) and methylarsonite ($n = 456$). Following clarifications requests, data providers confirmed that all analytical results for DMA were in the +5-oxidation state [DMA(V)] while for MMA not answer was provided by the data provider and the analytical results were considered as MMA(V). No data were received on thio- analogues thio-DMA(V), dithio-DMA(V), and thio-MMA(V).

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:

- 248 analytical results (124 on MMA and 124 on DMA) were excluded as part of a duplicated file.
- 248 analytical results (124 on MMA and 124 on DMA) were excluded as they refer to total diet studies (TDS) samples that were prepared as consumed before being analysed.³⁸
- Five analytical results for MMA with relatively high values were excluded as they were not confirmed by the data provider (four samples of meat-based dishes and one sample of ground coffee).
- Six analytical results for DMA with relatively high values were excluded as they were not confirmed by the data provider (six samples of meat-based dishes).
- Two analytical results (one for DMA and one for MMA) were excluded as they were reported in duplicate.
- Nine analytical results for DMA were excluded as they were generated from samples with sampling strategy reported as 'Suspect sampling' (four samples of 'Vegetables and vegetable products', three of 'Fruit and fruit products', one of 'Coffee, cocoa, tea and infusions' and one of 'Composite dishes').³⁹

Few of the samples submitted to EFSA as TDS samples were kept in the final data set. This refers to seven samples (five of 'Marinated/pickled fish', one of 'Smoked fish' and one of 'Canned/jarred fish') that were not processed before being analysed, and with a level of aggregation matching the level of classification of the individual samples. To ensure a proportionate representation of the individual samples and, therefore, an accurate use of 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 presence of relatively high LODs/LOQs might have a significant influence on the UB scenario. Therefore, an evaluation of the reported LOQs was performed to reduce the impact of high LOQs reported, but without compromising the number of analytical results (EFSA, 2018). After the assessment, it was decided not to apply LOQ cut-off values on any of the two data sets. In the case of DMA, in those categories with combination of quantified and left-censored data, the derived LB and UB estimations did not differ much. For MMA, for most of the food categories, 100% of the data were left-censored; for the very few categories with both quantified and left-censored data, no LOQ cut-off value was applied to keep all the samples in the final data set.

During the data cleaning, 264 analytical results on DMA and 254 on MMA were excluded. The final data set comprised 1260 analytical results for DMA and 988 for MMA, all expressed in whole weight and as $\mu\text{g As/kg}$. For a total of 654 analytical results on DMA and 653 on MMA, no information was available on whether they were submitted as $\mu\text{g As/kg}$ or $\mu\text{g DMA(MMA)/kg}$; these samples were assumed to be expressed as $\mu\text{g As/kg}$. For 36% and 22% of the analytical data on DMA and on MMA, respectively, information on recovery was not reported, and they were either submitted as not corrected for recovery or without information whether corrected or not. Uncertainty associated with these assumptions is discussed in the corresponding section.

As regards the sampling strategy (EFSA, 2013), 55% and 62% of the samples were reported as 'Selective sampling' for MMA and DMA, respectively, and as 'Objective sampling' for 12% (for both MMA and DMA). For the rest of the samples, no information was provided. It was decided to retain all samples regardless of the sampling strategy, except for those reported as 'Suspect sampling' (see above).

Concerning the analytical methods, the main reported detection method for DMA was mass spectrometry (87%), in most of the cases as Inductively Coupled Plasma Mass Spectrometry (ICP-MS), either coupled to liquid chromatography (72%) or just as ICP-MS. The lowest LOQ in the analysis of DMA was $2 \mu\text{g As/kg}$ in a fish sample. For MMA, the vast majority of the samples were also reported as detected by mass spectrometry (96%), also as ICP-MS, in most of the cases as LC-ICP-MS (90%). The lowest LOQ in the analysis of MMA was $1.6 \mu\text{g As/kg}$ in a sample of processed/preserved fish.

As shown in Figure 1, the analytical results in the final data set were collected in eight EU countries for DMA and in four for MMA. Most of the samples were collected in Italy (67% for DMA, 85% for MMA). All food samples were taken from the EU market regardless of their country of origin. Among those, only a small number of samples originated from non-EU countries (6% for DMA and 4% for MMA), while information on the country of origin was not reported in ~6% of the data reported for each of the two data sets. The number of samples per sampling year is presented in Figure 2.

³⁸Few samples reported either as pooled samples and/or TDS samples were kept in the final data set as they refer to food commodities that were not processed before being analysed (e.g. smoked salmon, pickles, canned fish). The number of samples used in the pooled samples was considered when estimating mean occurrence values (weighted mean).

³⁹Samples analysed to confirm or reject a suspicion of non-conformity, i.e. not random sampling.

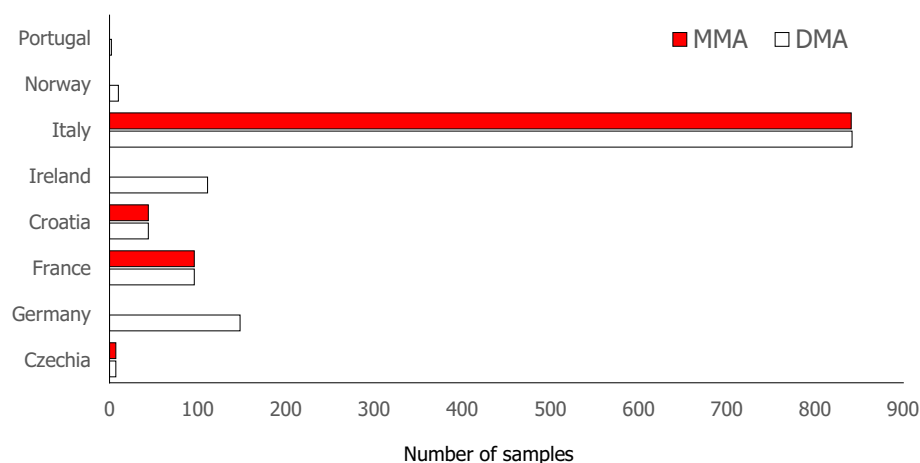


FIGURE 1 Distribution of analytical results reported for DMA and MMA across different EU countries (country of sampling; final cleaned data set).

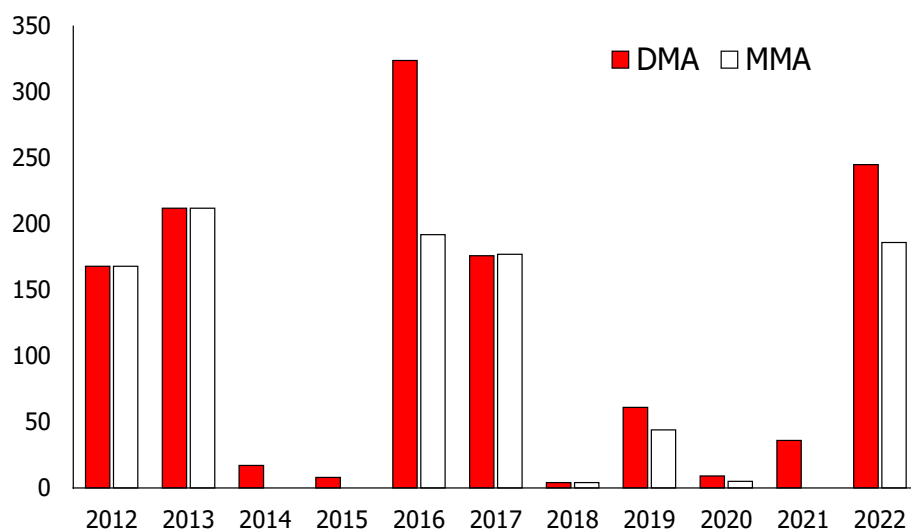


FIGURE 2 Distribution of analytical results reported for MMA and DMA by sampling year (final cleaned data set).

The left-censored data accounted for 44% of the analytical results on DMA, while in the case of MMA, the percentage increased up to 94%. 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 [Figures 3](#) and [4](#).

For DMA, as can be seen in [Figure 3](#), the highest number of quantified data were reported for samples codified as 'Grains and grain-based products', in particular for rice samples. Relatively high number of quantified data were also reported for seaweeds, included in the food category 'Vegetables and vegetable products', and for 'Fish, seafood, amphibians, reptiles and invertebrates' where DMA was found in different types of fish and seafood. For MMA ([Figure 4](#)), quantified data were almost exclusively reported for few samples of molluscs included in the FoodEx2 Level 1 food category 'Fish, seafood, amphibians, reptiles and invertebrates'. In the other food categories, such as 'Grains and grain-based products', almost if not all samples were reported as either below LOD or below LOQ. More details on the occurrence data on DMA and MMA are given below.

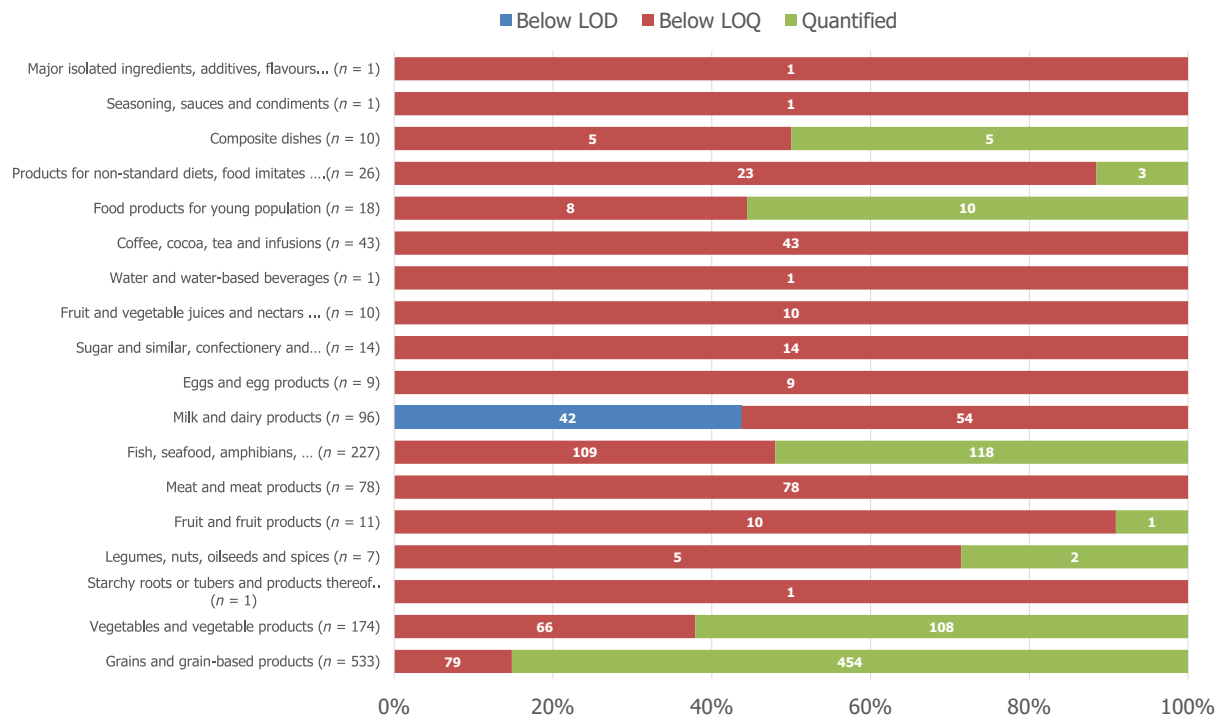


FIGURE 3 Overview of analytical results below LOD, below LOQ and quantified values in the final data set of DMA across the different food categories (FoodEx2 Level 1).

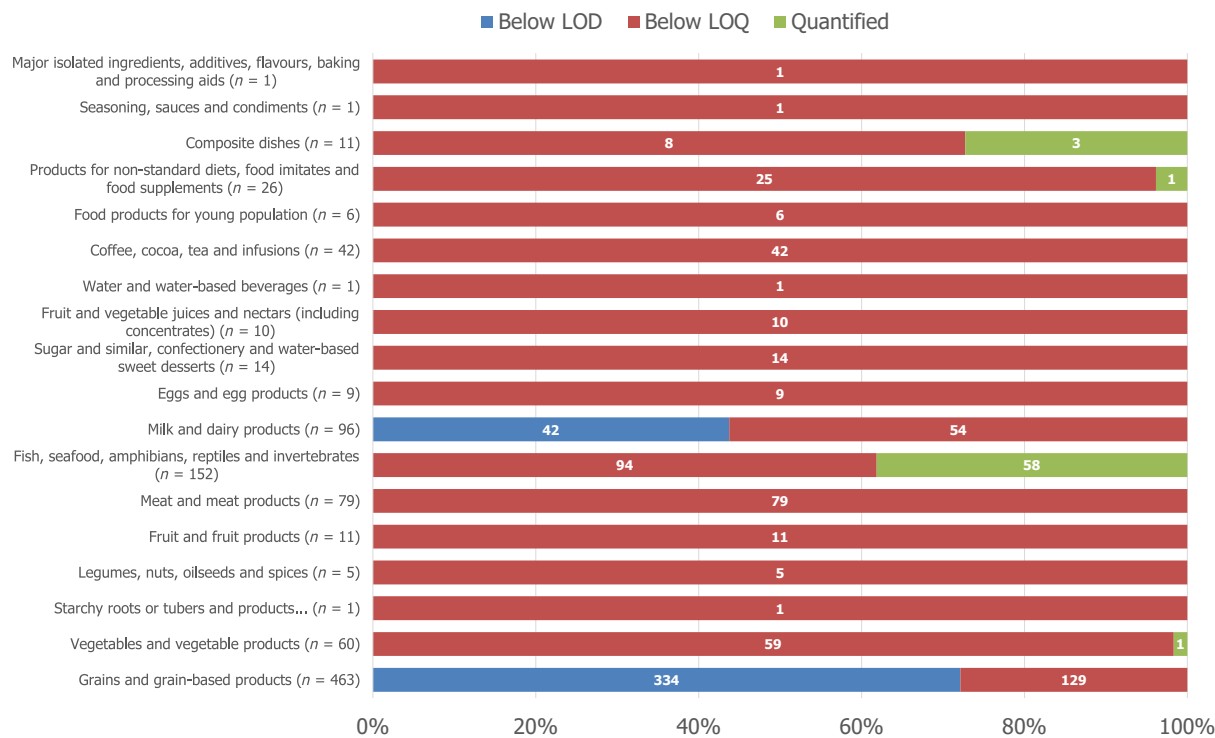


FIGURE 4 Overview of analytical results below LOD, below LOQ and quantified values in the final data set of MMA across the different food categories (FoodEx2 Level 1).

3.6.1.1 | Occurrence data on DMA

In the final data set of 1260 analytical results on DMA, a total of 18 food categories at FoodEx2 Level 1 were represented. The highest number of samples were reported for ‘Grains and grain-based products’ (n= 533), followed by ‘Fish, seafood, amphibians, reptiles and invertebrates’ (n=227) and ‘Vegetables and vegetable products’ (n=174). An overview of the number of analytical results, the proportion of left-censored data and the mean, median and 95th percentile (P95) concentrations at FoodEx2 Level 1 is presented in Table 8.

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

	N	%LC	Mean		P95 ^a	
			LB	UB	LB	UB
Grains and grain-based products	533	15	30.4	33.1	75.7	75.7
Vegetables and vegetable products	174	38	127.2	133.2	387.3	387.3
Starchy roots or tubers and products thereof, sugar plants	1	100	0.0	20.0	–	–
Legumes, nuts, oilseeds and spices	7	71	2.1	15.1	–	–
Fruit and fruit products	11	91	140.0	156.5	–	–
Meat and meat products	78	100	0.0	16.0	0.0	20.0
Fish, seafood, amphibians, reptiles and invertebrates	227	41	58.4	62.9	–	–
Milk and dairy products	96	100	0.0	7.6	0.0	20.0
Eggs and egg products	9	100	0.0	17.0	–	–
Sugar and similar, confectionery and water-based sweet desserts	14	100	0.0	17.4	–	–
Fruit and vegetable juices and nectars (including concentrates)	10	100	0.0	5.3	–	–
Water and water-based beverages	1	100	0.0	10.9	–	–
Coffee, cocoa, tea and infusions	43	100	0.0	14.8	–	–
Food products for young population	18	44	14.4	19.4	–	–
Products for non-standard diets, food imitates and food supplements	26	88	76.0	89.8	–	–
Composite dishes	10	50	417.5	427.5	–	–
Seasoning, sauces and condiments	1	100	0.0	10.9	–	–
Major isolated ingredients, additives, flavours, baking and processing aids	1	100	0.0	20.0	–	–

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

^aThe 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, seafood, amphibians, reptiles and invertebrates' was not calculated as this food category contains pooled samples.

The occurrence data were carefully examined and grouped at the appropriated FoodEx2 levels before being linked to the consumption data. Moreover, when no occurrence data were available for certain processed commodities identified by the CONTAM Panel as potentially relevant for the exposure to DMA, occurrence values were derived using the available data on raw primary commodities. Accordingly, the 439 analytical results reported for different types of rice were used to derive values for 'Rice and meat meal', 'Rice and vegetables meal', 'Rice pudding', 'Rice, meat, and vegetables meal with seaweed', 'Rice flakes', 'Rice flour', 'Rice porridge', 'Rice popped', and 'Rice drink', 'Fish soup with rice'. A similar approach with the available samples on 'Marine fish' ($n = 34$) was used to derive DMA levels in diverse fish-based commodities: 'Fish balls', 'Fish gratin', 'Fish pâté', 'Fish soup' and 'Prepared fish salad'. For 'Fish fingers, breaded', DMA levels were derived from the eight samples codified as 'Cods, hakes, haddocks' included in the samples of 'Marine fish'. To derive the DMA values for these processed commodities (see Table 9), processing factors and recipes were used as needed from the EFSA's Raw Primary Commodity model (EFSA, 2019), assuming that no losses of DMA occur during the processing. A food group named 'Canned/marinated/pickled seafood' was also created using the occurrence values reported for molluscs and crustaceans, together with one reported sample on unspecified canned seafood. Furthermore, to cover the relatively high amount of eating occasions reported as just 'Fish meat' without further details in the Comprehensive database (> 3000), an ad hoc food category was created grouping the 67 samples reported for different types of fish. As regards food for infants and young population, the few samples reported as 'Simple cereals which have to be reconstituted with milk or other appropriate nutritious liquids' were also used to cover the eating occasions of 'Cereals with an added high protein food which have to be reconstituted with water or other protein-free liquid' and those of 'Ready-to-eat cereal-based meal for children' reporting the presence of rice as ingredient.

Table 9 shows the occurrence data on DMA as they were used for the dietary exposure estimations. Overall, the highest levels of DMA were reported for few samples of salads that contain seaweeds in their composition ($n = 3$, LB = UB = 1356 µg As/kg). In fact, the other two food groups with the highest average levels of DMA were 'Algae and prokaryotes organisms' (dried) with LB–UB = 605–615 µg As/kg ($n = 112$), and 'Algae based formulations (e.g. Spirulina, chlorella)' with LB–UB = 104–118 µg As/kg ($n = 19$). Most of the data under 'Algae and prokaryotes organisms' were reported as brown seaweeds; however, they were all grouped as FoodEx2 Level 1 as the DMA average value for few samples of red seaweeds was within the range of concentrations reported for brown seaweeds. Additionally, at the level of consumption, the current eating occasions are in many cases just reported as unspecified seaweeds.

TABLE 9 Summary statistics (expressed as µg As/kg, weighted mean) of the levels of DMA in different food commodities as used for the dietary exposure estimations.

	N	%LC	Mean		HRP ^a	
			LB	UB	LB	UB
Grains and grain-based products						
Noodle, rice	6	0	26.8	26.8	27.2	27.2
Processed rice-based flakes ^b	439	4	39.2	39.9	71.6	71.6
Puffed/Extruded rice textured bread	31	35	32.6	43.5	50.2	50.2
Rice and similar-	439	4	33.5	34.1	79.2	79.2
Rice flour ^c	441	4	39.3	40.0	89.4	89.4
Rice porridge ^b	439	4	5.9	6.0	13.9	13.9
Rice, popped ^b	439	4	39.2	39.9	92.7	92.7
Crackers and breadsticks	9	89	1.2	11.9	0.0	10.9
Rusk	9	78	12.2	27.8	0.0	20.0
Vegetables and vegetable products						
Algae and prokaryotes organisms	116	9	143	145	449	449
Algae and prokaryotes organisms (dried) ^d	112	10	605	615	1706	1706
Aromatic herbs	6	83	5.0	21.7	0.0	20.0
Fish, seafood, amphibians, reptiles and invertebrates						
Molluscs	102	24	63.0	66.9	170	170
Unspecified Fish (meat)	67	69	19.9	28.3	40.0	40.0
Diadromous fish	19	42	4.9	10.3	10.0	10.9
Marine fish	34	71	36.5	45.0	12.0	20.0
Canned/jarred fish	18	61	10.5	14.7	–	–
Smoked fish	11	55	32.1	35.0	–	–
Marinated/pickled fish	5	0	83.7	83.7	–	–
Canned/marinated/pickled seafood ^e	123	36	53.0	57.1	170	170
Fish fingers, breaded ^f	8	63	3.2	6.4	0.0	5.2
Food products for young population						
Biscuits, rusks and cookies for children ^g	7	29	22.1	25.2	23.5	23.5
Cereals for infants or children, reconstituted	7	43	1.9	2.5	2.6	2.6
Products for non-standard diets, food imitates and food supplements						
Algae-based formulations (e.g. Spirulina, chlorella)	19	84	104	118	0.0	20.0
Rice drink ^b	439	4	4.6	4.7	10.9	10.9
Composite dishes						
Fish balls ^h	34	71	18.6	22.9	6.1	10.2
Fish gratin ^h	34	71	19.0	23.4	6.2	10.4
Fish pâté ^h	34	71	21.9	27.0	7.2	12.0
Fish soup ^h	34	71	17.1	21.1	5.6	9.4
Prepared fish salad ^h	34	71	17.5	21.6	5.8	9.6
Fish soup with rice ⁱ	439	4	23.9	29.3	31.3	36.6
Rice and meat meal ^b	439	4	8.3	8.4	19.6	19.6
Rice and vegetables meal ^b	439	4	9.9	10.1	23.4	23.4
Rice pudding ^b	439	4	3.7	3.8	8.7	8.7
Rice, meat, and vegetables meal ^b	439	4	5.0	5.1	11.7	11.7
Salads	3	0	1356	1356	–	–

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

^aHighest reliable percentile that can be estimated when applying the following minimum sample size for each percentile: five samples for the P50, 11 samples for the P75, 29 samples for the P90 and 59 samples for the P95. Percentiles for 'Marinated/pickled fish', 'Canned/jarred fish' and 'Smoked fish' were not calculated as they contain pooled samples.

^bOccurrence value derived using the 439 analytical results reported for rice and processing factors and recipes from the EFSA's Raw Primary Commodity model (EFSA, 2019).

^cOccurrence value derived using the 439 analytical results reported for rice and processing factors and recipes from the EFSA's Raw Primary Commodity model (EFSA, 2019) and two samples submitted to EFSA.

^dOccurrence value derived from the samples of Algae and prokaryotes organisms reporting moisture content.

^eOccurrence value derived using the data reported to EFSA for molluscs and crustaceans, together with one reported sample on unspecified canned seafood.

^fOccurrence value derived using the data reported to EFSA for 'Cods, hakes, haddocks' and processing factors and recipes from the EFSA's Raw Primary Commodity model (EFSA, 2019).

^gSamples of 'Biscuits, rusks and cookies for children' identified as rice cakes by the data providers.

^hOccurrence value derived using the 34 analytical results reported for 'Marine fish' and processing factors and recipes from the EFSA's Raw Primary Commodity model (EFSA, 2019).

ⁱOccurrence value derived using the 439 analytical results reported for rice, those reported for 'Fish meat; and processing factors and recipes from the EFSA's Raw Primary Commodity model (EFSA, 2019).

Rice samples were reported mainly as 'Rice grain, polished' ($n=221$, LB=UB=40.0 $\mu\text{g As/kg}$) and 'Rice grain, brown' ($n=110$, LB = UB=27.8 $\mu\text{g As/kg}$). All the different types of rice were grouped together as 'Rice and similar' ($n=439$; LB–UB=33.5–34.1 $\mu\text{g As/kg}$). For the immense majority of the rice samples (95%), Italy was reported as country of origin; the representativeness of these samples for the European population can be considered acceptable as Italy is the main rice producer in the EU and around two-thirds of rice consumed in Europe is grown in the EU. Additionally, it is accepted that DMA levels are typically higher in rice growth in temperate regions (e.g. Europe) than in rice from China and tropical regions (Carey et al., 2020; Dai et al., 2022).

Different levels of DMA were reported depending on the type of fish. The highest average levels were for the few samples of 'Marine fish' ($n=34$, LB-UB=36.5–45.0 $\mu\text{g As/kg}$), while no DMA was found in samples of 'Freshwater fish' ($n=7$). DMA was also quantified in 'Diadromous fish' ($n=19$; mean LB-UB=4.9–10.3 $\mu\text{g As/kg}$), in particular in all the 10 samples codified as Atlantic salmon. Other foods worth mentioning as regards their levels of DMA are those codified as 'Puffed rice textured bread' ($n=31$; mean LB–UB=32.6–43.5 $\mu\text{g As/kg}$). This refers to a food commodity already identified by EFSA as a relevant source of iAs (EFSA, 2014, 2021) and, more recently, also as containing DMA and the respective methylated thioarsenates thio-DMA(V) and dithio-DMA(V) (Colina Blanco et al., 2021, 2024). Similar commodities were reported as food products for young populations codified as 'Biscuits, rusks and cookies for children' ($n=7$; mean LB-UB=22.1–25.2 $\mu\text{g As/kg}$).

Summary statistics on the occurrence data on DMA codified at the different FoodEx2 levels are reported in Annex F.

3.6.1.2 | Occurrence data on MMA

For MMA also a total of 18 food categories at FoodEx2 Level 1 were represented in the final data set of 988 analytical results. However, in this case, only 63 samples reported quantified data (6%), most of them on molluscs (mainly mussels and oysters). The two categories with the highest number of samples were the same as for DMA, 'Grains and grain-based products' ($n=463$) and 'Fish, seafood, amphibians, reptiles and invertebrates' ($n=152$). An overview of the number of analytical results, the proportion of left-censored data, and the mean, median and 95th percentile (P95) concentration at FoodEx2 Level 1 is presented in Table 10.

TABLE 10 Summary of MMA occurrence data by food categories at FoodEx2 Level 1 (expressed as $\mu\text{g As/kg}$, weighted mean).

	N	% LC	Mean		P95 ^a	
			LB	UB	LB	UB
Grains and grain-based products	463	100	0.0	6.0	0.0	20.0
Vegetables and vegetable products	60	98	1.1	17.1	0.0	20.0
Starchy roots or tubers and products thereof, sugar plants	1	100	0.0	20.0	–	–
Legumes, nuts, oilseeds and spices	5	100	0.0	18.1	–	–
Fruit and fruit products	11	100	0.0	18.3	–	–
Meat and meat products	79	100	0.0	16.0	0.0	20.0
Fish, seafood, amphibians, reptiles and invertebrates	152	53	46.3	53.4	251	251
Milk and dairy products	96	100	0.0	7.5	0.0	20.0
Eggs and egg products	9	100	0.0	16.9	–	–
Sugar and similar, confectionery and water-based sweet desserts	14	100	0.0	17.3	–	–
Fruit and vegetable juices and nectars (including concentrates)	10	100	0.0	5.0	–	–
Water and water-based beverages	1	100	0.0	10.7	–	–
Coffee, cocoa, tea and infusions	42	100	0.0	14.2	–	–
Food products for young population	6	100	0.0	10.4	–	–
Products for non-standard diets, food imitates and food supplements	26	96	1.9	16.3	–	–
Composite dishes	11	73	88.5	103.0	–	–
Seasoning, sauces and condiments	1	100	0.0	10.7	–	–
Major isolated ingredients, additives, flavours, baking and processing aids	1	100	0.0	20.0	–	–

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

^aThe 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.

Since most of the data on MMA were left-censored, only specific exposure scenarios for consumers of selected commodities were conducted. It is important to note that no quantified levels of MMA were reported for the 411 samples of rice analysed; this in line with literature data that typically reports either no MMA in rice or at very low levels. Similarly, for

fish, all the samples ($n=35$) were also reported as left-censored data. However, considering the small number of samples reported to EFSA and that literature indicates the presence of MMA in some types of fish, the CONTAM Panel decided to make use of MMA data on fish from the literature. In particular, the data were retrieved from the 'CALIPSO fish and seafood' study conducted by French Institute for Agronomy Research (INRA) and the French Food Safety Agency (AFSSA) to assess the exposure of high consumers of seafood to trace elements, pollutants and omega 3 (Leblanc, 2006). In this study, most of the samples analysed were also left-censored data in line with EFSA data (85.5%). However, MMA was quantified in different types of fish and these data were used to conduct one specific scenario for consumers of fish meat. Similarly, MMA data on prawns/shrimps were retrieved from Zmozinski et al. (2015), as well as on processed few fish-based commodities from Hackethal et al. (2021). Table 11 shows an overview of the occurrence data used for the different exposure scenarios for MMA combining the data submitted to EFSA and those retrieved from the literature: data on fish meat, molluscs and crustaceans, and on processed/preserved fish. The highest mean levels are found for a few samples of smoked herrings ($n=20$; 397 $\mu\text{g As/kg}$), with also relatively high values in canned herrings ($n=20$; 152 $\mu\text{g As/kg}$). The measurements of these samples were reported by Hackethal et al. (2021). Among the samples of fish meat retrieved from the literature, the average levels of MMA seem to be different depending on the type of fish analysed. The highest average levels were reported for the group 'Miscellaneous demersal marine fishes' (e.g. ocean perch, anglerfish, monkfish, dories, etc.) for which an average value of 87.7 $\mu\text{g As/kg}$ was reported ($n=35$). Important to note the data submitted to EFSA for 27 samples of oysters, all quantified, with MMA average levels of 227 $\mu\text{g As/kg}$. Among the food categories with quantified data submitted to EFSA, 48 samples of mussels had an average concentration of 54.0–61.5 $\mu\text{g As/kg}$ (LB–UB). As done with DMA, occurrence values for a few processed commodities were derived using occurrence data on raw primary commodities (in this case from literature data), and processing factors and recipes from the EFSA's Raw Primary Commodity model (EFSA, 2019). Mean MMA values in these processed commodities varied from 2.7 $\mu\text{g As/kg}$ (LB = UB) in 'Fish fingers, breaded' to 13.5 $\mu\text{g As/kg}$ (LB = UB) in 'Fish pâté' (see Table 11).

Summary statistics on the occurrence data on MMA reported to EFSA and codified at the different FoodEx2 levels are described in Annex F.

TABLE 11 Summary statistics (expressed as $\mu\text{g As/kg}$, weighted mean) for MMA in food commodities as used to estimate dietary exposure in different exposure scenarios (food samples submitted to EFSA and food samples retrieved from the literature).

	N	% LC	Mean		Comments
			LB	UB	
Fish meat ^a					
Unspecified Fish (meat)	415	–	22.0		Leblanc (2006)
Diadromous fish	25	–	12.8		Leblanc (2006)
Miscellaneous coastal marine fishes	70	–	25.6		Leblanc (2006)
Miscellaneous demersal marine fishes	35	–	87.7		Leblanc (2006)
Flounders, halibuts, soles	70	–	14.3		Leblanc (2006)
Mackerel	20	–	78.0		Leblanc (2006)
Cods, hakes, haddocks	130	–	5.1		Leblanc (2006)
Herrings, sardines, anchovies	25	–	25.0		Leblanc (2006)
Tunas, bonitos, billfishes	40	–	21.0		Leblanc (2006)
Molluscs					
Unspecified molluscs	99	44	88.6	95.6	EFSA occurrence data
Clams, cockles, arkshells	21	90	2.9	17.0	EFSA occurrence data
Mussels	48	46	54.0	61.5	EFSA occurrence data
Oysters	27	0	227	227	EFSA occurrence data
Processed or preserved fish					
Canned herring	20 ^b	–	152		Hackethal et al. (2021)
Smoked herring	20 ^b	–	397		Hackethal et al. (2021)
Canned mackerel	1 ^c	–	82.0		Leblanc (2006)
Canned anchovies	2 ^c	–	19.0		Leblanc (2006)
Fish fingers, breaded ^d			2.7	2.7	From data on ‘Cods, hakes, haddocks’
Composite dishes ^e					
Fish balls	415	–	11.5		From fish meat data
Fish gratin	415	–	11.7		From fish meat data
Prepared fish salad	415	–	10.8		From fish meat data
Fish pâté	415	–	13.5		From fish meat data

TABLE 11 (Continued)

	N	% LC	Mean		Comments
			LB	UB	
Fish soup	130	–	10.6		From fish meat data
Crustaceans					
Shrimps and prawns	9	–	9		Zmozinski et al. (2015)

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

^aFish samples (raw) retrieved from the study report 'CALIPSO, Fish and seafood consumption study and biomarker of exposure to trace elements, pollutants and omega 3' (Leblanc, 2006). MMA values obtained from the analysis of different samples (N) pooled in composites samples representing different areas and provisioning places in France. For samples below LOD (0.007 µg As/kg) or below LOQ (0.02 µg As/kg), MMA was considered as being equal to half these limits.

^bThe MMA value was obtained from a single measurement of one composite sample consisting of 20 pooled samples from different locations and considering market share data (Hackethal et al., 2021). These samples are part of the first German TDS (BfR MEAL Study) and correspond to samples that were not further processed before being analysed ('Smoked herrings') or just shortly heated (few of the samples) in the case of the composite sample of 'Canned herring'.

^cIn this case, N refers to the number of composite samples. The exact number of samples analysed to derive the reported values was not available, but it was between one and five for each composite sample depending on the market shares of the different brands sampled (Leblanc, 2006).

^dOccurrence value derived using the data available for 'Cods, hakes, haddocks', and processing factors and recipes from the EFSA's Raw Primary Commodity model (EFSA, 2019).

^eOccurrence values derived using the average values for the 415 samples of Unspecified Fish (meat), and processing factors and recipes from the EFSA's Raw Primary Commodity model (EFSA, 2019).

3.6.2 | Occurrence data from the literature

In general, the occurrence data from the literature are consistent with those from the EFSA data set. The sample size is usually much smaller, and some studies include biological samples that might not be considered as common food products. The literature data, however, include food items such as mushrooms currently not represented in the EFSA data set. Furthermore, the literature data include arsenic species, such as MMA(III) and the thiolated organoarsenicals, which are not contained in the EFSA data set. This overview of the literature data is in three parts dealing with di-, mono- and tri/tetra-methylated arsenic species, which is the order of their relative abundance in food. Within each part, the major food types that contain these species are presented.

3.6.2.1 | Dimethylated small organoarsenic species in food

DMA(V) is the predominant small organoarsenic species in food. Among terrestrial foods, relatively high levels of DMA(V) are found in rice. In rice, DMA(V) is present in higher concentration than monomethylarsonic acid (MMA(V)), and, in some cases, its concentration may exceed that of iAs (Cheyns et al., 2017; Maher et al., 2018). The scientific literature reports a great variation of DMA(V) concentrations in rice, from < 2 µg As/kg up to 957 µg As/kg as reported in husked and polished rice grains from Argentinian cultivars (Quintero et al., 2014). Moreover, the contribution of DMA(V) to the total arsenic content in rice grain also varies widely, with values ranging from < 10% to above 70% (Farías et al., 2015).

Different factors have been identified in an attempt to explain the variation in DMA(V) levels across rice grown in different parts of the world. Although genotypic variation among cultivars could also play an important role, differences in arsenic speciation are attributed mostly to environmental factors such as temperature (temperate & tropical regions) and agronomic practices (aerobic & anaerobic conditions), but also to soil composition (silt, clay, organic carbon, etc.) and soil properties (grain size, porosity) (Dai et al., 2022; Maher et al., 2018; Zhao et al., 2013). Anaerobic conditions typically used for rice cultivation (flooded paddy fields) result in increasing percentages of DMA(V); microbial activity in the soil is mainly responsible of the methylation of inorganic arsenic under reducing conditions. DMA(V) levels are typically higher in rice grown in temperate regions (the greater part of Europe and the United States) than in China and tropical regions such as India, Thailand or Bangladesh (Carey et al., 2020; Dai et al., 2022). Around two-thirds of the rice consumed by the European population is grown in the EU. Japonica rice represents around 75% of the total European production and Indica rice the remaining 25%. Japonica varieties are typically consumed in Southern Europe (where they are produced), while Indica varieties are more popular in Northern Europe. The one-third of rice imported by Europe mainly refers to Indica rice, and it largely comes from Asian countries such as Myanmar, Pakistan, Thailand, Cambodia and India.⁴⁰

Mushrooms can also contain appreciable levels (1 µg As/g dry matter (d.m.) or higher) of DMA(V), with some species having 10% of their total As content present as DMA(V) (Braeuer & Goessler, 2019; Nearing et al., 2014; Šlejkovec et al., 1997). A study of 23 samples from eight species of edible mushrooms collected in China reported DMA(V) levels from < 0.02 to 1.04 µg As/g d.m with a mean of ca. 0.10 µg As/g d.m. (Zou et al., 2020).

Although seafoods generally contain high levels of arsenic, DMA(V) is not usually a dominant species. In fish, DMA(V) is often detected but the levels are generally low; for example, Nam et al. (2010) reported that bluefin and yellowfin tuna contained 0.04–0.21 µg As/g d.m., Chen and Jiang (2021) found DMA(V) levels of 0.03–0.14 µg As/g d.m. for three marine fish species and Guo et al. (2022) reported that 50 fish samples from eight coastal sites in China contained ca. 0.1–0.2 µg As/g

⁴⁰<https://agridata.ec.europa.eu/extensions/DashboardRice/RiceTrade.html#>.

d.m. In a study of arsenic species in food from Belgium markets, 53 samples of marine fish had DMA(V) levels from <0.005 to ca. $0.1 \mu\text{g As/g d.m.}$ (estimated from the reported wet mass As levels) (Ruttens et al., 2012). Other studies in Europe, however, have reported higher levels of DMA in different types of fish. Kalantzi et al. (2017) conducted arsenic speciation in sardines and anchovies collected from six coastal areas in Greece. Those authors reported DMA levels ranging between 0.072 and $0.956 \mu\text{g As/g d.m.}$, with the highest values reported in sardines. Similarly, Moreda-Piñeiro et al. (2011) analysed six marine fish species sampled from Spanish supermarkets, reporting mean DMA concentrations up to $1 \mu\text{g As/g d.m.}$ in sardines, and also relatively high mean concentrations in mackerel ($0.407 \mu\text{g As/g d.m.}$) and cod ($0.574 \mu\text{g As/g d.m.}$). Shellfish (molluscs and crustaceans) commonly have concentrations of DMA(V) higher (ca 2–3 fold) than those in fish (Guo et al., 2022).

DMA(V) is a usual, minor arsenic species in algae, although as compared to other food commodities algae contain relatively high levels. For example, Garcia-Salgado et al. (2012) investigated 12 species of edible algae with total As concentrations ranging from 23 to $126 \mu\text{g As/g d.m.}$, and reported DMA(V) levels from <0.01 to $0.9 \mu\text{g As/g d.m.}$ Furthermore, a study of arsenic species in 50 samples of algae from commercial harvesters in New England reported total As concentrations of 4 – $106 \mu\text{g As/g d.m.}$, with DMA(V) levels of 0.04 – $0.97 \mu\text{g As/g d.m.}$ (Taylor & Jackson, 2016). The highest DMA(V) levels were found in brown algae and the lowest in green algae.

The reduced form of DMA(V), namely dimethylarsinous acid, DMA(III), is a reactive, difficult to measure species, which is not detected in foods (Molin et al., 2015).

Thio-DMA has been recently reported in rice and rice-based commodities (Colina Blanco et al., 2021). In the same study, it is described that the commonly used analytical method for measuring small organoarsenic species can convert thio-DMA to DMA(V) (Colina Blanco et al., 2021). Therefore, reports in the scientific literature could be underestimating the thio-DMA content, with a concomitant overestimation of DMA(V). Using a method suitable for quantifying both thio-DMA and DMA(V), Dai et al. (2022) quantified these two species in ~250 rice samples from across six different continents. Based on the data from that study, Dai et al. (2022) proposed that 30% the measured DMA(V) concentration in rice could be considered as thio-DMA. Dithio-DMA(V) has also been reported in rice but only at trace levels from 54 samples of commercial rice, 14 contained dithio-DMA(V) up to a maximum concentration of $0.003 \mu\text{g As/g d.m.}$ (Colina Blanco et al., 2021). In contrast to DMA(V), the thio-DMA(V) species have so far not been reported in any food product other than in rice or rice-based commodities. It remains possible, however, that thio-DMA(V) is present in other food commodities, but it has not yet been detected owing to the above-mentioned conversion to DMA(V) during commonly used arsenic speciation methods.

The presence of thio-DMA(V) and dithio-DMA(V) in rice has taken on greater significance with the recent study showing that their concentrations increase markedly when rice is heated to make puffed rice cakes (Colina Blanco et al., 2024). The data indicate that when rice is heated, the DMA(V) originally present is mostly converted to dithio-DMA(V) and to a lesser extent thio-DMA(V). The authors proposed that S-containing compounds, such as cysteine, naturally contained in the rice thermally degrade to hydrogen sulfide, which then thiolates DMA(V). Furthermore, when the rice cakes were stored for 3 months, the dithio-DMA(V) mostly converted to thio-DMA(V) and DMA(V). The same study of Colina Blanco et al. (2024) also measured the arsenic species in 80 commercial samples of puffed rice cakes, and reported levels ($\mu\text{g As/g d.m.}$) of DMA(V) (0 – 0.11 , mean 0.013), thio-DMA(V) (0 – 0.26 , mean 0.034) and dithio-DMA(V) (0 – 0.34 , mean 0.037).

3.6.2.2 | Monomethylated small organoarsenic species in food

MMA(V) is a common, albeit minor species in rice. Its concentrations are generally in the range of $<5\%$ – 25% of DMA(V) concentrations. For example, Barnett et al. (2021) measured the arsenic species in 389 samples of white rice with a mean total As content $0.132 \mu\text{g As/g d.m.}$ and reported concentrations of $0.002 \mu\text{g As/g d.m.}$ and $0.062 \mu\text{g As/g d.m.}$ for MMA(V) and DMA(V), respectively. In the study of Colina Blanco et al. (2024), MMA(V) was detected (up to $0.04 \mu\text{g As/g d.m.}$) in 25 of the 80 samples of puffed rice cakes analysed. Those authors also reported traces of MMA(V) (0.001 – $0.002 \mu\text{g As/g d.m.}$) in 3 of 10 rice samples investigated.

MMA(V) has been reported in several species of mushrooms, usually at low levels although for some species, it is the dominant arsenical (Braeuer et al., 2020; Braeuer & Goessler, 2019). A study of 23 samples from eight species of edible mushrooms collected in China reported MMA(V) levels from <0.02 to $0.38 \mu\text{g As/g d.m.}$ with a mean of ca. $0.10 \mu\text{g As/g d.m.}$ (Zou et al., 2020).

Braeuer et al. (2018) showed that species of *Elaphomyces* mushrooms, with total As concentrations up to $660 \mu\text{g As/g d.m.}$, contain MMA(V) as their major arsenic species accounting for up to 85% of the total As. *Elaphomyces* mushrooms are generally not eaten – these results are mentioned to highlight the likelihood that such extreme As-accumulating ability will also be found for some edible mushroom species.

MMA(V) is not significant in fish with values usually less than $0.03 \mu\text{g As/g d.m.}$ (e.g. Ruttens et al., 2012). The levels of MMA(V) in shellfish are usually slightly higher than those in fish, but are still low; for example, Liu et al. (2022) measured 199 samples from eight molluscan species and reported a mean MMA(V) content of $0.1 \mu\text{g As/g d.m.}$, which represented only 0.1% of the total As content (arsenobetaine contributed 86% to the As total).

Algae can also contain MMA(V) although it usually makes only a minor contribution to the total As content. For example, from 26 samples of algae from commercial harvesters in New England, only seven contained detectable MMA(V) with the values ranging from 0.03 to $0.09 \mu\text{g As/g d.m.}$

In 2008, both MMA(III) and thio-MMA(V) were detected in a single carrot sample (Yathavakilla et al., 2008). MMA(III) is difficult to measure because it readily converts to MMA(V) in solution.

Recently, thio-MMA(V) together with dithio-MMA(V) were detected at trace levels (0.001–0.007 µg As/g d.m.) in about 10% of 80 samples of puffed rice cakes (Colina Blanco et al., 2024). It should be noted that the situation with the monomethylated species is similar to that described above for the dimethylated species, namely, MMA(III) and the thio-MMA(V) species are labile and will be readily converted to MMA(V) during the analytical procedure unless mild conditions are employed.

3.6.2.3 | Tri- and tetra-methylated organoarsenic species

The other small organoarsenic species reported in the literature are trimethylarsine oxide (TMAO) and tetramethylarsonium ion (TETRA). Although both species have been reported in rice (e.g. Hansen et al., 2011; Rahman et al., 2019), they are not common constituents of rice. Similarly, edible mushrooms also do not commonly contain TMAO or TETRA (Braeuer & Goessler, 2019; Zou et al., 2020), although some mushrooms of *Elasphomyces*, which are generally not eaten, contain TMAO up to 42 µg As/g d.m representing 28% of the total As content (Braeuer et al., 2018).

Fish and shellfish generally contain traces of TMAO and TETRA (Taylor et al., 2017), but there have been only rare reports (e.g. Shiomi et al., 1987) of them being present at significant levels. Neither of these compounds is a normal constituent of edible algae.

3.7 | Exposure assessment

Chronic dietary exposure to DMA and MMA was estimated following the methodology described in Section 2.5, using the occurrence data shown in Tables 9 and 11 in Section 3.6.1 and the consumption data from the dietary surveys described in Annex D.

3.7.1 | Dietary exposure to DMA

Table 12 shows a summary of the chronic dietary exposure estimates to DMA across 49 dietary surveys carried out in 22 European countries. The exposure estimates calculated for each dietary survey together with further details on the contribution of food groups to the exposure are presented in Annex G (Tables G1–G3).

TABLE 12 Summary statistics of the chronic dietary exposure assessment to DMA (µg As/kg bw per day) across European dietary surveys.

	Mean dietary exposure (µg As/kg bw per day)						
	N	Lower bound (LB)			Upper bound (UB)		
		Min	Median	Max	Min	Median	Max
Infants (> 12 weeks to < 12 months)	12	0.018	0.035	0.059	0.021	0.044	0.074
Toddlers (≥ 12 months to < 36 months)	15	0.019	0.034	0.130	0.022	0.042	0.157
Other children (≥ 36 months to < 10 years)	19	0.012	0.026	0.084	0.016	0.032	0.098
Adolescents (≥ 10 years to < 18 years)	21	0.007	0.015	0.048	0.008	0.017	0.054
Adults (≥ 18 years to < 65 years)	22	0.005	0.013	0.038	0.006	0.015	0.044
Elderly (≥ 65 years to < 75 years)	19	0.004	0.013	0.037	0.005	0.016	0.044
Very elderly (≥ 75 years)	14	0.007	0.011	0.033	0.009	0.014	0.039
Pregnant women	6	0.004	0.019	0.039	0.006	0.022	0.046
Lactating women	2	0.011	–	0.025	0.014	–	0.031
	95th percentile dietary exposure (µg As/kg bw per day) ^a						
	N	Lower bound (LB)			Upper bound (UB)		
		Min	Median	Max	Min	Median	Max
Infants (> 12 weeks to < 12 months)	12	0.069	0.100	0.251	0.080	0.119	0.308
Toddlers (≥ 12 months to < 36 months)	15	0.067	0.142	0.397	0.074	0.156	0.477
Other children (≥ 36 months to < 10 years)	19	0.058	0.097	0.258	0.065	0.108	0.310
Adolescents (≥ 10 years to < 18 years)	21	0.029	0.057	0.133	0.034	0.068	0.158
Adults (≥ 18 years to < 65 years)	22	0.021	0.058	0.109	0.025	0.067	0.136
Elderly (≥ 65 years to < 75 years)	19	0.017	0.053	0.126	0.020	0.062	0.149
Very elderly (≥ 75 years)	14	0.026	0.052	0.091	0.029	0.059	0.104
Pregnant women	6	0.018	0.067	0.109	0.021	0.077	0.121
Lactating women	2	0.042	–	0.062	0.050	–	0.078

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

The highest dietary exposure was estimated for the young population, in particular in one dietary survey for 'Toddlers', with LB–UB mean exposures of 0.130–0.157 $\mu\text{g As/kg bw per day}$ and LB–UB 95th percentile exposures of 0.397–0.477 $\mu\text{g As/kg bw per day}$. In the adult population,⁴¹ dietary exposure was similar across the different population groups with maximum LB–UB estimates of 0.038–0.044 $\mu\text{g As/kg bw per day}$ and 0.133–0.158 $\mu\text{g As/kg bw per day}$ for mean and 95th percentile exposures, respectively. The exposure estimates for 'Pregnant women' and 'Lactating women' were within those found in the adult population; the same was observed for the dietary exposure estimated in the only dietary survey on 'Vegetarians' (0.005–0.008 and 0.021–0.028 $\mu\text{g As/kg bw per day}$, LB-UB mean and 95th percentile exposures, respectively). Since the risk assessment on arsenic has traditionally been focused on iAs and/or total arsenic, no dietary exposure assessments for DMA were identified in the literature apart from the study from Hackethal et al. (2023). In this scientific paper describing the first German TDS (BfR MEAL Study), dietary exposure to DMA (median) was estimated for consumers of individual foods across different age groups. In that study, the highest median dietary exposure to DMA was reported following the consumption of plaice/sole for both children of 6–11 years (0.90 $\mu\text{g As/kg bw per day}$) and subjects ≥ 14 years (0.72 $\mu\text{g As/kg bw per day}$). Other relatively high exposures to DMA were estimated for consumers of cod liver, 0.65 $\mu\text{g As/kg bw per day}$ and 0.51 $\mu\text{g As/kg bw per day}$ in subjects ≥ 14 years and children 6–11 years, respectively.

Table 13 shows a summary of the average contribution to the dietary exposure to DMA of different food groups across population groups.

Overall, the main food contributors to the dietary exposure to DMA across population groups were 'Fish, seafood, amphibians, reptiles and invertebrates' and 'Grains and grain-based products', and additionally for part of the young population ('Infants', 'Toddlers'), 'Food products for young population' (all at FoodEx2 level 1). The food category 'Vegetables and vegetable products' despite comprising seaweeds, the commodities with the highest reported DMA values, were not identified as relevant contributors as their consumption is relatively low represented in the EFSA consumption. Due to the relatively small differences in the LB and UB exposure estimates, the contributions shown here for the LB are also applicable to the UB estimations.

In 'Infants', the main food contributors were 'Fish meat' (3%–78%, median 13%) and 'Cereals for infants or children, reconstituted' in the infant population (0%–72%, median 33%). In 'Toddlers', the most relevant contributor was 'Fish meat' (14%–77%, median 29%) followed by 'Rice' (11%–65%, median 29%), with 'Cereals for infants or children, reconstituted' being less relevant as compared to infants (see Table 13). Within the different types of fish meat, those codified as 'Marine fish' were by far the main food contributing to the dietary exposure to DMA, with a median contribution across 'Toddlers' of 25% and up to 77% average contribution in the dietary survey with the highest dietary exposure to DMA. In the age class 'Other children', 'Rice' became the most relevant average contributor (17%–72%, median 43%), but also 'Fish meat' kept its relevance in the dietary exposure to DMA (16%–61%, median 30%).

Overall, in the adult population ('Adults', 'Elderly', 'Very elderly'), again 'Fish meat' and 'Rice' were the most relevant food contributors to the dietary exposure to DMA, although it can be said that the impact of the consumption of 'Fish meat' was to some extent more important with higher medians and higher maximum contributions (see Table 13). Average contribution of 'Fish meat' in the age class 'Very elderly' went up to 92% with a median contribution across surveys of 47%. In the adult population also other food commodities became relevant for the exposure to DMA in some countries, such as 'Dried/smoked/salted fish' with contribution up to 27% in 'Elderly' age class (median 6%) and 'Molluscs' up to 36% (median 4%) in 'Adults'.

⁴¹In this scientific opinion, adult population refers to the population groups 'Adults', 'Elderly' and 'Very elderly'.

TABLE 13 Average contribution of selected food groups to the mean dietary exposure to DMA (% at the LB estimations) across population groups.

	Grains and grain-based products						Food products for young population			Fish, seafood, amphibians, reptiles and invertebrates								
	Rice			Puffed/extruded rice textured bread			Cereals for infants or children, reconstituted			Fish meat			Dried/smoked/salted fish			Molluscs		
	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max
Infants	5	18	46	0	0.0	2	0	33	72	3	13	78	0	0	4	0	0	0.9
Toddlers	11	35	65	0	0.4	10	0	4	26	14	29	77	0	1	7	0	0.1	8
Other children	17	43	72	0	0.3	12	0	0.1	3	16	30	61	0	2	8	0	0.6	21
Adolescents	20	44	71	0	0.4	8	0	0	8	10	31	60	0	2	9	0	2	46
Adults	16	32	70	0	0.3	2	0	0	1	17	31	63	0	6	25	0	4	36
Elderly	9	20	65	0	0.1	2	0	0	0	12	44	69	0	6	27	0	2	32
Very elderly	7	25	64	0	0.0	1	0	0	0	25	47	92	0	5	24	0	0.2	17
Pregnant women	23	40	76	0	0.1	1	0	0	0	13	23	56	0	3	15	0	6	38
Lactating women	22	–	43	0	–	3	0	–	0	22	–	50	4	–	16	0	–	7

3.7.1.1 | Rice-based infant formula scenario with DMA

Typically, no DMA is expected to be present in milk-based infant formula. However, recently there is an increasing consumption of alternative infant formulas such as soya protein- and rice protein-based formulas for infants with cow's milk protein allergy and/or lactose intolerance. Since rice is one of the best-known sources of DMA in the diet, the potential dietary exposure to DMA following the consumption of rice-based infant formula was assessed.

The work of Meyer et al. (2018) was used to obtain information on few samples of dry infant formula powder made of hydrolysed rice. DMA concentrations varied between 2 and 19 µg As/kg, with an average value of 7.6 µg As/kg (*n* = 7, expressed in all cases as dry matter). These occurrence values were used after applying a dilution factor of eight for the reconstitution of the dry formula powder. As consumption data, values of 200 and 260 mL/kg bw per day as conservative mean and high- level consumption were used, as recommended by the EFSA Scientific Committee for infants below 16 weeks of age (EFSA Scientific Committee, 2017).

Two exposure scenarios were considered. One scenario using the mean DMA concentration as typically used for chronic dietary exposure estimates (scenario 1). A second one, using the maximum DMA concentration reported to account for a situation where infants might consume for a relatively long period a highly contaminated product, as infants are expected to regularly consume the same brand and, in the short term, even the same batch (scenario 2). In both scenarios, the occurrence values were combined with both mean and high-level consumption. The different exposure estimates are shown in Table 14. Potential dietary exposure to DMA in infants following the consumption of rice-based infant formula could be up to 0.25 µg As/kg bw per day for high consumers when considering average DMA levels. If high presence of DMA is considered, the dietary exposure in high consumers could be up to 0.62 µg As/kg bw per day.

TABLE 14 Dietary exposure estimates to DMA via the consumption data of rice-based infant formula using default consumption data as recommended by the EFSA Scientific Committee.^a

	Dietary exposure to DMA (µg As/kg bw per day)	
	Mean exposure	High exposure
Scenario 1 ^b	0.19	0.25
Scenario 2 ^c	0.48	0.62

Abbreviation: DMA, dimethylarsinic acid.

^aFor the exposure estimations, default consumption values of 200 mL/kg bw per day (mean) and 260 mL/kg bw per day (high level) were used (EFSA Scientific Committee, 2017).

^bReported mean levels for DMA in seven samples of rice-based infant formula were used for the exposure estimations (7.6 µg As/kg d.m, 0.95 µg As/kg ready to eat).

^cThe maximum reported value for DMA across the seven samples of rice-based infant formula was used for the exposure estimations (19 µg As/kg d.m, 2.4 µg As/kg ready to eat).

Additionally, particular attention was paid to the dietary exposure to DMA of two groups of population based on the occurrence data available and the exposure estimations: consumers of fish meat and consumers of rice. To better understand the exposure to DMA in these subjects, the dietary exposure to DMA in these consumers ('consumers only') is shown below.

3.7.1.2 | Rice consumers

Dietary exposure to DMA was estimated for average consumers and high consumers (95th percentile exposure) reporting the consumption of different types of rice (Table 15). The range of consumption of rice (minimum–maximum) for average and high consumers (95th percentile consumption) across dietary surveys and population groups is also provided in Table 15.

Consumption data were extracted from the EFSA Comprehensive database considering the dietary surveys described in Annex D. DMA mean concentrations in rice used for the exposure estimations, 33.5–34.1 µg As/kg bw per day (LB–UB), were derived from the data submitted to EFSA as described in Section 3.6.1.1 (see Table 9).

Table 15 shows the range of chronic dietary exposures to DMA in different dietary surveys across population groups (minimum–median–maximum, LB–UB). Among average consumers of rice ('consumers only'), the highest mean exposure was identified in one dietary survey within the age class 'Other children' with estimates of 0.076–0.078 µg As/kg bw per day (LB–UB). Similarly, when assessing high consumers of rice ('consumers only'), the highest 95th percentile exposure was estimated in one dietary survey also in the age class 'Other children' (0.186–0.190 µg As/kg bw per day, LB–UB).

TABLE 15 Chronic consumption of different types of rice,^a and dietary exposure to DMA across different dietary surveys ('consumers only', mean and 95th percentile).

	Consumption across dietary surveys (grams/day) ^a		Dietary exposure (LB-UB) across dietary surveys (µg As/kg bw per day) ^a					
	Range (min–max)		Mean			95th percentile		
	Mean	95th percentile	Min	Median	Max	Min	Median	Max
Infants	1–12	2–20	0.007–0.007	0.039–0.040	0.058–0.059	0.028–0.028	0.056–0.057	0.105–0.107
Toddlers	1–21	2–35	0.015–0.015	0.033–0.034	0.063–0.063	0.038–0.039	0.086–0.087	0.166–0.169
Other children	2–41	4–83	0.010–0.010	0.028–0.029	0.076–0.078	0.031–0.031	0.070–0.071	0.186–0.190
Adolescents	2–55	7–86	0.006–0.006	0.018–0.018	0.035–0.036	0.017–0.018	0.045–0.046	0.090–0.091
Adults	2–54	5–100	0.003–0.004	0.014–0.015	0.028–0.029	0.012–0.013	0.033–0.034	0.068–0.069
Elderly	2–65	5–116	0.003–0.003	0.011–0.012	0.036–0.037	0.008–0.008	0.024–0.024	0.061–0.062
Very elderly	2–59	3–86	0.007–0.007	0.012–0.012	0.030–0.031	0.027–0.027	–	0.062–0.063
Pregnant women	6–76	29–115	0.005–0.005	0.015–0.016	0.043–0.044	0.018–0.018	0.031–0.032	0.095–0.097
Lactating women	14–20	40	0.013–0.013	–	0.014–0.014	–	–	0.030–0.030
Vegetarians	10	18	0.006–0.006	–	–	0.017–0.017	–	–

Abbreviations: LB: lower bound; UB: upper bound.

^aConsumption data were extracted from the EFSA Comprehensive database for the eating occasions reported as 'Rice and similar' - (FoodEx2 Level 4) excluding 'Rice grain, red', 'Indian rice grain', 'African rice grain' and 'Hybrid Nerica®') considering the dietary surveys described in Annex D.

^aMean and 95th percentiles across dietary surveys were only considered when at least five and 59 consumers, respectively, were available within a dietary survey and population group. Median was estimated when at least five dietary surveys within each population group were available with mean and 95th percentiles estimated.

3.7.1.3 | Fish consumers

Dietary exposure to DMA was estimated for average consumers and high consumers (95th percentile exposure) reporting the consumption of different types of fish meat (Table 16). The range of consumption of fish meat (minimum–maximum) for average and high consumers (95th percentile consumption) across dietary surveys and population groups is also provided in Table 16.

Consumption data were extracted from the EFSA Comprehensive database considering the dietary surveys described in Annex D. DMA mean concentrations for the different types of fish meat (unspecified fish meat, diadromous fish and marine fish) used for the exposure estimations were derived from the data submitted to EFSA as described in Section 3.6.1.1 (see Table 9).

Table 16 shows the range of chronic dietary exposures to DMA in different dietary surveys across population groups (minimum–median–maximum, LB–UB). Among average consumers of fish meat ('consumers only'), the highest mean exposure was identified in one dietary survey within the age class 'Toddlers' with estimates of 0.165–0.205 µg As/kg bw per day (LB–UB). Similarly, when assessing high consumers of fish meat, the highest 95th percentile exposure was estimated in one dietary survey also in the age class 'Toddlers' (0.415–0.511 µg As/kg bw per day, LB–UB). These dietary exposure estimates are in line with those reported by Hackethal et al. (2023) for consumers of different types of fish across different age classes.

TABLE 16 Chronic consumption of different types of fish^a and dietary exposure to DMA across different dietary surveys ('consumers only', mean and 95th percentile).

	Consumption across dietary surveys (grams/day) ^a		Dietary exposure (LB-UB) across dietary surveys (µg As/kg bw per day) ^b					
	Range (min–max)		Mean			95th percentile		
	Mean	95th percentile	Min	Median	Max	Min	Median	Max
Infants	2–34	6–63	0.014–0.019	0.047–0.067	0.155–0.192	0.046–0.058	–	0.332–0.409
Toddlers	3–46	8–105	0.015–0.019	0.063–0.084	0.165–0.205	0.043–0.053	0.169–0.215	0.415–0.511
Other children	5–60	16–131	0.016–0.020	0.046–0.061	0.095–0.117	0.048–0.059	0.107–0.132	0.245–0.302
Adolescents	7–83	19–149	0.007–0.009	0.030–0.040	0.069–0.086	0.022–0.027	0.072–0.090	0.121–0.149
Adults	6–112	18–181	0.006–0.008	0.017–0.022	0.056–0.070	0.018–0.023	0.060–0.074	0.117–0.144

(Continues)

TABLE 16 (Continued)

	Consumption across dietary surveys (grams/day) ^a		Dietary exposure (LB–UB) across dietary surveys (µg As/kg bw per day) ^b					
	Range (min–max)		Mean			95th percentile		
	Mean	95th percentile	Min	Median	Max	Min	Median	Max
Elderly	5–106	16–156	0.008–0.010	0.026–0.034	0.059–0.073	0.021–0.026	0.061–0.082	0.095–0.117
Very elderly	5–76	70–143	0.008–0.013	0.023–0.030	0.030–0.038	0.043–0.054	–	0.077–0.095
Pregnant women	37–81	80–125	0.013–0.019	0.023–0.030	0.033–0.043	0.043–0.053	–	0.065–0.080
Lactating women	34–57	130	0.013–0.020	–	0.026–0.034	–	–	0.053–0.074
Vegetarians	27–97		0.005–0.006			–		

Abbreviations: LB, lower bound; UB, upper bound.
^aConsumption data were extracted from the EFSA Comprehensive database for the eating occasions reported as ‘Fish (meat)’(FoodEx2 Level 2) considering the dietary surveys described in Annex D.
^bMean and 95th percentiles across dietary surveys were only considered when at least five and 59 consumers, respectively, were available within a dietary survey and population group. Median was estimated when at least five dietary surveys within each population group were available with mean and 95th percentiles estimated.

3.7.2 | Dietary exposure to MMA

As described in Section 2.5, the dietary exposure estimations for MMA mainly focused on different scenarios with ‘consumers only’ of the main food commodities for which quantified MMA data were available. The selected food commodities were ‘Fish meat’, ‘Molluscs’ and ‘Processed/preserved fish’. Additionally, the total dietary exposure in ‘consumers only’ considering all occurrence data on MMA was also assessed. The exposure estimates calculated for each dietary survey across population groups are presented in Annex G (Tables G4–G6).

Table 17 shows the mean and 95th percentile exposure for ‘consumers only’ of ‘Fish meat’, ‘Molluscs’ and ‘Processed/preserved fish’. For each population group and food 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 five and 59 consumers were available, respectively. The highest exposures were estimated for high consumers of fish meat in infants and high consumers of processed/preserved fish in the elderly age class, in both cases with MMA estimates of 0.342 µg As/kg bw per day. The most relevant foods contributing to this exposure were ‘Miscellaneous demersal marine fishes’ (e.g. monkfish) and ‘Canned herrings’. Among the consumers of molluscs, the highest mean and 95th percentile exposures were estimated in the adult population, with 0.088 µg As/kg bw per day and 0.185 µg As/kg bw day, respectively. Similar to the situation with DMA, for MMA there are almost no studies in the literature on dietary exposure in humans, also because MMA is present in only few foods and typically at relatively low levels. The German TDS (Hackethal et al., 2023) provided some exposure estimations (median) in consumers of specific commodities across different age groups. Overall, the maximum dietary exposures for some of the selected commodities are similar to those estimated in the current assessment. As an example, median dietary exposure of 0.18 µg As/kg bw per day in consumers of herrings or 0.25 µg As/kg bw per day in consumers of cod liver are reported as maximum estimates (Hackethal et al., 2023).

TABLE 17 Range of dietary exposure to MMA in ‘consumers only’ of ‘Fish meat’, ‘Molluscs’ and ‘Processed/preserved fish’ across dietary surveys.

	Dietary exposure (µg As/kg bw per day) ^a (minimum–maximum)					
	Fish meat		Molluscs		Processed or preserved fish	
	Mean exposure	95th exposure	Mean exposure	95th exposure	Mean exposure	95th exposure
Infants	0.006–0.124	0.013–0.342	–	–	0.044	0.146
Toddlers	0.006–0.077	0.019–0.239	0.006–0.018	–	0.003–0.102	0.117
Other children	0.008–0.066	0.023–0.133	0.007–0.086	–	0.003–0.046	0.006–0.046
Adolescents	0.004–0.036	0.011–0.090	0.003–0.076	0.011–0.016	0.002–0.035	0.011–0.012
Adults	0.003–0.041	0.010–0.082	0.002–0.071	0.012–0.185	0.001–0.064	0.144–0.236
Elderly	0.004–0.048	0.012–0.098	0.002–0.088	–	0.002–0.098	0.140–0.342
Very elderly	0.008–0.024	0.023–0.070	0.005–0.083	–	0.002–0.167	–
Pregnant women	0.011–0.044	0.031–0.066	0.001–0.003	–	0.002–0.103	–
Lactating women	0.017–0.019	0.046	–	–	0.047	–

^aFor ‘Fish meat’ and ‘Processed/preserved fish’, the MMA values were retrieved from the literature (see Table 11 for the details on how the occurrence values were derived). Dietary exposure for these commodities was estimated using the reported value. For ‘Molluscs’, MMA data submitted to EFSA were used for the exposure estimations; for simplicity only, the UB exposure estimates are shown.

To complement the dietary exposure estimations of individual groups of food commodities, dietary exposure was also estimated to cover the plausible situation where individuals are exposed to MMA through the consumption of more than one group of food commodities (e.g. a consumer of molluscs and fish meat). All the occurrence values available for MMA as shown in Table 11 Section 3.6.1.2 were used. Considering 'consumers only', the highest mean MMA exposure was 0.124 µg As/kg bw per day, and the highest 95th percentile MMA exposure 0.342 µg As/kg bw per day, in both cases in infants (Annex G, Table 4). These estimates are in line with those shown in Table 17 taking into account the consumption of only individual groups of food commodities.

3.8 | Risk characterisation

A risk characterisation could only be carried out for MMA(V) and DMA(V).

3.8.1 | Risk characterisation of DMA(V)

The CONTAM Panel selected the BMDL₁₀ of 1.1 mg DMA(V)/kg bw per day (equivalent to 0.6 mg As/kg bw per day) for induction of bladder tumours in a 2-year study with male rats (Wei et al., 1999) as a reference point (RP) for risk characterisation for this compound.

Table 18 below presents the MOE values resulting from a comparison of the RP derived for DMA(V) with exposure estimates across dietary surveys and age groups (see Section 3.7.1). Comparison of the RP to estimated exposure values results in MOE values that range from 150,000 (lowest minimum LB exposure across national consumption surveys) to 3800 (highest maximum UB exposure across national consumption surveys) for the mean exposure estimates, and from 35,300 (lowest minimum LB) to 1300 (highest maximum UB exposure) for the 95th percentile exposure estimates across dietary surveys and age groups.

As can be seen in Table 18, the calculated MOEs are below 10,000 in many of the dietary surveys, in particular for 95th percentile exposures.

TABLE 18 DMA (V) Margin of exposure for mean and P95 dietary exposure estimates.^{a,b}

Age group	MOE calculated from the mean dietary exposure						MOE calculated from the P95 dietary exposure					
	Minimum		Median		Maximum		Minimum		Median		Maximum	
	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
Infants	33,300	28,600	17,100	13,600	10,200	8100	8700	7500	6000	5000	2400	1900
Toddlers	31,600	27,300	17,600	14,300	4600	3800	9000	8100	4200	3800	1500	1300
Other children	50,000	37,500	23,100	18,800	7100	6100	10,300	9200	6200	5600	2300	1900
Adolescents	85,700	75,000	40,000	35,300	12,500	11,100	20,700	17,600	10,500	8800	4500	3800
Adults	120,000	100,000	46,200	40,000	15,800	13,600	28,600	24,000	10,300	9000	5500	4400
Elderly	150,000	120,000	46,200	37,500	16,200	13,600	35,300	30,000	11,300	9700	4800	4000
Very elderly	85,700	66,700	54,500	42,900	18,200	15,400	23,100	20,700	11,500	10,200	6600	5800
Pregnant women	150,000	100,000	31,600	27,300	15,400	13,000	33,300	28,600	9000	7800	5500	5000
Lactating women	54,500	42,900	–	–	24,000	19,400	14,300	12,000	–	–	9700	7700

Abbreviations: LB, lower bound; MOE, margin of exposure; P95, 95th percentile; UB, upper bound.

^aThe MOEs were rounded to the second figure for better readability.

^bMOEs below 10,000 are in bold.

The MOEs derived for DMA(V) in the different exposure scenarios on rice-based infant formula (see Section 3.7.1.1) were 3200 (assuming mean DMA concentrations and mean consumption), 2400 (assuming mean DMA concentrations and high consumption), 1250 (assuming maximum DMA concentration and mean consumption) and 970 (assuming maximum DMA concentrations and high consumption), respectively. All MOEs were lower than 10,000. The MOEs derived for DMA(V) in the specific exposure scenario for rice consumers (see Section 3.7.1.2) ranged from 200,000 (minimum, LB) to 7700 (maximum, UB) for average exposure and from 75,000 (minimum, LB) to 3200 (maximum, UB) for P95 exposure (high consumers), respectively, across dietary surveys. MOE were lower than 10,000 at the highest average exposures for 'Toddlers' and 'Other children', and at the highest P95 exposures in each of the population groups except for 'Lactating women' and 'Vegetarians'. The MOEs derived for DMA(V) in the specific exposure scenario for fish consumers (see Section 3.7.1.3) ranged from 100,000 (minimum, LB) to 2900 (maximum, UB) for average exposure and from 33,300 (minimum, LB) to 1200 (maximum, UB) for P95 exposure (high consumers), respectively, across dietary surveys. MOEs were lower than 10,000 at the highest average exposure in 'Infants', 'Toddlers', 'Other children', 'Adolescents', 'Adults' and 'Elderly', and at the highest P95 exposures in each one of the population groups.

The CONTAM Panel noted that MOEs $\leq 10,000$ raise a health concern but also noted the uncertainties associated with interpretation of the mode of action for carcinogenicity with regard to the extent to which DNA-reactive mechanisms contribute to DMA(V) genotoxicity as well as the role of genotoxicity in its carcinogenicity (see Section 3.1.3.3 on Genotoxicity).

3.8.2 | Risk characterisation of MMA(V)

The CONTAM Panel selected the BMDL₁₀ of 18.2 mg MMA(V)/kg bw per day (equivalent to 9.7 mg As/kg bw per day) for reduced body weight seen in a 2-year study with male rats (Arnold et al., 2003) as a reference point for risk characterisation for this compound.

Table 19 below presents the MOE values for the different age groups resulting from a comparison of the RP derived for MMA(V) with exposure estimates for the different age groups focussing on 'consumers only' of the dietary exposure via the main food commodities for which quantified MMA data were available (see Section 3.7.2). The selected food commodities were 'Fish meat', 'Molluscs' and 'Processed/preserved fish'. MOEs ranged from 3,233,000 (average exposure, minimum) to 28,000 (P95 exposure, maximum) for fish meat consumers, from 9700,000 (average exposure, minimum) to 52,000 (P95 exposure, maximum) for molluscs consumers and from 9700,000 (average exposure, minimum) to 28,000 (P95 exposure, maximum) for processed/conserved fish consumers. As all MOEs were well above 500, the CONTAM Panel did not identify a health concern from dietary exposure to MMA(V) for these consumers.

TABLE 19 MMA(V): Margins of exposure from average and P95 exposure for fish meat, molluscs and processed/preserved fish consumers.

	MOE											
	Fish meat				Molluscs				Processed or preserved fish			
	Average exposure		95th percentile exposure		Average exposure		95th percentile exposure		Average exposure		95th percentile exposure	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Infants	1,617,000	78,000	746,000	28,000	n.a.	n.a.	n.a.	n.a.	220,000	220,000	66,000	66,000
Toddlers	1,617,000	126,000	511,000	41,000	1,617,000	539,000	n.a.	n.a.	3,233,000	95,000	83,000	83,000
Other children	1,213,000	147,000	422,000	73,000	1,386,000	113,000	n.a.	n.a.	3,233,000	211,000	1,617,000	211,000
Adolescents	2,425,000	269,000	882,000	108,000	3,233,000	128,000	882,000	606,000	4,850,000	277,000	882,000	808,000
Adults	3,233,000	237,000	970,000	118,000	4,850,000	137,000	808,000	52,000	9700,000	152,000	67,000	41,000
Elderly	2,425,000	202,000	808,000	99,000	4,850,000	110,000	n.a.	n.a.	4,850,000	99,000	69,000	28,000
Very elderly	1,213,000	404,000	422,000	139,000	1,940,000	117,000	n.a.	n.a.	4,850,000	58,000	n.a.	n.a.
Pregnant women	882,000	220,000	313,000	147,000	9700,000	3,233,000	n.a.	n.a.	4,850,000	94,000	n.a.	n.a.
Lactating women	571,000	511,000	211,000	211,000	n.a.	n.a.	n.a.	n.a.	206,000	206,000	n.a.	n.a.

Abbreviation: n.a., not applicable.

3.9 | 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 has developed a road map that structures the risk assessment process in broader groupings, as well as subgroupings. Sets of questions have also been defined to help the collection of uncertainties. There are four overarching elements of the road map: (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 20–22. As an extension of (iv) a quantitative of overall uncertainty in the calculated MOEs for DMA(V) is performed. The overall conclusion in section is therefore based on the quantitative analysis in combination with qualitative consideration of remaining uncertainty.

3.9.1 | Identification and prioritisation of uncertainties

The uncertainties for small organoarsenic species are listed in Tables 20–22. Based on the occurrence data submitted to EFSA exposure could only be assessed for DMA and MMA, and because of the limited occurrence data for MMA, no classical exposure assessment was possible for that arsenic species. Also, it was noted that only one study was suitable for dose–response modelling for DMA(V) and MMA(V) each. At the level of the risk characterisation (MOE) the impact of identified uncertainties related to data gaps, as well as in the case of DMA(V) the extent to which genotoxicity contributed to carcinogenicity and the unclear mode of genotoxic action were regarded to be of high priority.

TABLE 20 Elements of the CONTAM road map and relevance for the uncertainty analysis – chemical characterisation and analytical methods, hazard identification and characterisation and risk characterisation.

Main group	Subgroup	Overarching questions	Description of uncertainty	Priority ranking ¹
Chemical composition and analytical methods	Chemical composition	Is there uncertainty associated with the dose in the critical studies used in the risk assessment?	Uncertainty in the applied dose in cases where the arsenic species is reported as ppm and the precise form of the dosing compound is not reported, e.g. DMA(V) as the acid or as the sodium salt	1
			Uncertainty in the applied dose in cases where the arsenic species is not a commercial product of specified purity. Example 1: An arsenic species has been synthesised, purified and characterised in the research lab, but criteria of purity are not provided. Example 2: An arsenic species has been produced in situ from an arsenic precursor (e.g. MMA(III) from diiodomethylarsine), and neither the quantity nor the purity of the target toxicant has been established.	1
	Analytical methods	Are the analytes being reliably identified and reported?	Some analytes could be assigned the wrong structure, e.g. MMA(V) reported as MMA(III); thio-DMA(V) reported as DMA(III), arsenic species reported without valency e.g. DMA or MMA Current methods might not be suitable for all arsenic analytes.	2
		Are the target analytes being reliably quantified?	Labile analytes might be transformed to other arsenic analytes during the sample collection and storage, and during the various stages of the analytical process	2
		Are different labs producing consistent and similar results?	Intra- and interlaboratory variability	2
Hazard identification and characterisation	ADME	Is there uncertainty in any aspect of ADME?	Yes. There is insufficient information for all aspects of ADME for all small organoarsenic species other than MMA(V) and DMA(V)	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?	Many of the studies are of non-standard design with a limited range of endpoints. However, the critical studies used to identify reference points are well designed and reported	1
	Genotoxicity	Is there uncertainty on the genotoxicity of the compounds?	Yes. In vitro, there is inadequate evidence for the genotoxicity of thio-DMA(V), adequate evidence for the genotoxicity of MMA(III) and DMA(III) and moderate evidence for MMA(V) and DMA(V). In vivo, there are no studies for MMA(III), DMA(III) and thio-DMA(V) and only inadequate and limited evidence for the genotoxicity of MMA(V) and DMA(V), respectively	2

TABLE 20 (Continued)

Main group	Subgroup	Overarching questions	Description of uncertainty	Priority ranking ¹
	Mode of action	Are there uncertainties on the MoA of the substance that could affect the conclusions of the risk assessment?	Yes. There is uncertainty regarding the contribution of DNA reactive mechanisms of action to the genotoxic effects of small organoarsenic species	2
		Is there uncertainty on the human relevance of the MoA identified in experimental animals?	There is also uncertainty regarding the relative importance of the different MOAs identified for carcinogenicity and whether thresholded mechanisms for carcinogenicity can be assumed. The approach to risk assessment of DMA(V) is based on the most conservative assumptions	3
	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?	Negligible. For MMA(V) a BMR of 10% for reduced bw was selected that is in line with international guidance. Likewise, for DMA(V), a BMR of 10% was selected as the default for quantal data given in EFSA guidance	0
		Are there uncertainties in the selection of the RP that are not covered by the BMD confidence interval e.g. is model uncertainty covered	Yes. Not all uncertainties related to the animal studies and dose–response analysis of the critical endpoints might be covered	2

¹0 – no or negligible uncertainty, 1 – uncertainty with low priority, 2 – uncertainty with medium priority, 3 – uncertainty with high priority.

TABLE 21 Elements of the CONTAM road map and relevance for the uncertainty analysis – occurrence and exposure assessment.

Main group	Sub-group	Overarching questions	Description of uncertainty	Priority ranking ¹
Occurrence data	Analytical measurements	Is there uncertainty due to the performance of the analytical method? This may include identification, sensitivity and recovery	The method might not be capable of specifically identifying the As species, e.g. it might provide only generic data, such as DMA as opposed to DMA(V), DMA(III) or thio-DMA(V)	2
			The separation of As species (e.g. by LC) is not sufficient to allow reliable quantification.	1
			Analyte recoveries following extraction and chromatography have not been assessed and compensated for. Mass balance data are not provided	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
			Missing information in reporting the occurrence data (e.g. analytical method)	1
			For a number of samples, there is uncertainty on whether the DMA and MMA concentrations were expressed as elemental arsenic or µg As species/kg. When information was not available, the data were assumed to be submitted as µg As/kg.	2
		Is there uncertainty in the information on sampling strategy	For around 40% of the samples reported for DMA and MMA, no information was provided on the sampling strategy. It cannot be excluded that, for some of these samples, random sampling was not followed.	1

(Continues)

TABLE 21 (Continued)

Main group	Sub-group	Overarching questions	Description of uncertainty	Priority ranking ¹		
Dietary exposure estimates methodology	Consumption data	Data reporting	Composite foods with no clear information about ingredients and their proportion	1		
			Overall, the occurrence data set for both MMA and DMA is rather small. There is uncertainty related to the levels reported for rice and, in particular, for the different fish species	2		
			Low number of reporting countries	1		
			Not optimal distribution of year of samplings (e.g. too many old data)	1		
			Extrapolation of data from one food category to others (deriving occurrence values for processed commodities using those reported in raw primary commodities)	2		
			Is there uncertainty in the occurrence data due to lack of data for potentially relevant major food categories?	Lack of data for potentially relevant major food categories for MMA	2	
			Is there uncertainty in the occurrence data due to left censorship and the substitution method	Percentage of left-censored data	1	
			Representativeness of the data	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	
	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				
	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 form of the food reported (powder/liquid/reconstituted, etc.)	Occurrence data submitted to EFSA refers to uncooked food while some consumption data might refer to cooked food.	1		
		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- to 3-day 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	

¹0 – no or negligible uncertainty, 1 – uncertainty with low priority, 2 – uncertainty with medium priority, 3 – uncertainty with high priority.

TABLE 22 Elements of the CONTAM road map and relevance for the uncertainty analysis – risk characterisation.

Main group	Subgroup	Overarching questions	Description of uncertainty	Priority ranking ¹
Risk characterisation	Uncertainty factors	Is there uncertainty in the selected (default or specific) UFs?	The uncertainty factor for MMA(V) includes, next to the default factor of 100 for toxicokinetics and toxicodynamics, an additional factor of 5 for deficiencies in the database.	1
	Critical margin of exposure	Is there uncertainty in the margin of exposure of low concern?	For DMA(V), a margin of exposure of 10,000 was considered to be of low concern based on EFSA (2005). This is regarded as a standard uncertainty	1
	Risk metric	Is there uncertainty in the calculated margin of exposure?	Yes. Uncertainty in the exposure estimates and the BMDs derived for the critical endpoints. Also, occurrence data assumed in µg As/kg are related to BMDs for DMA and MMA. The latter also assumes that DMA and MMA effectively is DMA(V) and MMA(V), respectively	2

¹0 – no or negligible uncertainty, 1 – uncertainty with low priority, 2 – uncertainty with medium priority, 3 – uncertainty with high priority.

3.9.2 | Quantification of uncertainties in the calculated margins of exposure for DMA (V)

A quantification of the uncertainty in the calculated margin of exposure was performed. This data-driven and conditional analysis is based on information of uncertainty described by the exposure and BMD estimates in the Opinion, i.e. LB and UB scenarios for the exposure and the relevant BMD credible interval. The quantitative analysis was carried out for DMA (V), but not MMA (V). For MMA (V), the most conservative MOEs results (i.e. exposure at UB vs. the BMDL) in Table 19 were all very large. In such a case, the analysis described below will also not raise a concern.

For DMA (V), Monte Carlo simulations were conducted to support a (data-driven) quantification of overall uncertainty. The uncertainty in the BMD for total urinary bladder tumour incidence in male rats, and the uncertainty for a given exposure scenario was characterised by a probability distribution. These distributions were then combined to a distribution for the margin for exposure. The probability of an MOE < 10,000 was then estimated for each population group and exposure scenario (the mean and 95th percentile of exposure).

3.9.2.1 | Technical description of the approach

A generalised extreme value distribution was used to approximate the non-parametric BMD uncertainty distribution generated by the EFSA BMD software. The estimation of this distribution, using the BMDL, BMD and BMDU as inputs, is described in detail in EFSA CONTAM Panel (2024) where this approach was also validated for consistency against results from directly using the distribution generated by the BMD software internally (posterior BMD distribution). The uncertainty distribution for the mean and 95th percentile of exposure was assumed to be uniform, respectively. The lower and upper limits of the distribution were estimated by consideration of MIN and MAX results across LB and UB scenarios. More details can be found in EFSA CONTAM Panel (2024). MOEs were estimated by Monte Carlo simulations by randomly combining N = 30,000 generated BMD and exposure values from the respective uncertainty distribution. Probabilities in Tables 23–25 correspond to the frequency of an MOE below 10,000 for each exposure scenario and population group.

3.9.2.2 | Results

Results in terms of the estimated probability of an MOE < 10,000 in Tables 23–25 for the different exposure scenarios covered in the Opinion are discussed below. The approximate probability scale recommended for harmonised use in EFSA (EFSA Scientific Committee, 2018) is consulted in this process.

3.9.2.2.1 | Dietary chronic exposure

As shown in Table 23, for the mean exposure, it is unlikely that the MOE is below 10,000 for ‘Toddlers’ (probability ≈ 0.15), and it is very unlikely that this is the case for ‘Infants’ and ‘Other children’ (probability ≈ 0.02–0.04), or any of the other population groups (probability < 0.01). However, considering the 95th percentile of exposure it is about as likely as not, or likely, that the MOE is below 10,000 for ‘Infants’, ‘Toddlers’ and ‘Other children’ (probability ≈ 0.6–0.7, Table 23). For remaining population groups, the conclusion of the result suggests that an MOE < 10,000 is unlikely or very unlikely (probability ≈ 0.05–0.20, Table 23).

TABLE 23 Probability of an MOE < 10,000 considering chronic dietary exposure to DMA (V) (Table 12).

Population groups	Mean exposure		95th percentile	
Infants	0.02	Very unlikely	0.65	About as likely as not
Toddlers	0.15	Unlikely	0.72	Likely
Other children	0.04	Very unlikely	0.59	About as likely as not
Adolescents	< 0.01	Very unlikely	0.20	Unlikely
Adults	< 0.01	Very unlikely	0.12	Unlikely
Elderly	< 0.01	Very unlikely	0.13	Unlikely
Very elderly	< 0.01	Very unlikely	0.08	Very unlikely
Pregnant women	< 0.01	Very unlikely	0.09	Very unlikely
Lactating women	< 0.01	Very unlikely	0.05	Very unlikely

Note: Estimated probabilities are classified by terms according to the approximate probability scale recommended for harmonised use in EFSA Scientific Committee (2018). The scale has been simplified at the extremes so that all probabilities below 0.10 and above 0.90 are classified as ‘very unlikely’ and ‘likely’, respectively. Probability estimates below 1% are reported as < 0.01.

3.9.2.2.2 | Other exposure scenarios

For rice-based infant formula a MOE < 10,000 it is very likely (or certain) with respect to both the mean and the high exposure scenario (probability ≈ 1.0, Table 24). However, for ‘Infants’, ‘Toddlers’ and ‘Other children’, the broader assessment of rice

consumption ('consumers only') indicates that it is very unlikely (probability ≤ 0.02) to unlikely (probability $\approx 0.1\text{--}0.3$) that the MOE is below 10,000 with respect to the mean and 95th percentile of exposure, respectively (Table 24). For other population groups, the conclusion is 'very unlikely' for both the mean and 95th percentile of exposure (probability ≤ 0.06 , Table 24).

TABLE 24 Probability of an MOE < 10,000 considering exposure to DMA from consumption of rice-based infant formula (Table 14), and from chronic consumption of different types of rice (consumers only) across population groups (Table 15).

Population groups	Mean exposure		High exposure or 95th percentile ^a	
Rice-based infant formula	0.98	Very likely	1.0	Very likely
Infants	< 0.01	Very unlikely	0.10	Unlikely
Toddlers	0.01	Very unlikely	0.28	Unlikely
Other children	0.02	Very unlikely	0.29	Unlikely
Adolescents	< 0.01	Very unlikely	0.05	Very unlikely
Adults	< 0.01	Very unlikely	0.01	Very unlikely
Elderly	< 0.01	Very unlikely	0.01	Very unlikely
Very elderly	< 0.01	Very unlikely	0.02	Very unlikely
Pregnant women	< 0.01	Very unlikely	0.06	Very unlikely
Lactating women ^b	< 0.01	Very unlikely	< 0.01	Very unlikely
Vegetarians ^b	< 0.01	Very unlikely	< 0.01	Very unlikely

Note: Estimated probabilities are classified by terms according to the approximate probability scale recommended for harmonised use in EFSA Scientific Committee (2018). The scale has been simplified at the extremes so that all probabilities below 0.10 and above 0.90 are classified as 'very unlikely' and 'likely', respectively. Probability estimates below 1% are reported as < 0.01.

^aFor rice-based infant formula, a high exposure scenario is assessed rather than the 95th percentile (see Table 14).

^bAs can be seen in Table 15, the LB and UB exposures are very close or the same for lactating women and vegetarians, respectively. In this case, the estimated MOE uncertainty distribution depends more or less fully on the BMD credible interval.

When specifically assessing fish consumption, and consumers only, the probability of a MOE < 10,000 with respect to the mean exposure is unlikely for 'Infants and 'Toddlers' (probability ≈ 0.20), while it is very unlikely for the remaining population groups (probability ≤ 0.07) (Table 25). For the 95th percentile of exposure, the probability of an MOE < 10,000 is about a likely as not for 'Infants', 'Toddlers' and 'Other children' (probability $\approx 0.5\text{--}0.6$, Table 25). It is unlikely or very unlikely for the other population groups (probability $\approx 0.06\text{--}0.15$, Table 25).

TABLE 25 Probability of an MOE < 10,000 considering exposure to DMA from chronic consumption of different types of fish (consumers only) across population groups (Table 16).

Population groups	Mean exposure		95th percentile	
Infants	0.19	Unlikely	0.59	About as likely as not
Toddlers	0.21	Unlikely	0.61	About as likely as not
Other children	0.07	Very unlikely	0.53	About as likely as not
Adolescents	0.02	Very unlikely	0.15	Unlikely
Adults	0.01	Very unlikely	0.13	Unlikely
Elderly	0.01	Very unlikely	0.09	Very unlikely
Very elderly	< 0.01	Very unlikely	0.10	Unlikely
Pregnant women	< 0.01	Very unlikely	0.06	Very unlikely
Lactating women	< 0.01	Very unlikely	0.08	Very unlikely
Vegetarians	< 0.01	Very unlikely	–	–

Note: Estimated probabilities are classified by terms according to the approximate probability scale recommended for harmonised use in EFSA Scientific Committee (2018). The scale has been simplified at the extremes so that all probabilities below 0.10 and above 0.90 are classified as 'very unlikely' and 'likely', respectively. Probability estimates below 1% are reported as < 0.01.

3.9.3 | Summary on uncertainties

Based on the quantitative uncertainty analysis, except for rice-based infant formula, it is unlikely or very unlikely that the MOE associated with any of the mean exposure scenarios is below 10,000. However, considering the 95th percentile of exposure, it is about as likely as not, or likely, that the MOE is below 10,000 for 'Infants', 'Toddlers' and 'Other children' with respect to the assessment of dietary exposure, as well as the specific assessment of fish consumption. Guided by the quantitative analysis, but also noting the identified uncertainties more broadly, the CONTAM Panel considers that it

is probable that the MOE is higher than 10,000 with respect to the mean exposure for most, if not all, population groups, and that the MOE is lower than 10,000 for the 95th percentile of exposure, at least for 'Infants', 'Toddlers' and 'Other children'.

4 | CONCLUSIONS

4.1 | General information

Small organoarsenic species contain methyl groups, but no other organic groups, bound to arsenic. Dimethylarsinic acid (DMA(V)) is by far the most abundant of these arsenic species in food, being reported in a range of samples with highest concentrations found in rice, and in algae and other seafoods.

The pentavalent oxo-analogues of small organoarsenic species are stable, both in food and in extracts of food, which makes their analysis relatively straightforward. The trivalent as well as the thio-containing small organoarsenic species are less stable in solution and convert readily to the pentavalent and slowly to the oxo-analogues, respectively. This lability greatly complicates their analysis.

4.2 | Toxicokinetics

- In experimental animals, MMA(V) and DMA(V) are well absorbed, distributed to various tissues and largely excreted unchanged in urine. They can, to a limited extent, undergo further methylation, while there is no evidence for demethylation.
- There are no data allowing estimation of absorption rates for other small organoarsenic species. One study with hamsters and rats showed efficient distribution of DMA(III), thio-DMA(V), dithio-DMA(V) (hamsters) and thio-MMA(V) (rats) to various organs, and urinary excretion of the pentavalent arsenic species following methylation of the monomethylated species and some interconversion of the pentavalent species.
- After a single oral dose in mice, MMA(III) was extensively methylated and more than 90% of the excreted dose in urine was DMA(V) and DMA(III).
- When MMA(V) and DMA(V) are ingested by humans, they seem to be well absorbed (> 75%), essentially being excreted unchanged in urine but also may undergo limited further metabolism.
- MMA(V) and DMA(V) have been shown to pass through the placenta and traces of DMA(V) have been detected in breast milk.
- In vitro studies with human cells indicate that the lower cellular uptake and transfer across intestinal barriers of pentavalent small organoarsenic species as compared to their trivalent forms is due to the negative charge of these species at physiological pH while trivalent species are neutral and more membrane permeable.
- Pentavalent species can be reduced to the corresponding trivalent form and there is in vitro evidence for presystemic thiolation and reduction of small organoarsenic species. The higher cellular uptake of the thio-compounds in cultured mammalian cells in comparison to their oxo-analogues can be explained by their lower polarity.

4.3 | Biomarkers

- Because small organoarsenic species are both contaminants in food and also metabolites of iAs and complex organoarsenic species, there is so far no arsenic species pattern in urine or blood that has been identified as a suitable biomarker for exposure to small organic arsenic species.

4.4 | Acute toxicity

- MMA(V), DMA(V) and TMAO are of low acute toxicity in laboratory animals. There are no data for the other small organoarsenic species.

4.5 | Repeated dose toxicity

- The limited data available on repeated dose toxicity in laboratory animals indicate that MMA(V) has adverse effects on the gastrointestinal tract, kidney, thyroid and liver and that DMA(III), DMA(V) and TMAO have effects on the urinary bladder and possibly on the kidney. There are no data for the other small organoarsenic species.

4.6 | Developmental and reproductive toxicity

- In developmental toxicity studies of MMA(V) and DMA(V) in rats and rabbits, developmental effects were only observed in the presence of maternal toxicity and no teratogenicity has been reported with either compound.

4.7 | Carcinogenicity

- Exposure of pregnant mice to MMA(III) resulted in tumours of the liver, lung and adrenals in male offspring and of the uterus and ovaries in female offspring.
- Two studies with MMA(V) found no increases in tumour incidences in a wide range of tissues in mice and rats.
- DMA(V) induces urinary bladder tumours in male and female rats.
- In mice, the results of different studies with DMA(V) are inconsistent, and do not provide convincing evidence of carcinogenicity.
- For TMAO, a single study in rats reported an increased formation of hepatocellular adenomas but no carcinomas and no tumours in other tissues.
- No carcinogenicity data are available for other small organoarsenic species.

4.8 | Genotoxicity

In the case of MMA(III) and DMA(III), there is evidence that they induce genotoxic effects *in vitro*. These effects include the induction of DNA single- and double-strand breaks, as well as clastogenic and aneugenic effects. Furthermore, they inhibit DNA repair mechanisms, promote the generation of ROS, generate oxidative damage to DNA and have the potential to induce cell transformation. Disruption of mitotic progression by altering the mitotic spindle apparatus may also lead to aneugenic as well as clastogenic effects. No *in vivo* genotoxicity studies are currently available.

- In the case of MMA(V) and DMA(V), the genotoxic effects are similar to those of the trivalent compounds although the evidence is less robust owing to the comparatively limited extent of investigation and some conflicting data. In the case of DMA(V) *in vivo* data show the induction of oxidative damage to DNA in various organs and one study reports the occurrence of DNA breaks in lymphocytes of orally exposed mice.
- Thio-DMA(V) presents distinct characteristics. It is highly cytotoxic *in vitro*, interferes with cytokinesis, inhibits PARP1 at low concentrations, yet it does not induce DNA breaks or oxidative damage to DNA. No *in vivo* genotoxicity studies are currently available.

4.9 | Mode of action

- The absence of mutagenic effects but the induction of chromosomal damage *in vitro* by MMA(III), DMA(III), MMA(V) and DMA(V) suggests that DNA breaks induced by oxidative damage to DNA, along with the inhibition of DNA repair, may be the pivotal events causing clastogenic effects.
- The *in vitro* genotoxic characteristics of DMA(V), coupled with the *in vivo* induction of oxidative DNA damage in various organs and DNA breaks in PBMC of orally exposed rodents, indicate a possible involvement of DNA damage in its carcinogenic effects.
- For MMA(III), no mode of action studies was identified with the exception of the genotoxicity studies. Since there is adequate evidence that MMA(III) is genotoxic *in vitro*, and despite no studies *in vivo*, the CONTAM Panel concluded that the mechanism of carcinogenicity is likely to involve genotoxicity.
- There are several plausible modes of action for the carcinogenicity of DMA(V), including genotoxicity as well as cytotoxicity followed by regenerative cell proliferation. There is uncertainty regarding the relative importance of the different MOAs, and whether thresholded mechanisms can be assumed.

4.10 | Observations in humans

- No human studies were identified on health outcomes related to concentrations of small organoarsenic species in food, but only related to MMA and DMA in urine. It is not possible to distinguish between ingested DMA and DMA formed by methylation of inorganic As or by catabolism of arsenosugars and arsenolipids. Therefore, studies reporting associations between MMA or DMA in urine and health outcomes in humans cannot be used for risk assessment for these compounds.

4.11 | Derivation of reference points and approach for risk assessment

- For MMA(V) decreased body weight as a result of observed diarrhoea following dietary exposure in rats was identified as the critical endpoint and a BMDL₁₀ of 18.2 mg MMA(V)/kg bw per day (equivalent 9.7 mg As/kg bw per day) was calculated as a reference point (RP).
- For DMA(V), an increase in urinary bladder tumours in rats following exposure via drinking water was identified as the critical endpoint and a BMDL₁₀ of 1.1 mg/kg bw per day (equivalent to 0.6 mg As/kg bw per day) was calculated as an RP.
- The toxicological data for other small organoarsenic species are insufficient to identify critical effects.
- Although the data available allow the derivation of reference points for MMA(V) and DMA(V), important data are missing, and therefore, the CONTAM Panel concluded that it is not appropriate to establish health-based guidance value but that the margin of exposure approach should be applied.
- The CONTAM Panel concluded that an MOE of ≥ 500 does not raise a health concern taking into account the default uncertainty factor of 100 for inter- and intra-species differences and an additional uncertainty factor of 5 to account for the deficiencies in the database.
- For DMA(V), there is convincing evidence that it is a rodent carcinogen. The mechanisms of genotoxicity and its role in carcinogenicity of DMA(V) are however not fully elucidated. In the absence of established MoAs for the genotoxicity/carcinogenicity of DMA(V), the Panel applied an MOE of 10,000 to the RP of 0.6 mg As/kg bw per day.

4.12 | Occurrence data

- One thousand and two hundred and sixty analytical results for DMA and 988 for MMA from sampling years 2012–2022 were available. All data were assumed to be reported for the pentavalent species when this information was not confirmed by the data providers.
- No confirmed data were received for DMA(III) or MMA(III).
- No data were received on thio-analogues thio-DMA(V), dithio-DMA(V) and thio-MMA(V).
- All data were converted into $\mu\text{g As/kg}$ before being used for dietary exposure assessment; when information was not available, the data were assumed to be submitted as $\mu\text{g As/kg}$.
- Data were collected in eight EU countries for DMA and in four for MMA; most of the samples were collected in Italy (67% for DMA, 85% for MMA).
- The left-censored data accounted for 44% and 94% of the analytical results on DMA and MMA, respectively.
- For DMA, the highest levels were reported in seaweeds and seaweed-containing products. Other commodities with relatively high levels of DMA were rice and rice-based commodities, and fish and seafood.
- For MMA, occurrence data submitted to EFSA were complemented with data from the literature (mainly on fish meat and processed/conserved fish) before estimating dietary exposure.
- Occurrence data from the scientific literature support the data submitted to EFSA for DMA(V) and MMA(V); they show that DMA(V) is commonly present in foods but at low levels, and MMA(V) is only occasionally reported, and then only as a trace constituent.
- Occurrence data for the other small organoarsenic species show that the levels are generally very low although occasional exceptions have been reported.
- Recent literature data for thio-DMA(V) suggest that application of a milder analytical procedure might reveal that this As species is more widespread than currently thought.

4.13 | Exposure assessment

- For DMA, the highest dietary exposure was estimated for the young population, in particular 'Toddlers', with LB–UB mean exposures of 0.130–0.157 $\mu\text{g As/kg bw per day}$ and LB–UB 95th percentile exposures of 0.397–0.477 $\mu\text{g As/kg bw per day}$.
- Overall, the main food contributors to the dietary exposure to DMA across population groups were 'Fish, seafood, amphibians, reptiles and invertebrates' and 'Grains and grain-based products', and additionally for part of the young population ('Infants, 'Toddlers'), 'Food products for young population' (all at FoodEx2 level 1).
- When considering the consumption of rice only, the highest mean and the highest 95th percentile exposure (high consumers) were identified in one dietary survey in the age class 'Other children' with estimates (LB–UB) of 0.076–0.078 $\mu\text{g As/kg bw per day}$ and 0.186–0.190 $\mu\text{g As/kg bw per day}$, respectively.
- When considering the consumption of fish meat only, the highest mean and the highest 95th percentile exposure (high consumers) were identified in one dietary survey in the age class 'Toddlers' with estimates (LB–UB) of 0.165–0.205 $\mu\text{g As/kg bw per day}$ and 0.415–0.511 $\mu\text{g As/kg bw per day}$, respectively.
- Potential dietary exposure to DMA in infants following the consumption of rice-based infant formula could be up to 0.25 $\mu\text{g As/kg bw per day}$ for high consumers when considering average DMA levels. If high presence of DMA is considered, the dietary exposure in high consumers could be up to 0.62 $\mu\text{g As/kg bw per day}$.

- For MMA, the highest exposures were estimated for high consumers of fish meat in infants and high consumers of processed/preserved fish in the elderly age class, in both cases with MMA estimates of 0.342 µg As/kg bw per day.
- Among the consumers of molluscs, the highest mean and 95th exposures to MMA were estimated in the adult population, with 0.088 µg As/kg bw per day and 0.167 µg As/kg bw per day, respectively.

4.14 | Risk characterisation

- A risk characterisation could only be carried out for DMA(V) and MMA(V).

4.14.1 | Risk characterisation of DMA(V)

- Comparison of the reference point of 0.6 mg As/kg bw per day with estimated exposure values results in MOE values that range from 150,000 (lowest minimum LB exposure across national consumption surveys) to 3800 (highest maximum UB exposure across national consumption surveys) for the mean exposure estimates, and from 35,300 (lowest minimum LB) to 1300 (highest maximum UB exposure) for the 95th percentile exposure estimates across dietary surveys and age groups. Calculated MOEs are below 10,000 in most of the dietary surveys, in particular for 95th percentile exposures.
- For DMA(V), the MOEs resulting from a comparison of the RP with exposure estimates from the scenarios on rice-based infant formula ranged from 3200 to 970 and lower than 10,000.
- The MOEs derived for DMA(V) in the specific exposure scenario for rice consumers were lower than 10,000 at the highest average exposures for 'Toddlers' and 'Other children', and at the highest P95 exposures in each of the population groups except for 'Lactating women' and 'Vegetarians'. The MOEs derived for DMA(V) in the specific exposure scenario for fish consumers were lower than 10,000 at the highest average exposure in 'Infants', 'Toddlers', 'Other children', 'Adolescents', 'Adults' and 'Elderly', and at the highest P95 exposures in each one of the population groups.
- The CONTAM Panel noted that these MOEs raise a health concern but also noted the uncertainties associated with interpretation of the mode of action for carcinogenicity with regard to the extent to which DNA-reactive mechanisms contribute to DMA(V) genotoxicity as well as the role of genotoxicity in its carcinogenicity.

4.14.2 | Risk characterisation for MMA(V)

- The reference point derived of 9.7 mg As/kg bw per day was compared with exposure estimates for the different age groups focussing on 'consumers only' of the dietary exposure via the main food commodities for which quantified MMA(V) data were available.
- The selected food commodities were 'Fish meat', 'Molluscs' and 'Processed/preserved fish'. The MOEs ranged from 3,233,000 (average exposure, minimum) to 28,000 (P95 exposure, maximum) for fish meat consumers, from 9700,000 (average exposure, minimum) to 52,000 (P95 exposure, maximum) for molluscs consumers and from 9700,000 (average exposure, minimum) to 28,000 (P95 exposure, maximum) for processed/conserved fish consumers. All MOEs were well above 500 for average and high consumers and thus do not raise a health concern.

4.15 | Overall uncertainty in the risk characterisation

- For MMA(V), a quantitative uncertainty assessment was not carried out considering the very large MOEs, none of which raises any possible concern.
- For DMA(V) based on the quantitative analysis, it is unlikely or very unlikely that the MOE associated with any of the mean dietary exposure scenarios is below 10,000. Considering the 95th percentile of exposure, it is about as likely as not, or likely, that the MOE is below 10,000 for 'Infants', 'Toddlers' and 'Other children' with respect to the broader assessment of dietary exposure, as well as the specific assessment of fish consumption. For rice-based infant formula, it is certain that the MOE is below 10,000 for both mean and high exposures.

5 | RECOMMENDATIONS

- There is a need for robust validated analytical methods for determining small organoarsenic species in food with a focus on MMA(V) and DMA(V), MMA(III) and DMA(III) and their respective thio-analogues.
- Certified reference materials especially for trivalent and thio-containing small organoarsenic species in products such as rice and seafood are required.
- Occurrence data on small organoarsenic species should be reported to EFSA expressed as elemental arsenic concentrations (e.g. µg As/kg food).

- Occurrence data on small organoarsenic species in breast milk are needed, in addition to more occurrence data on foods (e.g. rice, fish and seafood, rice-based infant formulas) to confirm the present exposure estimations and food contributors.
- There is a need for improved understanding of the human ADME of small organoarsenic species following ingestion and the human health implications.
- There is a need for experimental studies on developmental effects, reproductive toxicity and neurotoxicity for MMA(V) and DMA(V) and a general need for more toxicity data on small organoarsenic species other than MMA(V) and DMA(V).
- Further studies on genotoxic properties are needed for all small organoarsenic species as well as studies clarifying their mode of action.

ABBREVIATIONS

μM	micromolar
4NQO	4-nitroquinoline 1-oxide
8-oxodG	8-oxo-2'-deoxyguanosine
8-OHdG	8-hydroxydeoxyguanosine
AAS/AES	atomic absorption/atomic emission spectroscopy
ALS	amyotrophic lateral sclerosis
Art.	article
As	arsenic
As(III)	arsenite
As(V)	arsenate
As3mt	arsenic 3+ methyltransferase
AsH ₃	arsine
BER	base excision repair
BIOCONTAM	EFSA Biological Hazards Panel
BMD	benchmark dose
BMDL01	benchmark dose lower confidence limit of 1% extra risk
BMR	benchmark response
BNN	N-butyl-N-(4-hydroxybutyl)nitrosamine
BPDE	benzo(a)pyrene diolepoxide
BrDU	Bromodeoxyuridine
bw	body weight
CA	chromosomal aberration
CAS	Chemical Abstracts Service
CFA	colony forming ability
(CH ₃) ₂ AsH	dimethylarsine
CHO	Chinese Hamster Ovary
ConA	concanavalin A
CONTAM	EFSA Panel on Contaminants in the Food Chain
CP	cyclophosphamide
DDR	DNA damage response
DEN	diethylnitrosamine
DHE	dihydroethidium
DMA	dimethylated arsenic
DMAA	dimethylarsinate
DMA(III)	dimethylarsinous acid
DMA(V)	dimethylarsinate
DMA(III)	iododimethylarsine
DMBDD	N-bis(2-hydroxypropyl)nitrosamine
DMDTA(V)	dimethyldithioarsinic acid
dithio-DMA(V)	dimethylarsinodithioic acid
dithio-MA(V)	methylarsonodithioic acid
DMI	dimethylarsine iodide
DMMTA	dimethylmonothioarsenate
DN	double negative
DNA	deoxyribonucleic acid
DP	double positive
DSB	double-strand break
EC-HPLC	high-performance liquid chromatography-electrochemical detection
ED	electrochemical detection
EEC	European Economic Community
EGCG	epigallocatechin

EHEN	N-ethyl-N-hydroxyethylnitrosamine
ELISA	Enzyme-Linked Immunosorbent Assay
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organisation
FLARE	Fluorescent Advanced Oxidation Protein Products
FPG	formamidopyrimidine-DNA glycosylase
GD	gestation day
GSH	glutathione
GSSG	glutathione disulfide
GST-P	glutathione S-transferase positive
H ₂ O ₂	hydrogen peroxide
HBE	bronchial epithelial cells
HG	hydride generation
hOGG1	human 8-Oxoguanine DNA glycosylase 1
HPLC	high-performance liquid chromatography
HPLC-ICPMS	high-performance liquid chromatography-inductively coupled plasma mass spectrometry
HPLC-VG-AAS	high-performance liquid chromatography-vapour generation-atomic absorption spectroscopy
IARC	International Agency for Research on Cancer
iAs	inorganic arsenic
iAs(III)	arsenite
IC ₅₀ /IC ₇₀	Inhibitory concentration 50%/70%
ICPMS	inductively coupled plasma mass spectrometry
ICR	Institute of Cancer Research
i.e.	that is
i.p.	intraperitoneal
IST	immuno spin-trapping
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC ₅₀	lethal concentration 50%
LD ₅₀	lethal dose 50%
LOAEL	lowest observed adverse effect level
LOD	limit of detection
M	molar
MAs(III)	methyloxoarsine
Me ₃ As	trimethylarsine
ML	Maximum Level
mM	millimolar
MMA	monomethylated arsenic
MMA(III)	methylarsonous acid
MMA(V)	methylarsenate
MMMTA ^V	monomethylmonothioarsenate
MN	micronucleus
MSI	microsatellite instability
MTA ^V	monothioarsenate
Na	sodium
NAC	N-acetylcysteine
NBR	NCI-Black Reiter
NK	natural killer
nM	nanomolar
NOAEL	no observed adverse effect level
NP	nanoparticle
NRF2	Nuclear Factor E2-Related Factor 2
O	oxygen
OECD	Organisation for Economic Co-operation and Development
ODD	oxidative DNA damage
OGG1	8-oxoguanine DNA glycosylase
oxo-As(V)	oxidised arsenate
PARP(–1)	poly-(ADP-ribose) polymerase (1)
PBMC	peripheral blood mononuclear cell
PCNA	proliferating cell nuclear antigen
PI	propidium iodide
pKa	acid dissociation constant
PND	postnatal day

Ppb	parts per billion
RBC	red blood cell
ROS	reactive oxygen species
RONs	reactive oxygen and nitrogen species
S	sulfur
SCE	sister chromatid exchange
SOD	superoxide dismutase
SSB	single-strand break
TETRA	tetramethylarsonium ion
thio-As(V)	thio-arsenate
thio-DMA(V)	dimethylmonothioarsenate
thio-MMA(V)	thio-monomethylarsonic acid
thio-TMA	trimethylarsine sulfide
TK	thymidine kinase / toxicokinetics
TMA	Trimethylarsine
TMAO	trimethylarsine oxide
TPA	12-O-tetradecanoylphorbol-13-acetate
TTA(V)	trithioarsenate
UROtsa	human urinary tract epithelial cell line
URO-MSC	the MMA(III)-exposed UROtsa variant cells
V-FITC	V-fluorescein isothiocyanate
VG	vapour generation
WG	Working Group
WHO	World Health Organization
wt	wild type
XRCC1	X-ray cross complementing protein 1

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CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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ANNEXES

ANNEX A

Protocol for 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

BMD calculations

Annex C 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.

ANNEX D

Dietary surveys used for chronic dietary exposure assessment

The annex is provided as a separate Excel file containing the information on the food consumption data used for the chronic dietary exposure assessment to MMA and DMA, and is available on the EFSA Knowledge Junction community on Zenodo at: <https://doi.org/10.5281/zenodo.11500592>

ANNEX E

Raw occurrence data set

The annex is provided as a separate Excel file containing the raw occurrence data set for MMA and DMA in food, and is available on the EFSA Knowledge Junction community on Zenodo at: <https://doi.org/10.5281/zenodo.11500650>

ANNEX F

Occurrence data set for DMA and MMA

The annex is provided in a separate Excel file containing summary statistics for the occurrence data on DMA and MMA at different FoodEx2 levels, and is available on the EFSA Knowledge Junction community on Zenodo at: <https://doi.org/10.5281/zenodo.11500667>

ANNEX G

Dietary exposure estimations – MMA and DMA

The annex is provided as a separate Excel file containing the chronic dietary exposure estimations for MMA and DMA, and is available on the EFSA Knowledge Junction community on Zenodo at: <https://doi.org/10.5281/zenodo.11500675>

ANNEX H

Outcome of the public consultation

Annex H provides responses to the public consultation comments along with the original comments, and is available under the Supporting Information section on the online version of the scientific output.