Annex to: Update of the risk assessment of inorganic arsenic in food. doi:10.2903/j.efsa.2024.8488

Annex A Protocol for the update on inorganic arsenic (iAs) in food

The current protocol or strategy reports on the problem formulation and approach selected by the Panel on Contaminants in the Food Chain (CONTAM Panel) to update the previous risk assessment of iAs in food. The protocol is in accordance with the draft framework for protocol development for EFSA's scientific assessments (EFSA, 2020). This framework foresees that the extent of planning in the protocol (i.e. the degree of detail provided in the protocol for the methods that will be applied in the assessment) can be tailored to accommodate the characteristics of the mandate. Considering the timelines and the available resources, the CONTAM Panel applied a low level of planning.

A.1. Problem formulation

A.1.1 Objectives of the risk assessments

The objectives of the risk assessments are to assess the risk for adverse effects in humans associated with the dietary exposure to iAs in food.

In 2009, the CONTAM Panel published an opinion on arsenic in food (EFSA CONTAM Panel, 2009). This opinion is the starting point for the present assessment in which new data becoming available since then will be used to update the assessment. In 2020, the EFSA has assessed the chronic exposure to iAs (EFSA, 2021). This exposure assessment will be compared with the (updated) reference point for iAs to characterise the risk.

A.1.2 Target populations

The target population of the human risk assessment is the European population, including specific vulnerable groups yet to be identified.

A.1.3 Inorganic arsenic species and route of exposure

The risk assessment will focus on the dietary exposure to iAs.

A.1.4 Adverse effects and endpoints

The human risk assessment will address the adverse effects associated with the exposure to iAs as identified in the hazard identification step.

A.1.5 Identification of the risk assessment sub-questions

A series of sub-questions under each risk assessment pillar (i.e. hazard identification, hazard characterisation and exposure assessment) will be answered and combined for performing the risk assessment. The sub-question identified are reported in Table A.1.

Table A.1. Sub-questions to be answered for the risk assessment

Risk assessment step	No	Sub-questions
Hazard identification	1	What adverse effects are caused by pre-natal and early- life exposure to iAs in experimental animals?
Hazard identification	2	What adverse outcomes are associated with exposure to iAs in humans?
Hazard identification	3	Are iAs and its human metabolites genotoxic (*in vitro*, *in vivo* in animals and in humans)?
Hazard characterisation	4	Are iAs and its human metabolites carcinogenic in experimental animals?
Hazard characterisation	5	What is the ADME of iAs in humans?
Hazard characterisation	6	What is the difference in ADME of iAs between humans and experimental animals?
Hazard characterisation	7	What is the relevance of biomarkers for exposure and effect for assessing the hazard of iAs?
Hazard characterisation	8	What is the dose-response relationship between iAs and relevant endpoints in humans?
Hazard characterisation	9	What is the mode of action in humans that can explain the observed adverse effects by iAs?
Exposure assessment	10	What are the levels of iAs in food in Europe?[a]
Exposure assessment	11	What is the effect of processing on the levels of iAs in food?[a]
Exposure assessment	12	What are the consumption levels of foods contributing to the exposure to iAs among the European population?[a]
Exposure assessment	13	What is the estimate of exposure to inorganic arsenic from the diet in the European population?

ADME: absorption, distribution, metabolism and elimination

(a): These questions have already been answered in the EFSA report on chronic dietary exposure to iAs (EFSA, 2021) and will be integrated into the present assessment.

Studies on humans will be considered for the hazard identification and characterisation and animal studies will only be considered where relevant for the assessment (i.e. for evaluation of genotoxicity, carcinogenicity and transgenerational effects). The CONTAM Panel at its 118th Plenary meeting agreed that based on the abundance of human information available, the preferred use of human studies over animal studies (which is laid down in EFSA guidance) and the differential toxicokinetics of iAs in different omitting animal studies for sections other than those mentioned is appropriate. It was furthermore agreed that not a range of BMDs from individual studies should be identified but that a single Reference Point (RP) for the endpoint of concern should be identified.

The potential association between iAs exposure and the endpoints of interest for the human risk assessment will be evaluated. It will include an assessment of the dose-response relationship for the derivation of a chronic RP, and an evaluation of possible uncertainties, for example, those derived from consideration of the toxicokinetic and toxicodynamic properties of iAs and from considerations of the inter-species differences and intraspecies variability. The final step will be the comparison of the iAs exposure estimates (EFSA, 2021) to a health-based guidance value (HBGV, e.g. a tolerable intake) or the calculation of margins of exposure (MOEs).

A.2. Method for answering the sub-questions

The sub-questions formulated in Table A.1 will be answered by a comprehensive narrative approach. A literature search will be performed to identify primary research studies as well as reviews and meta-analyses relevant to the sub-questions formulated. In addition, the bibliography of the key full text papers will be checked for further potentially relevant studies. This technique is known as snowballing. The expertise of the working group will be used in deciding whether to pursue these further to complement the evidence collection.

To inform the sub-question related to the hazard identification and characterisation (**sub-questions 1 to 9),** all studies reporting associations with effects in humans (e.g. epidemiological studies), and in the respective *in vivo* studies in experimental animals that reported genotoxic or carcinogenic effects after exposure to iAs will be considered. The eligibility criteria related to the report characteristic are listed in Table A.2 (and apply to all sub-questions). The eligibility criteria related to study characteristics are listed in Tables A.3, A.4 and A.5 for studies in humans, in experimental animals and toxicokinetic studies.

The details of the studies will be reported in tables and discussed in the corresponding section of the Opinion. The experimental animal studies will be reported by: (i) animal species, (ii) endpoint, (iii) target compound(s) tested and (iii) study duration. The human epidemiological studies will be reported by: (i) endpoint, (ii) target compound(s) analysed and (iii) study design.

The selection of the scientific studies for inclusion or exclusion will be done by the relevant domain experts from the CONTAM WG on arsenic in food and the CONTAM Panel. It will be based on consideration of the extent to which the study is relevant to the assessment, and on general study quality considerations (e.g. sufficient details on the methodology, performance and outcome of the study, on dosing, substance studied and route of administration and on statistical description of the results), irrespective of the results. Major limitations in the information used will be documented in the scientific Opinions.

Table A.2. Eligibility criteria related to report characteristics (all sub-questions)

Language	In	English [(a)]
Time	In	Inorganic arsenic species and relevant organic metabolites: From 2009 onwards
Publication type	In	Peer-reviewed primary research studies (i.e. studies generating new data), systematic reviews, reviews, meta-analyses, extended abstracts, conference proceedings

(a): Studies in languages other than English might also be cited if considered relevant by the experts from the CONTAM WG on iAs or the CONTAM Panel.

Table A.3. Eligibility criteria for the selection of human epidemiological studies

Sub-questions 2, 7, 8		
Study design	In	Cross-sectional studies Cohort studies Case-control studies
	Out	Animal studies *In vitro* studies
Study characteristics	In	Duration of exposure > 6 months. For continuous outcomes > 100 subjects and for discrete outcomes > 5, Exposures to arsenic concentrations < 150 µg/L in drinking water or corresponding exposure level for arsenic biomarkers.
	Out	/
Population	In	All populations groups, all ages, males and females Study location: all countries
	Out	/
Exposure/ intervention	In	iAs intake per day As in drinking water iAs in urine and its metabolites Total urinary iAs (u-tiAs), i.e. sum of iAs and metabolites MMA and DMA Other iAs biomarkers that can be transformed into iAs intake
	Out	Intake of total As Total As in urine or blood, unless arsenobetaine could be excluded Studies on inhalation of arsenic Studies with occupational exposure
Specific outcome of interest	In	All endpoints
	Out	/

Table A.4. Eligibility criteria for the selection of toxicological studies in experimental animals and *in vitro* studies

Sub-question 1, 3, 4		
Study design	In	Experimental animal studies in mammals (e.g. rats, mice, monkeys, guinea pigs, mini pigs, rabbits, hamsters, dogs, cats, minks) *In vitro* studies in relevant systems (mammalian (including human)) primary cells and cell lines, subcellular interaction studies and bacterial cell lines used in genotoxicity studies
	Out	Human studies, studies in non-relevant species (e.g. non laboratory animals)
Study characteristics	In	Any study duration Any number of animals Any human culture cells/models
	Out	/
Population	In	Any age, males and females
	Out	/

Exposure/ intervention	In	Route of administration: Oral (feeding, gavage studies), *s.c.*, *i.p.*, *i.m.* OR inhalation or dermal Estimated exposure validated Number of doses: single or repeated administration Dose groups: ≥ 1 dose groups + control group
Specific outcome of interest	In	Genotoxicity, carcinogenicity, epigenetics (*in vitro*)
	Out	All others

Table A.5. Eligibility criteria for the studies on toxicokinetics

Sub-questions 4, 5, 6		
Study design / test system	In	*In vivo* studies in humans *In vivo* studies in experimental animals *In vitro* studies in human culture cells/models
	Out	/
Exposure/ intervention	In	Route of administration: Oral (feeding, gavage studies), *s.c.*, *i.p.*, *i.m. dermal, inhalation*
	Out	/
Specific outcome of interest	In	Any outcome related to the absorption, distribution, metabolism and elimination of the target compounds

Information about previous risk assessments by international bodies, chemistry, analytical methods, current EU legislation, previously reported occurrence data in food and exposure assessments (including time trends), as reported in the literature, will be gathered and summarised in a narrative way (supported by tables, if relevant) based on expert knowledge and judgement.

The general principles of the risk assessment process for chemicals in food as described by WHO/IPCS (2009) will be applied, which include hazard identification and characterisation, exposure assessment and risk characterisation. In addition, the following EFSA guidance documents pertaining to risk assessment will be followed for the development of the risk assessment:

- Guidance of the Scientific Committee on a request from EFSA related to uncertainties in Dietary Exposure Assessment (EFSA Scientific Committee, 2007),
- Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: General principles (EFSA Scientific Committee, 2009),
- Management of left-censored data in dietary exposure assessment of chemical substances (EFSA, 2010),
- Guidance of EFSA on the use of the EFSA Comprehensive European Food Consumption Database in exposure assessment (EFSA, 2011a),
- Overview of the procedures currently used at EFSA for the assessment of dietary exposure to different chemical substances (EFSA, 2011b),
- Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment (EFSA Scientific Committee, 2011),
- Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data (EFSA Scientific Committee, 2012a),

- Scientific Opinion on Risk Assessment terminology (EFSA Scientific Committee, 2012b),
- Update: Guidance on the use of the benchmark dose approach in risk assessment (EFSA Scientific Committee, 2017a),
- Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA Scientific Committee, 2019),
- Scientific Opinion on the guidance on the use of the weight of evidence approach in scientific assessments (EFSA Scientific Committee, 2017b),
- Guidance on the assessment of the biological relevance of data in scientific assessments (EFSA Scientific Committee, 2017c),
- Guidance on Uncertainty Analysis in Scientific Assessments (EFSA Scientific Committee, 2018),
- Guidance on Communication of Uncertainty in Scientific Assessments (EFSA, 2019),
- Guidance on the use of the benchmark dose approach in risk assessment (EFSA Scientific Committee, 2022)

A.2.1. Literature searches

The literature searches to inform the risk assessments on inorganic arsenic will be performed searching the following bibliographic databases or scientific citation research platforms:

1. PubMed

2. Web of ScienceTM (Core collection: Science Citation, Index, Emerging Sources Citation Index, Current Chemical Reactions, Index Chemicus)

The literature searches for studies relevant to inorganic arsenic will be performed by EFSA staff.

The output from the searched databases, i.e. the bibliographic references including relevant information, e.g. title, authors, abstract, will be exported into separate Endnote files, allowing a count of the individual hits per database. Files will then be combined, and duplicate records will be removed.

A.2.2. Integration of the lines of evidence for hazard identification and method to perform hazard characterisation

The final critical endpoints will be identified by integrating evidence from both human and experimental animal lines of evidence considering the respective level of confidence. A dose-response assessment will be performed on relevant adverse effects for the identification of chronic Reference points, e.g. no-observed-adverse-effect levels (NOAELs) or benchmark doses (BMDs) and its lower confidence limits (BMDLs) for a particular incidence of effect. The lowest relevant Reference Point will be considered for the possible derivation of an HBGV or to calculate the MOE.

A.3. Method to address the uncertainties in the risk assessment

The evaluation of the inherent uncertainties in the risk assessments on iAs will be performed based on the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA Scientific Committee, 2007), the report on 'Characterizing and Communicating Uncertainty in Exposure Assessment' (WHO/IPCS, 2008), the new guidance on uncertainties of the EFSA Scientific Committee (EFSA Scientific Committee, 2018) and the guidance on communication of uncertainty in scientific assessments (EFSA, 2019).

Recommendations will be included in the Scientific Opinion for the generation of additional data that could decrease the impact of the identified uncertainties on the conclusions of the risk assessment.

A.4. Approach for reaching risk characterisation conclusions

The general principles of the risk characterisation for chemicals in food as described by WHO/IPCS (2009) will be applied as well as the different EFSA guidance documents relevant to this step of the risk assessment (see Section A.1 above).

A.5. Public consultation

In line with EFSA's policy on openness and transparency, and in order for EFSA to receive comments on its work from the scientific community and stakeholders, EFSA engages in public consultations on key issues. Accordingly, the draft Opinion on inorganic arsenic will be subject to public consultation before their final adoption by the CONTAM Panel.

The comments received will be evaluated by the WG on iAs in food and by the CONTAM Panel and wherever appropriate taken into account for finalisation of the draft Opinion.

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