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Title: Fate and toxicological relevance of arsenic speciation in different rice types depending on the cooking mode

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Keywords: total and inorganic arsenic; DMA; ICP-MS; speciation analysis; cooked rice

Corresponding Author: Dr. Thierry GUERIN, PhD

Corresponding Author's Institution: French Agency for Food Safety

First Author: Thierry GUERIN, PhD

Order of Authors: Thierry GUERIN, PhD; Petru JITARU, Dr; Sandrine MILLOUR; Marco ROMAN, Dr; Kaoutar EL KOULALI; Laurent NOEL, Dr

Abstract: Rice is one of the most consumed foods in the world but also an important arsenic exposure source, especially for populations with traditionally rich rice-based diets. In this study, total (Ast), inorganic arsenic (Asi, the sum of As(III)+As(V)) and dimethylarsonic acid (DMA) species were determined in 37 samples of commercial rice collected in France. Inductively coupled plasma-quadrupole mass spectrometry (ICP-QMS) was employed for Ast determination whereas anion-exchange chromatography hyphenated to ICP-MS was used for speciation analysis of Asi and DMA. Ast levels in raw rice varied from 0.041 to 0.535 mg kg-1 whereas Asi (the most toxic species) varied from 0.025 mg kg-1 (in polished Basmati rice) up to 0.471 mg kg-1 (in organic rice duo). The corresponding daily intake and associated risk for health in France were estimated depending on the age group (children, adolescent and adults) and gender. The intake varied between 0.002 and 0.184 □g kg-1 b.w for Ast and 0.002 and 0.153 □g kg-1 b.w for Asi, which are well below that providing a minimal risk of chronic toxicity. Nevertheless, organic wholegrain rice may entail a significant risk for children in case of sole consumption at the expenses of polished rice. The effect of rice cooking/boiling by four different procedures was also investigated in terms of the overall toxicological risk related to As species. A partial removal Asi was observed, whereas no removal was seen for DMA. Pre-rinsing and boiling the raw rice by using an excess of water was proved to be the most efficient mode to obtain a significant Asi removal and further reduction of the toxicological risk for children, particularly for the white rice types. These findings can be useful to assess and mitigate the risk associated to arsenic exposure from rice consumption.

Dear Editor,

We have submitted electronically the manuscript entitled: "*Fate and toxicological relevance of arsenic speciation in different rice types depending on the cooking mode*" by Petru Jitaru, Sandrine Millour, Marco Roman, Kaoutar El Koulali, Laurent Noël and Thierry Guérin that we wish to publish as an original research article in *Journal of Food Composition and Analysis.*

Looking forward to future collaboration.

Yours sincerely,

Dr Thierry Guérin
Université Paris-Est
Anses, Laboratory for food safety / Chemical food contaminants department (chair)
F-94700 Maisons-Alfort, France

Novelty of the work

This manuscript reports the application of simple, fast and accurate analytical procedures for total and speciation analysis of arsenic in a rather large variety of rice samples commercialized in France. A deep statistical data treatment (non-parametric Kruskal-Wallis test) and the calculation of the Hazard Quotient allowed an accurate assessment of the risk associated with rice consumption by different populations groups divided function of age (children, adolescent and adults) as well and gender. In addition, using the same approaches of data treatment and interpretation, different rice cooking modes were investigated for a better understanding of the actual toxicological risk for children and adult population in terms of both inorganic and organic arsenic species. Although the arsenic levels reported in our study may be specific to the rice sample type analyzed (as they depend on soil properties, groundwater and irrigation water quality, etc.), the consistent statistical and toxicological output may allow the food authorities to better advice the consumers concerning the reduction of arsenic species (particularly the inorganic arsenic) in rice prior to consumption.

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HIGHLIGHTS

- A deep statistical data treatment (non-parametric Kruskal-Wallis test) and the calculation of the Hazard Quotient allowed an accurate assessment of the risk associated with rice consumption by different French populations groups
- Different rice cooking modes were investigated for a better understanding of the actual toxicological risk for children and adult population in terms of both inorganic and organic arsenic species.
- Advice the consumers concerning the reduction of arsenic species (particularly the inorganic arsenic) in rice prior to consumption.

Graphical Abstract

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Fate and toxicological relevance of arsenic speciation in different rice types depending on the cooking mode

Petru Jitaru[1], Sandrine Millour[1], Marco Roman[2], Kaoutar El Koulali[1], Laurent Noël[1,3] and Thierry Guérin[1*]

[1] Université Paris-Est, Anses, Laboratory for food safety, F-94700 Maisons-Alfort, France

[2] University Ca' Foscari of Venice, Department of Environmental Sciences, Informatics and Statistics, Via Torino 155, 30172 Venice Mestre, Italy

[3] Present address: The French Directorate General for food, Ministry of Agriculture, Agro-Food and Forestry, 75732 Paris Cedex 15

* corresponding author:

E-mail: thierry.guerin@anses.fr ; Tel: +33 1 49 77 27 11 ; Fax: +33 1 49 77 26 50

Abstract

Rice is one of the most consumed foods in the world but also an important arsenic exposure source, especially for populations with traditionally rich rice-based diets. In this study, total (As_t), inorganic arsenic (As_i, the sum of As(III)+As(V)) and dimethylarsonic acid (DMA) species were determined in 37 samples of commercial rice collected in France. Inductively coupled plasma-quadrupole mass spectrometry (ICP-QMS) was employed for As_t determination whereas anion-exchange chromatography hyphenated to ICP-MS was used for speciation analysis of As_i and DMA. As_t levels in raw rice varied from 0.041 to 0.535 mg kg^{-1} whereas As_i (the most toxic species) varied from 0.025 mg kg^{-1} (in polished Basmati rice) up to 0.471 mg kg^{-1} (in organic rice duo). The corresponding daily intake and associated risk for health in France were estimated depending on the age group (children, adolescent and adults) and gender. The intake varied between 0.002 and 0.184 μg kg^{-1} b.w for As_t and 0.002 and 0.153 μg kg^{-1} b.w for As_i, which are well below that providing a minimal risk of chronic toxicity. Nevertheless, organic wholegrain rice may entail a significant risk for children in case of sole consumption at the expenses of polished rice. The effect of rice cooking/boiling by four different procedures was also investigated in terms of the overall toxicological risk related to As species. A partial removal As_i was observed, whereas no removal was seen for DMA. Pre-rinsing and boiling the raw rice by using an excess of water was proved to be the most efficient mode to obtain a significant As_i removal and further reduction of the toxicological risk for children, particularly for the white rice types. These findings can be useful to assess and mitigate the risk associated to arsenic exposure from rice consumption.

Keywords: total and inorganic arsenic; DMA; ICP-MS; speciation analysis; cooked rice

1. Introduction

Arsenic (As) is a ubiquitous metalloid being present at trace levels in many environmental compartments; it enters the food chain mainly from the contaminated drinking water[1] and several largely consumed foodstuffs, such as fish and rice, the latter being an important contributor to As intake in countries with traditionally rice-based diets.[2,3] Arsenic levels in rice depend on the geographical location, growing/soil conditions and also on the level of contamination of the irrigation water.[4-7] Despite the relatively large panel of As species present in food, the rice accumulates mostly monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenite (As(III)) and arsenate (As(V)), the latter (inorganic) species being the most.[2,8] Given their similar toxicological properties, the sum of As(III) and As(V) is most cases refereed as inorganic arsenic (As_i). As_i is carcinogenic for humans;[1] acute exposure to high As_i levels can also cause vomiting, abdominal pain and diarrhea.[9] Chronic exposure to As_i can also cause skin lesions, diabetes, hypertension and cardiovascular diseases. MMA and DMA (the methylated metabolites of inorganic arsenic) are excreted in the urine and are considered less toxic than As_i; nevertheless, MMA and DMA have also been identified as possible cancer promoters and further studies are underway regarding their actual toxicity.[4] Several other arsenic species commonly present in rice, such as arsenobetaine (AsB), arsenocholine (AsC), trimethylarsine oxide (TMAO) and arseno-sugars are currently considered non-toxic.[2]

Taking into account the high health risk associated to arsenic poisoning, the European Food Safety Authority (EFSA) stated that an intake ranging from 0.3 to 8 μg kg^{-1} body weight (b.w.) per day should be used as a reference for characterizing As_i risk.[10,11] Similarly, the Agency for Toxic Substances and Disease Registry (ATSDR) provided a Minimal Risk Level (MRL) of daily intake between 0.3 and 20 μg kg^{-1} b.w. for individual As species (As_i, MMA and DMA), which is defined as the dose that is likely to lead no appreciable risk of adverse

non-cancer health effects over a specific duration of exposure.[12] In 2014, the Joint Expert Committee on Food Additives (JECFA)[13] recommended a maximum level for As_i in milled and parboiled rice of 0.2 mg kg^{-1} (no such limit is yet regulated for husked (brown) rice). Very recently, the European Commission regulated the maximum levels of As_i in different type of rice, with maximum levels (ML) ranging from 0.10 mg kg^{-1} for rice destined for infant foods up to 0.30 mg kg^{-1} for rice waffles, wafers, crackers and cakes.[14] Apart from the EU regulations, at an international level, the only existing regulatory limit for As in rice is applied in China (0.15 mg As_i kg^{-1}).[13]

The lack of international regulations in terms of health risk related to As exposure *via* food relates also on the difficulty in its accurate determination in biological matrices, especially at trace and ultra-trace levels. The most common analytical approach for As speciation analysis relies on the coupling of (anion exchange) high performance liquid chromatography (AE-HPLC) with the inductively coupled plasma-quadrupole mass spectrometry (ICP-QMS).[15,16] Despite the well-recognized ICP-QMS advantages as a detection technique for trace and ultra-trace elemental analysis, its application to As (monoisotopic, atomic mass of 75 amu) determination is still difficult because of several severe spectral interferences such as $^{40}Ar^{35}Cl$ and $^{40}Ca^{35}Cl$.[17,18] In addition, since As is mono-isotopic, the use of a primary method for its quantification such as isotope dilution-ICP-MS is not possible. In such circumstances, one of the main approaches of method validation for As determination (including speciation analysis) is based on the assessment of the accuracy profile.[19]

The aim of this study was to determine the concentration and the toxicological relevance of As_t, As_i and organic As species in different types of rice (types of grain, industrial processing and geographical origin) commonly consumed in France. For this task, previously developed and fully validated methods based on the accuracy profile were employed.[20] Additionally, the influence of four different cooking approaches on the concentration and toxicological

relevance of total and individual As species in rice in relation with children (3-10 years old) and adult population was addressed. These results can be useful to understand as well as mitigate the arsenic exposure related to the consumption of specific types of rice depending on the consumer profile.

2. Materials and methods

2.1. Reagents

Ultrapure water (18 MΩ cm) obtained by purifying distilled water using a Milli-Q^{TM} PLUS system combined with an Elix 5 pre-purification (Millipore SA, Saint-Quentin-en-Yvelines, France) was used throughout the study. The As concentration (As_t) of this ultra-pure water was ≤0.012 µg L^{-1}, which was considerably lower that the method limit of detection (MDL), hence it was considered As-free water.

Methanol (HPLC gradient grade), nitric acid (Suprapur. 67%) and hydrogen peroxide (Normapur, 30% m/m, used to oxidize As(III) to As(V) for As_i determination were purchased from VWR (Fontenay-sous-Bois, France).

For As_t measurements, an As(III) stock standard solution at 1000 mg L^{-1} (Analytika, Prague, Czech Republic) was used. Working standards for external calibration were prepared daily in 6% (v/v) HNO_3. For speciation analysis, standard solutions of individual As species (1000 mg L^{-1}, as As) were prepared from the following substances: sodium arsenate dibasic heptahydrate (≥ 98.0%), DMA (≥ 99.0%), AsB (≥ 95.0%) (Sigma Aldrich, Saint-Quentin-Fallavier, France), methylarsonic acid (≥ 98.0%), AsC bromide (≥ 98.0%), TMAO (≥ 98.0%) (all from Tri Chemical Laboratories, Yamanashi, Japan). A multi-species solution of As(V), MMA, DMA, TMAO, AsC at 1.0 mg L^{-1} and AsB at 3 mg L^{-1} was used as an intermediate stock standard solution for external calibration; the working standard solutions were prepared daily from this multi-species standard solution by appropriate dilution in ultra-pure water. A

standard solution of scandium (Sc) at 2.0 µg L^{-1} was used as internal standard (IS) for As_t determination. A multi-element solution (10 µg L^{-1}) prepared from a stock tuning solution (Agilent Technologies, Courtaboeuf, France) was used for ICP-MS optimization. All standard solutions were stored in the dark at 5 °C until analysis in order to prevent their degradation.

2.2. Reference materials and samples

Two certified reference materials (CRMs), namely TORT 2 (lobster hepatopancreas, National Research Council Canada), certified for As_t and BC 211 (rice powder, Institute for Reference Materials and Measurements, Geel, Belgium) certified for DMA and As_i were used in this study for quality control.

Fifty-four raw rice samples were initially selected for this study. The samples having the same grain type, origin and industrial treatment were pooled so that a total of 37 composite samples were finally obtained and consequently analyzed. The samples were selected according to the brand, the type of grain (short, medium, long and extra-long), industrial pre-treatement (white or polished, brown, wholegrain, steamed, parboiled) and origin (Thailand, India, Burma, Surinam, Japan, Himalaya, France (Camargue, Languedoc), Italy and USA). The rice samples analysed in this study represent the 7 groups of rice types mostly consumed in France: *Basmati*, *Thai*, *White*, *White for risotto*, *Organic semi-wholegrain duo*, *Three-rice mix* and *Wholegrain rice*. Each sample/composite was milled prior to the preparation step (digestion or extraction).

2.3. Instrumentation

A Multiwave 3000 closed-vessel microwave digestion system (Anton-Paar, Courtaboeuf, France) equipped with 80 mL quartz vessels (80 bar operating pressure) was used for samples digestion in view of the As_t determination. The analysis was carried out using an ICP-quadrupole MS (ICP-QMS) model 7700x from Agilent Technologies (Courtaboeuf, France)

equipped with a third-generation Octopole Reaction System (ORS^3); Helium was used as collision gas to alleviate the spectral interferences. The ICP-QMS was equipped with an autosampler (ASX 500 model 510, CETAC, Omaha, Nebraska, USA) for automated sample introduction. Daily optimization was carried out to obtain maximum sensitivity while minimizing oxides (CeO^+/Ce^+) and doubly-charged (Ce^{2+}/Ce^+) levels (<2%). More details regarding the instrumental settings and data acquisition parameters are given in Table 1.

As_i and DMA speciation analysis was carried out by ionic exchange chromatography (IEC, Ultimate 3000) coupled to a $X\text{-}Series^{II}$ ICP-QMS equipped with a concentric nebulizer and impact bead spray chamber (both instruments from Thermofisher Scientific, Courtaboeuf, France). The chromatographic separation was achieved using an IonPac AS7 ion exchange column (250 × 4 mm; 10 µm particles). An IonPac AG7 guard column and an automated injection valve (100 µL injection loop) were used throughout (see also Table 1). All the digest or extract samples were filtered using 0.45 µm polyvinylidene fluoride (PVDF) syringe filters (Millipore, France).

2.4. Samples preparation and analytical procedures

2.4.1. Rice cooking/boiling

For each of the 7 groups of rice types plus a steamed white rice, the samples with the highest concentration of As_t were selected to study the fate of As species during various cooking (boiling) modes. The following cooking modes were employed in this study:

A: non-rinsed rice was boiled in a volume of (ultrapure) water approximately 3 fold its weight until complete absorption of the boiling water;

B: 80 g of rice were placed in a 500 mL beaker and then rinsed six fold with ultra-pure water; the rinsing water was discarded and the rice was further boiled in a volume of ultrapure water approximately 3 fold its weight until complete water absorption ;

C: identical to mode B, except for the fact that the rice was boiled in a volume of ultrapure water six fold its weight; the excess of the boiling water was discarded;

D: raw rice (unwashed) was placed in an open colander over a pot of boiling ultrapure water and steamed (without lid);

The cooked/boiled rice samples were freeze-dried and milled prior to the analyses for determination of As_t and arsenic species.

2.4.2. Total arsenic determination

A method developed previously in our laboratory that was also accredited by the French Committee of Standardization (Cofrac)[19] was used for total As determination.[21] Briefly, 0.3 g of dry weight sample was precisely weighed in a quartz vessel and then thoroughly mixed with 3 mL of concentrated HNO_3 and 3 mL ultrapure water. The mixture was then submitted to microwave heating in a closed system during 7 min following the temperature program reported elsewhere.[21] After cooling at room temperature, the extract solutions were quantitatively transferred into 50 mL polyethylene flasks and then 100 µL of IS solution were added to achieve a final concentration of 2 µg L^{-1} of Sc; ultrapure water was finally added to the digested samples to a final volume of 50 mL. As_t concentration in the digests was determined by ICP-QMS (Agilent) using an in-house validated method on the basis of the accuracy profile procedure.[22,23] Briefly, the quantification was performed by external calibration (5 points) in the range 0-50 µg L^{-1}. The limit of quantification (LOQ) for As_t determination was 0.002 mg kg^{-1} of fresh matter for a typical sample weight of 0.3 g and a final volume of 50 mL.

2.4.3. Arsenic speciation

As speciation analysis was carried out by using a method previously developed in our laboratory with slight modifications.[20] Briefly, 0.15 g of freeze-dried sample were mixed with

10 mL of a H_2O_2:H_2O mixture (1:9 ratio, v/v) directly into the microwave digestion vessels (H_2O_2 was used here to oxidize As(III) to As(V)). The mixtures were then heated at 80°C for 6 min. After cooling at room temperature, the extracts were quantitatively transferred into 50 mL polyethylene flasks and after filling to 50 mL with ultrapure water they were centrifuged at 3500 rpm for 5 min. The supernatants were then filtered through syringe filters. Separation of As species was carried out by anion exchange chromatography (AEC) during a total chromatographic run of 5 min.[20] External calibration (6 points) using peak area was employed for species quantification (PlasmaLab software of the ICP-MS instrument was used for peaks' integration).

The concentrations were calculated after blanks subtraction only if the Internal Quality Controls (IQCs) were satisfactory in compliance with the ISO/IEC 17025 standard (2005).[24] LOQ (0.020 mg kg^{-1}) was assessed based on the accuracy profile approach[22,23], as the concentration level where at least one of the limits of the tolerance interval intersects the acceptability limit whereas the limit of detection (LOD) is calculated as the half of LOQ.[25]

2.5. Uncertainty calculation and quality control

The expanded uncertainty (U_c) of the results reported in this study was calculated as twice the combined uncertainty (u_c) that was estimated as the standard deviation characterizing the intermediate precision (S_R) was calculated for duplicate analysis during ten days for As_i and 12 days for DMA, during approximately a time span of 3 months (validation by means of accuracy profile), as explained elsewhere.[26-28] S_R was weighed by the number of analyses (n=1 in this case work) and a coverage factor k=2 (95%) was used (eqn. 1).

$$U = k \times u_c = 2 \times \frac{S_R}{\sqrt{n}} \qquad (1)$$

To calculate the uncertainty of each result (X), the relative standard deviation in terms of intermediate precision (CV_R) was used (eqn. 2, n=1 in this case).

$$U = 2 \times \frac{CV_R}{100} \times X \qquad (2)$$

In this study, CV_R was 16% for As_i and DMA and 15% for As_t.

Several IQCs were set up in this study in order to ensure the results reliability.[29] Data were valid only when all the acceptance criteria were satisfied. Briefly, an IQC concerning the calibration step relied on the achievement of a correlation coefficient (r^2) $\geq$ 0.995 when using a 6 points calibration curve. For As_t determination, IS was monitored to assess the instrumental drift and matrix effects. In most cases (93%) IS was recovered between 80% and 120%. A middle-range standard solution containing 2 µg L^{-1} of As_i and 5 µg L^{-1} of DMA was also systematically analyzed every eight samples (and also at the end of the sequence) in order to assess the instrumental drift; the deviation of the concentration of this standard solution compared to the theoretical value was $\leq$ 20%.

Method trueness was also assessed according to the FD V03-115 procedure[27] by analyzing in parallel with each batch of samples BC 211 (for speciation analysis) and TORT-2 (for As_t determination) CRM (see Table 2). A result was considered reliable when its value was comprised in the confidence interval (CI) calculated on the basis of the certified value ($X_{certified}$) of the CRM as following (eqn. 3):

$$CI = X_{certified} \pm \left[k \times \frac{CV_R \times X_{certified}}{100 \times \sqrt{N}} \right] \qquad (3)$$

where:

CI: confidence interval, k=3 (p=99%), N=11

As can be seen in Table 2, the data obtained for BC 211 and TORT-2 fitted within the CI.

A multi-species standard solution at a concentration level of the LOQ was also thoroughly analyzed to assess the data reliability at very low levels. This control was applied for the

247 samples with concentration < 2×LOQ and it was considered satisfactory if the measured
248 concentration was comprised within the CI (expressed here in terms of intermediate
249 repeatability) obtained during the method validation (by assessing the accuracy profile). Most
250 of the data (80%) obtained by analysis of the control standard solution (LOQ level) fell well
251 within the confidence interval for As_i, DMA and As_t.

252 A number of 23 blanks obtained in parallel either with the digestion (As_t) and extraction (As_i
253 and DMA) of rice samples were analyzed in the same conditions as the corresponding
254 samples to monitor the cross-contamination and memory effects for As_t determination. All
255 speciation blanks showed levels below the LOQ, whereas for As_t, 83% of the blanks were <
256 LOQ.

257 Finally, for quality control purposes, 10% of samples were analyzed in duplicate to assess the
258 repeatability and the batch-specific errors. In all cases, RSD<20% were obtained for duplicate
259 analyses for As_i, DMA and As_t determination, respectively.

260
261 ***2.6. Statistical data treatment***

262 For statistical data treatment, the values LOD/2 (5 μg kg^{-1}) and LOQ/2 (10 μg kg^{-1}) were
263 assigned to the data <LOD and <LOQ, respectively. The non-parametric Kruskal-Wallis test
264 was then applied to check for statistical differences in As speciation between rice types.

265 The toxicological relevance of As species in the samples was assessed by characterizing their
266 associated risk for health. Based on the Hazard Quotient (HQ) concept, the species-specific
267 risk was defined as the human exposure (daily *per capita* intake in μg kg^{-1} b.w.) of the
268 population divided by the corresponding MLR according to the formula[30]:

269 $$HQ_{Asi/DMA} = \frac{Intake_{Asi/DMA}}{MRL_{Asi/DMA}} \qquad (4)$$

270 The intake was calculated from the average daily consumption of rice in France obtained
271 during the INCA surveys (data available for 2006-2007)[31-33] and the average b.w. of the

French population (data available for 2002-2003),[34] see Table 3. Since consumption data were distinct for polished and wholegrain rice, the following classification was adopted: Basmati, White, Steamed White, Thai, wild rice and White risotto were considered as polished; brown, wholegrain and semi-wholegrain rice were considered as wholegrain. Consistently, the consumption of duo and trio mixtures was estimated based on the combination of their components. Distinct consumption and b.w. values were considered for three age groups: children (3-10 years), adolescents (11-17 years) and adults (18-79 years) and corrected for gender using population distribution in 2002/2003 (Table 3).

Given the very low (sub-toxic) levels of As measured in all samples, the MRLs for chronic (>1 year) oral exposure to humans were considered as the more appropriate toxicological reference, and any comparison between samples must be intended in view of "how far" they are to lead a minimum toxic effect. According to the ATSDR, the following MRLs were adopted: 0.3 μg kg^{-1} b.w. for As_i and 20 μg kg^{-1} b.w. for DMA.[11,12.] To evaluate the effect of the various cooking procedures in terms of the overall As-related toxicological relevance of each rice type, a combined risk level was calculated based on the species-specific HQs, as follows (eqn. 5):

$$Combined\ risk\ (\%) = \left[1 - (1\text{-}HQ_{Asi}) \cdot (1\text{-}HQ_{DMA})\right] \times 100 \qquad (5)$$

As represented in Figure 1, the combined risk can be adopted as a measure of the overall "distance" of a sample from a potential toxic effect (the up-right borders of B) in the multivariate space defined by the HQ of individual As species. Consistently, as much as the combined risk of a sample approaches 100%, as closest is at least one As species to lead a minimum toxic effect. This assessment was carried out separately for females and males, and for the three age groups reported above. The Welch's t-test was adopted to check for statistical difference between the combined risk of each rice type cooked/boiled following the various procedures, and the corresponding raw cereal (α=10%, two-tailed test).

3. Results and discussion

3.1. Assessment of total arsenic and its speciation in raw rice

3.1.1. Total arsenic

As_t concentrations in the samples analyzed in this study showed a large variation, spanning between 0.041 mg kg^{-1} for long-grain white organic Basmati rice from India up to 0.535 mg kg^{-1} for a duo of long-grain organic rice from France (Table 4). The lowest levels were found in Basmati rice, ranging from 0.041 to 0.129 mg kg^{-1}. Lower but still consistent levels were found in a three-rice mix (unknown origin) (0.301 mg kg^{-1}), a short-grain rice for risotto from France (0.280 mg kg^{-1}), a whole-grain rice (steamed, parboiled or steamed black rice, 0.215 mg kg^{-1}) and a steamed wholegrain long-grain rice from Uruguay (0.234 mg kg^{-1}). These data are consistent with the levels reported for French rice from the 'Camargue' region (0.280 mg kg^{-1})[35] and with those obtained in previous studies.[36] For instance, in a Canadian study (2009-2010)[37] the mean level of total As (0.241 mg kg^{-1}) in brown rice (range, 0.050 to 0.386 mg kg^{-1}) was higher than that in white rice (mean, 0.136 mg kg^{-1}; range; 0.040 to 0.190 mg kg^{-1}). Likewise, a study carried out by the US Food and Drug Administration (FDA) in 2013 on 1300 samples of rice and rice products found that As_t was at the low end of the range in instant rice, and at the high end in brown rice.[38] Since it appeared that As_t was substantially removed from brown rice during the industrial polishing process, the higher As concentrations in brown rice suggest that the contaminant is attached to the bran or the surface of the rice grain.[39-41]

3.1.2. Arsenic speciation analysis

In all of the samples analyzed in this study, the predominant arsenic species were As_i and DMA (Figure 2). Because of their difference in toxicity, the results will be discussed separately for As_i and organic arsenic species (represented here by DMA).

3.1.2.1. Inorganic arsenic species

For the batch of samples analyzed in this study, large variations in As_i concentrations were observed (Figure 3). The lowest levels were found in Indian white long-grain organic Basmati rice (0.025 mg kg^{-1}) whereas a maximum level (0.471 mg kg^{-1}) was measured in the organic long-grain rice duo from France (see Table 4). For the other rice samples, As_i concentration ranged between 0.080 and 0.160 mg kg^{-1} in white long-grain rice (n = 5) and from 0.099 to 0.173 mg kg^{-1} in Thai white long-grain rice (organic or conventional, n = 6), levels which are compatible with those reported elsewhere.[36,39]

It is also interesting to note that As_i level was highly correlated to As_t concentration (Figure 4). In our study, As_i fraction (compared to As_t) varied between 50% (three-rice mix) up to approximately 100% in a risotto rice, which is comparable with another study.[42]

Among the polished rice samples analyzed in this study, two samples solely showed a higher level compared to the Maximum Level (ML) of 0.2 mg kg^{-1} as regulated by the European Comission.[14] Among the wholegrain rice samples analyzed in this study, one sample solely (organic rice duo, As_i level = 0.471 mg kg^{-1}) exceeded the ML.

3.1.2.2. Organic arsenic species

Among the two organic As species intended to be determined in this study, DMA solely was quantified in 23 of the 37 samples; its levels ranged between 0.030 mg kg^{-1} (steamed white long Italian rice) and up 0.109 mg kg^{-1} for wild brown, red and steamed white rice of unknown origin. In white rice for risotto, DMA levels ranged from 0.027 to 0.057 mg kg^{-1}, whereas only one sample (Basmati rice) showed a quantifiable DMA level (0.033 mg kg^{-1}).

Based on the relative fraction of As_i and DMA with respect to As_t, the rice could be classified into two categories, namely As_i- and DMA- rice types.[43] In this respect, all samples analyzed in our study can be classified as the *As_i type*.

3.1.3. Toxicological relevance of arsenic speciation

Compared to their respective MRLs (Figure 4), the levels of As_i and DMA in raw rice were typically 10 fold lower (HQ≤0.1) than the value at which a minimum toxic effect is expected to appear. The much lower MRL of As_i with respect to that of DMA makes it two orders of magnitude more toxicologically relevant at the levels found in the analyzed rice samples, so that A_{si} is the dominant hazard. However, consumption patterns may significantly affect the resulting risk: wholegrain rice types have lower HQs than polished rice due to the much lower consumption (see Table 3), in spite of their higher level of A_{si}. Still, it must be pointed out that assuming a sole consumption (equivalent to the total) of the organic rice duo, the resulting HQ_{Asi} would be potentially significant for children (1.02±0.20), and relatively high also for adolescents and adults (0.62±0.12 and 0.46±0.09, respectively). Children are the mostly exposed age group due to the high relative consumption of rice (0.65 g day^{-1} kg^{-1} b.w., gender corrected average) with respect to adolescents and adults (0.40 and 0.29 g day^{-1} kg^{-1} b.w., respectively, gender corrected average), but only 0.6% is wholegrain (2.2% and 3.2% for adolescents and adults, respectively). Thus, it appears appropriate not to provide wholegrain rice to children from the point of view of As toxicity.

3.2. Influence of different cooking/boiling modes on arsenic speciation

A comparison of As_t and A_s species concentration in raw and cooked/boiled rice in ultra-pure water by using four different modes (see Figure 5) was also carried out in this study. It is interesting to note that a significant loss of As_t and As_i was accounted in cooked rice, whereas no DMA leaching was seen with any of the cooking/boiling modes discussed previously (see

also Table 5). Indeed, approximately 20% of As_t and up to 40% of As_i was lost for most of the samples when cooking mode A was applied. Similarly, mode B contributed to a loss of approximately 30% of As_t and up to about 50% of As_i, depending on the rice type. It appears also that rinsing helped to remove up to 16% of As_i and 20% for total arsenic depending on the rice type. For instance, the rinsing was very effective either in terms of As_t and As_i for white rice and white risotto whereas it was scarcely effective for the other rice types. Furthermore, boiling rice in ultrapure water with a pre-rinse step (mode C) removed between 32% to 70% of As_t and 32% to 80% of As_i (depending on the rice type). For all samples, this was equivalent to 52% of and of 53% As_i, which is in agreement with other published data.[44] This confirms that water soluble As species can be removed by discarding the boiling water. Although the effect of pre-rinsing is ineffective for some types of rice, the boiling method is effective in reducing As_t and As_i levels, providing that the water itself is not contaminated. Finally, the steaming cooking mode (D) contributed to a removal of 21% of As_t and 26% for As_i for all samples, with large variations depending on the rice type.

The effect of cooking on the toxicological relevance of As speciation was assessed by calculating a combined risk (MRLs-relative, %) for the raw and cooked product according to the various preparation procedures, for three age groups and distinct by gender (see Figure 5). As discussed above, the raw white rice types entail a greater combined risk for health (between 51±10% for white risotto/female children and 10±2% for basmati/male adults) mainly due to the much higher consumption with respect to wholegrain rice (combined risk ~1 order of magnitude lower). Apart of one single non-significant exception (steamed white rice cooked by mode A), all cooking procedures led to a reduction of the combined risk for all rice types, that is the consequence of a loss of As_i. The most relevant effect, statistically significant for almost all rice types, was noticed for cooking procedure C, which reduced the combined risk of the raw cereals by approximately 3 fold on average. Notably, that was the

only cooking procedure capable of reducing significantly the toxicological score of the Three-rice mix and Wholegrain rice. The cooking mode B was still effective to reduce significantly the toxicological score of some rice types (Basmati, White, Thai, White risotto and Organic duo), but to a less extent. The cooking modes A and D were significant only for Thai, White risotto and Organic duo. As expected, the combined risk for steamed white rice is not significantly affected by further cooking. The cooking procedures tested in this work have therefore the potential to reduce the level of As_i in rice, particularly by rinsing and discarding the boiling water. The toxicological relevance of this effect may vary with the rice type, particularly depending on the consumption patterns, being more beneficial for children and females being the mostly exposed age group and gender.

4. Conclusions

Assessment of total, inorganic and organic arsenic levels in different varieties of raw and boiled rice commercialized in France is proposed in this study. For all samples investigated here, the most abundant species was the inorganic arsenic, which is the species of major concern in terms of toxicity. It is worth to underline that the results reported here may be specific to each rice sample type studied, as the level of arsenic in rice depends on soil properties, harvesting time, groundwater and irrigation water quality, surrounding industrial activity, etc. Hence this manuscript cannot draw a comprehensive conclusion regarding the arsenic contamination of rice found on France market. Nevertheless, this study allows a better understanding of the potential toxicological risk for children and adult population in terms of (inorganic and organic) arsenic species during rice cooking by different boiling modes. Partial removal either of total and inorganic arsenic from rice can be achieved by selecting the appropriate cooking mode. Our results indicate that pre-rinsing the rice and discarding the boiling water leads to a consistent removal of arsenic, mainly in inorganic form. This study

may allow the food authorities to better advice the consumers concerning the reduction of inorganic arsenic in rice prior to consumption.

5. Acknowledgements

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Figures captions

Figure 1. Graphical representation of the method used to calculate the combined risk.

Figure 2. Chromatogram obtained for the analysis of a raw rice extract solution by AE-HPLC hyphenated to ICP-QMS.

Figure 3. Correlation between As_i and As_t in terms of concentration (mg kg^{-1} ± U_c)

Figure 4. Biplots of As_i and DMA concentration (mg kg^{-1}) in different types of raw rice (a-d), and corresponding HQs calculated for adults and children (e-h), value ± σ. Solid and dotted lines represent the LOQ (20 mg kg^{-1}) and LOD (10 mg kg^{-1}), respectively, and the corresponding estimates of HQ (the dashed line represents the regulated maximum level of As_i in polished or white rice).

Figure 5. Effect of various rice cooking procedures (A-D) on the As toxicological risk with respect to the raw cereal, estimated for children (3-7 years), adolescents (11-17 years) and adults (18-79 years) depending on the gender. The risk was calculated by combining the HQ_{Asi} and HQ_{DMA}, and expressed as percentage (log scale, value ± σ); 100% corresponds to the occurrence of a minimum toxic effect. The asterisks mark statistically significant differences with respect to the raw cereal.

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Table 1. Instrumental **ICP-MS** operating parameters

Total arsenic determination by Agilent 770 ICP-MS	
Power	1400 W
Nebulizer type	MicroMist
Plasma gas flow rate (Ar)	15 L min^{-1}
Auxiliary gas flow rate (Ar)	1±0.1 L min^{-1} (depending on daily optimization)
Nebulizer argon flow	1±0.1 L min^{-1} (depending on daily optimization)
He gas flow rate (CRC)	4.3 mL min^{-1}
Integration time	3 s
Sampling/skimmer cones	Nickel
Arsenic speciation by AE-HPLC coupled to X-Series^{II} ICP-MS (Thermo Fisher)	
ICP-MS parameters	
Plasma power	1450 W
Plasma gas flow	15 L min^{-1}
Auxiliary gas flow	0.9 ± 0.1 L min^{-1} (depending on daily optimization)
Nebulizer gas flow	0.9 ± 0.1 mL min^{-1} (depending on daily optimization)
Isotopes/masses monitored (m/z)	75 (^{75}As); 77 ($^{40}Ar^{37}Cl$)
Dwell time	500 ms
HPLC parameters	
Analytical column	IonPac AS7 (250 x 4 mm. 10 µm particles, Dionex)
Guard column	IonPac AG7 (50 x 4 mm. 10 µm particles, Dionex)
Flow rate	1.35 mL min^{-1}
Mobile phase A	0.8×10^{-3} mol L^{-1} HNO_3 (0.8 mM) in 1% MeOH (pH = 3.8)
Mobile phase B	500×10^{-3} mol L^{-1} HNO_3 (500 mM) in 1% MeOH (pH = 1.4)
Gradient	0 - 3 min: 99% A 3 - 5 min: 10% A 5 - 12 min: 80% A 12 -12.5 min 99% A

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Table 2. As_t, As_i and DMA reference and measured concentrations (mg kg^{-1}) measured in BC 211 and TORT-2 CRM.

Species	**BC 211**		**TORT-2**
	As_i	**DMA**	**As_t**
Reference value [a]	0.124 ± 0.060	0.119 ± 0.038	21.6 ± 5.2
Measured [b]	0.121 ± 0.010 (n=11)	0.120 ± 0.004 (n=11)	21.6 ± 1.4 (n=8)

[a] mean value ± confidence interval

[b] mean value ± U (the number of replicates is given in the brackets)

Table 3. Population studied (number), average b.w. and rice consumption in France used to calculate the intake of As species.[Error! Bookmark not defined.]

	Children (3-10 years)	**Adolescents** (11-17 years)	**Adults** (18-79 years)	**Gender**
French population (2002/2003)	3,113,311	2,902,375	21,834,14	Females
	2,960,987	2,778,796	23,164,768	Males
Average b.w. (2002/2003) kg	24.41	50.56	63.15	Females
	24.89	54.87	77.19	Males
Rice consumption in France (2006/2007) g day^{-1} per person	0.09	0.45	0.65	Wholegrain
	15.77	20.36	19.80	White

12 **Table 4.** As_t, As_i and DMA concentrations (mg kg^{-1}± U_c[a]) measured in raw rice by ICP-MS (As_t)
13 and IEC-ICP-MS (As_i and DMA), respectively.

Rice type	Grain	Origin	As_t	As_i	DMA	As_i + DMA	Mass balance (%)[b]	As_i/As_t (%)
White Basmati	Extra long	Himalaya	0.048 ± 0.010	0.034 ± 0.007	0.010 ≤ x < 0.020	0.034 ± 0.007	71 ± 21	71 ± 21
	Extra long	India	0.074 ± 0.026	0.055 ± 0.011	< 0.010	0.055 ± 0.011	74 ± 30	74 ± 30
	Long	India	0.044 ± 0.015	0.034 ± 0.007	< 0.010	0.034 ± 0.007	77 ± 31	77 ± 31
	Long	Himalaya	0.129 ± 0.009	0.115 ± 0.023	< 0.010	0.115 ± 0.023	89 ± 19	89 ± 19
	Long	India	0.088 ± 0.018	0.050 ± 0.010	0.033 ± 0.011	0.083 ± 0.015	94 ± 26	57 ± 27
	Long	India	0.041 ± 0.008	0.025 ± 0.005	< 0.010	0.025 ± 0.005	61 ± 17	61 ± 17
Organic white Basmati	Long	India	0.052 ± 0.010	0.036 ± 0.007	0.010 ≤ x < 0.020	0.036 ± 0.007	69 ± 19	69 ± 19
	Long	Himalaya	0.126 ± 0.025	0.097 ± 0.020	0.010 ≤ x < 0.020	0.097 ± 0.020	77 ± 22	77 ± 22
	Long	India	0.070 ± 0.014	0.041 ± 0.008	< 0.010	0.041 ± 0.008	59 ± 16	59 ± 16
Wholegrain Basmati	Extra long	Himalaya	0.079 ± 0.016	0.049 ± 0.010	< 0.010	0.049 ± 0.010	62 ± 18	62 ± 18
Basmati duo (white and wholegrain)	Long	India	0.067 ± 0.013	0.042 ± 0.008	0.010 ≤ x < 0.020	0.042 ± 0.008	63 ± 17	63 ± 17
White	Long	Italy	0.116 ± 0.023	0.104 ± 0.021	0.010 ≤ x < 0.020	0.104 ± 0.021	90 ± 25	90 ± 25
		Camargue, France	0.205 ± 0.041	0.160 ± 0.032	0.059 ± 0.019	0.219 ± 0.037	107 ± 28	92 ± 30
		Italy	0.110 ± 0.022	0.101 ± 0.020	< 0.010	0.101 ± 0.020	92 ± 26	92 ± 26
		Surinam	0.139 ± 0.028	0.080 ± 0.016	0.056 ± 0.018	0.136 ± 0.024	98 ± 26	58 ± 28
		NA	0.167 ± 0.033	0.121 ± 0.024	0.042 ± 0.013	0.163 ± 0.027	98 ± 25	72 ± 27
Steamed white	Long	Italy	0.181 ± 0.036	0.153 ± 0.031	0.030 ± 0.010	0.183 ± 0.033	101 ± 27	85 ± 29
Steamed and organic white	Long	Europe	0.120 ± 0.024	0.109 ± 0.022	0.010 ≤ x < 0.020	0.109 ± 0.022	91 ± 26	91 ± 26
Thai white	Long	Thailand	0.241 ± 0.048	0.173 ± 0.024	0.058 ± 0.013	0.231 ± 0.027	96 ± 22	72 ± 23
	Long	Thailand	0.146 ± 0.029	0.112 ± 0.022	0.041 ± 0.013	0.153 ± 0.026	105 ± 27	77 ± 29
	Long	Thailand	0.173 ± 0.035	0.134 ± 0.027	0.050 ± 0.016	0.184 ± 0.031	106 ± 28	77 ± 30
Thai organic white	Long	Thailand	0.143 ± 0.028	0.101 ± 0.020	0.047 ± 0.015	0.148 ± 0.025	103 ± 27	71 ± 29
	Long	Borders of Burma	0.133 ± 0.027	0.099 ± 0.020	0.035 ± 0.011	0.134 ± 0.023	101 ± 27	74 ± 29
Thai perfumed white	Long	Thailand	0.213 ± 0.042	0.148 ± 0.030	0.054 ± 0.017	0.202 ± 0.034	95 ± 25	69 ± 27
White risotto	Short	Italy	0.197 ± 0.039	0.146 ± 0.029	0.040 ± 0.013	0.186 ± 0.032	94 ± 25	74 ± 26
		Languedoc, France	0.280 ± 0.056	0.237 ± 0.047	0.048 ± 0.016	0.285 ± 0.050	102 ± 27	85 ± 29
		NA	0.137 ± 0.027	0.107 ± 0.021	0.027 ± 0.009	0.134 ± 0.023	98 ± 35	78 ± 27
		Camargue, France	0.190 ± 0.038	0.200 ± 0.040	0.057 ± 0.018	0.257 ± 0.044	135 ± 36	103 ± 38
		Japan	0.195 ± 0.039	0.172 ± 0.034	0.047 ± 0.015	0.219 ± 0.037	112 ± 29	88 ± 32
Rice mix (72% steamed white + 14% wholegrain red + 14% wild)	Long	Europe or Thailand	0.121 ± 0.024	0.087 ± 0.017	0.021 ± 0.007	0.108 ± 0.018	89 ± 23	72 ± 25
Three-rice mix (13% wild brown + 14% red + 73% steamed white)	Long	NA	0.301 ± 0.060	0.151 ± 0.030	0.109 ± 0.035	0.260 ± 0.046	86 ± 23	50 ± 24
Three-rice mix (steamed white/wholegrain red/wild black)	Long	black rice from the United States	0.118 ± 0.023	0.083 ± 0.017	0.010 ≤ x < 0.020	0.083 ± 0.018	70 ± 20	70 ± 20
Organic rice duo (semi	Long	Camargue,	0.535 ± 0.107	0.471 ± 0.094	0.045 ± 0.014	0.516 ± 0.095	96 ± 26	88 ± 27

wholegrain + red)		France						
Organic rice duo (semi wholegrain + red)	Long	Camargue, France	0.311 ± 0.062	0.281 ± 0.041	0.046 ± 0.015	0.327 ± 0.041	105 ± 25	90 ± 26
Steamed black wholegrain	Medium	Italy	0.234 ± 0.046	0.214 ± 0.043	0.033 ± 0.011	0.247 ± 0.044	106 ± 28	91 ± 30
Steamed wholegrain	Long	Uruguay	0.215 ± 0.043	0.123 ± 0.025	0.086 ± 0.027	0.209 ± 0.037	97 ± 26	57 ± 28
Parboiled wholegrain	Long	Italy	0.227 ± 0.045	0.177 ± 0.035	0.056 ± 0.018	0.233 ± 0.039	103 ± 25	78 ± 29

[a:] calculated based on method intermediate reproducibility
[b] mass balance = $[As_i + DMA] / [As_t]$;
NA: not available

Table 5. As_t, As_i and DMA concentrations (mg $kg^{-1} \pm U_c$[a]) measured in cooked/boiled rice by ICP-MS (As_t) and AE-HPLC-ICP-MS (As_i and DMA), respectively.

Rice type	Grain	Origin	Cooking mode	As_t	As_i	DMA	As_i+DMA[a]
White	Long	France	raw	0.205 ± 0.041	0.160 ± 0.032	0.059 ± 0.019	0.219 (107)
			A	0.179 ± 0.036	0.120 ± 0.024	0.062 ± 0.020	0.182 (102)
			B	0.157 ± 0.038	0.094 ± 0.019	0.068 ± 0.022	0.162 (103)
			C	0.120 ± 0.029	0.058 ± 0.012	0.063 ± 0.020	0.121 (101)
			D	0.196 ± 0.029	0.112 ± 0.022	0.065 ± 0.021	0.177 (90)
Steamed white	Long	Italy	raw	0.181 ± 0.036	0.153 ± 0.031	0.030 ± 0.010	0.183 (101)
			A	0.189 ± 0.038	0.165 ± 0.033	0.034 ± 0.011	0.200 (106)
			B	0.159 ± 0.038	0.145 ± 0.029	0.028 ± 0.009	0.173 (109)
			C	0.123 ± 0.030	0.104 ± 0.021	0.025 ± 0.008	0.129 (105)
			D	0.159 ± 0.024	0.142 ± 0.028	0.029 ± 0.009	0.171 (108)
Thai white	Long	Thailand	raw	0.241 ± 0.048	0.173 ± 0.024	0.058 ± 0.013	0.231 (96)
			A	0.167 ± 0.033	0.102 ± 0.020	0.047 ± 0.015	0.148 (89)
			B	0.138 ± 0.033	0.091 ± 0.018	0.049 ± 0.016	0.140 (101)
			C	0.125 ± 0.030	0.064 ± 0.013	0.058 ± 0.019	0.122 (98)
			D	0.153 ± 0.023	0.097 ± 0.014	0.052 ± 0.012	0.149 (97)
White Basmati	Long	Himalaya	raw	0.129 ± 0.009	0.115 ± 0.023	< 0.010	0.115 (89)
			A	0.083 ± 0.017	0.077 ± 0.015	< 0.010	0.077 (93)
			B	0.064 ± 0.015	0.064 ± 0.009	< 0.010	0.064 (100)
			C	0.050 ± 0.012	0.035 ± 0.007	< 0.010	0.035 (70)
			D	0.090 ± 0.014	0.073 ± 0.015	< 0.010	0.073 (81)
White risotto	Short	France	raw	0.280 ± 0.056	0.237 ± 0.047	0.048 ± 0.016	0.285 (102)
			A	0.204 ± 0.041	0.161 ± 0.032	0.046 ± 0.015	0.205 (100)
			B	0.149 ± 0.036	0.127 ± 0.025	0.047 ± 0.015	0.174 (117)
			C	0.091 ± 0.022	0.044 ± 0.009	0.043 ± 0.014	0.087 (96)
			D	0.197 ± 0.030	0.156 ± 0.031	0.047 ± 0.015	0.203 (103)
Organic rice duo	Long	France	raw	0.535 ± 0.107	0.471 ± 0.094	0.045 ± 0.014	0.516 (96)
			A	0.321 ± 0.064	0.298 ± 0.042	0.043 ± 0.010	0.341 (106)
			B	0.306 ± 0.073	0.286 ± 0.057	0.042 ± 0.013	0.328 (107)
			C	0.162 ± 0.039	0.120 ± 0.024	0.037 ± 0.012	0.157 (97)
			D	0.316 ± 0.047	0.292 ± 0.058	0.041 ± 0.013	0.333 (105)
Three-rice mix (13% wild brown+ 14% red + 73% steamed white)	Long	NA	raw	0.301 ± 0.006	0.151 ± 0.030	0.109 ± 0.035	0.260 (86)
			A	0.305 ± 0.061	0.140 ± 0.028	0.123 ± 0.039	0.262 (86)
			B	0.264 ± 0.063	0.136 ± 0.027	0.108 ± 0.034	0.244 (92)
			C	0.173 ± 0.042	0.074 ± 0.015	0.087 ± 0.028	0.161 (93)
			D	0.299 ± 0.045	0.136 ± 0.027	0.112 ± 0.036	0.248 (83)
Steamed black wholegrain	Medium	Italy	raw	0.234 ± 0.046	0.214 ± 0.043	0.033 ± 0.011	0.247 (106)
			A	0.226 ± 0.045	0.198 ± 0.040	0.027 ± 0.009	0.225 (100)
			B	0.211 ± 0.051	0.199 ± 0.040	0.033 ± 0.010	0.232 (110)
			C	0.113 ± 0.027	0.095 ± 0.019	0.021 ± 0.007	0.116 (103)
			D	0.212 ± 0.023	0.192 ± 0.038	0.023 ± 0.008	0.215 (101)

[a] calculated based on method intermediate reproducibility

[b]: the values in the brackets indicate the fraction compared to As_t

Figures

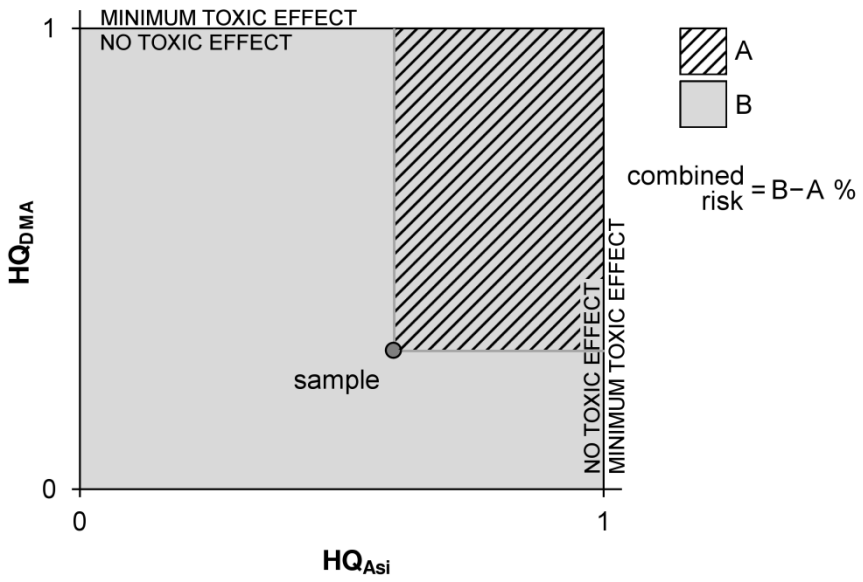

Figure 1

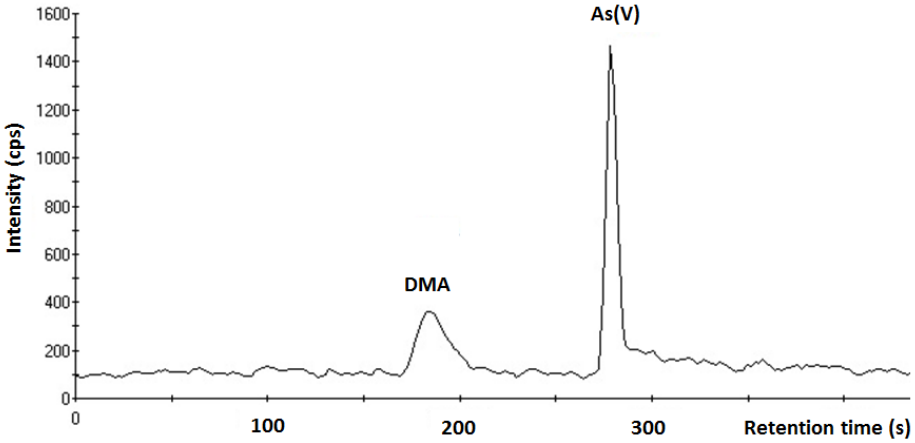
As(V)
DMA
Intensity (cps)
1600
1400
1200
1000
800
600
400
200
0
0
100
200
300
Retention time (s)

Figure 2

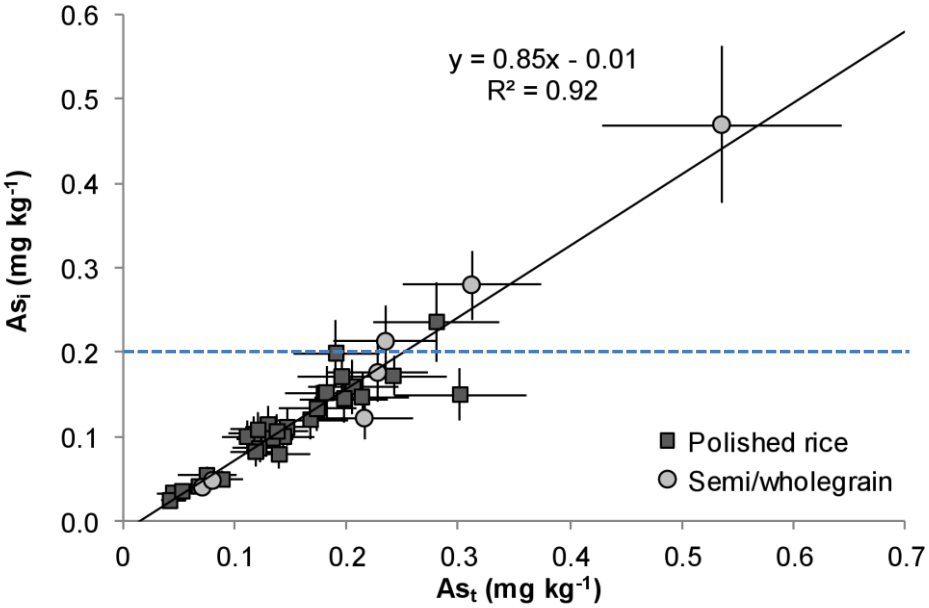
y = 0.85x - 0.01
R² = 0.92
Polished rice
Semi/wholegrain
As_i (mg kg-1)
As_t (mg kg-1)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7

Figure 3

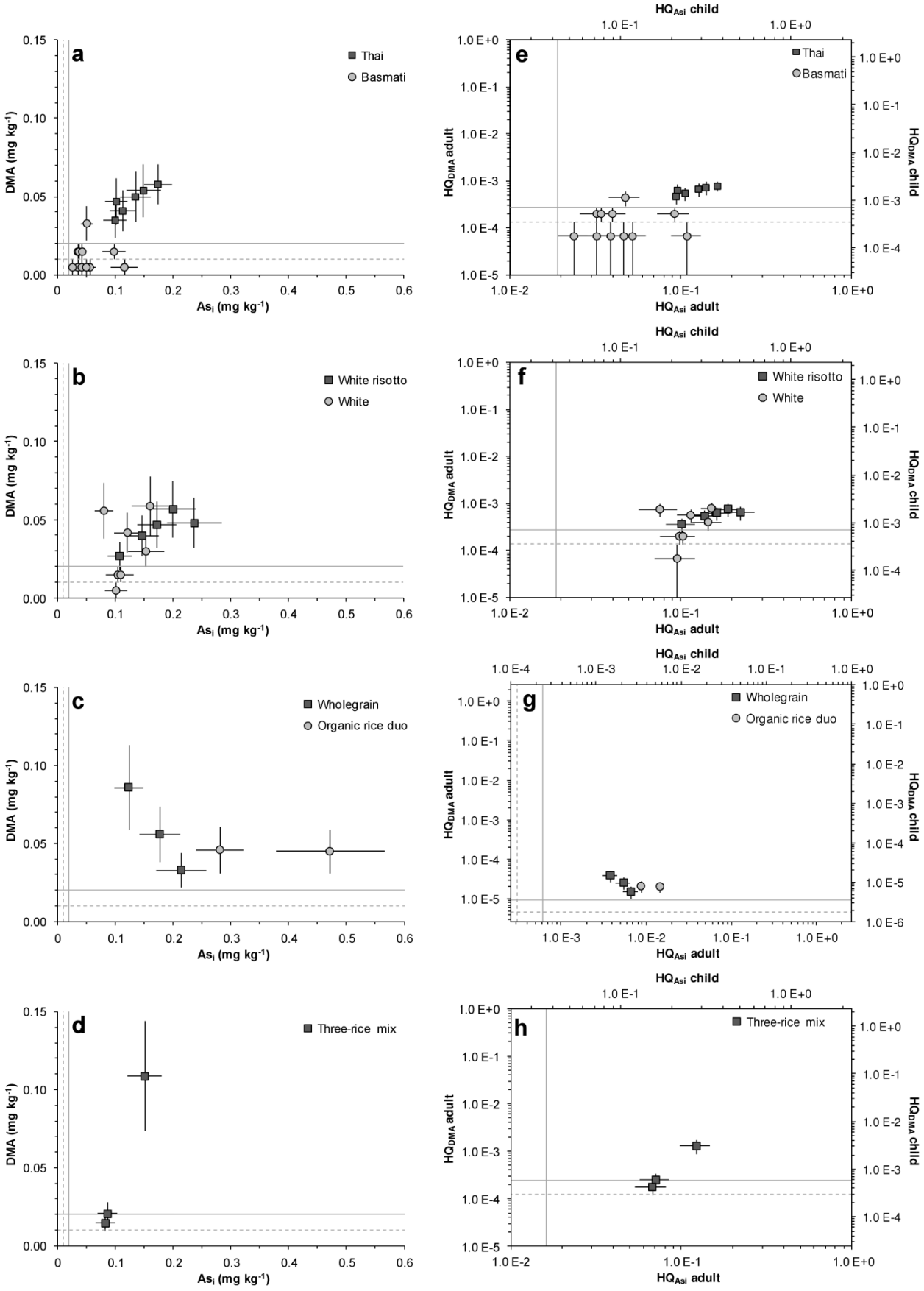
a
Thai
Basmati
DMA (mg kg-1)
Asi (mg kg-1)
b
White risotto
White
c
Wholegrain
Organic rice duo
d
Three-rice mix
e
HQAsi child
HQDMA adult
HQDMA child
HQAsi adult
f
g
h

Figure 4

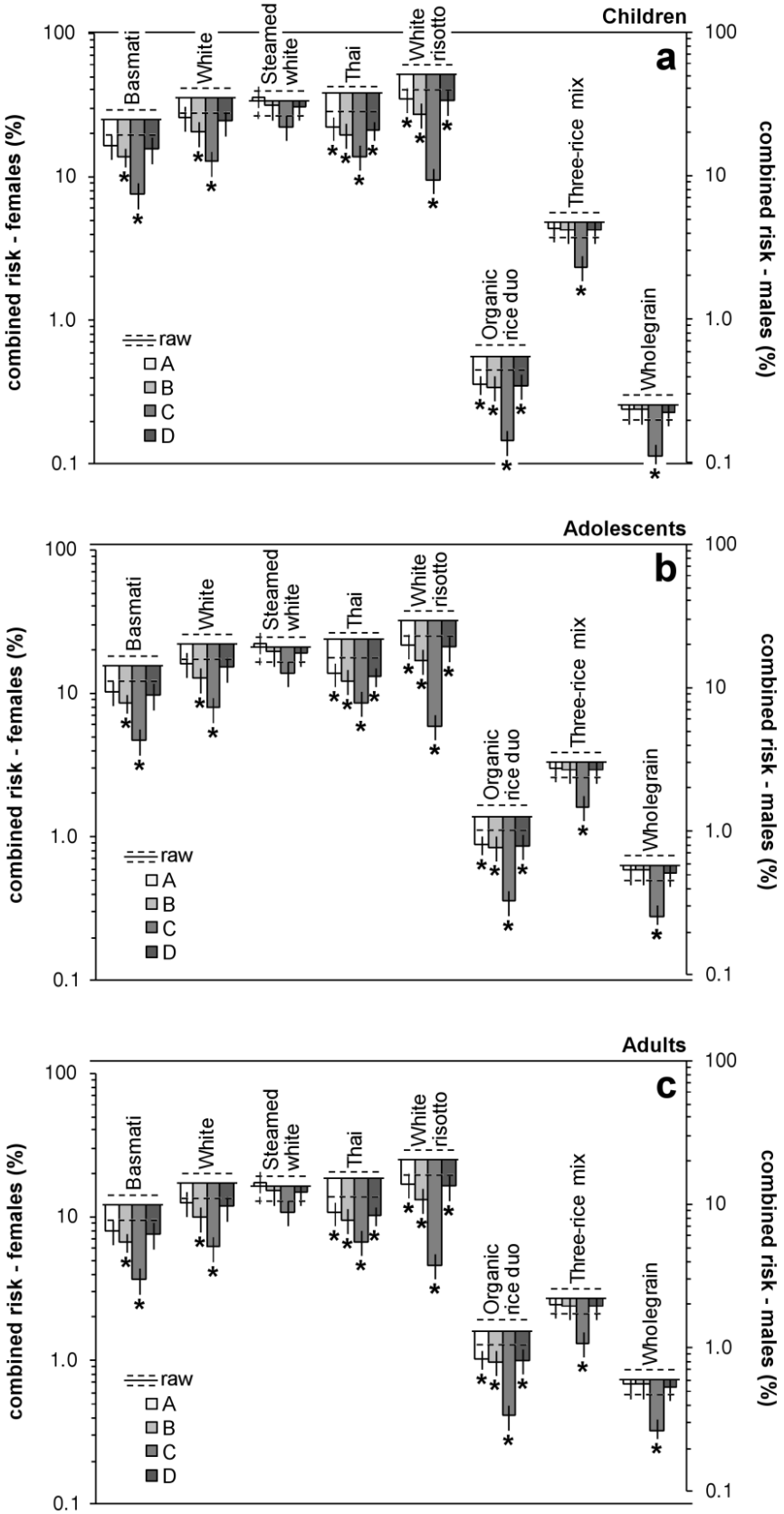
Children
a
Adolescents
b
Adults
c
combined risk - females (%)
combined risk - males (%)
100
10
1.0
0.1
Basmati
White
Steamed white
Thai
White risotto
Organic rice duo
Three-rice mix
Wholegrain
raw
A
B
C
D

Figure 5