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**World Health
Organization**

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DISCUSSION PAPER ON POSSIBILITY TO DEVELOP A CODE OF PRACTICE FOR THE PREVENTION AND REDUCTION OF ARSENIC CONTAMINATION IN RICE

In order to assist the Committee on how to proceed further with the development of a Code of Practice for the Prevention and Reduction of Arsenic Contamination in Rice, Codex members and Observers are invited to consider the summary and recommendations in paragraphs 104-105.

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Introduction

1. The 5th Session of the Committee on Contaminants in Food (CCCF) agreed to initiate new work to elaborate maximum levels (MLs) for arsenic in rice through an electronic Working Group lead by China (REP11/CF, paras. 60-64).
2. The 6th Session of CCCF discussed the proposed draft MLs for arsenic in rice and the related recommendations in the working document. The Committee agreed that a discussion paper considering the possibility to develop a Code of Practice for the Prevention and Reduction of Arsenic Contamination in Rice should be prepared for consideration by the next session of the CCCF and that an electronic Working Group chaired by China and co-chaired by Japan would prepare the discussion paper (REP12/CF, paras. 50-65).
3. It is the purpose of this paper to discuss the possibility of developing a Code of practice for the Prevention and Reduction of Arsenic Contamination in Rice in the 7th Session of the CCCF.
4. China and Japan prepared the draft, with comments from Australia, Canada, Colombia, Indonesia, the Philippines, the United Kingdom, the United States of America, and the International Council of Grocery Manufacturer Associations. A list of countries and NGOs that joined the EWG can be found in the Annex.

Section 1 Physical and chemical properties of arsenic

5. The International Programme on Chemical Safety (IPCS) summarizes briefly the physical and chemical properties of arsenic as follows (1).
6. Arsenic is a metalloid widely distributed in the earth's crust and present at an average concentration of 2 mg/kg. It occurs in trace quantities in rocks, soil, water and air. Arsenic can exist in four valency states: -3, 0, +3 and +5. Under reducing conditions, arsenite (As(III)) is the dominant form; arsenate (As(V)) is generally the stable form in oxygenated environments. Elemental arsenic is not soluble in water. Arsenic salts exhibit a wide range of solubilities depending on pH and the ionic environment.

Section 2 Sources of arsenic in the environment

- General environment

7. The IPCS also describes the sources of arsenic in the environment (1). Arsenic is present in more than 200 mineral species, the most common of which is arsenopyrite. It has been estimated that about one-third of the atmospheric flux of arsenic is of natural origin. Volcanic action is the most important natural source of arsenic, followed by low-temperature volatilization. Inorganic arsenic of geological origin is found in groundwater used as drinking water in several parts of the world, for example Bangladesh. Elemental arsenic is produced by reduction of arsenic trioxide (As₂O₃) with charcoal. As₂O₃ is produced as a by-product of metal smelting operations. It has been estimated that 70% of the world arsenic production is used in timber treatment as copper chrome arsenate (CCA), 22% in agricultural chemicals, and the remainder in glass, pharmaceuticals and non-ferrous alloys. Mining, smelting of non-ferrous metals and burning of fossil fuels are the major industrial processes that contribute to anthropogenic arsenic contamination of air, water and soil. Historically, use of arsenic-containing pesticides has left large tracts of agricultural land contaminated. The use of arsenic in the preservation of timber has also led to contamination of the environment.

- Paddies environment

8. According to A.A. Meharg and F.J. Zhao (2), arsenic sources to paddies can be divided into natural and anthropogenic. Natural inputs are sub-divided into inherent soil arsenic, and any arsenic carried in additionally through flooding (aqueous and sediment), and wet and dry atmospheric deposition. Some of the major rice growing regions of the world suffer natural geological elevations in arsenic. Depending on prevailing climatic and hydrological conditions, soils and sediments, surface waters, ground waters and air can become enriched in arsenic where these geological conditions prevail. The typical example of natural elevation is the Holocene tracts of the Bengal Basin which are higher in arsenic than the Pleistocene terraces of the same region. Ten Holocene and ten Pleistocene paddies dispersed over Bangladesh were studied (3), and it was found that the Holocene soils had approximately five-fold more total arsenic than the Pleistocene soils. A similar situation is found for ground waters from Holocene versus Pleistocene sediments (4). This difference has major consequences for arsenic levels in Bangladeshi rice (3, 5).
9. Scientific papers have reported natural elevations in arsenic in paddies in other regions. According to A.A. Meharg and F.J. Zhao (2), ultimately, the bulk of all paddy soil arsenic will be of geogenic weathering origin, either due to soil formation from local bedrock, or from sediment carried in from upstream. Atmospheric deposition is unlikely to be a major source of arsenic (6, 7), except in rice grown on soils near metal smelters, which may be contaminated by aerosol emissions.
10. Anthropogenic sources are multitudinous and highly variable and can be considered in the following classes (2):
 - (a) Non-point source industrial/urban pollution for paddies downstream of large population centres;
 - (b) Use of fertilizers and organic manures contaminated with arsenic¹;
 - (c) Point-source industrial pollution;

¹ Many fertilizers may contain trace levels of arsenic. Contaminated should not be interpreted as equivalent to trace levels of arsenic.

- (d) Use of arsenic pesticides; and
- (e) Contamination of irrigation water.

Section 3 Deposition, transformation and transportation of arsenic

- Overview of deposition and transformation in the environment

11. The IPCS overviews briefly the deposition and transformation of arsenic compounds in the environment (1).
12. Arsenic is released into the atmosphere and exists mainly adsorbed on particulate matter. These particles are dispersed by the wind and are returned to the earth by wet or dry deposition. Arsines emitted from soils or sediment after microbial biosynthesis undergo oxidation in the air, reconvertng the arsenic to non-volatile forms, which settle back to the ground. Dissolved forms of arsenic in the water column include arsenate, arsenite, methylarsonic acid (MMA) and dimethylarsinic acid (DMA). In well-oxygenated water and sediments, nearly all arsenic is present in the thermodynamically more stable pentavalent state (arsenate). Arsenite and arsenate species can interchange oxidation state depending on the reduction-oxidation (redox) potential (Eh), pH and biological processes. Some arsenic species have an affinity for clay mineral surfaces and organic matter and this can affect their environmental behaviour. There is potential for arsenic release when there are fluctuations in Eh, pH, soluble arsenic concentration and sediment organic content. Many arsenic compounds tend to adsorb to soils, and leaching usually results in distribution over only short distances in soil.
13. Additionally, in the case of volatile Arsines, it is understood that their stability is reasonably high with atmospheric lifetime being hours rather than seconds. Correspondingly therefore, they can be widely dispersed in the atmosphere (8, 9).
14. Three major modes of arsenic biotransformation have been found to occur in the environment: redox transformation between arsenite and arsenate, the reduction and methylation of arsenic, and the biosynthesis of other organoarsenic compounds, such as arsenosugars and arsenobetaine. There is biogeochemical cycling of compounds formed from these processes (Figure 1).

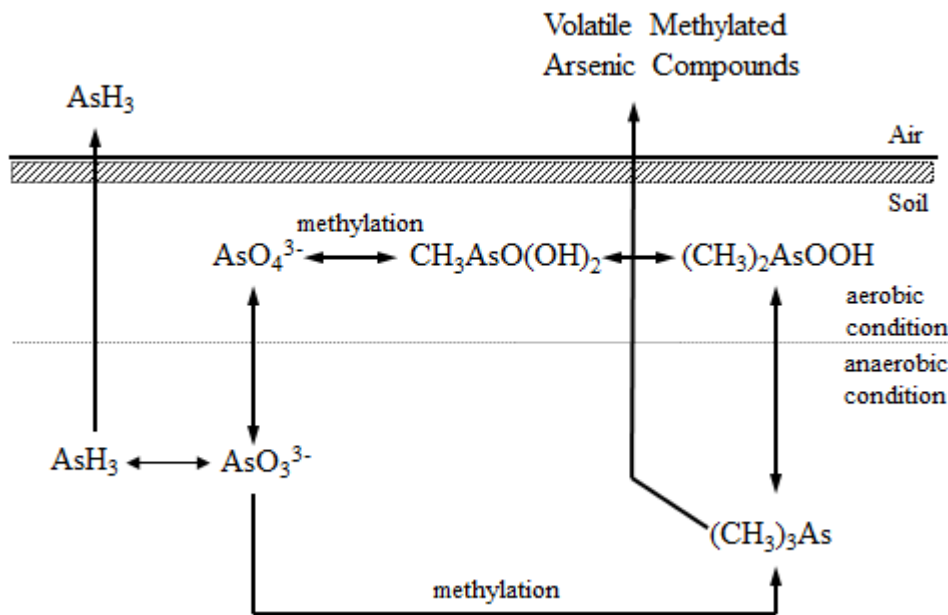


Figure 1: Biogeochemical cycling of arsenic in the rice paddy (modified from McBride (10))

- Arsenic transformation in rice paddies

15. Rice is generally a crop grown under flooded conditions though it can be grown aerobically. Flooding makes anaerobic conditions and consequently lower the redox potential (Eh) in paddy soil. On the other hand, draining water in paddy fields generates aerobic conditions and raises the Eh immediately. This variation of the Eh in paddy soil influences the redox state of arsenic as well as other elements (Fe, Mn, S, etc.) (Figure 2).

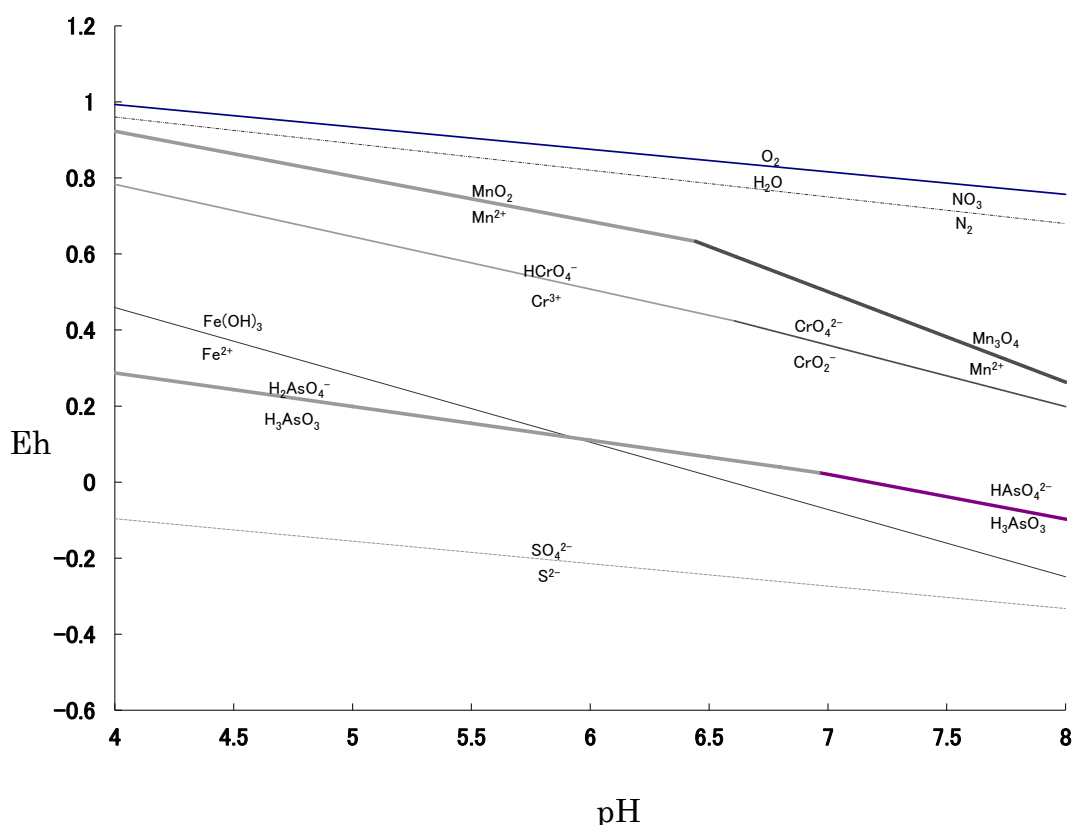


Figure 2: Eh and pH diagram for As, Fe and other elements in soil

16. In a study of paddy fields (11), the Eh of soil pore water² (soil solution) at depths of 0.1-0.2 m (the depth of the rhizosphere³ of rice plants) varied profoundly compared with the Eh at 1.0 m. The Eh values at 0.1-0.2 m continuously decreased from around 600 mV to below 0 mV after flooding. The low Eh values at the depths of 0.1-0.2 m in the flooded paddy fields returned to high values (400-600 mV) immediately after draining water and returning the fields to a non-flooded condition. Those profound Eh variations during the flooding cycle affected dissolved Fe, Mn and As concentrations in soil pore water. Soil pore water above depths of 1.0 m contained higher concentrations of Fe, Mn and As than irrigation water during the flooded period. These results suggest these ions are released to the soil pore water from submerged paddy soil (11).

17. In a number of As-contaminated paddy soils from Bangladesh and China were incubated under flooded conditions (12), pore-water Eh had dropped to below 200 mV and the arsenic concentration in the pore water increased and more than 80% of the arsenic mobilized into the pore water was in the form of arsenite. The increase in arsenite in flooded paddy soil increases arsenic availability to rice plants. The concentrations of arsenic in the pore water in the Bangladeshi paddy soils were 2–5 mg/L, whereas those in the Chinese paddy soils were 0.5–0.8 mg/L. These results indicated that the Bangladeshi paddy soils included in this study, which were contaminated due to Holocene sediments and/or long-term irrigation of groundwater with high concentration of arsenic, have a greater arsenic lability than the Chinese soils in this study, which were contaminated by mining or natural geological sources.

² Soil pore water is water occurring in the small openings, spaces, and voids between particles of unconsolidated soil. The water is held in place in the portion of the vadose water zone between the roof zone and the water table by entrapment, ionic attraction, and capillary or adhesive forces. Measuring the concentration of contaminants in the water is generally useful for assessing bioavailable contaminants. Soil solution is soil pore water collected by a sampling device buried in soil operating at negative pressure.

³ The rhizosphere is the zone of soil immediately surrounding plant roots, in which the roots influence physical and chemical reactions and microbiological activity in the soil.

18. Two main reasons have been proposed to explain why arsenic solubility increases as conditions in flooded paddy soils become more anaerobic (2). First, some soil-adsorbed iron oxides/hydroxides are reduced and released into the solution phase, a process called reductive dissolution. Since these iron oxide minerals are an important host phase of arsenic in soil, their dissolution also releases the sorbed arsenic into the solution phase. It is often observed that arsenic mobilization in flooded soils is accompanied by parallel Fe mobilization (11, 12). Studies show that the ratio of As to Fe released can vary widely among different soils, reflecting the amount of arsenic sorbed by easily reducible iron oxides/hydroxides (11, 12). The type and property (e.g., the degree of crystallinity) of iron oxides/hydroxides have a strong influence on the rate of reductive dissolution and arsenic mobilization. Second, soil-adsorbed arsenate may be reduced to arsenite in the soil solid phase during flooding and, since arsenite is less strongly adsorbed on soil, it has a greater tendency to partition into the solution phase. A scientific paper showed that As(V) was predominant in the soil solid phase with As(III) accounting for only about 15% in two paddy soils before flooding. After flooding and as the Eh decreased, the proportion of As(III) increased to 60–80% at the Eh of -100 mV. In comparison, arsenic speciation in the solution phase was dominated by As(III) in both flooded and non-flooded conditions (13).

19. Arsenite sorbed on the soil solid phase is much more easily desorbed into the solution phase than arsenate (13-15). The distribution coefficient (solution phase conc./solid phase conc.) for arsenite increases rapidly with increasing pH from 5.5 to 7, whereas for arsenate, the increase is apparent only at $\text{pH} > 7$.

20. The low Eh associated with continuous flooding also cause reduction of sulphate ions to sulphide ions. The sulphide ions can react with As and precipitate out of soil solution as arsenic sulphide in sewage sludge (16), sediments (17, 18) and soils (19). The precipitation of arsenic sulphide, in turn, lowers the As concentration in the soil solution, and may thereby lower the amount of As bio-available to rice plants.

21. Additionally, in a study, there were significant differences in the absorption by rice of arsenic species from soil pore water. Between arsenite, arsenate, MMA and DMA, uptake of inorganic species was more than ten times higher than uptake of organic species, and arsenite uptake was considerably higher than arsenate uptake; e.g. at concentrations of 0.053 mmol/L arsenate in irrigation water, the uptake rates of arsenite, arsenate, MMA and DMA were 147, 126, 12.7, and 5.7 nmol/(g-fresh weight·h), respectively (20).

- Molecular mechanism of arsenic transportation from soil to rice

22. Rice roots are able to absorb each arsenic species from the soil pore water. The absorption of arsenate and arsenite by rice plants differs greatly in molecular mechanisms.

23. Arsenate is a chemical analogue of phosphate, since the two elements belong to the same group in the Periodic Table. For this reason, arsenate will compete with phosphate for transport by phosphate plant membrane transporters. Phosphate effectively suppresses arsenate uptake in hydroponic cultures of rice, which is attributable to competition between the two anions for the same transporter (20). There are 13 genes named *OsPht1;1*-*OsPht1;13* which encode the putative high-affinity phosphate transporter proteins in the rice genome (21). In rice plants over-expressing the phosphate transporter gene *OsPht1;8*, arsenate uptake was much enhanced (22), indicating that *OsPht1;8* has a high affinity for both phosphate and arsenate and is probably involved in the uptake and long-distance transport of phosphate and arsenate in rice (22, 23).

24. Arsenate taken in by rice roots is, however, reduced in the root cells to arsenite rapidly (24, 25), and the dominant arsenic species in the rice root is arsenite (26-28). Furthermore, in flooded soil, arsenite is the main arsenic species taken up by rice roots, along with a small amount of DMA, and the phosphate/arsenate transport pathways ultimately contribute little to arsenic accumulation in rice (22).

25. As mentioned in "Phosphates" in para. 51, addition of phosphate fertilizers increased arsenic accumulation in rice grain, probably due to the competitive adsorption of phosphates to arsenic-binding soil constituents such as iron oxides/hydroxides (14, 29, 30). This is one of the important mechanisms that lead to increased bioavailability of arsenic to the rice plant.

26. Arsenite is transported into rice by a silicate transporter, and added silicates decrease arsenite transport into rice. The mechanism of the Si-mediated decrease in arsenite uptake was elucidated by Ma et al. (26, 31, 32), who identified the rice Si transport proteins OsNIP2;1 (Lsi1)⁴ and Lsi2⁵. The processes of Si and As transport from the external medium to the stele involve the influx of silicic acid and arsenite by Lsi1 and then the efflux of them towards the stele, regulated by Lsi2. Moreover, Lsi2 can play a more important role in controlling As accumulation in rice shoots and grain than Lsi1. Si-mediated decreases in As uptake can be explained by competitive inhibition between Si and arsenite. Therefore, Guo et al. (33, 34) has reported that the addition of silicate inhibited As accumulation in rice when arsenate was used as the As form; yet the effect was not attributable to a direct competition between Si and arsenate since they do not share the same transporters. The observations can now be properly explained by the involvement of Lsi2 in arsenic uptake and translocation. Arsenate taken up by rice roots is reduced rapidly in the root cells to arsenite (24, 25), which is transported towards the xylem through the effluxer Lsi2 and is subject to competitive inhibition from Si (28). Thus pre-treatment with Si can inhibit As translocation to shoot (33, 34). The efficient Si uptake pathway in rice also allows inadvertent passage of arsenite, thus explaining why rice is efficient in accumulating As.

Section 4 Toxicity and exposure for arsenic

27. The risk assessment on inorganic arsenic in foods was conducted in the 72nd meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and its results were issued in the monograph in 2011 (35).

28. According to the JECFA evaluation, the lower limit on the benchmark dose for a 0.5% increased incidence of lung cancer (BMDL_{0.5}) was determined from epidemiological studies to be 3.0 µg/kg bw inorganic arsenic per day (2-7 µg/kg bw per day based on the range of estimated total dietary exposure) using a range of assumptions to estimate exposure to inorganic arsenic from drinking water and food.

29. JECFA concluded that the great majority of inorganic arsenic exposure occurs through naturally contaminated groundwater — through drinking water, through water used in food preparation, and through water used to irrigate food crops, particularly rice. Paddy rice may also contain relatively high levels of arsenic at low soil arsenic levels due to the high availability of arsenic in flooded soils. Reported mean dietary exposure to inorganic arsenic in the USA and various European and Asian countries ranged from 0.1 to 3.0 µg/kg bw per day. The proportion of inorganic arsenic exposure arising from food relative to water increases as the concentration of inorganic arsenic in the water decreases. At the lower end of the exposure range, food can also be a major contributor to inorganic arsenic exposure. For certain regions of the world where concentrations of inorganic arsenic in drinking-water exceed 50–100 µg/l, some epidemiological studies provide evidence of adverse effects. There are other areas where arsenic concentrations in water are elevated (e.g. above the WHO guideline value of 10 µg/l) but are less than 50 µg/l. In these circumstances, there is a possibility that adverse effects could occur as a result of exposure to inorganic arsenic from water and food, but these would be at a low incidence that would be difficult to detect in epidemiological studies.

30. A recent study in China showed that the weekly inorganic arsenic intake is about 4.95 µg / kg bw, and about 60% is from rice (36).

Section 5 Scope on possible development of a Code of Practice for the Prevention and Reduction of Arsenic Contamination in Rice

31. The General Standard for Contaminants and Toxins in Food and Feed (GSCTFF) states as follows in Section 1.3.1 in the Preamble:

- Contaminant levels in food shall be as low as reasonably achievable through best practice such as Good Agricultural Practice (GAP) and Good Manufacturing Practice (GMP) following appropriate risk assessment;
- To ensure that adequate action is taken to reduce food contamination, a code of practice shall be elaborated comprising source related measures and GAP as well as GMP in relation to the specific contamination problem;
- The effect of the actions to reduce contamination shall be assessed by monitoring, survey programs and more specialized research programs, where necessary; and
- A broad approach shall be applied taking into account all relevant information that is available, for developing recommendations and control measures.

32. Thus, the concentration of arsenic in rice should be as low as reasonably achievable and a relevant code of practice should be developed in line with the GSCTFF. The 72nd JECFA indicated in its monograph on arsenic that general strategies for reducing human exposure to arsenic from foods include reducing arsenic uptake into food crops, increasing the proportion of less toxic organic forms relative to inorganic arsenic in food crops and reducing the arsenic content of foods by processing, preparation or cooking methods. A.A. Meharg and F.J. Zhao introduced strategies for producing low arsenic rice to minimize arsenic accumulation (2).

⁴ OsNIP2;1 (Lsi1) is a major influx transporter for silicic acid in rice. It is localised to the distal side of plasma membranes in the exodermal and endodermal cells of rice roots.

⁵ Lsi2 is an efflux carrier of silicic acid localized to the proximal side of the plasma membranes in the same cells of rice roots.

33. Therefore, the scope of possible development of a Code of Practice for the Prevention and Reduction of Arsenic Contamination in Rice should extend to the following measures taking into consideration the strategies mentioned and scientific facts in the above sections:

- Source directed measures;
- Agricultural practices (use of agricultural materials, control of irrigation water and selection of cultivars);
- Processing and cooking;
- Monitoring of effectiveness of measures.

Section 6 Measures for prevention and reduction

Section 6.1 Source directed measures

34. The main objective of the Code of Practice for Source Directed Measures to reduce Contamination of Food and Feed with Chemicals (CAC/RCP 49-2001) is to increase awareness of sources of chemical contamination of food and feed, and of source-directed measures to prevent such contamination. The code of practice requires that national food or feed control authorities should inform relevant national authorities and international organizations of potential or actual food or feed contamination problems and encourage them to take appropriate preventive action.

35. The code of practice recommends that the relevant national authorities and international organisations should be informed about actual and potential food or feed contamination problems and should be encouraged to take measures to:

- control emissions of pollutants from industry, e.g. the chemical, mining, metal and paper industries, and also from weapons testing;
- control emissions from energy generation (including nuclear plants) and means of transportation;
- control the disposal of solid and liquid domestic and industrial waste, including its deposition on land, disposal of sewage sludge and incineration of municipal waste;
- control the production, sale, use and disposal of certain toxic, environmentally-persistent substances;
- where possible, replace toxic environmentally-persistent substances with products that are more acceptable from the health and environmental points of view; and
- blacklist the areas concerned, i.e. prohibit the sale of foods and feeds derived from these polluted areas and advise against the consumption of such foods or use of such feeds, where agricultural land is heavily polluted due to local emissions.

36. In the case of arsenic, sources include air deposition, irrigation water (As-tainted groundwater/reclaimed waste water) and soil with geogenic origin of arsenic (volcanic ash soils). If these sources result in significant elevation of arsenic in rice, measures should be taken. The above measures can generally be used as source-directed measures for reducing arsenic contamination in rice. The source-directed measures including their applicability for arsenic should be discussed in the CCCF in view of:

- whether the measures are technically or economically feasible;
- whether the arsenic levels in paddies reflect previous or ongoing pollution; and
- whether the any measures specific to arsenic should be developed.

Section 6.2 Agricultural Measures

37. Arsenic is toxic not only to humans but also to plants including rice. One of the examples of toxicity of arsenic to rice plants is known as "straight-head disease". Various agricultural measures aimed at alleviating growth inhibition from straight-head disease in arsenic-contaminated rice paddies have been investigated, and provide relevant information to reducing more subtle instances of arsenic contamination in rice.

- Effect of the Use of Agricultural Materials⁶

38. Agricultural materials are applied to fields as sources of plant nutrition and/or soil amendments. Some materials are effective at reducing arsenic concentration in rice while other materials promote arsenic uptake, leading to high arsenic concentrations in rice. The type of agricultural materials used for soil amendment affects arsenic solubility, speciation of arsenic in the soil, and uptake of arsenic by the rice plant.

⁶ In this paper, "agricultural materials" mean fertilizers such as N, P, K including other elements and soil amendments such as lime including compost, manure etc.

• Iron-containing materials

39. It has been widely reported that supplemental iron-containing materials effectively suppress arsenic dissolution from soil solids (37). Three possible mechanisms for the suppression include creation of a high redox potential in the soil (38); sorption of arsenic on/in the iron-containing materials (39); and precipitation of low solubility arsenic compounds.

40. As mentioned in Figure 2 (Eh and pH diagram), the redox potential of iron species is relatively high compared to that of arsenic species. When iron-containing materials such as iron oxides are applied to soils, the decrease of Eh under flooding conditions is slowed. Thus, addition of iron materials can suppress the reduction of arsenate to arsenite and the dissolution of arsenic from soil solids (38).

41. Second, in general, arsenate is strongly adsorbed by most mineral constituents of soils, such as various oxides/hydroxides of iron or aluminium, aluminosilicate clay minerals and manganese oxides, whilst arsenite exhibits a limited affinity for most soil minerals except iron oxides/hydroxides (40).

42. Amorphous iron oxides/hydroxides adsorb greater amounts of arsenate or arsenite than their crystalline counterparts due to larger specific surface areas and adsorption sites such as iron hydroxyl groups (14). In an abiotic column test of different iron-containing materials (41), the adsorption maxima for arsenite and arsenate were, respectively, 1.75 mol kg⁻¹ and 1.28 mol kg⁻¹ for ferrihydrite, 0.35 mol kg⁻¹ and 0.24 mol kg⁻¹ for goethite, and 0.11 mol kg⁻¹ and 0.08 mol kg⁻¹ for hematite. Table 1 indicated that: 1) Arsenate was adsorbed more largely and strongly to ferrihydrite, which is amorphous, than to goethite and hematite, which are crystalline; 2) Arsenite was adsorbed more than arsenate on all types of iron oxides/hydroxides, regardless of whether they were crystalline or amorphous; and 3) in all cases, despite being adsorbed more extensively, arsenite desorbed more easily than arsenate.

43. In an experiment conducted in flooded paddies, it was observed that application of FeCl₃·H₂O (25 mg Fe kg⁻¹ soil) markedly lowered the water soluble arsenic levels in soil and the arsenic concentrations in husked rice (by 25% and 9.2% respectively), and the iron-supplemented plants grew faster than plants grown under ordinary conditions (42).

44. As described in para. 18, in soil incubation tests under flooded (anaerobic) conditions, arsenic solubility increases as a result of the reduction of iron oxides/hydroxides and reduction of arsenate. However, recent pot experiments in Japan revealed that the use of iron-containing material containing sulphur can lead to the formation of orpiment (As₂S₃) and/or arsenophrine (FeAsS) in strongly reductive conditions. Since orpiment and arsenophrine are poorly soluble, use of the iron-sulphur soil amendments to produce insoluble arsenic compounds may lower availability of soluble arsenite in soil pore water (unpublished data). Investigations into the effect of soil amendments which combine iron and sulphur will be followed by large scale experiments in paddies or concrete frames in the future in Japan.

45. As a summary, iron-containing materials are effective in alleviating arsenic dissolution from soil solids and arsenic uptake by rice plants.

46. In an experiment with potted rice plants and flooded soils, the addition of bivalent ionized iron (Fe²⁺) promoted iron oxide plaque formation on root surfaces; reduced the toxic effect of arsenic on the growth of rice; resulted in reduced arsenic concentration in rice grain and straw; and increased grain yield (29, 43). The amount of ammonium oxalate extractable iron plaque correlated negatively with arsenic concentrations in rice grain and straw and positively with grain yields, though the correlations were not significant at the 0.01 level. Based on these observations, it was suggested that iron oxide plaque adsorbed arsenic and lowered arsenic contamination in rice, but there was no quantitative evaluation of the significance of this effect (Table 2) (29).

47. In a pot experiment, using an arsenic-contaminated water supply, addition of 0.1% and 0.5% amorphous iron oxides/hydroxides to soil enhanced iron plaque formation on the root surfaces, increased arsenic concentration on root surface, improved plant growth and reduced the arsenic concentration in rice plants (39).

48. Although most scientific and published papers indicate that iron supplementation suppresses As uptake in rice plants, there are a few scientific and published papers that suggest that iron plaque cannot act as a barrier to the uptake of arsenic to rice plants from soil pore water (44, 45) and that iron plaque may be a sink of arsenic for uptake by rice plants (45, 46).

49. Additionally, most commercially available agricultural iron containing materials contain impurities such as calcium silicate and phosphate, and most manure contains phosphate and/or iron. Research is needed to assess if the presence of these impurities impact arsenic on kinetics in soil, and to verify whether iron plaques may, under certain conditions, act as a reservoir of arsenic for uptake by rice. Also, adding iron may affect regulatory compliance for water quality regulations in some areas, like California, USA, which may affect regional ability to use iron supplements.

• Phosphates

50. Phosphates significantly decrease the adsorption of arsenite and arsenate on iron oxides/hydroxides in soil. In a study without use of plants, the fraction of arsenic bound to amorphous iron oxide was reduced substantially in the presence of 100 $\mu\text{mol/L}$ total phosphate at pH 4.0, namely, from over 95% to about 80% in case of arsenate, and from 75% to undetectable in case of arsenite (14). Similarly, at the same concentration of phosphate, arsenate and arsenite sorption on goethite was decreased from over 90% to about 65% and 15% respectively (14). The effect of phosphate on arsenate adsorption was greater at high pH than at low pH, whereas the opposite trend was observed for arsenite (14, 47). Additions of phosphate enhanced arsenic reduction to arsenite under anaerobic conditions, possibly due to increased desorption of arsenate, which subsequently facilitated the reduction process in the soil solution (19).

51. In an experiment using rice plants in pots with flooded soils, addition of phosphate fertilizers was found to increase arsenic accumulation in rice, probably as a result of increased desorption from the soil solid phase. The amount of arsenic adsorbed to iron oxide plaque on the rice roots also was lower (21% on average) in the presence of added phosphates (0-50 mg-phosphorus equivalent/kg-soil). Furthermore, the adverse effect of arsenic on grain yield was aggravated by phosphate addition. Higher doses of arsenic (15-30 mg-arsenic equivalent/kg-soil) combined with phosphate (50 mg-phosphorus equivalent/kg-soil) resulted in maximum reduction of grain yield, indicating that in this experiment, the added phosphate enhanced arsenic availability to the plant and thereby inhibited plant growth (see Table 2) (29).

52. It has also been suggested that low phosphate may increase the formation of iron plaque, leading to greater sequestration of arsenate (48). The overall effect of phosphate (as a balance between iron plaque formation and arsenate desorption by phosphate) on arsenic accumulation in rice grain still needs to be investigated in the field across soils with different phosphate levels.

53. On the other hand, phosphates will compete with arsenate for plant membrane transporters. The uptake rate of arsenate by rice plants from an incubating solution containing 0.05 mmol/L of arsenate decreased significantly ($P < 0.001$) with increasing phosphate concentration in the solution. High arsenate influx of 171.2 nmol/(g-fresh weight·h) was found in the absence of phosphate in the solution, which was reduced by 9%, 30%, 53%, 66%, 80%, and 88% at the addition of 0.01, 0.025, 0.05, 0.1, 0.25, and 0.5 mmol/L phosphate to the solution respectively. In contrast, the uptake rate of arsenite was, under the same test conditions, independent of phosphate concentration (20).

54. In evaluating the overall impact of phosphate on arsenic in rice, it should be noted that arsenate is likely to be reduced to arsenite under flooded conditions, as described in para. 15. Once arsenate displaced by phosphate from the solid phase is reduced to arsenite in soil pore water, competitive suppression of arsenic uptake by phosphate cannot be expected since arsenite is not taken up by roots via the phosphate transporter (2). Indeed, a recent study showed that the phosphate transport pathway made little contribution to the arsenic concentration in rice plants grown in flooded soil (22).

• Silicates

55. Silicic acid is a natural ligand in soils that can compete with the adsorption of arsenic, particularly arsenite. In an experiment, use of silicates increased arsenic concentration in soil solution (49).

56. The presence of 1 mM silicic acid decreased arsenite adsorption on goethite by 40% (50). Addition of sparingly soluble SiO_2 gel to a flooded soil considerably increased the concentrations of arsenite and, to a lesser extent, arsenate in the soil solution (49).

57. On the other hand, as described in para. 26, increased silicates availability decreased arsenite accumulation in rice by suppressing arsenite uptake via the silicic acid/arsenite transporters.

58. By using hydroponic experiments (33), a strong suppressing effect of silicates on rice plant arsenic uptake was shown. Application of external silicates lowered the arsenic concentrations in rice shoots and roots significantly. In addition, silicates significantly lowered shoot phosphorus concentration and shoot phosphorus uptake (33).

59. Further study found that the addition of silicates to the pre-treatment or uptake solution significantly lowered shoot and root arsenic concentrations and shoot phosphorus concentrations. Therefore, both internal and external silicates suppressed the uptake of arsenic and phosphorus. Arsenic uptake kinetics showed that the effect of silicates on arsenite uptake is not caused by direct competition with arsenic for transporters (34). It is likely due to the effect of silicates on arsenite translocation from root to shoot, which is facilitated by the Lsi2 protein.

60. These findings were supported by soil-based experiments conducted in Germany (51) and England (49) with rice plants in pots. By using five kinds of soil with As levels ranging from 5.0 to 15.1 mg/kg (51), there was a close positive relationship between Fe and As concentrations in the soil solution, suggesting that the major part of As was from the iron oxides through reductive dissolution. Indigenous silicic acid in the soil solution suppressed As uptake (51). Soils with high plant available Si contents had relatively low plant As content.

61. In another experiment with potted rice plants (49), addition of silica gel (20 g of SiO_2 / kg of soil) to soil decreased total arsenic concentrations in straw and grain by 78% and 16%, respectively. In the same experiments, Si addition decreased the concentration of inorganic arsenic in grain by about 59% but increased the DMA concentration by 33%. Since Lsi2 does not transport MMA or DMA (49), there is no competitive effect of silicic acid on the accumulation of MMA or DMA in rice shoots, unlike for arsenite.

62. Therefore, applying Si fertilizers to rice crops may be an effective way of mitigating the problem of excessive transfer of As from paddy soil to rice grain. However, field trials are still lacking on the effect of Si fertilizers on As accumulation.

• **Organic materials⁷**

63. Dissolved organic matter can compete with As for adsorption sites at mineral surfaces, thereby decreasing arsenate and arsenite adsorption and enhancing arsenic release to soil pore water (5, 52). Input of organic matter including biogas slurry and dried distillers grain significantly increased the release of arsenite in paddy soil, and enhanced total arsenic accumulation in rice plants (53, 54).

64. On the other hand, stable organic matters may also serve as binding agents, thereby reducing arsenic mobility. Flooded paddy soil usually contains significant amounts of stable organic matter, as a result of anaerobic soil conditions that limit the degradation of organic matter (55). Humic substances and other stable organic materials usually have affinity with arsenic, due to ligand exchange reactions with COOH and phenol/catechol OH functional groups. Recent studies provide direct evidence for the ability of arsenic to complex with humic substances (As-SH) (56, 57).

65. Methylated arsenic species usually account for 10%-90% of total arsenic in the soil or rhizosphere (54, 58). The addition of organic matter stimulates the growth of various indigenous microbes, including arsenic methylators that facilitate arsenic methylation and volatilization from soil, and the volatilization ability varied with type of organic matter (54, 59, 60). The application of biogas slurry and dried distillers grain to paddy soil also resulted in increased arsenic methylation in paddy soil, and increased accumulation of methylated arsenic species in rice plants and grains (53, 54). Methylated arsenic species were more liable to transport from shoots and roots to grains than inorganic arsenic (54, 61).

66. In summary, the application of organic matter to soil with elevated As should be handled cautiously. For example, flowering stage is critical, as arsenic uptake at this stage directly influences arsenic transport to the grain (61, 62). Therefore, application of organic matter at this stage should be avoided, since As absorbed on iron plaque can be released with the addition of organic matter and is made available for methylation, uptake and translocation into the grain at this critical growth stage in terms of As in rice grain (46, 61, 62).

67. In addition, the land application of manure from livestock fed arsenicals may be a contributor to arsenic in paddy soils in spite of no direct evidence of paddy field contamination by Roxarsone (63, 64).

• **Summary about the application of agricultural materials**

68. Iron containing soil amendments, which are generally low in cost, can lower arsenic concentration in soil pore water due to their ability to adsorb arsenic. If silicates are insufficient in the paddy soil, the use of silicates may decrease arsenic concentration in rice due to competition between silicates and arsenite.

69. There are few experiments in the paddy fields, so control measures based on laboratory experiments should be tested in each region.

- **Effect of the control of irrigation water**

70. Growing rice "aerobically" lowers the solubility of arsenic and, as a result, decreases the bioavailability of arsenic to rice plants. Arsenic concentrations in different rice tissues are markedly lower in aerobic conditions than in anaerobic conditions, and arsenic concentrations in the rice plant follow the order of straw > husk > rice grain (65).

71. The inorganic arsenic percentage in rice grains is different in anaerobic and aerobic conditions. Based on one study, rice grain from anaerobic conditions has a lower percentage of inorganic arsenic relative to total arsenic. However, the concentration of inorganic arsenic in anaerobically grown rice was 2.6 to 2.9-fold higher than in aerobically grown rice (65).

72. Partial drainage of fields, either periodically or throughout rice growth (42), and growing rice on raised beds (66) have been used to reduce arsenic toxicity to the rice plant, and these practices also reduce the arsenic concentrations in rice grain and straw (Table 3). In alternate wetting and drying (AWD) which is a kind of partial drainage of fields, irrigation water is applied to obtain flooded conditions after a certain number of days have passed after the disappearance of ponded water (67). Although the effect of adopting AWD on arsenic uptake by rice has not been tested under field conditions, a recent field study showed that arsenic concentration in rice grain in intermittently flooded paddy is 41% lower than that in continuously flooded paddy (68).

73. The yield for rice grown under aerobic conditions tends to be smaller than for rice grown in flooded conditions, possibly due to damage to plants by pathogens and nematodes, and the lower bioavailability of nutrients such as phosphorous (69). There is a potential for rice grown on raised beds and/or aerobic conditions in some agricultural circumstances to result in lower yields and significantly increase the likelihood and severity of some diseases, especially blast disease in rice (67, 70, 71).

⁷ In this paper, the term, "organic materials", means fertilizers or composts derived from animals, manures, plants, or others such as sludge (biosolids, sewage biosolids, or sewage-driven biosolids).

74. On the positive side, much effort is currently being directed towards “aerobic” rice production methods as water availability is becoming physically and economically limiting in some rice growing regions (72). In addition, aerobic conditions controlled by partial drainage of fields affect rice yield much less than growing rice in significantly arsenic-contaminated soil with conventional flooding practices (Table 3).

75. Cultivating paddy rice in partial drainage conditions is good practice in view of lowering arsenic accumulation without impacting rice yields if soil in paddy fields is significantly contaminated by arsenic. The question is when and how long farmers should maintain “aerobic conditions” throughout the rice cultivation period. Recent papers provide information on partial drainage practices (Table 4). One study found that draining water after flowering decreases arsenic concentrations in rice grains and husks compared with continuous flooding and that flooding after flowering increases arsenic concentrations in rice tissues compared with aerobic treatment. This suggests that arsenic in rice grain and husk is derived from both the uptake before and after flowering (49). Another study found that keeping paddy soil submerged from 3 weeks before heading (emergence of rice panicles) to 3 weeks after heading lowers the redox potential (Eh) in paddy soil, and consequently markedly increases arsenic concentrations in rice grain, while during the period from planting to 3 weeks before heading, the lower Eh in paddy soil does not affect arsenic accumulation in rice grain (73).

76. The study by Arao et al. suggests that it is important that farmers maintain paddy soil in oxidative conditions from several weeks before heading/flowering to several weeks after heading/flowering⁸ by controlling water levels in the field. Submerging paddy soil after rice heading/flowering should be avoided since lowering the Eh in paddy soil after heading/flowering increased inorganic arsenic concentrations in rice grain (Table 4) (73).

77. Most published papers (49, 65, 73) on controlling As uptake by controlling flooding are based on the uptake of arsenic in rice plants grown in pots in greenhouses, with the exception of a few reports (42, 66). Data are lacking on arsenic uptake in rice grown under field conditions. A rice growing study in paddy fields and concrete frames is being conducted currently in several sites in Japan to assess the effects of controlling irrigation water during periods before/after rice heading/flowering. However, the available data suggest that controlling the water levels during the period of heading/flowering in paddy fields is a practical farming method to control the redox potential (Eh) in paddy soil.

78. A potential negative effect of using aerobic growth to limit As uptake is an increase in cadmium uptake. The scientific literature (73, 75) indicates that flooding paddy fields is effective in reducing cadmium concentrations in rice grain, while aerobic conditions in paddy soil lead to increased mobilization of cadmium and increased cadmium uptake in rice. Keeping paddy fields flooded during the period from 15 days before heading/flowering to 25 days after heading reduced the cadmium concentration in brown rice (76). These results suggest that it may be difficult to decrease arsenic and cadmium concentrations in rice grain simultaneously by means of water control measures alone. In Japan, a new study will be launched soon investigating whether arsenic and cadmium concentration in rice grains can be lowered simultaneously by controlling irrigation water and by using a rice cultivar with low cadmium uptake (77), along with agricultural materials.

- Effect of varietal selection

79. Arsenic in rice plants is also under genetic control. It was first reported that several quantitative trait loci (QTLs) are linked to arsenic in rice in 2008 (78). A number of recent field studies have indicated substantial genetic variation in grain on total arsenic concentration, as well as the concentration of arsenic species (79-86) (Table 5).

80. Field experiments conducted at two sites in Bangladesh (Faridpru and Sonargaon) found 4-4.6 fold variation in total grain arsenic (0.16~0.74 mg kg⁻¹ at Faridpru, 0.07~0.28 mg kg⁻¹ at Sonargaon) among 76 cultivars consisting of Bangladeshi landraces and parents of permanent mapping populations. Although the two field sites had different levels of arsenic contamination, highly significant cultivar differences were detected and a significant correlation ($r = 0.802$) in grain arsenic of 76 cultivars was observed, indicating stable genetic differences in arsenic accumulation (80). Considering the concentration of total arsenic in whole grain samples, the local landraces with red bran had higher arsenic levels than those with brown bran. The higher arsenic levels in the red bran rice did not result exclusively from higher As in the bran layer, since the same percentage of grain arsenic was removed during polishing of all the cultivars. Furthermore, arsenic speciation results indicated that there was a significant difference in the percentage of inorganic arsenic between the subgroups of rice cultivars and a significant subgroup by field site interaction. The contribution of genetic variation to the percentage of inorganic arsenic in rice grains was identified (80).

81. A recent study was made over a 3-year period (2004, 2005, and 2007) of genetic variation in 25 rice cultivars. The study was conducted at one field site in Stuttgart, Arkansas, USA, in a paddy with moderate arsenic concentrations under continuously flooded field conditions (83). Concentrations of total grain arsenic and arsenic species varied widely among the different cultivars. Arsenic concentration and speciation were mostly dependent on genotype, which accounted for about 70% of the variation in total grain arsenic. However, there were also significant contributions from genotype by year (17.1%) and year effects (variation between years) (3.5%), which were likely impacted by annual differences in environmental conditions such as temperature and local soil characteristics (83).

⁸ Heading is the time when the panicle begins to exert from the boot. Heading may take over 10 to 14 days. Flowering generally begins upon panicle exertion or on the following day. Consequently, flowering is considered synonymous with heading (74). In Arao's study, heading days had continued about 1 week, but it's difference have no impact on As and Cd concentrations in rice. The difference between heading and flowering in rice is also no matter in actual practice for controlling irrigation water in paddy fields in Japan.

82. In a study of 10 rice cultivars grown in Japan in pot experiments with high arsenic soil or in field trials with low arsenic soil, the variability in total and inorganic grain arsenic and dimethylarsinic acid among cultivars was statistically significant, and the variability in inorganic arsenic was smaller than one in total arsenic (86). The genetic diversity in As accumulation and As speciation in rice grains was investigated using a world rice core collection (WRC) comprising 58 accessions grown over a 3-year period, and approximately 3-fold difference in the grain As concentration was found between the lowest and highest WRC cultivar. Concentrations of total As, inorganic As, and DMA were significantly affected by genotype, year, and genotype-year interaction effects. Among the WRC accessions, Local Basmati and Tima (indica type) were identified as the cultivars showing the lowest stable total As and inorganic As concentrations (87). However, rice cultivars grown in pots with high arsenic soil contained much higher grain arsenic than the same cultivars grown in a paddy field with a low arsenic soil.

83. Recently, Norton et al. (2012) (82) reported a study at six field sites (one each in Bangladesh and China, two in Arkansas, USA, and two in Texas, USA) comparing flooded and non-flooded treatments on 312 genetically diverse common rice cultivars. They found that there was a 3-34 fold range of total arsenic contamination in grain within each field with significant differences in grain arsenic across the four main rice subpopulations. The genetic variations in grain arsenic at the different sites were large, and are comparable with those found in many other studies (79, 80, 83, 84). These studies indicate that cultivars with low arsenic concentration in grain could be developed through breeding, although effects for year, location and flooding management were also statistically significant, suggesting that breeding strategies must take into account environmental factors (82).

84. On the other hand, in a different Norton et al. study (79), when comparisons of more cultivars were made across wider environmental context in three countries (two field sites each in Bangladesh (76 cultivars), India (89 cultivars) and China (84 cultivars)), the genetic variation of As conc. in grain was stable across two sites in both Bangladesh and India, but not across the two Chinese sites. The difference in the genetic variation of As concentration in grain may be due to similar sources of arsenic contamination between the two sites in India and Bangladesh, but different sources at Chinese sites.

85. In the same study, a subset of 13 cultivars grown at all six field sites was also used to evaluate the contribution of genotype, environment and genotype × environment interaction to grain As. The results indicated that the largest factor in determining grain arsenic levels was the environment (field sites), followed by genotype × environment interaction and genotype (79).

86. Considering the fact that not only the source of arsenic contamination, but also field management practices and other soil properties like baseline arsenic level could cause grain arsenic variation, it is not surprising that different genotypes behaved differently at different field sites. Similar to the results of Norton et al. (80), genotype had a significant effect on the percentage of inorganic arsenic, but the environmental influence was greater (79). These results emphasize the importance of environmental impact on total arsenic and its speciation in rice grain.

87. Additionally, Tuli et al. (2010) (84) studied environmental effect on grain arsenic in field trials of 90 cultivars at three locations in West Bengal, India. Although the total arsenic levels in soils at three sites were similar at about 10 mg/kg, the grain total arsenic levels in the 90 rice varieties ranged from 0.079 to 2.70 mg/kg. Grain inorganic arsenic also varied widely among the cultivars. Ahmed et al. (2011) (85) also investigated the effect of genotype, environment, and genotype × environment interaction by using 38 Bangladeshi cultivars grown at ten research stations across diverse agro-ecological zones in Bangladesh. Environment accounted for 69% – 80% of the observed variability on total arsenic concentration in grain, while genotype and genotype × environment interaction accounted for only 9% – 10% and 10% – 21% of the variation, respectively.

88. There is a possible relationship between flowering⁹ time and As concentrations in grain, but it may not be consistent across field sites (and years) (82). Pillai et al. (83) reported a significant positive correlation between the concentration of DMA in grain and the number of days to heading⁹. There was also a significant positive correlation between inorganic arsenic concentration in grain and days to heading in one season. Further studies relating flowering time and time to heading to arsenic concentration in grain are needed to elucidate the mechanisms.

89. Moreover, inorganic arsenic correlated strongly with total arsenic among a subset of 40 cultivars grown in Bangladesh and China (82), indicating that inorganic arsenic can be used as a marker of total arsenic in rice grain, however the regression slope can vary from region to region.

⁹ Heading – is the time when the panicle begins to exert from the boot. Heading may take over 10 to 14 days due to variations within tillers on the same plant and between plants in the field. Agronomically, “heading date” is defined as the time when 50 percent of the panicles have at least partially exerted from the boot. Flowering refers to the events between the opening and closing of the spikelet (florete) and lasts for 1 to 2 1/2 hours. Flowering generally begins upon panicle exertion or on the following day and is consequently considered synonymous with heading (74).

90. In summary, these results indicate that there is large genetic diversity in As concentrations in rice grain and that selecting cultivars that accumulate less As in grain is one of the promising methods for As reduction. In addition to studies comparing different cultivars, QTL methods may be useful for the development of new cultivars with low arsenic accumulation through marker-assisted breeding. However, other factors like the environment, location, flooding management, and genotype-environment interaction can significantly influence grain arsenic accumulation. Therefore, breeding for low arsenic traits must take into account environmental factors and bred cultivars must be widely tested for different agronomic conditions to identify the optimum conditions under which low concentration of arsenic in grain can be achieved.

Section 6.3 Measures on processing and cooking

- Effect of processing

91. There are several studies that indicate that arsenic in rice can be decreased by polishing (milling) brown rice. Torres-Escribano et al. (88) found a higher inorganic arsenic concentration in brown rice compared with white rice, which might indicate that part of the arsenic is attached to components of the bran. Narukawa et al. (89) prepared rice flours from materials in the following series, 100% brown rice, 90% (90% of the grain remaining, 10% of the outer layers milled off), 70% (30% milled off), and 50% (50% milled off), and then analysed total, inorganic and organic arsenic in each flour and total arsenic in bran (10% of outer layers milled from brown rice). The results showed that the highest total arsenic and inorganic arsenic concentrations were found in unpolished brown rice flour and the total arsenic and inorganic arsenic concentrations in the other three flours decreased with the increasing degree of polishing. The concentration of total arsenic in the bran was much higher than that in the brown rice flour (Table 6). However, the relative amounts of each arsenic species in different fractions were not different. Consequently, polishing brown rice to obtain white rice may lead to a substantial decrease in total and inorganic arsenic concentration.

92. Similar findings were reported by the CFSA laboratory in China (Table 7) (90). The total arsenic content of a set of unpolished rice samples from different regions ranged from 83-739 ng g⁻¹ (mean = 255 ng g⁻¹), while the total arsenic content of a set of polished rice samples ranged from 33-437 ng g⁻¹ (mean = 143 ng g⁻¹). The inorganic arsenic concentration of unpolished rice samples ranged from 71-567 ng g⁻¹ (mean = 209 ng g⁻¹), while the inorganic arsenic concentration of polished rice samples ranged from 28-217 ng g⁻¹ (mean = 108 ng g⁻¹). DMA ranged from below the limit of detection to 156 ng g⁻¹ in unpolished rice and from below the limit of detection to 128 ng g⁻¹ in polished rice. All samples had MMA below the limit of detection except for one polished rice sample from Jiangxi, China, with an MMA concentration of 17 ng g⁻¹.

93. However, the reduction in total arsenic in polished rice compared with unpolished rice is mainly due to a decrease in inorganic arsenic. The percentage of inorganic arsenic was higher in unpolished rice than in polished rice, which was similar to that Meharg et al. (91) reported. The percentage of DMA was greater in polished rice than in unpolished rice when the recovery in unpolished rice and polished rice were close to each other. The drop in inorganic arsenic with polishing was generally greater than that of organic arsenic.

- Effect of cooking

94. There are a number of studies focused on how cooking rice in contaminated water affects the contents of arsenic in the cooked rice. In a study (88), the cooking process mimicked one of the processes normally applied in Spanish households: boiling in water with an initial rice to water ratio of 1:4, until all the liquid has evaporated. The cooking water was spiked with various concentrations of arsenate ranging from 0.1 to 1 mg/L to emulate the concentrations of arsenic that might be found in water from arsenic-endemic areas. After cooking, the inorganic arsenic concentration ranged in the analyzed rice samples between 0.428 µg g⁻¹dw (0.1 µg mL⁻¹ in the cooking water) and 3.89 µg g⁻¹dw (1.0 µg mL⁻¹ in the cooking water) with a mean rice retention of 89±13% of the arsenic from the cooking water. Comparable results were also reported by Ackerman et al. (92), who found 89-105% retention of arsenic from cooking water that was used in a ratio of rice to water of 1:1 to 1:4. It is found that when cooking water was contaminated with arsenic, it increased the concentration of both total and inorganic arsenic in the cooked rice(93). Also, it is found that cooking rice with arsenic contaminated water could raise the arsenic concentration from 0.14 mg/kg to 0.37 mg/kg (94, 95). While the aforementioned studies mainly focused on the retention of arsenic by rice from contaminated water, other investigations tested the effects of cooking rice in uncontaminated water. Sengupta et al. (2006) (96) tested the three major rice cooking procedures followed globally. Using low arsenic water (arsenic < 0.003 mg/L), the traditional method of the Indian subcontinent (wash until clear; cook with rice to water ratio of 1:6; discard excess water) removed up to 57% of the arsenic from uncooked rice containing arsenic at 0.20-0.54 mg/kg. Approximately half of the arsenic that had been removed was associated with the wash water and half was found in the discard water. With low arsenic water, the contemporary method of cooking unwashed rice at a rice-to-water ratio of 1:1.5-2.0 until no water remains did not modify the arsenic content. However, preliminary washing until clear removed 28% of the rice arsenic. The results were not influenced by the water source (tubewell, dug well, pond or rain), cooking vessel (aluminium, steel, glass or earthenware), or the absolute weight of rice or volume of water.

95. Raab et al. (97) systematically investigated total arsenic and inorganic arsenic in different rice types, i.e., two varieties of basmati, one wholegrain and one polished were of Indian origin, which had undergone various forms of cooking in non-contaminated water. The effects of rinse washing, low water volume (rice to water ratio 1:2.5) and high water volume (rice to water ratio 1:6) cooking, as well as steaming, were investigated. Rinse washing was effective at removing about 10% of the total and inorganic arsenic from basmati rice, but was less effective for other rice types. Cooking rice in a large volume of water (6: 1, water: rice) had the greatest effect with regards to lowering arsenic levels in cooked rice. Specifically, it preferentially reduced the inorganic arsenic content by 45% of that in the raw rice, when combined with rinse washing. This study indicates that rinse washing and a high volume of non-contaminated cooking water are effective in reducing the arsenic content of cooked rice, specifically the inorganic component. However, it is important to note that rice is fortified in some countries, and that washing may remove fortification nutrients.

96. In summary, cooking and preparation methods can decrease arsenic levels in foods. It is important to use water that has low levels of arsenic for cooking and washing rice (or preparing food in general). Rinse washing and using a high volume of low arsenic cooking water are effective in reducing the arsenic content of cooked rice, if excess water is discarded. If cooking water is contaminated with arsenic, adsorption by the food may occur, leading to elevated arsenic levels. Cooking rice with arsenic contaminated water can actually contribute even more to total dietary arsenic exposure from rice than that from the arsenic present in the rice itself. More research may be needed to determine if and how different cooking procedures affect total arsenic and arsenic species in rice.

Section 7 Monitoring of effectiveness of measures

97. The measures for reducing arsenic in rice should not only be selected based on scientific evidence taking into consideration other legitimate factors¹⁰ but also be monitored by appropriate ways to examine its effectiveness. There are several ways to monitor the effectiveness depending on characteristic of the measures. Since the measures examined in this discussion paper are classified into three types, namely source directed measures, agricultural measures, and processing and cooking measures, the ways to monitor the effectiveness are characterized as follows.

- For source directed measures

98. As mentioned in para. 34, the food or feed control authorities should inform relevant national authorities of potential or actual food or contamination problems and encourage them to take appropriate action, especially measures to the anthropogenic sources. Since the source directed measures would result in decreased levels of contamination in the long term, the food or feed control authorities should watch continuously the actions such as establishment of limits to restrict emission arsenic to the environment and results of monitoring arsenic levels in environment.

99. If contamination of agricultural land is limited in small areas caused by industrial pollution, it may be feasible to conduct countermeasure such as soil amendment or replacement¹¹. In that case, it may be good practice to monitor the arsenic concentration in the soil before and after countermeasures. On the other hand, if agricultural land or ground waters are widely contaminated by natural source, non-point source or previous activities, widely monitoring arsenic concentrations in soil or irrigation water is necessary.

- For agricultural measures

100. Introducing agricultural measures to reduce arsenic contamination in rice is generally more practical and feasible, especially in areas contaminated by natural sources. The agricultural measures should be examined for its effectiveness in the paddy fields in regions. Generally, it takes a considerable amount of time for new agricultural methods to be widely adopted by rice farmers. Therefore, it is important to educate farmers with indicating the need for measures to reduce arsenic in rice and the effectiveness of the recommended measures.

101. Once agricultural measures are widely adopted by farmers, the level of arsenic in rice is expected to decrease. The effectiveness of agricultural measures should be monitored by occurrence data of arsenic in rice grain.

102. To examine the ability of each agricultural measure to reduce arsenic contamination in rice, changing the experimental conditions in field trials may provide useful information to determine whether a measure should be adopted as good agricultural practice. However, there are large uncertainties about the extent to which farmers may implement the measures.

- For processing and cooking measures

103. Studies investigating As levels in rice after implementation of processing and cooking measures can provide useful information about the effectiveness of the measures in reducing concentrations of As in rice. However, such studies are not sufficient by themselves for estimating the effectiveness of these measures in reducing dietary exposures to arsenic.

¹⁰ Statement of principles concerning the role of science in the Codex decision-making process and the extent to which other factors are taken into account (Appendix to the Procedural Manual of the Codex Alimentarius Commission).

¹¹ There have also been successful studies in pot tests of the ability of ferns to phytoremediate arsenic-contaminated soil (98, 99).

Summary and Recommendations

-Summary

104. Discussions on measures for reduction of arsenic in rice and monitoring of its effectiveness based on the scientific papers in Section 6 and 7 are summarised as follows.

• Source directed measures

- (a) The code of practice for source directed measures to reduce contamination of food and feed with chemicals (CAC/RCP 49-2001) can generally be used as source directed measures for arsenic in rice.
- (b) The use of soil and/or irrigation water contaminated with unusually high levels of arsenic should be avoided, although this may be difficult in countries where arsenic contamination is ubiquitous in soil or water. If soils are highly contaminated, it may not be possible to grow rice with safe levels of As.
- (c) If a source directed measures are to be developed, technical or economic feasibilities, and specific extent of arsenic pollution of soil and water in each region should be taken into consideration.
- (d) From a food security point of view, there may be difficulties in introducing source directed measures when arsenic contamination in soil is ubiquitous in the country. However, source directed measures should still be applied wherever possible, e.g. avoiding the use of heavily contaminated As-contaminated soil and/or irrigation water.

• Use of agricultural materials

- (a) Most iron containing materials effectively lower arsenic levels in rice and they are relatively cheap. Then, they can be regularly used. However, a few findings suggest the iron plaques may actually act as a reservoir for arsenic. This is an area requiring further investigation.
- (b) Phosphates significantly decrease the adsorption of arsenite and arsenate on iron oxides/hydroxides in soil, and therefore may increase arsenic levels in soil pore water in paddies. The ability of phosphates to compete with arsenate uptake is not an important factor affecting arsenic levels in rice, because arsenite is the primary form of arsenic taken up by rice plants. Countries may need to weigh the benefits of using phosphate fertilizers against any risks of increased arsenic levels in rice.
- (c) If silicates are insufficient in the paddy soil, the use of silicates may decrease arsenic concentrations in rice by preventing arsenite uptake and transport. However, further research is required to investigate the use potential to use silicates under various field conditions.
- (d) Organic matter can increase arsenic release from the soil solid phase to soil pore water in the paddy soil. Organic matter may also stimulate microbe-mediated arsenic methylation, potentially leading to an increase in methylated arsenic species in rice plants. This is an area requiring further research.
- (e) Organic fertilization immediately after flowering should be avoided, as much As adsorbed on iron plaque can be released upon organic matter application, and lead to As uptake and transport into grain.
- (f) There are few experiments in the paddy fields, so control measures based on laboratory experiments should be tested in fields in each region to ensure the validity of results.

• Control of irrigation water

- (a) If possible, paddy soil should be maintained in oxidative (non-flooded) conditions from several weeks before and several weeks after heading/flowering of rice to reduce arsenic concentrations in rice grain.
- (b) Submerging paddy soil after heading/flowering of rice ears should be especially avoided since lowering the Eh in paddy soil after heading/flowering causes increased inorganic arsenic concentrations in rice grain.
- (c) Flooding controls may need to be modified when cadmium is a co-contaminant with arsenic. It is important to note that anaerobic conditions (i.e. flooding) favour uptake of arsenic, whereas aerobic conditions (draining) favour the uptake of cadmium. Therefore, where a paddy is contaminated with both arsenic and cadmium, it may be necessary to combine control of irrigation water with use of agricultural materials and/or varietal selections.
- (d) Flooding control of irrigation water also has impacts on disease and pest pressure as well as crop yield in some agricultural circumstances. Therefore, the feasibility of controlling irrigation water as a means of reducing arsenic uptake will vary from country to country.

• Selection of cultivars

- (a) Low arsenic cultivars can be selected, if growing conditions allow, since there is substantial genetic diversity in arsenic accumulation in rice grain. It is very important to combine high yield under aerobic production while maintaining quality and low inherent arsenic accumulation in the same cultivars.

- (b) The appropriate selection of low arsenic cultivars should include careful consideration of environmental conditions like variation by year, location, flooding management and genotype-environment interactions.
- (c) Breeding for low arsenic traits must take into account environmental factors and bred cultivars must be tested for different agronomic conditions to identify the optimum conditions under which low grain arsenic can be achieved.

- **Processing and cooking**

- (a) White rice has lower arsenic concentrations than brown rice. National authorities could consider optional advice for consumers to choose white rice to reduce arsenic dietary exposure. However, whole grains are a source of nutrients such as iron, magnesium, selenium, B vitamins, and dietary fibre, and whole-grain intake may reduce the risk of such conditions as cardiovascular disease, overweight, and type 2 diabetes (USDA/HHS, 2010); therefore the risk and benefit of consumption advice for whole grain versus white rice should be balanced by each country.
- (b) Cooking and preparation methods can decrease arsenic levels in foods. It is important to use water that has low levels of arsenic for cooking and washing rice.
- (c) Rinse washing and using a high volume of non-contaminated cooking water followed by discarding excess cooking water are effective in reducing the arsenic content of cooked rice. However, in some countries, rice is fortified with nutrients, and washing may reduce nutrient fortification, so that the benefits of washing must be balanced against the benefits of fortification.

- **Monitoring of effectiveness of measures**

- (a) The concentration of arsenic in contaminated paddies and rice and its products should be monitored before and after implementation of countermeasures. If agricultural land or ground waters are widely contaminated by natural sources, non-point source or past activities, monitoring arsenic concentrations in soil or irrigation water is necessary.
- (b) The effectiveness of agricultural measures should be monitored by occurrence data of arsenic in rice grain.
- (c) Field trial may provide useful information on the effectiveness of agricultural practices in reducing arsenic. However, there are large uncertainties about the extent to which farmers may implement the measures. Therefore, education of farmers is an important measure to be addressed.

- **Recommendations**

105. Based on the summary, the EWG recommend that:

- (a) The CCCF should decide whether or not a CoP for the prevention and reduction of arsenic in rice be developed, taking into consideration its necessity, data gap, etc.
- (b) If the CCCF decides to develop a CoP,
 - i. The Committee should agree to the following:
 - The CoP should be based on scientific evidence and available information including data from field experiments; and
 - Feasibility and regional differences in agricultural production should be considered.
 - ii. The committee should discuss issues below in order to establish its scope and content:
 - Source directed measures for arsenic contamination
 - whether the existing source directed measures adopted by the Codex are applicable to arsenic from the view of technical and economic feasibility including food security;
 - if applicable, also to discuss whether specific issues need to be included for application of these measure to arsenic (for example, regional differences of arsenic pollution of soil, water and air; natural or anthropogenic sources; etc.); and
 - whether there are any measures specific to arsenic.
 - Agricultural practices
 - whether it is feasible to include agricultural practices in a CoP and how (e.g. as an explanatory note).
 - Processing and cooking practices
 - whether it is appropriate to include processing and cooking practices in a CoP;
 - if it is appropriate, whether negative effect of decreasing nutrition is also taken into consideration; or
 - if it is not appropriate, whether there are other ways, such as guide to consumers.

- (c) If the CCCF decides not to develop a CoP,
- i. The Committee should discuss whether it can develop “[principle / policy / other appropriate title to be proposed]”¹² for developing a CoP” as an alternative. Its purpose and contents are as follows:
- Purpose is to encourage members to conduct research and survey for reducing arsenic in rice in order to obtain necessary, scientific information for developing a CoP in Codex and/or in member’s territories; and
 - The [principle / policy / other appropriate title to be proposed] will contain policies and necessary considerations on possible measures to reduce arsenic contamination in rice and a list of required research and survey. It may contain measures, where there is sufficient information to support their effectiveness in reducing arsenic in rice, and such source directed measures as those for preventing pollution of soil, water, and air by arsenic.
- (d) CCCF members are encouraged to conduct research and studies on the following:
- Occurrence in major rice-producing regions;
 - Effectiveness of agricultural measures under field conditions;
 - Role of iron plaque on roots to adsorption of arsenic and subsequent release during root turnover and harvest;
 - Role of silicates in the transport of arsenic in roots;
 - Impact of impurities of agricultural materials on arsenic solubility and dissolution in soil;
 - Translocation of absorbed arsenic to shoots and grains;
 - Development of rice cultivars with low arsenic grain, and/or proportionally more organic arsenic, by using QTL analysis and marker-assisted breeding;
 - Concentration and ratio of organic/inorganic arsenic;
 - Environmental and cultivation conditions that affect concentrations/ratios of organic/inorganic arsenic;
 - Effect of non-flood and/or partial-flood production practices on inorganic arsenic and cadmium in rice grain.

¹² An appropriate title should be determined through the discussion in the CCCF.

Table1: Chemical analysis of ferrihydrite, goethite, hematite column sand comparing conditions at experiment initiation and termination (41)

Type of iron (hydr)oxide	Arsenic species	Surface area [m ² g ⁻¹]	Iron content [mmol kg ⁻¹ sand]	Loading [mmol-As kg ⁻¹ sand]	Initial Arsenic [μmol]	Desorbed Arsenic [μmol]	Arsenic in effluent 1 days after [μmol L ⁻¹]	Arsenic in effluent 2 days after [μmol L ⁻¹]	Arsenic in effluent 10 days after [μmol L ⁻¹]
Ferrihydrite	Arsenite	2.60	92.2	3.01	60.3	0.9	2	4	2
		2.60	92.2	14.99	299.9	193.4	470	610	240
	Arsenate	3.26	114.6	4.91	98.2	5.8	8	28	4
		3.26	114.6	5.92	118.4	15.3	28	74	8
Goethite	Arsenite	1.06	132.5	1.23	24.6	10.2	29	35	11
		1.06	132.5	3.02	60.2	37.8	530	360	20
	Arsenate	1.06	132.5	1.46	29.1	3.3	15	15	5
		1.06	132.5	2.12	42.4	14.4	180	80	10
Hematite	Arsenite	0.37	137.0	0.20	3.9	2.9	10.4	12.4	3.2
		0.37	137.0	0.57	11.1	8.8	220	80	5
	Arsenate	0.37	137.0	0.33	6.8	4.3	70	25	5
		0.37	137.0	0.47	8.9	7.6	285	30	5

The figures were picked up from line graphs in Figure 1 in the paper.

All types of iron hydroxide coated sand.

Table 2: Effects of iron and phosphate containing materials on arsenic uptake into rice grain and straw (29)

(a) study results

Additive amount (mg/kg)	Arsenic ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$)	0	0	0	0	15	15	15	15	30	30	30	30
	Iron ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)	0	50	0	50	0	50	0	50	0	50	0	50
	Phosphate (KH_2PO_4)	0	0	50	50	0	0	50	50	0	0	50	50
Total Arsenic conc. in grain (mg/kg)	0.197 e	0.194 e	0.208 e	0.215 e	0.564 c	0.371 d	0.619 bc	0.611 bc	0.636 b	0.615 bc	0.708 a	n.a.*	
Total Arsenic conc. in straw (mg/kg)	0.301 e	0.295 e	0.304 e	0.313 e	3.24 d	3.75 d	4.70 c	5.29 b	5.77 b	5.47 b	6.21 a	6.14 a	
Grain yield (g/pot)	46.8 b	47.8 ab	48.9 a	47.8 ab	31.1 c	41.7 b	20.9 e	41.7 b	9.1 f	9.7 f	3.1 g	0	
Ammonium oxalate extractable iron from iron oxide plaque of rice roots (mg/kg)	5690 bc	6310 a	4810 de	5130 d	5120 d	5390 cd	4790 de	4970 de	4980 de	6060 ab	4520 e	5130 d	
Ammonium oxalate extractable arsenic from iron oxide plaque of rice roots (mg/kg)	18.9 h	15.2 h	18.7 h	18.6 h	269 e	185 g	226 f	185 fg	639 a	503 b	427 c	339 d	

Mean values in a line having same letter do not differ significantly at the 5% level by Duncan's multiple range test.

* n.a. means "not analyzed."

(b) study condition

Soil characteristics	Soil texture – SiL, pH - 6.5, C/N = 9.3, total As - 4.1 mg/kg, total soil iron oxide – 7300 mg Fe/kg
Water management	Pots were watered regularly to maintain 3-4 cm standing water in each pot.

Table 3: Effect of growing rice under flooded and aerobic conditions on arsenic concentrations in rice grain and straw and grain yield

Study	Total arsenic concentration				Grain yield (unit)		
	Soil (mg/kg dried base)	Grain (mg/kg)		Straw (mg/kg)		Flooded	Aerobic
		Flooded	Aerobic	Flooded	Aerobic		
Duxbury & Panaullah (2007)(66) Field study; Arsenic gradient in Bangladeshi farmer's fields from 20 years of use of irrigation water from shallow tube wells. "Aerobic" means rice was grown on raised beds.	11.6	0.54	0.26	7.3	1.1	(kg/ha)	
	26.3	0.53	0.28	9.7	1.2	8920	7770
	39.5	0.38	0.34	9.9	3.2	8110	8240
	57.6	0.34	0.36	11.5	3.9	6230	6970
						2990	5210
Xu et al. (2008)(65) Pot experiment in glass house; Arsenic (arsenite and arsenate) added. "Aerobic" means 70% of soil's water holding capacity.	Control 15.1	1.0	0.09	13	1	(g/pot)	
	+As ^{III} 15.1+10	2.5	0.18	26	3	8.0	9.2
	+As ^V 15.1+10	2.2	0.15	30	2	6.0	8.0
						6.2	10.2
Xie & Huang (1998)(42) Arsenic-contaminated site; "Aerobic" means maintaining moist conditions after a few days of flooding, Eh~260 mV.	Control 68	0.65	0.49	48 (flag leaf)	18 (flag leaf)	(kg/ha)	
						5500	6600

Table 4: Effects of irrigation water control between flooded and drained condition on the arsenic concentration in rice grain and straw

(a) Study Results			As conc. under different conditions of irrigation water							
T. Arao et al (2009)(73)	As conc. in soil	Sample/Analyte	F1 (mg/kg)	F2 (mg/kg)	PF1 (mg/kg)	PF2 (mg/kg)	PF3 (mg/kg)	Ae1 (mg/kg)	Ae2 (mg/kg)	
	Soil A 25 mg/kg (dry weight)	Grain, inorganic As	0.45	0.42	0.22	0.32	0.49	0.11	0.12	
		Grain, total As	0.95	0.92	0.30	0.36	0.55	0.11	0.10	
		Inorg./total As ratio	0.47	0.46	0.73	0.89	0.89	1.0	1.2	
		Straw, total As	27.3	29.5	15.9	11.7	18.4	1.8	1.1	
	Soil B 48 mg/kg (dry weight)	Grain, inorganic As	0.35	0.30	0.15	0.36	0.39	0.11	0.10	
		Grain, total As	1.7	1.7	0.59	0.60	1.26	0.17	0.14	
		Inorg./total As ratio	0.21	0.18	0.25	0.60	0.31	0.64	0.71	
		Straw, total As	26.2	26.7	17.0	18.1	23.2	5.0	0.9	
	R. Y. Li et al (2009)(49)	As conc. in soil	Sample/Analyte	F (mg/kg)		PF1 (mg/kg)	PF2 (mg/kg)	Ae (mg/kg)		
		11.6 mg/kg (dry weight)	Grain, inorganic As	0.58		0.42	0.3	0.1		
			Grain, total As	1.3		0.63	0.28	0.07		
Inorg./total As ratio			0.45		0.67	1.1	1.4			
Straw, total As			23		13	4.8	0.5			

The figures were picked up from bar graphs in Figure 2 in the paper.

(b) Study conditions	Irrigation water control in experiments
<p>T. Arao et al (2009)</p> <p>Pot experiments in green house;</p> <p>Soil conditions:</p> <p>Soil A, 25 mg/kg total As, 0.56 mg/kg total Cd, 1.6% total C, 0.15% total N, pH of 5.6;</p> <p>Soil B, 48 mg/kg total As, 0.66 mg/kg total Cd, 3.4% total C, 0.32% total N, pH of 5.5;</p> <p>Eh measurement, soil water collected by a soil-water sampler at a depth of 0.1 m in each pot.</p> <p>Rice growing records:</p> <p>transplanting in pot (14th May), heading of rice (1st-6th August),</p>	<p>7 irrigation water control (heading day is the beginning of the last heading day in each pot.)</p> <p><u>[Flooded conditions]</u></p> <p><u>F1</u>: flooding throughout entire growth period.</p> <p><u>F2</u>: flooding from transplanting to 3 weeks after heading.</p> <p><u>[Partially flooded conditions]</u></p> <p><u>PF1</u>: flooding from transplanting to heading.</p> <p><u>PF2</u>: flooding from transplanting to 3 weeks before heading and from heading to 3 weeks after heading.</p> <p><u>PF3</u>: flooding from transplanting for 2 weeks and from 3 weeks before heading to 3 weeks after heading.</p> <p><u>[Aerobic conditions]</u></p> <p><u>Ae1</u>: flooding from transplanting to 3 weeks before heading.</p> <p><u>Ae2</u>: flooding from transplanting for 2 weeks.</p>
<p>R. Y. Li et al (2009)</p> <p>Pot experiments;</p> <p>Soil condition, 11.6 mg/kg total As, 1.42% total C, 0.13% total N, pH of 5.2, silty clay loam;</p> <p>Eh measurement, the electrode being inserted at approximately 0.01 m below the soil surface.</p>	<p>4 irrigation water control:</p> <p><u>[Flooded conditions]</u></p> <p><u>E</u>: flooding throughout the entire rice growth period;</p> <p><u>[Partially flooded conditions]</u></p> <p><u>PF1</u>: flooding from transplanting to flowering period;</p> <p><u>PF2</u>: flooding after flowering period; and</p> <p><u>[Aerobic conditions]</u></p> <p><u>Ae</u>: aerobic throughout the entire rice growth period.</p>

Table 5: Varietal differences of arsenic contamination in rice

(a) Study Results

No.	Arsenic conc. in soil or soil solution		Cultivar		Arsenic contamination in rice								
			genetic characteristic (Japonica or Indica)	number of cultivars tested	analyte (total-As or inorganic-As)	conc. in grain			conc. in straw				
						sample	min (mg/kg)	median (mg/kg)	max (mg/kg)	min (mg/kg)	median (mg/kg)	max (mg/kg)	
1	total-As 6.44±0.24 mg/kg in soil with addition of sodium arsenite on some level	Control		5	total-As	brown	0.24	0.28	0.31	5.8	7.0	7.8	
						polished	0.14	0.18	0.23				
			with 10 mg-As/kg		5	total-As	brown	0.31	0.51	0.53	19	20	21
							polished	0.28	0.33	0.42			
			with 20 mg-As/kg		5	total-As	brown	0.38	0.61	0.67	25	27	30
							polished	0.32	0.49	0.58			
			with 30 mg-As/kg		5	total-As	brown	0.47	0.59	0.75	49	61	72
							polished	0.43	0.54	0.65			
2-1	inorganic-As 3.0 µg/L in soil solution	Japonica		10 [*]	total-As	brown	0.11	0.14	0.17				
					inorganic-As		0.08	0.11	0.13				
2-2	inorganic-As 63.7 µg/L and DMA 20.5 µg/L in soil solution	Japonica		10 [*]	total-As	brown	1.9	2.5	3.1				
					inorganic-As		0.14	0.20	0.24				
3-1	29.6±7.2 mg/kg in soil and 198±31 µg/L in a tubewell water			72	Total-As	brown	0.16	0.39	0.74				
3-2	10.3±2.2 mg/kg in soil and 331±13 µg/L in a tubewell water			76	Total-As	brown	0.07	0.17	0.28				

3-3	17.9±4.0 mg/kg in soil and 131±8.8 µg/L in a tubewell water		80	Total-As	brown	0.11	0.36	0.84			
3-4	6.3±1.3 mg/kg in soil and 14.9±4.1 µg/L in a tubewell water		79	Total-As	brown	0.05	0.27	0.73			
3-5	65.6±2.5 mg/kg in soil and 602±314 µg/L in a tubewell water		80	Total-As	brown	0.27	0.41	0.75			
3-6	64.6±4.7 mg/kg in soil and 218±86 µg/L in a tubewell water		77	Total-As	brown	0.37	0.57	0.85			
4	Total-As 13.8 mg/kg in soil with 5 times of 600 ml of a solution 0.4 mg-As L ⁻¹ (Na ₃ AsO ₄ · 12H ₂ O)		6	Inorganic-As	brown	0.15	0.22	0.35			
				Total-As	brown	0.32	0.35	0.69	11.3	19.2	14.2
5-1	Total-As 18.3±1.2 µg/g in soil	Japonica Indica	3	Total-As	brown	0.28**	0.40**	0.56**			
5-2	Total-As 18.3±1.2 µg/g in soil	Japonica Indica	3	Total-As	brown	0.46**	1.32**	1.48**			
5-3	Total-As 5.0±0.3 µg/g in soil	Japonica Indica	3	Total-As	brown	0.10**	0.16**	0.18**			
5-4	Total-As 5.0±0.3 µg/g in soil	Japonica Indica	3	Total-As	brown	0.36**	0.44**	0.54**			
6	Total-As 1.4 mg/kg in soil (extracted with 1M-HCl)	Japonica Indica	58	Total-As	brown						
				2009		0.08	0.19	0.33			
				2008		0.03	0.10	0.18			
				2007		0.08	0.18	0.30			
				Inorganic-As	brown						
				2009		0.06	0.15	0.27			
2008		0.01	0.05	0.16							
2007		0.05	0.11	0.24							

7		Japonica Indica	8	Total-As	polished	0.24	0.48	0.55			
8-1	As 5.9 mg/kg in soil	Japonica(8) Indica(13)	21	Total-As 2005 2004	polished	0.27 0.19	0.48 0.42	1.83 0.86			
8-2	As 5.9 mg/kg in soil	Japonica(3) Indica(7)	10	Total-As 2007 2004	polished	0.27 0.27	0.38 0.46	0.63 0.60			
				Inorganic-As 2007 2004	polished	0.09 0.09	0.13 0.12	0.15 0.15			
9-1	As 14±0.3 mg/kg	Japonica Indica	312		brown	0.19	0.44	0.90			
9-2	As 65±2 mg/kg	Japonica Indica	295		brown	0.36	0.66	1.27			
9-3	As 5±1 mg/kg (2007) 4±2 mg/kg (2006)	Japonica Indica	352 346	2007 2006	brown	0.03 0.10	0.21 0.36	1.04 0.99			
9-4	As 3±1 mg/kg	Japonica Indica	377		brown	0.17	0.62	1.68			
9-5	As 2±0.2 mg/kg	Japonica Indica	374		brown	0.01	0.04	0.13			

* 9 non-glutinous cultivars and 1 glutinous cultivar.

** The figures were picked up from bar graphs in Figure 1 in the paper.

(b) Study Conditions

No.	Testing location		Soil characteristics					Water management (Eh variations in periods of heading or flowering)	Author, years
	Country or region	pot or field	Soil types	Soil texture*	pH	C/N	others		
1	Bangladesh	Pot (glasshouse)		SiCL	7.4			3-4 cm water from soil level was maintained throughout the growth period.	• M. Azizur Rahman et al. (2007) (100)
2-1	Japan	Field	Fluvisols		5.9	12		Full irrigation was applied until grain harvesting after mid-season drainage.	• M. Kuramata et al. (2011) (86)
2-2	Japan	Pot (glasshouse)	Fluvisols		6.5	10		Full irrigation was applied until grain harvesting.	
3-1	Faridpur (Bangladesh)	Field		SiL	8.1	14		continually flooded condition	• G. J. Norton et al. (2009) (79, 80)
3-2	Sonargaon (Bangladesh)	Field		SiCL	7.1	10		alternative wet-dry cycles	
3-3	De Ganga (India)	Field						continually flooded condition	
3-4	Nonaghata (India)	Field						continually flooded condition	
3-5	Chenzhou (China)	Field							
3-6	Qiyang (China)	Field							
4	China	Pot (glasshouse)		Loam	6.49			flooded condition (a layer of water about 2-3 cm above the soil surface)	• W. J. Liu et al. (2006) (101)
5-1	USA	Field	Dewitt silt loam	SiCL				Saturated condition (maintaining soil moisture at or above the field capacity)	• B. Hua et al. (2011) (102)

5-2	USA	Field	Dewitt silt loam	SiCL				Flooded condition from about the five-leaf stage to full maturity	
5-3	USA	Field	Dewitt silt loam	SiCL	5.9			Saturated condition (maintaining soil moisture at or above the field capacity)	
5-4	USA	Field	Dewitt silt loam	SiCL	5.9			Flooded condition from about the five-leaf stage to full maturity	
6	Japan	Field	Gray lowland soil		5.9	12		Flooded condition except mid-summer drainage in early July	• Kuramata et al. (2013) (87)
7	China	Field							• X-L. Ren et al. (2006) (103)
8-1	USA	Field	Dewitt silt loam		5.9			Flooded condition until 1 week before harvest	• T. R. Pillai et al. (2010) (83)
8-2	USA	Field	Dewitt silt loam		5.9			Flooded condition until 1 week before harvest	
9-1	Bangladesh	Field			8.2			Flooded condition until when a majority of the cultivars had flowered and then the field was dried until harvest.	• G. J. Norton et al. (2012) (82)
9-2	China	Field			6.3			Flooded condition until when a majority of the cultivars had flowered and then the field was dried until harvest.	
9-3	Arkansas (USA)	Field	Dewitt silt loam		5.5 (2007)			A flood was applied at five-leaf stage and drained 15-20 days after all the cultivars had flowered. Then the field was dried until harvest.	
9-4	Texas (USA)	Field	League clay		5.5			Flush irrigation until plants reached an average 18 cm height, and then flooded condition.	
9-5	Texas (USA)	Field	League clay		5.5			Flush irrigation was continued to keep the root damp but not saturated.	

* Abbreviations mean as follows: "SiCL", silky clay loam; "SiL", silt loam.

Table 6: Concentrations of Total As and As Species in Rice with Different Degrees of Polishing^a (89)

DP%	Concentrations (mg/kg)						Total As	ext. ratio%
	As(III)	As(V)	i-As	DMAA	sum			
100	0.145	0.011	0.156	0.006	0.161	0.173	93.3	
90	0.089	0.003	0.092	0.005	0.097	0.107	90.0	
70	0.064	0.002	0.066	0.005	0.071	0.079	89.5	
50	0.051	0.001	0.052	0.001	0.056	0.063	89.6	
bran						0.725		

^a DP%, degree of polishing; 100, bran rice (non-polishing); 90, milling 10% of outer layers from brown rice (10% polishing); 70, 30% polishing; 50, 50% polishing; bran, 10% of outer layers milled from brown rice; i-As, sum of As(III) and As(V); sum, sum of all As species; ext. ratio, extraction ratio(sum/total As * 100)

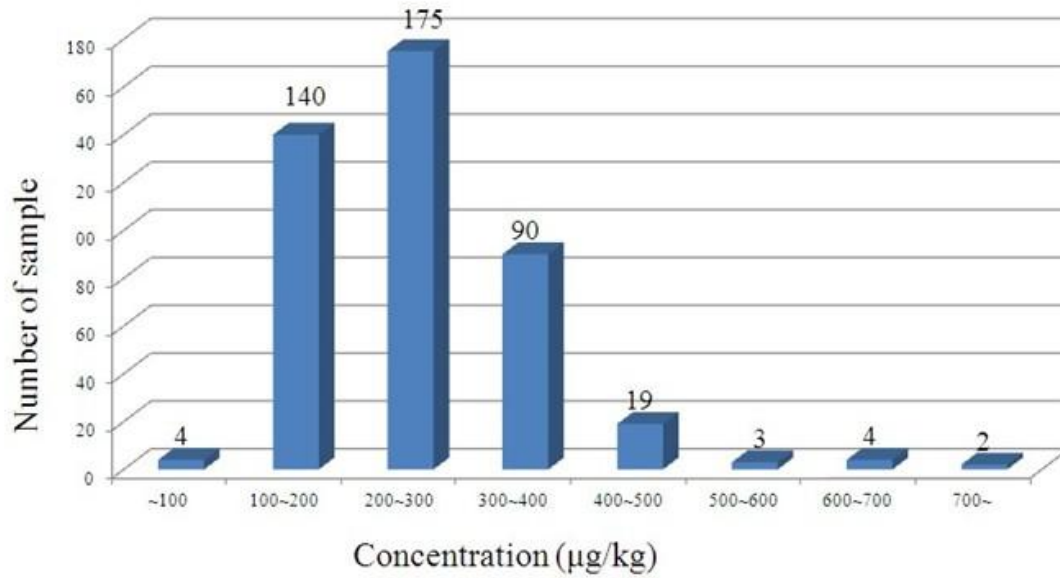
Table 7: Concentrations of Total As and As Species in unpolished and polished Rice from different provinces of China

rice source	grain fraction	DMA ($\mu\text{g}/\text{kg}$)	MMA ($\mu\text{g}/\text{kg}$)	Arsenite+ arsenate ($\mu\text{g}/\text{kg}$)	species sum ($\mu\text{g}/\text{kg}$)	total digest As ($\mu\text{g}/\text{kg}$)	organic As%	inorganic As%	extraction efficiency%
Fujian	unpolished rice	16	-	120	136	147	11	82	93
	polished rice	13	-	108	122	142	9	76	85
Chongqing	unpolished rice	26	-	133	158	184	14	72	86
	polished rice	22	2	131	155	171	14	77	91
Guangdong	unpolished rice	19	-	169	188	202	9	84	93
	polished rice	16	-	131	147	161	10	81	91
Henan	unpolished rice	51	-	153	204	216	24	71	94
	polished rice	23	-	121	145	185	12	65	78
Zhejiang	unpolished rice	59	-	195	255	277	21	70	92
	polished rice	32	-	120	153	190	17	63	81
Liaoning	unpolished rice	30	-	154	187	199	15	77	94
	polished rice	17	-	109	128	173	10	63	74
Jiangxi	unpolished rice	42	-	247	290	309	14	80	94
	polished rice	24	-	135	160	175	14	77	91
Jiangsu	unpolished rice	23	-	155	178	187	12	83	95
	polished rice	19	-	66	85	90	21	73	94
Sichuan	unpolished rice	22	-	183	206	218	10	84	94
	polished rice	15	-	82	97	103	15	80	94
Hunan	unpolished rice	23	-	265	288	308	7	86	94
	polished rice	17	-	107	24	142	12	75	87

Hubei	unpolished rice	32	-	203	235	246	13	83	96
	polished rice	25	-	106	131	137	18	77	96
Guangxi	unpolished rice	28	-	260	289	302	9	86	96
	polished rice	26	-	118	144	151	17	78	95
Yunnan	unpolished rice	20	-	175	195	200	10	88	98
	polished rice	15	-	65	81	85	18	76	95
Anhui	unpolished rice	30	-	225	255	263	11	86	97
	polished rice	26	-	140	166	178	14	78	93
Jilin	unpolished rice	50	-	288	377	426	12	68	88
	polished rice	28	-	115	143	152	18	75	94

“Rice source”: showing the original location of the rice samples collected. “Extraction efficiency%”: showing the total arsenic concentration in the extraction solution from the rice sample as the ratio of total arsenic concentration of the rice sample.

Sample number of each total As concentration range in brown rice



Sample number of each total As concentration range in white rice

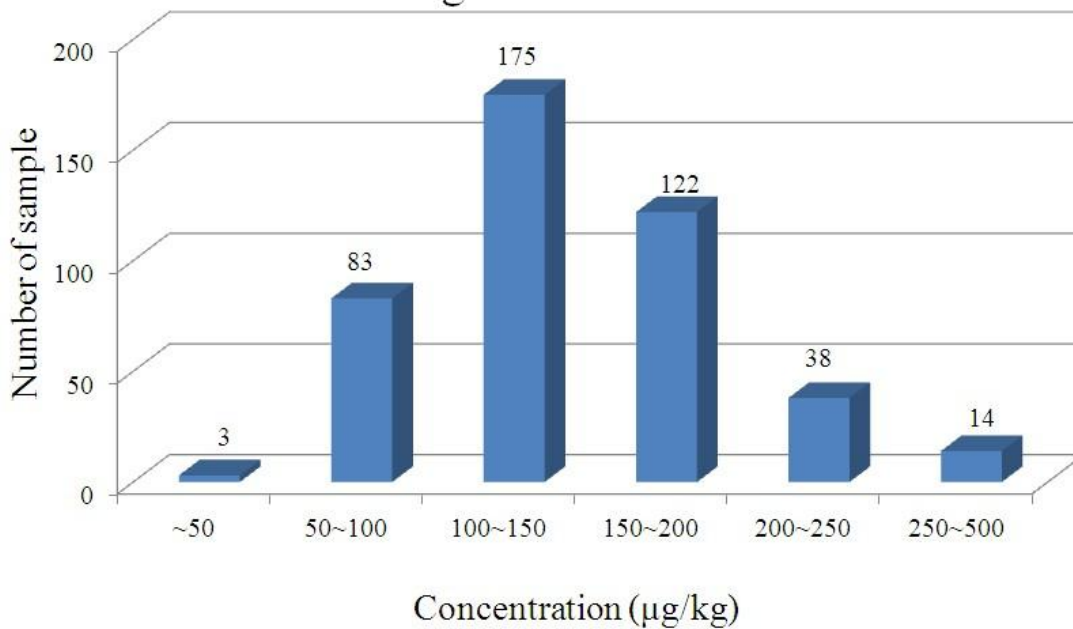


Figure 3: The distribution of sample numbers of each concentration in brown and white rice

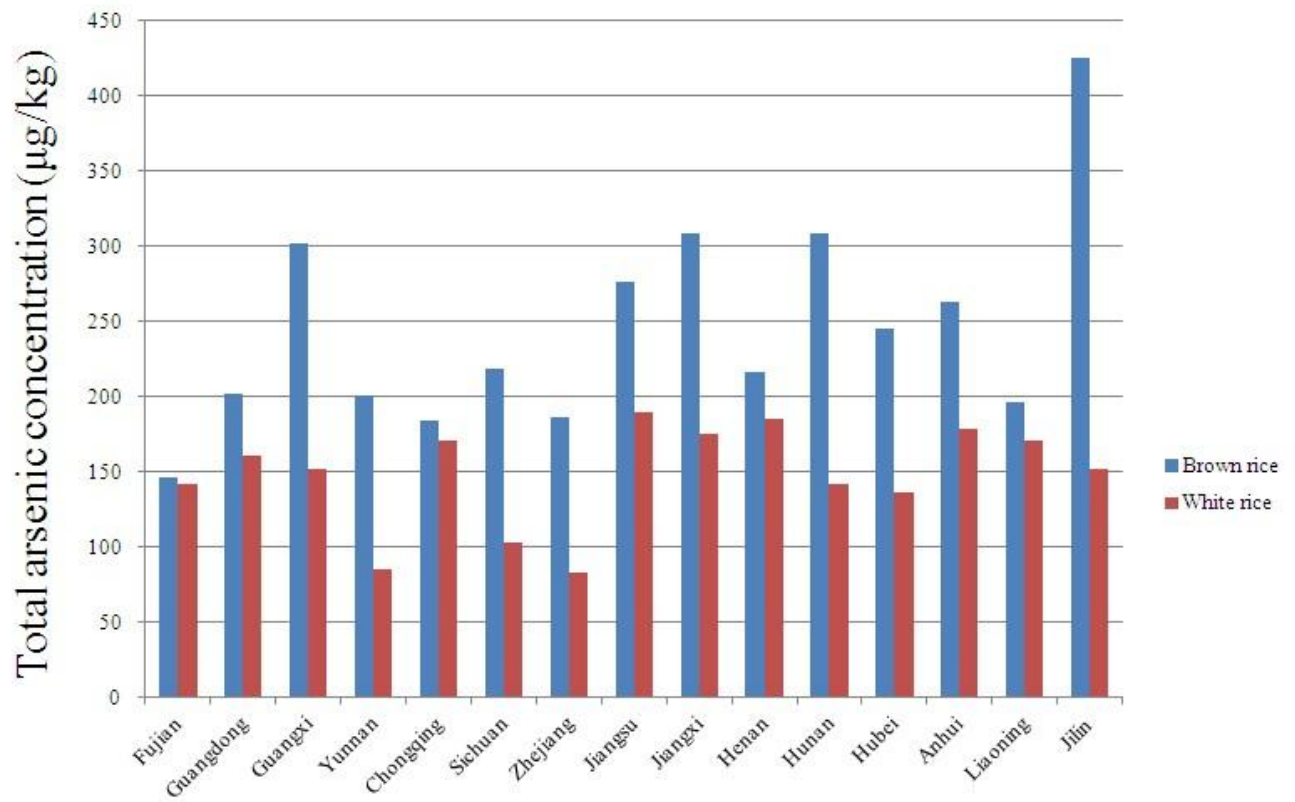


Figure 4: The concentration of total arsenic in unpolished rice and polished rice in different provinces of China

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