Universidad Miguel Hernández
Escuela Politécnica Superior de Orihuela

EVALUACIÓN DEL CONTENIDO DE CONTAMINANTES EN LA ALIMENTACIÓN INFANTIL

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Evaluación de la exposición de contaminantes en la alimentación infantil.

Tesis Doctoral realizada por Amanda Ramírez Gandolfo, licenciada en Ciencia y Tecnología de los Alimentos, en el Departamento de Tecnología Agroalimentaria de la Universidad Miguel Hernández de Elche, para la obtención del grado de Doctor.

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HACEN CONSTAR

Que el presente trabajo ha sido realizado bajo nuestra dirección y recoge fielmente la labor realizada por la licenciada en Ciencia y Tecnología de los Alimentos Amanda Ramírez Gandolfo para optar al grado Doctor.

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CERTIFICA:

Que da su conformidad a la lectura de la Tesis Doctoral presentada por Amanda Ramírez Gandolfo, titulada "*Evaluación del contenido de contaminantes en la alimentación infantil*" que se ha desarrollado dentro del programa de doctorado "Ciencias y Tecnologías Agrarias y Alimentarias" de este departamento, bajo la dirección del Prof. Dr. Ángel A. Carbonell Barrachina, del Prof. Dr. Francisco M. Burló Carbonell y de la Prof.Dra. Maria Concepción Castaño Iglesias, la cual consideran conforme en cuanto a forma y contenido para que sea presentada para su correspondiente exposición pública.

Y para que conste a los efectos oportunos firmo el presente certificado en Orihuela a _____ de _____ de _____.

Fdo.: Dr. José Ramón Díaz Sánchez

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Resumen

En los últimos años la EFSA (*European Food Safety Autority*) ha evaluado la exposición a elementos traza potencialmente tóxicos, llegando a la conclusión que son los niños menores de 3 años los que presentan mayores riesgos de exposición. El arsénico inorgánico se ha relacionado directamente con el arroz, cereal básico en el primer año de vida. En la presente Tesis Doctoral se determinan o establecen los contenidos de dichos contaminantes en alimentos infantiles a base de arroz. El arsénico es el principal contaminante presente en este tipo de productos, con concentraciones máximas entre 150 y 620 µg/kg. En los productos de origen vegetal, las especies arsenicales inorgánicas superaron el 60 %. Se ha establecido una relación entre los contenidos de arsénico y arroz de los productos analizados. La estimación de la exposición por la ingesta de estos productos toma valores cercanos al doble entre los niños que siguen una dieta a base de productos sin gluten y aquellos que siguen una dieta con gluten.

Abstract

In the last year, the European Food Safety Authority (EFSA) evaluated the exposition to the metalic trace elements and concludes that babies have high exposition risks. Inorganic arsenic is directly related with rice and basic cereals for babyfood in the first year.

The content of this thesis determine the presence of these contaminants in infant rice products. Arsenic is the main contaminant present in this kind of product, with maximal concentration of 150 to 620 µg/kg depending on the nature of the product.In the vegetal product, more than 60% are from inorganic species. We observe an obvious relation between the content of arsenic with the quantity of rice in the analyzed products. In consuming these products, people which get celiac disease have a twice higher exposition of arsenic inorganic.

Prólogo

Este documento se ha elaborado siguiendo la normativa de la Universidad Miguel Hernández de Elche para la "Presentación de Tesis Doctorales como un conjunto de publicaciones", y se ha dividido en las siguientes partes:

1. INTRODUCCIÓN, en la que se presenta el tema de la Tesis y los antecedentes del trabajo realizado.

2. OBJETIVOS, MATERIALES Y MÉTODOS, en la que se plantean los objetivos de la presente Tesis Doctoral, así como los materiales y métodos utilizados para llevarlos a cabo.

3. PUBLICACIONES, que incluye los siguientes artículos:

3.1 Burló F., Ramírez-Gandolfo A., Signes A., Parvez H., Carbonell-Barrachina A.A. (2012). Arsenic contents in Spanish infant rice, pureed infants foods and rice. *J. Food. Sci.* 77: 15-19.

3.2 Carbonell-Barrachina A.A., Ramírez-Gandolfo A., Xiangchun W., Norton G.J, Burló F., Deacon C. and Meharg A.A.(2012). Essential and toxic elements in infant foods from Spain, UK, China and USA. *J. Environ. Monit.* 14:2447-2445.

3.3 Carbonell-Barrachina A.A., Ramírez-Gandolfo A., Xiangchun W., Norton G.J, Burló F., Deacon C. and Meharg A.A.(2012).Inorganic arsenic contents in rice-based infant foods from Spain, UK, China and USA. *Environ. Pollut*. 163:77-83

3.4 Ramírez-Gandolfo A., Haris P.I., Munuera S., Castaño-Iglesias C., Burló F., Carbonell-Barrachina A.A. (2012). Ed. Nova Publishers. Chapter 7: Occurrence of inorganic arsenic in rice-based infant foods: soil-rice-infant relationship. pp 155-172. Arsenic: sources, environmental impact, toxicity and human health a medical geology prespective; New York, ISBN: 978-1-6281-320-1.

4. RESULTADOS y DISCUSIÓN.

5. CONCLUSIONES.

6. BIBLIOGRAFÍA, se recogen únicamente las referencias que aparecen citadas en el texto de la presente Tesis Doctoral, aunque también lo estén en las publicaciones adjuntas.

Esta Tesis Doctoral cumple con la normativa de la Universidad Miguel Hernández de Elche para "La obtención del título de Doctor de la Universidad Miguel Hernández".

ÍNDICE

1. INTRODUCCIÓN

1-INTRODUCCIÓN

1. ELEMENTOS TRAZA EN LA ALIMENTACIÓN

1.1 Arsénico

El arsénico (As) es un elemento químico, clasificado como metaloide dado su comportamiento metálico y no metálico. Se localiza en la tabla periódica dentro del grupo V (**Imagen 1**), se puede encontrar en forma de especies orgánicas e inorgánicas y en diferentes estados de oxidación.

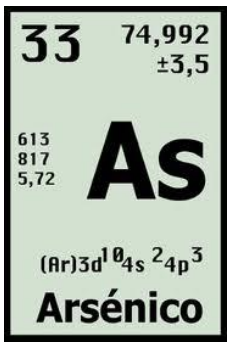

Imagen 1. Representación del arsénico en la tabla periódica

El **arsénico inorgánico (As-i)** es considerado la forma más toxica; los compuestos inorgánicos pentavalentes de As son menos tóxicos que los trivalentes, ya que la afinidad por los grupos tiol (-SH) de las proteínas es menor (Kreppel *et al*., 1993).

En cuanto al **arsénico orgánico (As-o)** no se encuentra de forma natural, es sintetizado mediante reacciones de metilación. A pesar de presentar menor toxicidad que las especies inorgánicas, las especies orgánicas pueden llegar a producir el mismo efecto si la exposición es prolongada y considerable (Domínguez Carmona, 2009).

Las formas orgánicas de As suelen estar presentes en la naturaleza en concentraciones menores que las especies inorgánicas. Su proporción se ve incrementada como resultado de reacciones de metilación catalizadas por la actividad enzimática de microorganismos (bacterias y mohos) y algas. Las reacciones de metilación se llevan a cabo tanto en condiciones aerobias como anaerobias. En ausencia de oxigeno predomina la formación de arsina (gas). Las formas orgánicas dominantes son el ácido dimetilarsínico (ADMA) y el ácido monometilarsónico (AMMA), donde el As está presente en ambos casos como As pentavalente (V)(Hasegawa *et al*., 1999).

Se han encontrado compuestos orgánicos complejos derivados del As; como arsenobetaína, arsenocolina, arsenolípidos y arsenoazúcares, en organismos marinos (OMS, 1998). Las reacciones de metilación producidas en algas suelen producir este tipo de complejos, siendo la arsenobetaina la especie arsenical predominante en peces.

La **arsina** (AsH_3) (**Imagen 2**) es un gas y es el compuesto arsenical con mayor toxicidad; por ello en 1986 el Comité de Expertos de la Organización Mundial de la Salud (OMS) estableció un límite admisible para la arsina en locales de trabajo en USA de 0,05 mg/L, siendo este el nivel máximo establecido como no peligroso.

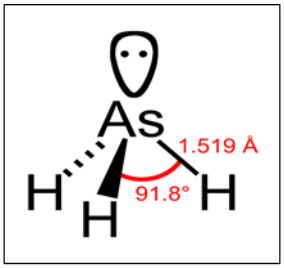

Imagen 2. Estructura molecular de arsina

1.1.1 Origen de la contaminación por arsénico

El As es un elemento que se encuentra de forma natural en la corteza terrestre, en forma de arseniatos, arsenitos y óxidos de arsénico. Las fuentes contaminación por As pueden deberse a:

- procesos naturales, tales como erupciones volcánicas, meteorización e incluso del ciclo biológico en el cual intervienen microorganismos, plantas, animales y humanos, y
- procesos derivados de la actividad humana, como es la emisión de residuos en industrias mineras, uso de plaguicidas y fertilizantes.

Por tanto, la situación geográfica es un indicativo de las zonas de riesgo de contaminación por As.

La entrada del As en la cadena alimentaria se origina básicamente a través de la contaminación del suelo (Williams *et al.*, 2007a). Las principales vías de contaminación por As en el suelo son:

- La **calidad del agua** de riego, haciendo referencia la cantidad de As presente en el agua. Los suelos de Bengala Occidental (India) fueron regadas durante muchos años con agua contaminada por As (Meharg *et al.*, 2003) (**Imagen 3)**. Posteriormente, Gosh *et al.* (2004) demostraron la presencia de elevadas concentraciones de este metaloide en el suelo en dicha zona.

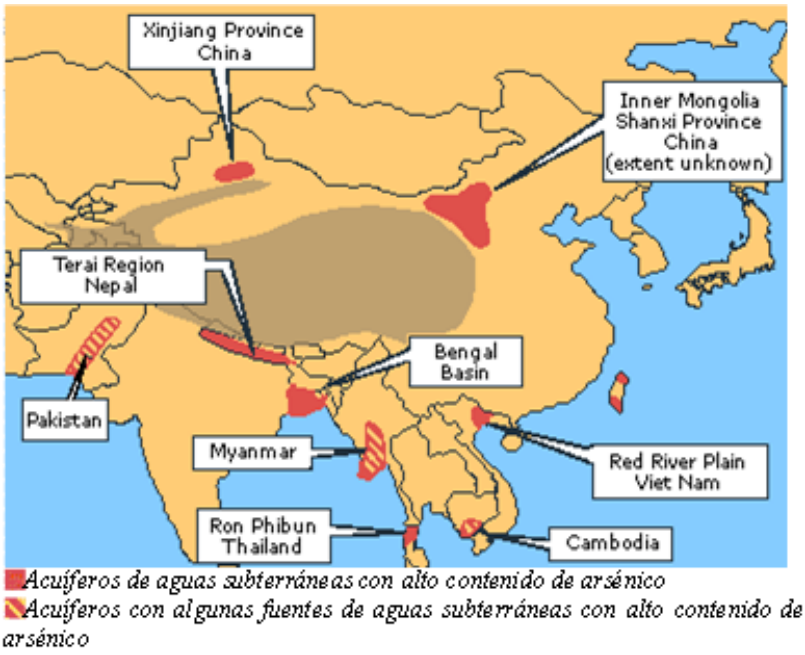

Imagen 3. Mapas acuíferos con altos contenidos de arsénico en Asia (Aguado-Alonso J., 2009)

- Utilización de **productos fitosanitarios** con un alto contenido en As bajo sus diferentes especies. El uso productos fitosanitarios en el cultivo aportan una elevada concentración de As al suelo e incluso al agua. En la actualidad se limita el contenido de este metaloide en dichos productos con el fin de controlar la contaminación del suelo. Algunas de las funciones de dicho metaloide como tratamiento fitosanitario son: coadyuvante de otros principios activos, insecticida, herbicida y defoliante.

La cantidad de As que presenta el suelo depende de diversos factores, principalmente los que determinan su capacidad de absorción y retención; entre ellos cabe mencionar: textura, contenido de sesquiterpenos y presencia de otros compuestos que interfieren en su absorción, por ejemplo la arcilla es el principal material absorbente de As (Carbonell Barrachina *et al.*, 1995).

1.1.2 Arsénico en la cadena alimentaria

El As ha sido detectado en la mayoría de los alimentos; sin embargo, la especie arsenical varía en función del alimento. El porcentaje del As-i con respecto al arsénico total (As-t) presente en los alimentos varía desde el 0 % en peces de agua salada al 75 % en otros productos de origen vegetal (CEPA, 1993).

Los principales factores que influyen en el contenido de As en el alimento son:

- La *concentración de arsénico* en el suelo, la presencia de otros iones, la forma química en que se presenten estos iones, el pH, (Marín *et al*., 1993).
- El *uso de fertilizantes fosfatados* con niveles altos de As que aumentan la absorción vegetal (Schroeder *et al.*, 1966)
- La *capacidad de acumulación* de las plantas (Aspiazu *et al.*, 1987)
- *Temperatura y el potencial redox* del suelo (Merry *et al*., 1986a,b).

Las plantas que crecen en suelos contaminados con As pueden contener elevados niveles de este metaloide, especialmente en las raíces (Grant & Dobbs, 1977; Wauchope & Mcwhorter, 1977; Carbonell-Barrachina *et al*., 1995). Las plantas absorben As a través de las raíces y de la masa foliar, y éste es transportado a través del xilema/floema y se acumula en las diferentes partes del vegetal. Meharg *et al.* (1990) afirmaron que la absorción de metales pesados limita el mecanismo de transporte vegetal, quedando acumulados principalmente en las raíces. Afirmación corroborada en 2002 por Abedin *et al.* con el estudio que confirma que las raíces son la parte de la planta que más As acumula.

El As tiene dos vías de entrada en la planta:

- **absorción radicular**, por contaminación del suelo y/o agua. El As es transportado por el xilema hasta la hojas y a través del floema vuelve a la raíz, lugar donde se acumula (Wauchope, 1983), y
- **absorción foliar**, por la aplicación de tratamientos fitosanitario, normalmente es la especie orgánica. El As es transportado desde las hojas a las raíces a través del floema.

El transporte de este metaloide a través de la planta se produce en pocas horas, principalmente en el estado de oxidación +5 (Wauchope, 1983). Se trata de un proceso rápido que normalmente finaliza con la acumulación en los órganos de reserva de la planta (Marín *et al*., 1993).

Se han realizado diferentes estudios sobre el contenido de As en alimentos. Martí *et al.* (2008) analizaron los metales pesados (As, Cd, Hg y Pb) en diferentes productos alimentarios de origen español. Los resultados muestran que los alimentos con mayor

contenido fueron los de origen marino, con una media de 350 µg As-t/kg producto y el arroz 180 µg As-t/kg producto.

Por otro lado, Signes *et al.* (2008a) estudiaron el contenido de As-t en alimentos de Bengala Occidental (India), mostrando un mayor contenido por tratarse de cultivos procedentes de un área geográfica con suelos y aguas de riego altamente contaminadas por As. Algunos ejemplos son: arroz con cascarilla 496 ± 14 µg/kg, rábano 167 ± 5 µg/kg, zanahoria 121 ± 2 µg/kg y arroz descascarillado 120 ± 10 µg/kg.

1.1.3 Metabolismo del arsénico

La intoxicación por As en humanos se da principalmente por la ingesta de alimentos que presentan elevadas concentraciones del metaloide; por lo que es importante tener en cuenta las vías metabólicas y de eliminación de este elemento para comprender el riesgo real que supone para los humanos.

La digestión y absorción intestinal es un proceso complicado e influenciado por muchos factores que determinan el paso de los elementos al torrente sanguíneo. Se habla de la **biodisponibilidad** para determinar la cantidad de sustancia susceptible para pasar al torrente sanguíneo. La absorción intestinal del As depende de la forma en la que se presenta, ya que presentan diferentes propiedades físico-químicas; por ejemplo los arsenitos son más hidrosolubles, y por tanto su absorción es mayor que otras formas de As, esta característica también influye en su eliminación. Se ha observado que la biodisponibilidad del As-i es mayor a la del As-o (Domínguez Carmona, 2009). Todo indica que la biodisponibilidad del As-i a partir del arroz es alta, del orden del 90 % (Ackerman *et al.*, 2005). Por otro lado, el termino **bioaccesibilidad** hace referencia a la proporción del As presente inicialmente en un alimento que es liberado en la fracción acuosa dentro del tracto gastrointestinal. Laparra *et al.* (2005) estudiaron la bioaccesibilidad del As(III) y As(V) presentes en arroz cocido. Tras una simulación de la digestión gastrointestinal, la bioaccesibilidad del As-i se estimó en el rango 63-93 %. Consecuentemente, parece razonable indicar que tanto la bioaccesibilidad como la biodisponibilidad del As-i en el arroz cocido son elevadas. Sin embargo, se necesitan más estudios para evaluar la bioaccesibilidad y biodisponibilidad del As en diferentes tipos de matrices alimentarias.

Cuando este elemento alcanza el torrente sanguíneo es captado por los leucocitos, aunque si la dosis es elevada, el As acaba almacenándose en los hematíes y se distribuye a través del organismo llegando al hígado, pulmones, bazo, piel y riñón. El As se une a las proteínas y a grupos sulfhídrilo inhibiendo la acción de las enzimas, impidiendo así el correcto funcionamiento celular e incluso afectando a la estructura del ADN, por hipometilación (Sciandrello *et al.*, 2004). De ahí que sea considerado como un compuesto cancerígeno con factor 1 (IARC, 1987).

La principal vía metabólica de detoxificación del As es la metilación; a partir de este proceso se originan compuestos como los ácidos monometilarsónico, dimetilarsínico o trimetilarsónico. Por tanto, el As-i se transforma en As-o; se trata de una respuesta protectora del organismo, aunque estudios recientes indican que estos compuestos alteran los procesos de reparación del ADN y por tanto no tiene la función que se pensaba (Domínguez Carmona, 2009).

La eliminación del As, al igual que la absorción, depende de la forma en la que esté se presente. Por ejemplo, el 70 % del As-o se elimina por vía urinaria en 24 horas sin haber sufrido transformación (Domínguez Carmona, 2009), de ahí que presente una toxicidad menor a las formas inorgánicas. Existen otras vías de excreción como son la biliar, que lleva a la excreción por las heces, como ocurre con el As no absorbido, también la vía epitelial y a través de las uñas y pelo. Gibson *et al.* en 1982 observaron un aumento en los niveles de As en lactantes de menos de un año, mediante el análisis del cabello, tan pronto como se introdujeron alimentos en su dieta en sustitución de la leche materna. El principal problema de salud reside en la acumulación de este metaloide por una exposición prolongada y dosis elevadas.

La exposición prolongada al As llega a desencadenar un cuadro clínico crónico y puede manifestarse en:

- piel: desarrollando cáncer de piel, hiperqueratosis y melanosis (**Imagen 4**);
- hígado: desarrollando cirrosis y ascitis;
- pulmón: puede desencadenar un cáncer de pulmón cuando el arsénico es inhalado;
- sistema circulatorio: desarrollo de la enfermedad del pie negro por necrosis en las extremidades, y

- malformaciones en el feto, puesto que se trata de un agente teratógeno.

En la **Imagen 4** se pueden observar los efectos de la exposición crónica al As en Bengala Occidental (India), zona afectada por la contaminación del agua subterránea, como se ha comentado anteriormente. Se considera como el mayor envenenamiento de la historia con cerca de 100 millones de afectados. El problema radica en que el agua de riego y potable está altamente contaminada por As-i, llegando a cultivos como el del arroz, con alta capacidad de retención del As-i. Además el arroz es la base de la alimentación de la zona, supone un 60 %, por lo que contribuye de forma significativa a la ingesta dietética de As-i (Signes *et al.*, 2008a).

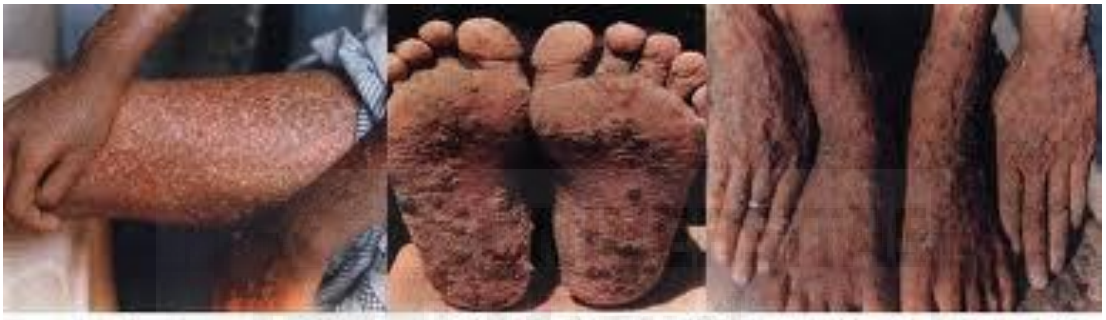

Imagen 4. Hiperqueratosis y melanosis producida por la exposición arsénico en el Oeste de Bengala

Dados los efectos que produce en la salud el As-i la FAO/OMS en conjunto en 1989 establecieron un Ingesta Semanal Tolerable Provisional (ISTP) para el As-i de 15 µg/(kg peso x semana).

1.2 Cadmio

El cadmio (Cd) es un metal clasificado como uno de los metales más tóxicos. Se encuentra dentro del grupo IIB de la tabla periódica (**Imagen 5**), junto a otros elementos como el cinc y mercurio. El cadmio se presenta en su forma iónica (en mayor medida), formas inorgánicas y formas orgánicas, por la unión a estructuras complejas como las proteínas (WHOIPCS, 1992a). El cadmio presenta mayor estabilidad en su forma iónica con un estado de oxidación Cd^{+2}.

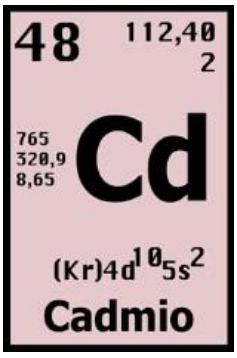

Imagen 5. Representación del cadmio en la tabla periódica

Las principales especies sólidas que se encuentran en el suelo se presentan en la forma de óxido de cadmio (CdO), carbonato de cadmio ($CdCO_3$). El cadmio también está presente en el aire, ya sea en forma de partículas o gases como puede ser el cloruro de cadmio ($CdCl_2$). La solubilidad del mismo en el agua depende

de la forma en la que se encuentre, por lo que puede aparecer disuelto en el mismo o bien se deposita en los sedimentos.

La toxicidad del cadmio es independiente de la forma en la que se encuentre, de ahí que se halla establecido tanto un ISTP como un límite máximo (LM), sin hacer distinciones entre sus formas o especies, cuyo valor es 7 µg/ (kg peso x día) para el caso del ISTP, así como un LM variable en función del producto desde 0,05 mg/kg en carne y hortalizas (CE 1881/2006) hasta 3 mg/kg en especias (RD 2242/1984).

1.2.1 Origen de la contaminación por cadmio

El Cd es un metal que se encuentra de forma natural en la corteza terrestre, principalmente en forma de sulfuro de cadmio formando parte de la greenockita. Además aparece asociado a otros metales como cinc, cobre y plomo. Las fuentes contaminación por Cd pueden deberse a:

- procesos naturales, tales como la erosión y erupciones volcánicas (Pacyna *et al*., 2001)
- procesos derivados de la actividad humana, como es la emisión de residuos en industrias mineras, uso de plaguicidas, sobre todo fosfatados, y derivados del uso de pinturas y pilas.

El cadmio liberado llega a alcanzar el agua, aire y suelo, donde se acumula y entra en la cadena alimentaria. El cadmio presente en el suelo tiene una fuerte influencia con los procesos derivados de la actividad humana; por otro lado, estudios evidencian que las emisiones atmosféricas no influyen de manera significativa en el contenido de Cd en el suelo (Bak *et al.,* 1997). Se puede presentar en formas solubles en agua o insoluble (complejos inorgánicos u orgánicos), los principales factores que determinan la movilidad del Cd en el suelo son:

- *Concentración de aniones de cloro*, aumentan la movilidad de Cd.
- A *pH ácidos* predominan las formas Cd^{+2}, aumentan la solubilidad del mismo teniendo mayor movilidad.
- *Concentración de materia orgánica*, suele interactuar con los iones de Cd^{+2} reduciendo la movilidad (OCDE, 1994).

Otras de las vías de entrada del Cd en la cadena alimentaria es a través del agua, ya sean aguas subterráneas o superficiales (utilizadas en el riego de cultivos), incluso el agua marina. En el caso de aguas superficiales o subterráneas, la forma en la que se encuentre el cadmio es esencial para comprender su distribución. Las formas solubles son arrastradas y distribuidas, mientras que las insolubles se acumulan formando parte del cadmio del suelo. En el caso del medio marino, hay que tener en cuenta la erosión producida en el mismo, llegando a suponer una gran cantidad de cadmio presente en este medio. Por lo que, en este caso la principal fuente de cadmio viene determinada por procesos naturales.

1.2.2 Cadmio en la cadena alimentaria

El cadmio se ha detectado en alimentos de diverso origen, se bioacumula en animales marinos (ATSDR, 1999). También se ha observado contenidos importantes en productos de origen vegetal, en zonas cuyos suelos presentan cantidades elevadas de dicho metal. La principal fuente de exposición al Cd es a través de la alimentación, siendo los cereales y hortalizas de hoja las fuentes más importantes, aunque también contribuyen de forma significativa los organismos marinos.

La principal vía de entrada del Cd en las plantas es a través del suelo. Smith (1994) afirmó que el factor más importante que determina la absorción del cadmio en la planta es el pH; aunque el potencial redox, la temperatura, el contenido en calcio, el contenido en materia orgánica y la calidad del agua son otros de los factores que también influyen. La forma de cadmio que más solubilidad comporta, y por ello mayor disponibilidad, es en su forma iónica (Cd^{+2}). Por otro lado, se ha observado una mayor disponibilidad del cadmio en suelos arcillosos (He & Singh, 1994).

El Cd es absorbido a nivel radicular, por difusión o en competencia con el transporte selectivo de calcio, su movilidad dentro de la planta es reducida por ello suelen encontrarse concentraciones mayores en raíces, sin embargo se encuentra bastante en hortalizas de hoja, esto se debe a que una pequeña parte del Cd absorbido es transportado a través del xilema a diferentes punto de la planta, siendo las hojas y el tallo el reservorio aéreo principal de este metal (Chan & Shoback, 2004).

En cuanto al contenido en animales de origen marino, se ha observado una asimilación de alrededor de un 40 % de Cd en el fitoplancton (Neff, 2002), por lo que la

principal vía de entrada de este metal es a través del consumo del mismo. Se han encontrado valores de asimilación entre 70-88 % en almejas, las cuales se alimentan de diatomeas (Neff, 2002). Sin embargo los valores descienden de forma considerable en el pescado blanco, con una bioasimilación del Cd entre 0,1-1 % (Harrison & Klaverkamp, 1989).

Se han realizado diferentes estudios sobre el contenido de Cd en alimentos. Los cereales son el principal grupo de alimentos de origen vegetal que más concentración de Cd presentan. Según el documento de evaluación realizado por la FAO/OMS (2004) en conjunto, se observó que el arroz procedente de Japón contenía una media de 0,061 mg/kg frente a la media de 0,017 mg/kg en el resto de países a estudio, por otro lado para el trigo se han observado valores 0,054 mg/kg y en verduras 0,012-0,04 mg/kg.

En productos de origen marino, las concentraciones varían desde 0,20 mg/kg en moluscos hasta 1,38 mg/kg en ostras.

1.2.3 Metabolismo del cadmio

La digestión y absorción intestinal es un proceso complicado e influenciado por muchos factores que determinan el paso de los elementos al torrente sanguíneo. En general la absorción de Cd en el sistema digestivo es baja, del orden de 5-10 %, esto se debe a que el Cd queda retenido en la mucosa intestinal por la unión a las metalotioneinas. La descamación de la mucosa intestinal hace que se elimine el Cd a través de las heces (Min *et al.*, 2008). Sin embargo, dosis elevadas de Cd hace que el mismo alcance el torrente sanguíneo. En este punto el Cd ya sea en su forma libre o unido a la metaloliniteina es transportado a los principales órganos reservorios, entre 40-80 % de Cd se encuentra en el hígado y riñón.

El principal efecto tóxico tras una exposición prolongada a dicho metal es una disfunción renal, la cual conlleva una alteración del fósforo y calcio, aumentando la excreción de calcio lo que implica no solo un mayor riesgo de formación de cálculos renales sino consecuencias a nivel óseo por una baja mineralización potenciando una osteoporosis, así como fragilidad ósea (aumento de fracturas) (EFSA, 2010).

Además, al igual que el As-i, el cadmio fue considerado como agente cancerígeno dentro del grupo I por la IARC (1993), actúa mediante dos mecanismos sobre el ADN:

- Promueve el estrés oxidativo, favoreciendo la producción de radicales libres.

- Impide la reparación de ADN aunque no se han descrito los medios de actuación se cree que tiene una relación directa con los mecanismos de acción de las enzimas reparadoras del ADN, cambiando la estructura básica en la síntesis proteica.

Dados los efectos del cadmio en la salud humana, la FAO/OMS en conjunto estableció en 1993 un ISTP 7 µg/(kg peso x semana).

1.3 Mercurio

El mercurio (Hg) es un metal pesado, se encuentra en la tabla periódica junto al cadmio y cinc dentro del grupo IIB (**Imagen 6**). Se puede presentar en formas orgánicas o inorgánicas, a diferencia del arsénico su especie más toxica se encuentra en forma orgánica, metilmercurio. En su estado elemental es insoluble tanto en agua como el medios grasos, mientras que el metilmercurio es liposoluble, pudiendo atravesar la barrera hematoencefalica (Sanfeliu *et al.,* 2003).

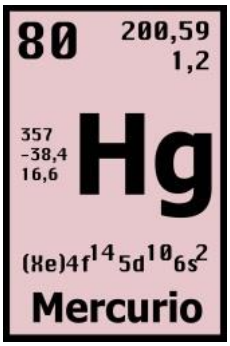

Imagen 6. Representación del mercurio en la tabla periódica

De forma natural encontramos el mercurio en sus formas inorgánicas formando sales como; el sulfuro de mercurio (II) (HgS), oxido de mercurio (II) (HgO), la cuales presentan mayor solubilidad en agua que su forma elemental. Las formas orgánicas surgen de la transformación del mercurio a través de reacciones de metilación llevadas a cabo por microrganismos, dando lugar al metilmercurio. El 90 % de mercurio presente en animales marinos se encuentra bajo esta forma, a diferencia de otros alimentos de origen vegetal este tipo de alimentos llevan un control más riguroso sobre el contenido en mercurio (EFSA, 2012).

1.3.1 Origen de la contaminación por mercurio

El Hg se encuentra de forma natural en el medio. Sin embargo la actividad industrial ha propiciado la extensión y movilización del mismo en los diferentes ecosistemas. Por otro lado, el mercurio se encuentra en constante transformación entre sus formas inorgánicas y orgánicas. Las formas inorgánicas contribuyen a su sedimentación y presencia en el suelo, en cambio las formas orgánicas entran en la cadena trófica siguiendo su ciclo.

Las principales fuentes de contaminación por mercurio son:

- Derivadas de procesos naturales, tales como la erosión y actividad volcánica.

- Derivadas de la actividad humana, producida por la industria minera, ya sea primaria o como subproducto del refinamiento de la industria del cinc, oro y plata, supone un 83 % de la contaminación, uso de fungicida y herbicida (EFSA, 2004).

De tal manera que el Hg alcanza el agua y el suelo, la principal vía de entrada de este metal en la cadena alimentaria es el agua (EFSA, 2012). El mercurio en el suelo se suele presentar precipitado en forma de $Hg(OH)_2$ lo que reduce su disponibilidad para la planta. La disponibilidad de este metal en el medio acuático es mayor, sobre todo en sus formas orgánicas. A través de las algas las formas orgánicas de mercurio entran en la cadena trófica, llegando incluso a biomagnificarse.

1.3.2 Mercurio en la cadena alimentaria

El medio marino es el principal medio de bioacumulación de este metal, sobre todo en su forma orgánica (metil-mercurio o dimetilmercurio), diversos estudios han encontrado altos niveles de este contaminante en peces. A pH elevados la transformación del Hg-i a formas orgánicas predomina en forma de dimetilmercurio. El mercurio se bioacumula; de este modo se observa mayores valores de Hg-o en animales marinos de mayor edad del orden de 180 µg/kg, ocurre lo mismo en función de la escala nivel trófico del animal (Watras *et al.* 1998). Estudios realizados por Storelli *et al.* (2002) muestran que a mayor tamaño del animal mayor contenido en Hg; sin embargo, se ha observado valores altos en peces de menor tamaño asociado al nivel de contaminación local. El contenido en mercurio encontrado en animales marinos varía entre especies, Kumar *et al.* (2010) reportaron valores de 0,11-1,76 mg/kg.

Otros de los alimentos que acumulan mercurio son los cereales 3 µg/kg, los productos lácteos y derivados 6 µg/kg y la carne 10-20 µg/kg.

1.3.3 Metabolismo del mercurio

En función del estado en el que se encuentre el mercurio varía la absorción, metabolismo y eliminación del mercurio; por ejemplo el mercurio en su estado elemental Hg no se absorbe a nivel intestinal, siendo eliminado a través de las heces sin presentar toxicidad alguna (Rowland *et al.,* 1997). Las formas inorgánicas presentan menor disponibilidad que las formas orgánicas 15 % frente 80-90 % respectivamente, una vez los

compuestos de Hg alcanzan el torrente sanguíneo se acumula en diferentes órganos; el Hg-i presenta mayor solubilidad que las formas orgánicas de ahí que se elimine a través de la orina.

El metilmercurio es la forma más toxica, presenta un rango de absorción del 80-90 %. Además, una vez alcanza el torrente sanguíneo es capaz de atravesar las barreras hematoencefalicas por su conjugación con la cistina, produciendo efectos adversos en el sistema nervioso central (SNC).

Los órganos reservorios del mercurio son:

- Hueso, el Hg-i puede llegar a sustituir el calcio.
- Hígado.
- Vías de eliminación secundaria, como el pelo y uñas.
- SNC, el Hg-o es capaz de atravesar la barrera hematoencefalica llegando a acumularse en cerebro.

En función del grado de exposición el metilmercurio almacenado en el cerebro se oxida y se acumula de forma crónica (Kanai *et al.,* 2003; Sakamoto *et al.,* 2004). La vía de eliminación de este compuesto puede ser a través de vías de eliminación secundarias, como el pelo, y vías de eliminación primarias, como las heces mediante las sales biliares y orina aunque en un porcentaje menor.

El efecto más importante de la exposición al Hg se produce principalmente sobre desarrollo nervioso fetal (WHO, 2004). Produce efectos negativos cognitivos, en memoria, visuales, en el desarrollo del habla. En adultos, una exposición crónica puede conducir a efectos no solo en el sistema nervioso sino también en el gastrointestinal, respiratorio y reproductivo (EFSA, 2012).

Dados los efectos producidos de este metal en el ser humano, la FAO/OMS en conjunto en 1978 establecieron una Ingesta Semanal Tolerable Provisional (ISTP) de 1,6 µg/(kg peso x semana) para el metilmercurio y 5 µg/(kg peso x semana) para el mercurio total.

1.4 Plomo

El plomo (Pb) es un elemento químico, considerado como metal pesado, que se encuentra dentro del grupo IVA de la tabla periódica (**Imagen 7**). Se encuentra de forma natural en el medio, rara vez aparece en su estado elemental y el compuesto más común es sulfuro de plomo (II) (PbS).

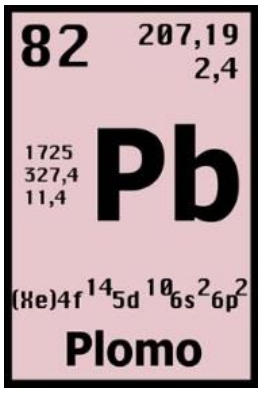

Imagen 7. Representación del plomo en la tabla

En la naturaleza el estado de oxidación predominante es Pb^{+2}; las formas inorgánicas como fosfato de plomo (II) ($Pb_3(PO_4)_2$) y carbonato de plomo (II) ($PbCO_3$) presentan mayor solubilidad. Los compuestos orgánicos derivan de la alquilación y se producen a través de la síntesis industrial. En 2006, el plomo inorgánico (Pb-i) fue considerado como un agente cancerígeno dentro del grupo 2A por la IARC, el mecanismo de acción es similar al del As-i, estas formas inorgánicas presentan una alta afinidad por los grupos tiol (-SH) de proteínas y enzimas; entre ellas, las reparadoras de ADN.

La solubilidad del plomo elemental es baja, al igual que ciertas formas inorgánicas como sulfuro de plomo y fosfato de plomo, normalmente aparece disuelto en agua en forma de carbonato de plomo. La solubilidad del plomo en agua aumenta a pH bajos y bajos niveles de sales, de ahí que en aguas blandas y acidas se encuentren mayores cantidades de dicho metal (US ATSRD, 2007).

1.4.1 Origen de la contaminación por plomo

La actividad humana es la principal causa de contaminación atmosférica del plomo. La industria metalúrgica y minera así como la industria del combustible han provocado una movilización del mismo en los diferentes ecosistemas. Desde la prohibición del uso de plomo en la producción de combustibles en 1970, se ha logrado reducir el plomo ambiental desde 0,02 g/m^3 a 0,005 g/m^3 en la UE (EMEP, 2005). En la actualidad la adición de tetraetilo de plomo en la industria metalúrgica de metales no ferrosos supone la principal fuente de contaminación (Pacyna, 2001).

Este metal alcanza alimentos a través de suelo y aire sobre todo en zonas cercanas a altos niveles de emisión de Pb. El suelo es el principal reservorio de este metal pesado, llegando a contener una media de 10-70 mg/kg en la Unión Europea (UE) frente al 0,03 µg/L en agua salada y 0,08 µg/kg en agua dulce (Millot *et al.,* 2004). Las principales fuentes

de contaminación derivan de la actividad humana (US ATSDR, 2007). La movilidad del plomo es reducida suele alcanzar el agua subterránea por lixiviado. Los factores que determinan la disponibilidad del plomo son el pH (a menores valores aumenta la solubilidad) y el contenido en materia orgánica (a mayor contenido menor disponibilidad).

La absorción del plomo en la planta se produce de forma predominantes a nivel foliar (US ATSDR, 2007), tan solo un 0,13 % del Pb presente en el suelo queda disponible para la planta. Gzyl *et al.* (1995) determinaron un contenido de 10,7 mg/kg de masa seca en las raíces frente al 129 mg/kg de masa seca en hojas. Por tanto, la principal fuente de contaminación en plantas se produce de forma aérea.

El Pb en el medio marino suele encontrase como $PbSO_4$ y su disponibilidad depende de la forma en la que se encuentre (mayor disponibilidad en forma iónica), pH y salinidad (US ASTDR, 2007). El plomo inorgánico (Pb-i) es transformado por microorganismos a plomo orgánico (Pb-o) normalmente en forma de tetrametilo de plomo, la forma más toxica junto con el trialquilo de plomo superando la toxicidad de formas inorgánicas (UNEP, 2008). El plomo no se biomagnifica (US ATSDR, 2007) puesto que se acumula en hueso y/o cáscara.

1.4.2 Plomo en la cadena alimentaria

El plomo encontrado en los alimentos difiere notablemente en los grupos de alimentos, la EFSA (2010) reportó que el contenido medio en plomo en verduras era de 0,049 mg/kg, en productos de confitería 0,004-0,226 mg/kg, los cereales, la carne y pescado son los productos que más contribuyen de forma general a la exposición dietética en la UE con contenidos 0,269 mg/kg; 0,114 mg/kg; 0,550 mg/kg, respectivamente. Los valores de Pb más altos observados en productos de origen vegetal han sido en especias llegando a alcanzar 379 mg/kg (EFSA, 2010).

En Reino Unido se ha observado una reducción en el contenido de Pb en la cadena trófica, vinculada con la restricción de uso del mismo en la industria del combustible (Larsen *et al.,* 2002).

En Polonia, la exposición dietética a este metal se vincula al consumo de bebidas (17 %), pan (16 %) y verdura (16 %). Por otro lado, se atribuye un mayor porcentaje a carne con un contenido medio 1,63 mg/kg y cereales.

En España, la principal fuente de plomo deriva de productos vegetales como la fruta y hortalizas (Moreiras & Cuadrado, 1992).

1.4.3 Metabolismo del plomo

Intervienen varios factores en la absorción, metabolismo y eliminación del plomo en el organismo. La absorción del plomo varía desde 40-70 %, los factores que determinan dicha variación son (James *et al.*, 1985; Rabinowitz *et al*., 1980):

- Factores fisiológicos, como: en edades tempranas mayor absorción alrededor 60-70 % (Skerfving & Bergdahl, 2007); condiciones digestivas: en sujetos en ayunas se observa mayor absorción, el estado nutricional del hierro adecuado y la ingesta simultánea con calcio; reducen la absorción del plomo.
- Factores físico-químicos, como tamaño de la partícula y la especiación del plomo; mayor absorción de especies orgánicas.

Todavía no se ha descrito es el mecanismo de absorción de estos compuestos en el intestino, se trata de una de las actuales vías de investigación. Calderón-Salinas et al., (1999) afirmaron que el Pb^{+2} comparte la vía de entrada celular con el calcio (Ca^{+2}), mediante transporte activo, a través de los canales de calcio.

Diversos estudios han afirmado que la absorción del Pb es mayor en niños que en adultos (Heard & Chamberlain, 1982; James *et al.,* 1985; Rabinowitz *et al.,* 1980); sin embargo, estudios realizados por Gulson *et al.* (1997) mostraron que a partir de 6 años se absorbe una cantidad similar a la de los adultos. Por otro lado, la ingestión de otros alimentos puede llegar a reducir la absorción del plomo en un 60 %, estudios realizados por James *et al.* (1985) y Heard & Chamberlain (1982) en adultos mostraron esa reducción. La absorción de plomo en los niños se ve afectada por el estado nutricional de hierro (Watson *et al.,* 1986). Un bajo nivel de hierro aumenta la absorción de plomo (Barany *et al.,* 2005). Además el calcio reduce de forma considerable la absorción del plomo, tanto el niños como adultos (Mahaffey *et al.*, 1986;. Ziegler *et al.,* 1978).

Una vez el plomo es absorbido y alcanza el torrente sanguíneo es captado por los eritrocitos, un 96-99 % (Manton *et al.,* 2001; Smith *et al.,* 2002), los cuales los transportan a diferentes órganos del cuerpo. La vía de entrada del plomo a las células es a través del canal de calcio (Ca^{+2}), se trata un competidor directo del mismo (Calderón-Salinas *et al.,* 1999).

En adultos, el 90 % del plomo se encuentra en el hueso, frente al 70 % en niños (Fleming et al, 1997). Otros órganos reservorios son por orden decreciente: hígado, riñones, páncreas, ovario, bazo, próstata y cerebro (Barry, 1975; Gross *et al.,* 1975).

Los efectos que produce una intoxicación al plomo son osteoporosis, disfunción neurológica; como parálisis por su acción sobre las neuronas (el plomo orgánico produce la necrosis y el plomo inorgánico degeneración anóxica), infertilidad y riesgo de aborto, entre otros.

La principal vía de excreción del plomo es a través de la orina por difusión pasiva; otros mecanismos de eliminación es a través de la heces mediante las sales biliares (Ishihara & Matsushiro, 1986) y jugo pancreático (Ishihara *et al.,* 1987), uñas y sudor (Stauber *et al.,* 1994).

Dados los efectos que produce el plomo en la población en 1983 la OMS estableció un ISTP para el plomo de 25 µg/(kg peso x semana).

1.5 Seguridad Alimentaria

En los últimos años la Autoridad Europea de Seguridad Alimentaria (EFSA)-Panel de contaminantes de la cadena alimentaria, ha realizado una evaluación sobre la presencia de metales pesados en la alimentación, llegando a la conclusión general que los niños eran el grupo población con mayor riesgo de exposición por varios motivos: (i) órganos inmaduros en desarrollo y (ii) relación ingesta/peso. Dicha evaluación se estableció de total prioridad dado el vacío legal en el control de metales pesados en la alimentación infantil.

En 2004 la EFSA evaluó la exposición al mercurio y metilmercurio en la población de la UE mediante la alimentación, las conclusiones de dicho documento fueron:

(a) el metilmercurio presenta una toxicidad mayor que otras especies de mercurio.

(b) en general en la UE la exposición en la población adulta no llega a superar la Ingesta Semanal Tolerable Provisional (ISTP).

(c) los niños tiene mayor riesgo de superar el ISTP.

En 2009 la EFSA publicó su evaluación sobre la problemática del arsénico en la alimentación infantil, en la cual revisó la dieta de la población de la Unión Europea (UE) y afirmó que:

(a) el nivel de arsénico inorgánico debe reducirse.

(b) los niños menores de tres años de edad son los más expuestos al As-i, variando su ingesta diaria entre 0,50 -2,66 mg/kg de peso corporal.

(c) la exposición dietética al As-i en los bebés está directamente relacionada con el consumo de productos basados en el arroz.

En 2009 (EFSA) también se publicó la evaluación sobre el cadmio, las conclusiones que derivan de dicho documento son:

(a) se asocia estadísticamente la relación entre la exposición al cadmio y un mayor riesgo de cáncer de pulmón, vejiga y mama.

(b) la exposición al cadmio se encuentra próxima a los valores del ISTP, con una media de consumo de 2,3 mg/ (kg peso x semana).

(c) la principal fuente de exposición asociada deriva de cereales, frutos secos y semillas de oleaginosas.

(d) la principal fuente en lactantes deriva de los preparados comerciales a base de cereales.

En 2012 (EFSA) se publicó la evaluación sobre el plomo, las conclusiones que derivan del mismo son:

(a) la media de exposición al plomo en la UE es de 0,68 µg/(kg x día) considerablemente menor al ISTP 25 µg/(kg peso x semana).

(b) en niños se ha estimado una exposición mayor de 1,32 µg/(kg x día)- superando dosis de referencia para la aparición de síntomas de efectos tóxicos ($BMDL_{01}$ 0,5 µg/(kg peso x día).

(c) la exposición al plomo debe reducirse.

2. PROBLEMÁTICA DEL ARSÉNICO EN EL ARROZ

2.1 Arsénico en arroz

El arroz es un cereal básico en la alimentación de muchas culturas; su aporte calórico hace que sea un alimento importante en la dieta y, por ello, se considera el principal cereal destinado a la alimentación. De hecho, su producción es predominante respecto a otros granos.

La producción de arroz en la UE está dominada por Italia (36,3 %) y España (21,5 %), siendo los principales proveedores de este cereal a escala europea. España es, por tanto, uno de los principales proveedores de arroz para uso alimentario en la Unión Europea, incluyendo alimentos infantiles.

El grano de arroz está cubierto por los tegumentos de la espiguilla, que se conocen como cáscara o cascarilla. En la elaboración industrial se elimina la cascarilla, el salvado y el germen quedando únicamente el endospermo o albumen, que puede ser de varios tipos: translucido, opaco o bien de fractura mixta, conociéndose como grano pelado. Estas denominaciones sirven para su diferenciación comercial.

El arroz se cultiva en condiciones casi permanentes de inundación. Los suelos inundados ofrecen un ambiente único para el crecimiento y nutrición del cultivo de arroz. La principal causa de la acumulación de As en el arroz es su modo de cultivo; el arroz se cultiva en condiciones de inundación, anaerobiosis (**Imagen 9**) que comportan una elevada disponibilidad del As para las plantas (Signes *et al.*, 2008b).

Imagen 9. Cultivo de arroz en anaerobiosis

Bajo las condiciones de cultivo del arroz el arsenito es la forma dominante, mientras que arseniato, ADMA y AMMA están presentes en cantidades poco significativas. El arsenito es la especie más móvil y, por tanto, muestra un alto transporte activo hacia el interior de las raíces, siendo el transporte de ADMA y AMMA los más bajos. Las plantas de arroz son plantas adaptadas para crecer en suelos inundados, las cuales oxigenan su rizosfera mediante la formación de placas de oxi-hidróxidos de hierro. La formación de placas de hierro está condicionada a la presencia de fósforo, bajas concentraciones de éste dan lugar a

un incremento en la formación de placas de hierro. Estas placas se encargan de secuestrar el As y, por tanto, cuanto más As quede unido a las placas de hierro menor será la translocación de As a otros tejidos en la planta. La formación de placas varía en función de la variedad de arroz, por tanto es un factor a tener en cuenta, ya que según la variedad la cantidad de As en la planta varía.

La mineralización del suelo también juega un papel importante, ya que puede regular la concentración de las especies de As en el suelo mediante la formación de sales de As de baja solubilidad (Meharg, 2004).

El contenido de arsénico en el arroz está también relacionado con factores ambientales; el contenido de As en el suelo es un factor dominante, así lo demuestran los estudios realizados por Williams *et al.* (2007a). Dentro de un mismo país se encuentra cultivos con diferentes contenidos de As, dada la diferencia regional el contenido de As. El equipo de Meharg *et al.* (2009) analizó el contenido de As en arroces de diferente origen, llegando a la conclusión que en general el arroz de origen español presenta un alto contenido en As (**Figura 1**). Sin embargo, de todas las áreas investigadas hasta la fecha el arroz cultivado en Cádiz y Sevilla presenta unos valores de As inferiores al resto (Williams *et al.*, 2007b; Meharg *et al.*, 2009).

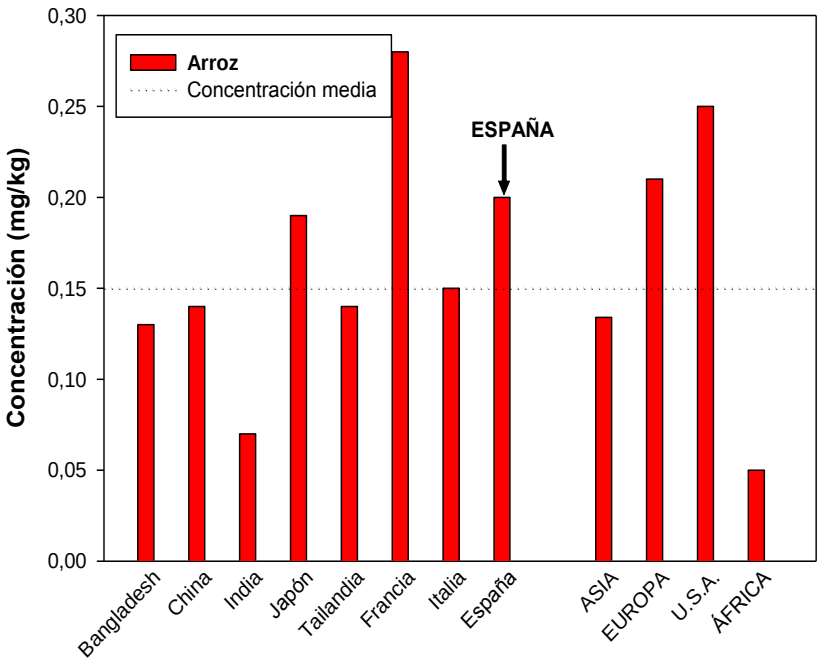

Figura 1. Concentración de arsénico total en arroz de diferentes países (Meharg *et al.*, 2009)

Ciertas prácticas agronómicas pueden afectar de modo significativo la disponibilidad del As y merecen ser estudiadas. Por ejemplo, el cultivo aeróbico del arroz está empezando a ser considerado en la Unión Europea con el objetivo de mejorar la eficiencia en el uso del agua y los fertilizantes nitrogenados. Comparado con la inundación continua de los campos de arroz, una gestión aeróbica reduce la asimilación de As por las plantas, y por tanto, reduce el contenido de As en el grano comestible (Arao *et al.*, 2009). Sin embargo, esta práctica abre nuevos temas de debate sobre la capacidad de adaptación de las variedades de arroz tradicionales europeas a la elevada disponibilidad de oxígeno, y por tanto, hay dudas sobre el rendimiento y calidad del arroz resultante. La incorporación de estiércol o paja seca ha demostrado que promueve la metilación del As, la cual a su vez reduce significativamente la toxicidad de este contaminante (Mestrot *et al.*, 2009).

2.1.1 Procesado de arroz

En la **Figura 2** se presenta el diagrama de flujo del procesado industrial del arroz. El objetivo principal del procesado industrial del arroz es adaptar el cereal para consumo humano. El descascarillado, indispensable en la producción de arroz integral, y mondado-pulido para generar arroz blanco (sin salvado).

Tras la **recepción** de la materia prima, se produce la **limpieza** eliminando impurezas mediante una corriente de aire para eliminar los deshechos de bajo peso y tamices vibratorios de diferente tamaño de poro.

El grano tras ser recolectado contiene 19-23 % de humedad. En dichas condiciones el almacenamiento del grano supone un riesgo importante al ataque de insectos y microbiológico, por lo que se debe reducir el contenido de humedad hasta alcanzar un 13 %.

Los tipos de **secado** de arroz pueden ser:

a) móvil: mediante la inyección de aire caliente en lecho fluidizado o columnas secadoras en donde el arroz se encuentra en movimiento, o

b) estacionario: en silos donde el arroz se mantiene estacionario y se le inyecta aire caliente o bien con aire del ambiente.

Tras finalizar con la etapa de secado, los granos de arroz pueden ser almacenados en silos durante meses.

La siguiente etapa es el **descascarillado** del *arroz paddy o con cascara.* Se trata de quitar la cascara que envuelve el grano de arroz. Como muestra la **Imagen 10,** el grano de arroz está cubierto por una cáscara, formada por lignina (20-25 %), celulosa (35-40 %) y hemicelulosa (15-20 %). Actualmente se estudia la capacidad de producción de etanol de dicho subproducto mediante hidrolisis enzimática (Piñeros *et al.*, 2010).

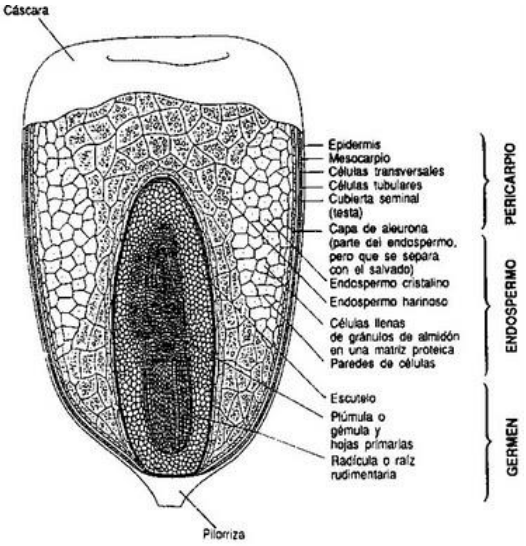

Imagen 10. Estructura grano de arroz. Owen Fenema R. (1984)

El descascarillado industrial consiste en eliminar el 95 % de la cáscara mediante la fricción con rodillos de goma, el producto obtenido se denomina **arroz marrón o arroz integral**, el cual conserva el salvado (pericarpio) y el germen

El siguiente paso es el **mondado**, consiste en el eliminar el salvado, es decir el resto de capas que cubren el grano (cutícula, epicarpio, endocarpio, testa y aleurona). Dicha etapa supone una importante pérdida nutricional, en cuanto al contenido en vitaminas, minerales y fibra, de ahí que el salvado de arroz sea utilizado en el sector nutracéutico y farmacéutico en la formulación de complementos alimentarios.

El germen es la parte del embrión con mayor contenido en grasas, se justifica porque es la reserva energética para su desarrollo. El grano se debe pulir para evitar el enranciamiento durante el almacenamiento, obteniendo así el **arroz blanco o pulido** mediante la fricción a presión con rodillos de piedra o acero.

Recientemente se ha introducido una etapa en el proceso de elaboración del arroz, el **vitaminado,** consiste en la inmersión de los granos de una solución rica en vitaminas.

Finalmente se **clasifica** el grano en función de los parámetros de calidad, se **dosifica** y **envasa**. El balance de materia supone una pérdida de un 15 % peso en el producto final (arroz blanco).

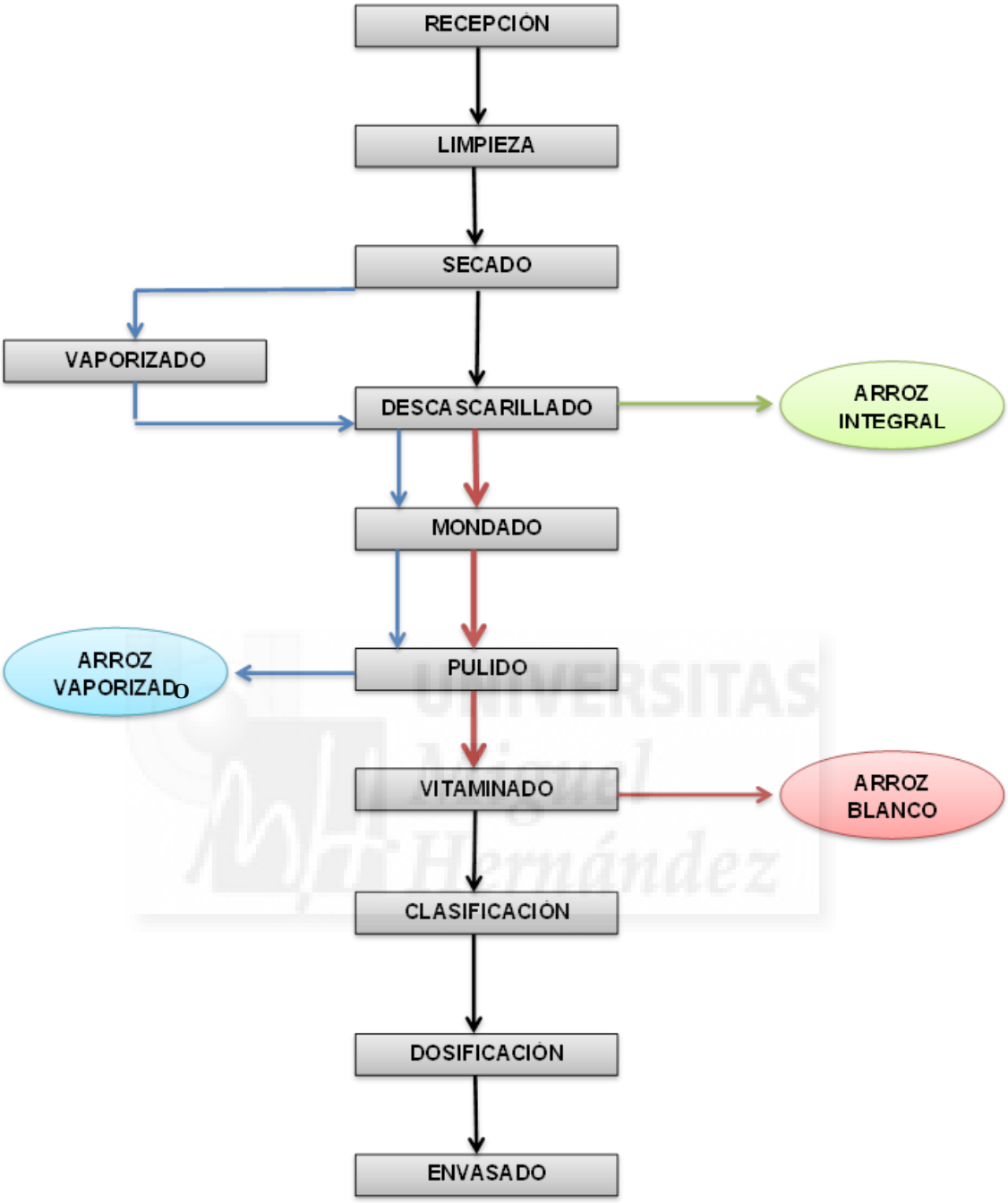

Figura 2. Diagrama de flujo procesado industrial del arroz

En base a las diferencias en el procesado del grano de arroz se pueden distinguir diferentes tipos de arroz (**Imagen 11**):

- **Arroz integral,** es el arroz que resulta de la eliminación de la cáscara y conserva el salvado.
- **Arroz blanco,** es el arroz pulido que no contiene ni la cáscara ni el salvado, suele pasar por la etapa vitaminado para potenciar su valor nutricional
- **Arroz vaporizado**, se trata de un arroz que se ha sometido tratamiento térmico de 60 °C mediante vapor a alta presión, previo al descascarillado. Con esta técnica se consigue que una gran parte de las vitaminas y los minerales pasen de la cáscara al grano, por lo que se evita la etapa de vitaminado, ya que conseguimos un grano de arroz rico en nutrientes.

Imagen 11. Tipos de arroz

El contenido de As en el arroz está fuertemente influenciado por el procesado de elaboración del arroz, la contaminación puede reducirse mediante la optimización del pre-procesado de arroz y su cocción final (Signes *et al.*, 2008c). Por ejemplo, el lavado y remojo de arroz puede reducir significativamente los niveles de As-t. La reducción en el contenido As-t en los alimentos puede ocurrir por pérdidas por; (i) solubilización al medio de cocción o bien por (ii) volatilización, debido a tratamientos de calor intenso durante un largo periodo. Además, las especies tóxicas pueden ser transformadas a otras durante la preparación, reduciendo la toxicidad.

Sun *et al.* (2008a) mostraron con sus estudios que el salvado de arroz tiene una mayor concentración de As-i que el grano, por lo que el pulido o descascarillado del arroz para obtener el arroz blanco conlleva a una disminución sustancial del As-i. Al mismo tiempo, Signes *et al.* (2008b) compararon dos procesos de descascarillados de arroz: (i) húmedo, el descascarillado se produce tras una cocción del arroz, obteniéndose arroz sancochado y (ii) seco, descascarillado mecánico, obteniendo arroz descascarillado denominado “atab” en India. Se concluyó que el método seco era el más aconsejable cuando

el agua está contaminada con As; sin embargo, el remojo se debe utilizar cuando la calidad del agua es adecuada, ya que permite la solubilización de parte del As, reduciendo así la cantidad de dicho elemento (Signes *et al.*, 2008a). Diversos estudios demuestran que parte del As del agua de cocción puede pasar al arroz llegando a aumentar la concentración de éste en un 10 % e incluso hasta un 35 % (Alam *et al.*, 2005).

Por otro lado, la contaminación por As está relacionada con los procesos de fabricación, en los que interviene el arroz como materia prima (Carbonell-Barrachina *et al.*, 2002). Devesa *et al.* (2008) revisaron los efectos de los tratamientos térmicos sobre la concentración de As-t y sobre la especiación de As en alimentos. En general, los cambios no fueron excesivamente importantes, pero pueden llegar a serlo cuando se emplean temperaturas elevadas, tales como las alcanzadas en la superficie de los alimentos durante las operaciones de fritura o cocción a la plancha (Torres-Escribano *et al.*, 2008).

2.2 Alimentos infantiles a base de arroz

El arroz es el principal ingrediente en los alimentos infantiles. Encontramos arroz en productos comercializados a partir de los 4 meses formando parte de papillas de cereales sin gluten, papillas de arroz y en galletas especiales para la dentición. A partir de los 6 meses encontramos el arroz en papillas combinadas con carne, como arroz con pollo e incluso recetas típicas como la paella. A partir de los 8 meses, el arroz sigue presente en combinación con el pescado. A lo largo de primer año de vida, se podría decir que el arroz está presente como base en la alimentación infantil.

La oferta de productos en el mercado español a base de arroz es amplia, además nos podemos encontrar con al menos 5 marcas diferentes que ofrecen el mismo tipo de productos. En la **Tabla 3**, se muestra la lista de ingredientes que figuran en la etiqueta de cinco marcas diferentes de papillas de cereales sin gluten y multicereales. Según las normas de etiquetado CE 13/2000, sabemos que aparecen en orden decreciente de concentración.

FORMULACIÓN CEREALES		
	Sin gluten	**8 Cereales**
CB1	Harina (96 %): hidrolizado de arroz y maíz, arroz y maíz	Harina (trigo, cebada, centeno, maíz, sorgo, arroz, avena y mijo)
CB2	Harina de cereales de dextrina (56 %): arroz y maíz	Harina de cereales dextrina (70 %): trigo, arroz, cebada, centeno, maíz, mijo, sorgo y avena
CB3	La harina de los cereales parcialmente dextrina (94 %): arroz, maíz y tapioca	Harina de cereales parciales dextrina (96 %): trigo, maíz, arroz, cebada, avena, centeno, sorgo y el mijo
CB4	Harina de hidrolizado (96 %): arroz, maíz y tapioca	Harina de hidrolizado (94 %): trigo, maíz, arroz, avena, cebada, centeno, el sorgo y el mijo
CB5	Cereales (61 %): harina de arroz y harina de maíz	Harina de cereales (66 %): trigo, cebada, centeno, maíz, arroz, avena, sorgo y el mijo

Tabla 3. Lista de ingredientes de alimentos infantiles de cereales comercializados en España.

En la **Tabla 4** se presenta la información del etiquetado de potitos a base carne y pescado, a diferencia del caso anterior se especifica la proporción de arroz que figura en la fórmula.

Además, existen otras formulaciones a base de arroz como:

- Papillas de arroz únicamente, es decir supone 100 % de arroz
- Leche de arroz, comercializado en tiendas especiales, sustituye a la leche en casos de intolerancia a la lactosa.
- Galletas de arroz o combinadas con otros cereales, como maíz para galletas recomendadas a partir de 4 meses.

Alimento Infantil	Formulación etiqueta
Arroz con merluza	Agua de cocción, las patatas, la merluza (12 %), arroz (7 %), cebolla, aceite de oliva (1,2 %), aceites vegetales, el apio, la sal
Puré de guisantes con arroz y merluza	Agua de cocción, los guisantes (20 %), merluza (9 %), arroz (7 %), cebolla, aceite de oliva (2 %), jugo de limón, la sal
Arroz con pollo	Agua de cocción, el pollo (15 %), arroz (6 %), zanahorias, almidón de maíz, tomate, cebolla, aceite de oliva (0,8 %), aceites vegetales, apio, sal
Zanahoria, arroz y pollo	Caldo de pollo (45 %), zanahoria (3,4 %), pollo (10 %), arroz (5 %), cebolla, almidón de maíz, aceite de oliva (0,5 %), aceites vegetales, perejil, sal, laurel

Tabla 4. Lista de ingredientes de potitos a base de arroz comercializados en España.

2.3 Arsénico en alimentos infantiles

La EFSA (2009) determinó que los productos a base de cereales son la vía predominante de exposición a As-i en la UE, siendo el arroz uno de los principales contribuyentes, debido a su alto contenido en As-t. Los niños se encuentran en mayor riesgo de exposición a los contaminantes alimentarios, como el As (EFSA, 2009; Meharg *et al.*, 2008a, c), dada la relación ingesta-masa corporal. Además las investigaciones indican que los receptores de los niños pequeños son más sensibles al As-i que los adultos (Fängström *et al.,* 2009).

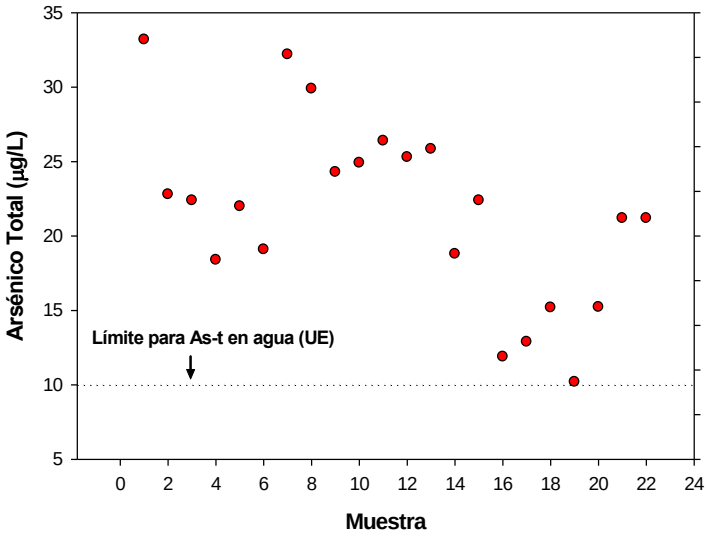

Figura 3.Arsénico total en leche de arroz (Meharg, 2008 a).

En los últimos ocho años, se ha estudiado productos infantiles elaborados a base de arroz (Meharg *et al.*, 2008c) y leche de arroz (Meharg *et al.*, 2008a) del Reino Unido, demostrando la presencia de niveles elevados de As, tanto As-t como As-i, llegando a sobrepasar los límites marcados por la UE para el agua potable (10 mg As-t/L)(**Figura 3**). En la actualidad, la legislación europea no ha establecido niveles máximos de As-i en alimentos. Es imprescindible que

se marque un límite, especialmente en alimentos que producen riesgos emergentes, como los alimentos infantiles basados en el arroz. En consecuencia, un objetivo fundamental de la Seguridad Alimentaria es asegurar que la exposición al As-i en niños se reduce al mínimo.

Es importante tener en cuenta en la formulación de alimentos infantiles el origen del arroz, la cantidad del ingrediente, procesado del arroz, así como el procesado del producto transformado a base de arroz (**Figura 4**). La etapa crítica en este proceso es el secado, la temperatura alcanzada en la superficie del alimento es elevada, pudiendo producir cambios en las especies arsenicales (Torres-Escribano *et al.* 2008). Una de las vías abiertas de investigación sería el estudio de las especies arsenicales el proceso de elaboración de alimentos infantiles.

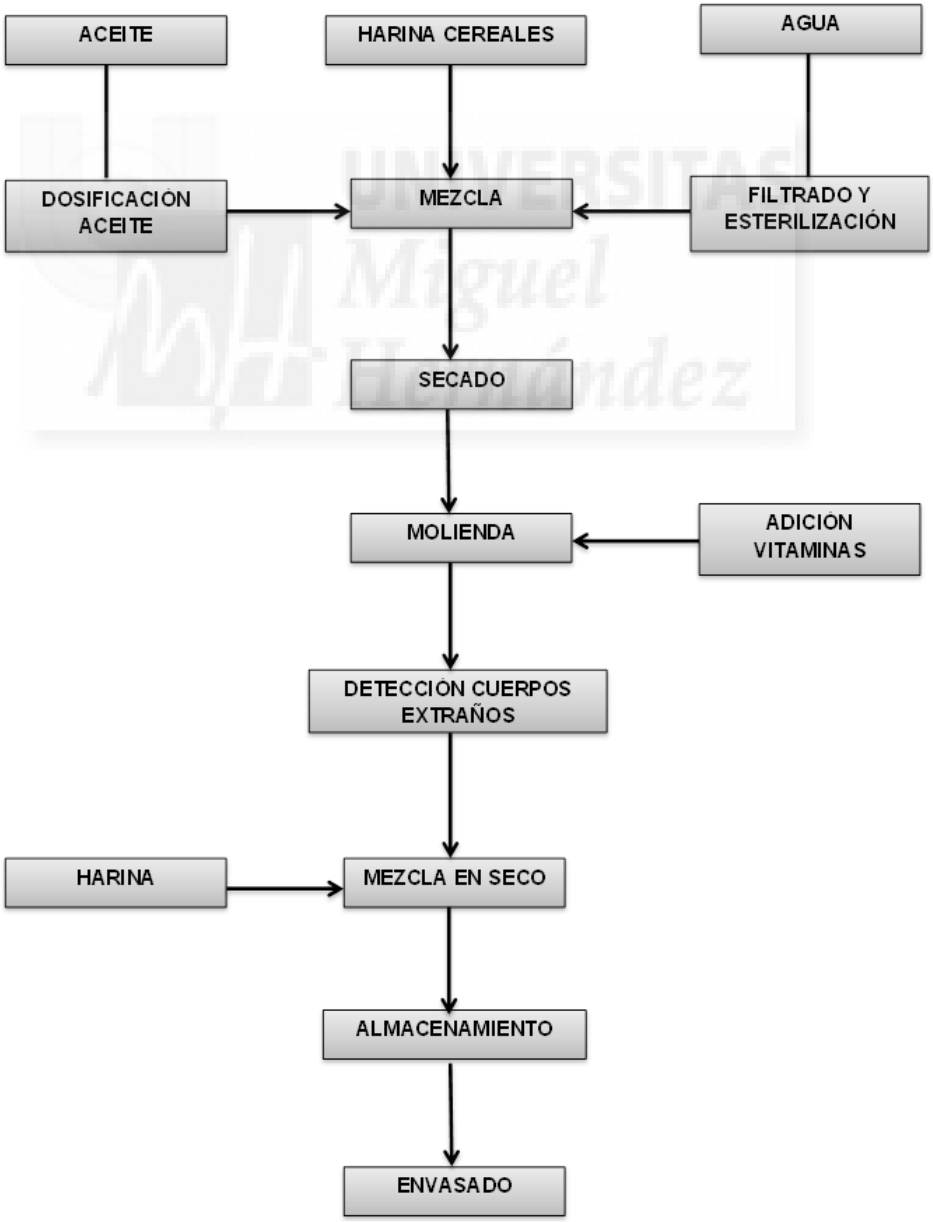

Figura 4. Diagrama de flujo del proceso de elaboración de papilla de cereales.

3. ALIMENTACIÓN INFANTIL

3.1 Alimentación en el lactante

En los primeros meses de vida del lactante su alimentación se basa en la leche materna; de forma natural la madre produce la leche con los nutrientes necesarios para el correcto desarrollo del lactante. Es el alimento ideal, fiel reflejo de la naturaleza que establece un vínculo especial entre madre-hijo. Sin embargo, en algunos casos no se puede alimentar correctamente al bebé, por ello las empresas dedicadas a la alimentación infantil intentan adaptarse mediante fórmulas de inicio o leche artificial, imitando lo más fielmente posible la leche de la madre. La ESPGHAN (*European Society for Pediatric Gastroenterology Hepatology and Nutrition*), establece unas normas sobre los requisitos de las fórmulas de inicio en cuanto al contenido en minerales, como el sodio, calcio, proteínas e hidratos de carbono; de este modo la Directiva CE 141/2006 establece las normas legales que debe cumplir los preparados infantiles (**Tabla 1**).

Requerimientos según Directiva 141/2006/CE fórmulas de inicio	
Energéticos	
Parámetro	**Valores min-max (kcal/100ml)**
Energía	60-70
Macronutrientes	
Parámetro	**Valores min-max (g/100kcal)**
Proteínas	1,8-3
Lípidos	4,4-6
Hidratos carbono	9-14
Lactosa	4,5
Micronutrientes	
Parámetro	**Valores min-max (mg/100kcal)**
Sodio	20-60
Potasio	60-160
Cloro	50-160
Calcio	50-140
Fósforo	25-90
Magnesio	5-15
Hierro	0,3-1,3
Cinc	0,5-1,5

Tabla 1. Tabla resumen de los requisitos exigidos por la Directiva 141/2006 en la producción y comercialización de fórmulas de inicio.

Ya sea una opción u otra (materna o leche artificial), la leche se convierte en el principal alimento en el primer año de vida. A partir de los 6 meses de vida las necesidades energéticas del lactante aumentan un 32 % (Serra *et al.,* 2004.), siendo necesario completar la alimentación con otros alimentos (Monte *et al.*, 2004); los primeros alimentos que se introducen son los cereales sin gluten y la fruta. A pesar de no tener una justificación nutricional, en muchas ocasiones se adelanta este proceso a los 4 meses con el fin de ofrecer diferentes sabores y texturas.

En algunas ocasiones, los alimentos para lactantes son enriquecidos en microelementos como hierro (Fe) y cinc (Zn) con el fin de cubrir las necesidades nutricionales, sin embargo existe discusión sobre la estabilidad y biodisponibilidad de los mismos (Zand *et al.,* 2011)

3.2 Introducción de alimentos en el lactante

El proceso de introducción de alimentos en el primer año de vida es complejo, y a pesar de guiarse por la norma de la ESPGHAN, hay ciertas diferencias en función de las indicaciones de cada pediatra. Si bien es cierto que la base de la introducción de alimentos es la unidad y progresión.

A partir de los **4 meses** se puede empezar a introducir los cereales sin gluten y la fruta (con algunas excepciones). Los cereales sin gluten son unos preparados deshidratados a base de arroz y maíz, se trata de los primeros cereales introducidos. Su idoneidad se caracteriza por su carácter antialergénico y fácil digestión (Meharg *et al.*, 2008b.; Mennella *et al.,* 2006). Además, las galletas elaboradas a base de estos cereales son propicias para favorecer el proceso de dentición, que suele comenzar a partir de esta etapa.

En cuanto a los potitos de frutas, las primeras semanas se recomienda los preparados de un único ingrediente ya sea comercial o casero, como manzana, plátano, pera o naranja. Éstas son las primeras opciones, mientras el resto de frutas se introducen de forma progresiva, excepto la fresas (tras el primer año de vida). En muchas ocasiones este potito viene preparado con una base de cereales.

A partir de los **5 meses** se recomienda la mezcla de frutas e incluso el acompañarlos con derivados lácteo, como los yogures de frutas. En esta etapa se ha establecido la nueva

textura (triturado) así como los nuevos sabores. Se suelen combinar los sabores, multi-frutas, cereales y derivados lácteos.

A los **6 meses**, se recomienda suplementar la fuente proteica con carne blanca, además de acompañarlo con verduras. Al igual que con la fruta se empieza con la patata y zanahoria, de forma progresiva se introducen el resto. En este momento, la alimentación del bebé empieza a verse enriquecida con nuevos sabores, la mezcla de carne blanca con verduras o cereales (arroz y pasta) supone un buen momento para planificar la dieta del lactante, establecer pautas de alimentación, horarios y hábitos.

Los cereales sin gluten se sustituyen por los multicereales, de forma progresiva con el objetivo de que a partir de los **7 meses** se complete el proceso de adaptación a cereales con gluten como trigo, centeno, avena, cebada y sorgo. Una vez alcanzado los 7 meses de vida, se puede completar el menú con carne roja. Además de forma ocasional se pueden introducir algunas legumbres.

El alimento estrella de esta etapa, que favorece el proceso de dentición (más acentuado en esta etapa), es el pan. Se trata del mayor estímulo que se presenta al niño, por un lado se fomenta el desarrollo psicomotor, aparecen nuevas texturas y calma el malestar producido por la dentición.

El pescado blanco, es el siguiente alimento a introducir, a partir de los **8 meses** se diversifica la fuente proteica. Se trata de una etapa de transición, el pescado es un sabor fuerte que suele producir rechazo. Las indicaciones pediátricas recomiendan cubrir las necesidades proteicas mediante la toma de carne y pescado diaria. El proceso de adaptación al nuevo sabor se considera hasta los 9 meses.

A los **10 meses** se plantea un nuevo marco dentro de la alimentación infantil, a partir de este momento es indispensable la planificación del menú del bebé, combinando carne, verduras, legumbres, pescado y cereales, con el fin adaptarse a las necesidades nutricionales. La distribución de los macronutrientes es muy similar a la de los adultos, seguir las pautas de la dieta mediterránea sería una opción adecuada. Es el momento propicio para inculcar un hábito alimentario correcto, por ejemplo 3 tomas de frutas y verduras al día, 1 toma de cereales o legumbres, 1 toma carne de ave, pescado o carne roja y 2 tomas de lácteos. Las variaciones en la alimentación hasta el año de vida son pocas.

Una vez pasado el **año** de vida se introducen de forma progresiva el resto de alimentos: huevos, fresas, frutos secos. Además se empieza a cambiar la textura de triturados a sólidos, comienza la etapa de adaptación de la alimentación del lactante a la del niño.

Las indicaciones mencionadas anteriormente son las descritas por pediatras en función de las necesidades nutricionales en el primer año de vida (AEP, 2006).

La evolución del bebé en esta etapa es muy importante, llega incluso a triplicar su peso, de ahí que las necesidades energéticas sea de 108 kcal/kg/día en los primeros 6 meses a 96 kcal/kg/día de los 6 a 12 meses. En cuanto a la distribución de macronutrientes es 50-54 % grasa, 36-40 % de hidratos de carbono, 7 % de proteínas, en la primera etapa, dicha distribución va evolucionando hasta alcanzar cierta similitud con las recomendaciones en adulto (55 % hidratos de carbono, 30 % grasa y 15 % proteínas).

3.3 Necesidades nutricionales

En el primer año de vida el peso del lactante se llega a triplicar, el desarrollo físico es tan importante que las necesidades energéticas se duplican, un 30 % de la energía va dirigida al crecimiento (Mataix *et al.,* 1995), al final del año el lactante no solo consume todos los alimentos (con algunas excepciones), sino que además se establecen las 5 tomas diarias.

En el proceso de desarrollo un aspecto muy importante es cubrir tanto las necesidades en macronutrientes como micronutrientes (**Tabla 2**), ya que a pesar de tener requerimientos menores cumplen funciones importantes en el organismo (Zand *et al.,* 2011).

El calcio es necesario para el desarrollo de los huesos, es la base durante el crecimiento, los expertos afirman que un aporte adecuado durante la infancia reduce las probabilidades de enfermedades óseas en el futuro. Por otro lado, la ingestión de calcio reduce de forma considerable la absorción de plomo en el intestino (Mahaffey *et al.,* 1986;. Ziegler *et al.,* 1978). Los lácteos y derivados son los alimentos con mayor contenido en este mineral, siendo indispensables en la dieta de los niños.

Los recién nacidos presentan una reserva de hierro importante, la cual abastece en los primeros 6 meses de vida los requerimientos. A partir de los 6 meses se requiere cubrir con la dieta las necesidades de este micronutriente. El hierro interviene en diversos procesos

en el organismo como en la síntesis de hemoglobina y síntesis de ADN, entre otros. Un estado nutricional del hierro correcto reduce la absorción del plomo (Barany *et al.*, 2005). Los alimentos con mayor contenido en hierro, que pueden ser consumidos en esta etapa son el pollo, legumbres y cacao.

El fósforo junto al calcio, forman parte de la estructura del hueso en forma de hidroxiapatita, por lo que se trata también de un mineral muy importante en el crecimiento y desarrollo.

El magnesio, es uno de los minerales más importantes en la contracción del músculo, trasmisión sináptica junto al calcio, además interviene en la formación del hueso junto al fósforo y calcio. Dicho micronutriente tiene una vinculación importante en el desarrollo psicomotor y neurológico del lactante. Para alcanzar las necesidades se deben consumir alimentos como cacao y arroz.

Otro de los minerales que interviene de forma directa en el crecimiento y desarrollo es el cinc, actúa en la síntesis de proteínas además de ser considerado como antioxidante natural. Por otro lado, el cinc reduce la absorción del cadmio aumentando la capacidad de retención de la mucosa intestinal (Foulkes, 1985). Una fuente importante de cinc son los cereales (trigo, maíz) y legumbres, principalmente lentejas.

El cobre tiene un papel fundamental en la producción de hemoglobina y mantenimiento del sistema muscular y óseo, los cereales y legumbres aportan una cantidad alta de dicho nutriente.

El selenio tiene diversas funciones, entre ellas actúa de forma directa sobre los radicales libre, además de neutralizar metales pesados como el arsénico, cadmio, mercurio o plomo (Zeng *et al.,* 2005). Estudios realizados por Rossman *et al.* (2007), mostraron una reducción en el arsénico inorgánico, acompañado por un aumento de dimetilarsenico y selenio en la orina. Otros estudios mostraron que el selenio aumenta la metilación del arsénico inorgánico (Verret *et al.* 2005; Son *et al.* 2008).

El sodio y potasio son los cationes más importantes en la osmo-regulación celular, la proporción correcta de ambos componentes permite que el mecanismo celular sea adecuado así como mantener el equilibrio hídrico. El cloro también interviene en la osmo-regulación hídrica, actúa sobre el sistema sodio/potasio.

Por último, el yodo interviene en el metabolismo energético ya que forma parte de la tiroxina, hormona secretada por la tiroides que además actúa de forma conjunta con la hormona del crecimiento.

Ingesta diaria recomendada en niños < 4 años			
	0-6 meses	6-12 meses	1-4 años
Energéticos (kcal)	650	950	1250
Proteínas (g)	14	20	23
Calcio (mg)	500	600	800
Hierro (mg)	8	10	16
Fósforo (mg)	240	300	480
Magnesio (mg)	60	75	125
Cinc (µg)	4000	5000	8000
Cobre (µg)	480	600	960
Sodio (mg)	184	230	368
Cloro (mg)	280	350	560
Potasio (mg)	312	390	624
Selenio (µg)	5,2	6,5	10,4
Cromo (µg)	0,4	0,5	0,8
Manganeso (µg)	30	37,5	60
Molibdeno (µg)	1,2	1,5	12
Yodo (µg)	40	50	70

Tabla 2. Tabla resumen de la ingesta diaria recomendada en macronutrientes y micronutrientes en niños menores de 4 años (Rojas-Rodriguez E., 2008).

Una dieta variada y equilibrada es la mejor forma para cubrir las necesidades tanto en macronutrientes como micronutrientes. Hasta los 6 meses de edad es difícil planificar la dieta del lactante, dada la limitación de alimentos permitidos. Pasada esta etapa es muy importante empezar a planificar las tomas y establecer hábitos.

3.4 Casos especiales

En ciertas ocasiones las indicaciones pediátricas y recomendaciones pueden verse alteradas. Durante la etapa infantil se suelen dar las siguientes situaciones:

- **Enfermedad gastrointestinal aguda o crónica**, causada normalmente por un rotavirus. El principal síntoma es la diarrea, en ocasiones acompañado con fiebre. El tratamiento dietético es restringir ciertos alimentos pasando a una dieta blanda, en la que la principal fuente de hidratos de carbono es el arroz, llegando incluso a cuadruplicar la cantidad en comparación con la alimentación del niño sano.

- **Intolerancia temporal o crónica a la lactosa**, en ciertas ocasiones el lactante puede desarrollar una intolerancia a la lactosa temporal por la inflamación de intestino delgado causado por una enfermedad gastrointestinal vírica. Rara vez se observa una intolerancia a la lactosa crónica, el bebé cuando nace produce una mayor cantidad de lactasa (enzima hidrolitica de la lactosa) que en la etapa adulta. En estos casos, se suele dar leche sin lactosa, se trata de un preparado en el cual se ha eliminado dicho azúcar o bien basado en otras fuentes de azúcares como el arroz.
- **La celiaquía** es una enfermedad gastrointestinal crónica, se presenta por una inflamación del intestino en presencia de gluten (NIDDK, 2008). El único tratamiento para la enfermedad celíaca es una dieta sin gluten, lo que supone no comer alimentos que contienen trigo, centeno y cebada, siendo la base de los cereales el arroz y maíz, alrededor de un 1 % de los niños es celiaco.

3.5 Legislación en alimentos infantiles

El control de seguridad alimentaria en el procesado industrial de los alimentos infantiles, sigue un patrón similar independientemente del producto a estudiar. Se lleva a cabo el control tanto en contaminantes químicos como microbiológicos. El control de contaminantes químicos se produce principalmente en las materias primas:

- **Análisis residuos de pesticidas**, el Reglamento CE 141/2006 establece que el valor de residuos de pesticidas en la alimentación infantil debe ser nulo. Aunque admite un margen considerado por contaminación cruzada, estableciendo un límite máximo de residuos (LMR) menores a 0,01 mg/kg para determinadas materias activas como; propineb y propileno tiourea, el resto de materias no deben sobrepasar el 0,01 mg/kg.
- **Micotoxinas**, el Reglamento CE 1881/2006 establece los límites de micotoxinas en alimentos infantiles; por ejemplo 10 µg/kg para el caso de patulina en compota de frutas, 0,1 µg/kg en el caso de aflatoxina B1 y 0,5 µg/kg para la ocratoxina A en cereales y derivados.
- **Metales pesados**, no existe un límite máximo definido, además no se hace distinción entre las especies. Actualmente un asunto de prioridad en la UE es fijar un límite para el arsénico inorgánico (As-i). Actualmente, el único valor de referencia que se puede tener para el control de As-i en alimentos infantiles es la ingesta semanal tolerable provisional (ISTP), descrita por FAO/OMS en 1989, que define 15 µg/(kg peso x

semana), tanto para niños como para adultos. En la actualidad la AESAN (Agencia Española de Seguridad Alimentaria y Nutrición) en colaboración con la EFSA están trabajando en el establecimiento de un límite máximo para el contenido de As-t y As-i en arroz y alimentos a base de arroz. Los niveles que se manejan son 300 y 200 µg/kg, respectivamente.

2. OBJETIVOS
MATERIALES Y MÉTODOS

2a-OBJETIVOS

La seguridad alimentaria en la alimentación infantil es una prioridad por las autoridades de la UE, regular el control de metales pesados en este tipo productos y estipular un nivel máximo de los mismos, es el objetivo que se ha marcado la EFSA.

En esta tesis doctoral se han planteado objetivos fundamentales para una primera evaluación de la situación:

1. Determinar el contenido de cuatro metales pesados: (i)arsénico, (ii) cadmio, (iii) mercurio y (iv) plomo en alimentos infantiles a base de arroz comercializados en diferentes países para niños menores de 12 meses.
2. Establecer el contenido de As-i en alimentos infantiles a base de arroz comercializados en diferentes países para niños menores de 12 meses.
3. Comparar el contenido de arsénico en productos a base de cereales sin gluten y con gluten comercializados en España.
4. Estimar la exposición dietética durante los primeros 12 meses de vida tanto al arsénico y sus especies inorgánicas, como al resto de metales pesados por el consumo de alimentos infantiles a base de arroz para niños menores de 12 meses.
5. Determinar el contenido en diversos nutrientes de interés nutricional: (a) calcio, (b) sodio, (c) hierro, (d) cobre, (e) manganeso, (f) cinc, (g) selenio, (j) cromo, (k) niquel y (l) cobalto en alimentos infantiles a base de arroz comercializados en diferentes países para niños menores de 12 meses.

2b-MATERIALES Y MÉTODOS

Para alcanzar los objetivos marcados se utilizaron los siguientes materiales y métodos:

1er OBJETIVO:

Se analizaron 22 muestras de productos infantiles a base de cereales; (i) 13 muestras de "cereales sin gluten", (ii) 9 muestras de "multicereales" de 7 marcas comerciales, 14 muestras de potitos infantiles; (iii) 10 a base de carne y (iv) 4 a base de pescado y 4 muestras de productos especiales; (v) 2 muestras de "productos anti-diarreicos", (vi) 1 muestra de "leche sin lactosa" y (vii) 1 muestra "postre a base de cereales". Todas las muestras presentaban arroz en su formulación y fueron adquiridas en diferentes mercados a nivel mundial; 14 muestras procedían de China, 5 muestras de Estados Unidos (USA), 5 muestras de Reino Unido (UK) y 7 muestras de España.

El procedimiento analítico seguido para la determinación de arsénico, cadmio y plomo fue mediante una digestión acida (HNO_3) en microondas y cuantificación en ICP-MS 7500. En el caso específico de la determinación de mercurio, se procedió a una digestión con peróxido de hidrogeno y posterior cuantificación por espectrometría de absorción atómica (AAS) Perkin Elmer Aanalyst 300) a una longitud de onda de 253,7 nm.

2^{ndo} OBJETIVO:

Se analizaron 46 muestras en total: 22 muestras de productos infantiles a base de cereales; (i) 13 muestras de "cereales sin gluten", (ii) 9 muestras de "multicereales" de 7 marcas comerciales, 19 muestras de potitos infantiles; (iii) 11 a base de carne y (iv) 8 a base de pescado y 5 muestras de productos especiales; (v) 2 muestras de "productos anti-diarreicos", (vi) 1 muestra de "leche sin lactosa" y (vii) 2 muestras de "postre a base de cereales". Todas las muestras presentaban arroz en su formulación y fueron adquiridas en diferentes mercados a nivel mundial; 14 muestras procedían de China, 5 muestras de Estados Unidos (USA), 5 muestras de Reino Unido (UK) y 7 muestras de España.

El procedimiento analítico seguido para la determinación de arsénico total fue mediante una digestión acida (HNO_3) en microondas y cuantificación en ICP-MS 7500. Para la determinación de algunas de sus especies; arsénico inorgánico (As-i), como ácido dimetilarsénico (ADMA) se procedió igualmente con una digestión acida (HNO_3) en microondas y cuantificación en HPLC-ICP-MS 1000 con una precolumna de 11,2 mm, 12-20 µm).

3er OBJETIVO:

Se analizaron 35 muestras en total: 10 muestras de productos infantiles a base de cereales; (i) 5 muestras de "cereales sin gluten", (ii) 5 muestras de "multicereales" de 5 marcas comerciales, 4 muestras de potitos infantiles; (iii) 2 a base de carne y (iv) 2 a base de pescado, comercializadas en el mercado español.

El procedimiento analítico seguido para la determinación de arsénico, cadmio y plomo fue mediante una digestión acida (HNO_3) en baño de arena en seco y se cuantificó mediante espectrometría de absorción atómica acoplado a un generador de hidruros (AAS-HG) mediante el uso de 0,1% $NaBH_4$, 0,4% NaOH y HCl a una longitud de onda de 183,7 nm.

4^{o} OBJETIVO:

Para la estimación de la ingesta de contaminantes en este grupo poblacional se tuvo en cuenta tanto las recomendaciones establecidas por la EPSGAN (*European Society for Pediatric Gastroenterology Hepatology and Nutrition*), como las propias de los fabricantes de los productos analizados. Diferenciando las 4 etapas (4, 6, 8 y 12 meses) principales que presentan modificaciones en la alimentación del lactante. A los 4 meses se consideró; 4 tomas de leche (160 ml) con 20g de cereales sin gluten, a los 6 meses; 3 tomas de leche (160 ml) con 20g de cereales sin gluten y 50g de potito a base de carne, a los 8 meses; 2 tomas de leche (160ml) con 30g de cereales con gluten o multicereales, 75g de potito a base de carne y 75g de potito a base de pescado, finalmente a los 12 meses; 2 tomas de leche (160ml) con 30g de cereales con gluten o multicereales, 100g de potito a base de carne y 100g de potito a base de pescado.

5º OBJETIVO:

Se analizaron 22 muestras de productos infantiles a base de cereales; (i) 13 muestras de "cereales sin gluten", (ii) 9 muestras de "multicereales" de 7 marcas comerciales, 14 muestras de potitos infantiles; (iii) 10 a base de carne y (iv) 4 a base de pescado y 4 muestras de productos especiales; (v) 2 muestras de "productos anti-diarreicos", (vi) 1 muestra de "leche sin lactosa" y (vii) 1 muestra "postre a base de cereales". Todas las muestras presentaban arroz en su formulación y fueron adquiridas en diferentes mercados a nivel mundial; 14 muestras procedían de China, 5 muestras de Estados Unidos (USA), 5 muestras de Reino Unido (UK) y 7 muestras de España.

El procedimiento analítico seguido para la determinación de elementos traza esenciales, tales como cobre, manganeso, cinc, selenio, cromo, niquel y cobalto fue mediante una digestión acida (HNO_3) en microondas y cuantificación en ICP-MS 7500. En el caso específico de la determinación de calcio, hierro y sodio, se procedió a una digestión acida y posterior cuantificación por espectrometría de absorción atómica (AAS) Perkin Elmer Aanalyst 100) a una longitud de onda de 422,37 nm; 248,3 nm y 589 nm, respectivamente).

3. ARTÍCULOS

3-ARTÍCULOS

3.1 Burló F., Ramírez-Gandolfo A., Signes A., Parvez H., Carbonell-Barrachina A.A. (2012). Arsenic contents in Spanish infant rice, pureed infants foods and rice. *J. Food. Sci.* 77: 15-19.

Arsenic Contents in Spanish Infant Rice, Pureed Infant Foods, and Rice

Francisco Burló, Amanda Ramírez-Gandolfo, Antonio J. Signes-Pastor, Parvez I. Haris, and Ángel A. Carbonell-Barrachina

Abstract: It seems there is a positive correlation between rice content and arsenic level in foods. This is of extraordinary importance for infants below 1 y of age because their diet is very limited and in some cases is highly dependent on rice-based products; this is particularly true for infants with the celiac disease because they have no other option than consume gluten-free products, such as rice or corn. Arsenic contents were significantly higher ($P < 0.001$) in gluten-free infant rice (0.057 mg kg^{-1}) than in products with gluten, based on a mixture of cereals (0.024 mg kg^{-1}). Besides, especial precaution must be taken when preparing rice-based products at home, because arsenic content in Spanish rice was high, with levels being above 0.3 mg kg^{-1} in some cases.

Keywords: baby food, celiac disease, daily intake, dietary exposure, food safety, gluten

Practical Application: From the data presented in this manuscript, it seems imperative that legislation on maximum residues of arsenic in food should be available as soon as possible to protect consumers worldwide. Research is needed to identify or breed rice cultivars with low accumulation of arsenic in the grain; otherwise the rice percentage in infant foods should be reduced.

Introduction

In 2009, the European Food Safety Authority (EFSA 2009) reviewed the diet of the European Union (EU) population and stated that; (1) inorganic arsenic (i-As) level should be reduced, (2) children under 3 y of age are the most exposed to i-As, varying their daily intake from 0.50 to 2.66 mg kg^{-1} of body weight, and (3) dietary exposure to i-As in babies was directly related to the intake of rice-based products. It was determined that the cereal-based products were the predominant route of exposure to i-As in the EU; rice is the main contributor due to its high content of total arsenic (t-As). Rice has higher grain As contents than other cereals (for example wheat and barley), as it is much more efficient in accumulating soil As (Williams and others 2007). A recent study demonstrated that people consuming large quantities of rice have an elevated exposure to i-As (Cascio and others 2011).

Both inorganic and organic As (o-As) species occur in the environment. Generally, the (i-As) forms are thought to be more toxic, both acute and chronic, than the organic o-As ones ; i-As is a chronic exposure carcinogen (NRC 2001; IARC 2004), and children are particularly susceptible to it (Vahter 2009). Early-life exposure to extremely low contents of i-As in drinking water has been linked to increased infant morbidity and mortality (Rahman and others 2009).

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Precooked, milled rice is a dominant carbohydrate source to weaning babies up to 1 y of age due to its material properties, tastelessness, low allergen potential, and nutritional value, including the absence of gluten (Meharg and others 2008b). The dependence on rice for infants with food intolerance is even higher; for instance, rice is the basic cereal for infants with the celiac disease (Meharg and others 2008a, 2008b).

One-year-old weaned babies' hair As content was found to be 10-fold greater than 1-mo-old infants. Infant formulae-fed babies had higher hair As contents than breast-fed counterparts, with cereals being the most elevated source of As in weaning diets (Gibson and Cage 1982).

Gluten-free infant rice is introduced in the infant's diet at the age of 4 mo. Later at month 6, the infant body is ready to enjoy more complex foods, such as cereals with gluten (wheat, oat, and so on). Finally, pureed infant foods containing meat (mainly chicken) and fish (mainly hake) are introduced at 6 and 8 mo of age, respectively (Fenwick 2005).

Recently, Ljung and others (2011) studied the essential and toxic elements in infant formula and infant foods and reported As values ranging from 0.33 to 1.58 $\mu g\ L^{-1}$ and 1.0 to 33 $\mu g\ kg^{-1}$, respectively. These authors concluded that rice-based products contained elevated As concentrations. Before, the contents of As in rice milk (Meharg and others 2008a), baby rice (Meharg and others 2008b), and other rice-based products (Sun and others 2009) were quantified and found to be elevated in products marketed in the UK.

Considering the elevated levels of As in rice-based infant foods, these require especial attention. The two objectives of this study were to compare the following: (1) the contents of As in infant cereals (with and without gluten), pureed infant foods (with fish or with meat), and in homemade rice-based infant foods and (2) the daily intake of As in infants from 4 to 12 mo of age.

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Materials and Methods

Instrumentation

Determination of t-As was performed with a Unicam Model Solaar 969 (Unicam Ltd., Cambridge, U.K.) atomic absorption spectrometer equipped with a continuous hydride generator Unicam Solaar VP90 (AAS-HG). Other equipment used included a sand bath (Falc, Treviglio, Italy), model BS 70, a muffle furnace (Hobersal, Barcelona, Spain), model 12 PR/300 series 8B, a grinder (Moulinex, Valencia, Spain) with a maximum power of 180 W, and a hot air oven (Selecta, Barcelona, Spain).

Reagents

Ultrapure deionized water (18 MΩ) was used for the preparation of the reagents and standards. All glassware was treated with 10% HNO_3 for 24 h and then rinsed 3 times with deionized water before use. All chemicals were of trace element grade. Commercial standard salt from arsenic trioxide (Panreac, Barcelona, Spain) was used for the preparation of standards. Magnesium nitrate, $MgNO_3$ and MgO (Panreac, Barcelona, Spain) were used in the ashing solution and 65% HNO_3 (Merck, Germany) during the digestion of samples. Hydrochloric acid, 37% HCl (Merck, Germany), 99% IK (Rectapur, Leuven, Belgium), and 99% ascorbic acid (Panreac, Barcelona, Spain) were used to adjust pH and as reducing agents. For the generation of H_3As, $NaBH_4$ (Panreac, Barcelona, Spain) solution was prepared by dissolving 1.4% of $NaBH_4$ powder in a 0.4% NaOH solution and filtering through Whatman nr 42 paper (Whatman Intl. Ltd., Kent, U.K.). Fresh $NaBH_4$ solutions were prepared daily.

Sample collection and preparation

Commercial infant rice/cereals (powdered baby-grade rice or cereals), pureed infant foods, and rice samples were obtained (in triplicate) in pharmacies and national supermarket chains in the city of Alicante (Spain) during 2010 and were analyzed for content of t-As. The products under analysis were:

- 10 infant rice/cereals samples (to be prepared using infant formula) from 5 commercial brands (CB1, CB2, CB3, CB4, and CB5) but with two different formulations;
  - 5 gluten-free samples (rice and corn);
  - 5 samples of cereals with gluten (mixtures of wheat, barley, oat, rye, sorghum, and millet);
- 4 samples of pureed infant foods (2 hake- and 2 chicken-based samples); and
- 21 rice samples that can be classified into 3 categories: 4 samples of paddy rice (with husk), 6 sample of brown rice (with bran layer), and 6 samples of white rice (without husk and bran layers).

In general, gluten-free infant rice is intended for consumption since 4 mo of age while infant cereals (mixture of as many as eight cereals) are recommended for infants of 6 or more mo of age. Most products are available worldwide and are produced by major manufacturers of infant foods. All infant rice/cereals samples were in powdered form while pureed infant foods were a mixture of cooking water, rice, and other ingredients, mainly vegetables.

From the labeling of the products, it can be ascertained that foods were made either in Spain or in other parts of the EU, but no specific information on the source of the rice grain or cereals was indicated in the products labels.

The samples of rice were studied to check the safety of the homemade infant foods based on rice.

Samples of rice and infant foods were dried in a hot air oven (Selecta, Barcelona, Spain) at 70 °C until constant weight; 96 h were needed for the infant foods. Then, samples were ground in a domestic grinder (Moulinex, Valencia, Spain) and the resulting powder was vacuum packed and kept at 4 °C until analysis. Analyses were run in triplicate.

Quantification of t-As

All samples were analyzed for t-As by Hydride Generation Atomic Absorption Spectrometry (HG-AAS). A 1-g portion of dried, ground, and homogenized sample was weighed and digested using the method previously described by Muñoz and others (2000). Calibration standards were prepared using the same HCl concentration of the samples and certified materials. The instrumental conditions used for the hydride generation were; reducing agent: 1.4% (m/v) $NaBH_4$ in 0.4% NaOH at a flow of 5 mL min^{-1}; HCl solution: 10% (v/v) at 10 mL min^{-1}; carrier gas: argon, at 250 mL min^{-1}; while the conditions for the atomic absorption spectrometer were: wavelength: 183.7 nm; spectral bandpass: 0, 5 nm; intensity of the hollow cathode lamp 8 mA; air/acetylene flame.

In each analytical batch, at least one reagent blank and one internationally certified reference material (CRM) were included to assess precision and accuracy for chemical analysis. The CRM (rice flour, NIST SRM 1568a) used for testing this analytical method was provided by CYMIT Química S.L. (Barcelona, Spain) and produced by the US National Institute of Standards and Technology. The quantified t-As content in this CRM was 0.288 ± 0.010 mg kg^{-1}, which is 99.3% recovery of its certified value of 0.290 ± 0.030 mg kg^{-1}.

Statistical analyses

All data were subjected to analysis of variance (ANOVA) and the Tukey's least-significant difference multi-comparison test to determine significant differences among samples (food type). The statistical analyses were performed using SPPS 14.0 (SPSS Science, Chicago, Ill., U.S.A.).

Results and Discussion

Arsenic concentrations

Arsenic contents in Spanish infant rice samples are summarized in Table 1; values ranged from 0.009 ± 0.001 to 0.080 ± 0.005 mg kg^{-1} in the sample from CB2 and the gluten-free sample from the same company, respectively.

Table 1– Arsenic concentration (mg kg^{-1}) in Spanish infant rice/cereals.

	As concentration (mg kg^{-1})		As concentration (mg kg^{-1}) in the rice used
Infant rice/cereals	Rice (gluten-free)	Cereals (with gluten)	Gluten-free infant rice
CB1	0.043 ± 0.002*b†	0.033 ± 0.001b	0.045
CB2	0.080 ± 0.005a	0.009 ± 0.001d	0.142
CB3	0.077 ± 0.003a	0.020 ± 0.004c	0.082
CB4	0.043 ± 0.004b	0.016 ± 0.002c	0.045
CB5	0.044 ± 0.001b	0.039 ± 0.002a	0.072
MEAN	0.057 ± 0.014	0.024 ± 0.009	0.077 ± 0.028

*Values are the mean of 3 replicates; the ± values represent the standard errors.
†Values followed by the same letter, within a column, were not significantly different at $P < 0.001$.
CB = commercial brand.

During the period from 4 to 6 mo of age, gluten-free foods are introduced in the baby's diet to supplement breast milk (Fenwick 2005). These products are mainly based on rice and corn, and sometimes they contain tapioca as well.

The t-As concentration was dependent on the product brand and the food type. Always the t-As concentration was higher in gluten-free products (mean of 0.057 ± 0.005 mg kg^{-1}) compared to samples of infant cereals with gluten (mean of 0.024 ± 0.009 mg kg^{-1}). This finding was probably related to the different percentage of rice used in the formulations (Table 2); gluten-free items had a mean rice content of 80.6 ± 9.1% while the rice percentage in products with gluten was about 10% to 15% (the percentage was not specified in the labels but ingredients were arranged by their quantity).

Other factors could also have played an important role: the rice cultivar (Norton and others 2009), the geographical origin (Meharg and others 2009), and the rice processing (Signes and others 2008). As previously mentioned, no clear reference is made on the products labeling regarding the geographical origin of the rice used. However, it can be ascertained that infant rice products were either manufactured in Spain or the EU and using Spanish or European rice varieties. Considering the labeled rice percentages and the t-As contents found, the t-As content of the rice used in the manufacturing of the gluten-free products would have ranged from 0.045 to 0.142 mg kg^{-1} (Table 1). These values agreed quite well with recent literature on t-As content in rice from different Spanish regions. Meharg and others (2009) studied rice from Valencia and Tarragona and t-As ranged from 0.050 to 0.820 mg kg^{-1}, with a median of 0.140 mg kg^{-1}, while Torres and others (2008) reported values of 0.098 to 0.406 mg kg^{-1}, with a mean of 0.201 mg kg^{-1} in rice grown in Spain (Torres and others 2008).

The t-As content in the pureed infant foods ranged from 0.049 to 0.479 mg kg^{-1} (Table 3), with significant differences ($P < 0.001$) being found between meat- and fish-based products.

Meat-based infant foods are introduced in the baby's diet at 6 mo, when the baby is ready to explore new flavors and needs an extra-energy input (Fenwick 2005). Fish-based infant foods are introduced later, approximately at month 8, when the baby is already used to eat simple solid foods (Fenwick 2005).

Table 2–Formulation (%) of Spanish infant rice/cereals under study.

Infant rice/cereals	Labeled formulation: Rice (gluten-free)	Labeled formulation: Cereals (with gluten)
CB1	Flour (96%): hydrolyzed rice, corn, and rice	Flour (wheat, barley, rye, corn, sorghum, rice, oat, and millet)
CB2	Flour of dextrin cereals (56%): rice and corn	Flour of dextrin cereals (70%): wheat, rice, barley, rye, corn, millet, sorghum, and oat
CB3	Flour from partially dextrin cereals (94%): rice, corn, and tapioca	Flour of partial dextrin cereals (96%): wheat, corn, rice, barley, oat, rye, sorghum, and millet
CB4	Hydrolyzed flour (96%): rice, corn, and tapioca	Hydrolyzed flour (94%): wheat, corn, rice, oat, barley, rye, sorghum, and millet
CB5	Cereals (61%): rice flour and corn flour	Cereal flour (66%): Wheat, barley, rye, corn, rice, oat, sorghum, and millet

CB = commercial brand.

Considering a mean t-As content in Spanish rice of 0.140 mg kg^{-1} (Meharg and others 2009) and the fact that rice was present in the analyzed infant foods in percentages ranging from 5% to 7% (Table 4), the t-As coming from rice would range from 0.007 to 0.010 mg kg^{-1}, and will represent only 3.3% and 14.4% of the t-As content in fish- and meat-based infant foods, respectively. Consequently, in these products other As sources are expected.

The first extra source of As could be fish. Considering the percentages of hake (9% to 12%) and the t-As contents in the fish-based infant foods, the t-As contents in hake used would range between 2.5 and 4.0 mg kg^{-1}. These values agreed with previously t-As contents reported in the literature. Falcó and others (2006) found t-As content of 3.22 to 4.55 mg kg^{-1} in hake purchased in Catalonia (Spain), and Perelló and others (2008) quantified the t-As content in hake cooked using different methods: raw 2.086 mg kg^{-1} fresh weight (fw), fried 2.906 mg kg^{-1}, and grilled 3.281 mg kg^{-1}.

Arsenic residues have been found in meat products; for instance, Polatajko and Szpunar (2004) reported values of 0.143 mg kg^{-1} in chicken meat. Similar values were also reported by Perelló and others (2008) in chicken cooked using different methods: raw 0.110 mg kg^{-1} fw, fried 0.128 mg kg^{-1}, grilled 0.092 mg kg^{-1}, and roasted 0.131 mg kg^{-1}. Consequently, some As could also come from the chicken meat.

Finally, vegetables are used in the manufacturing of all pureed infant foods under study and they could accumulate significant quantities of As under specific situations. However, the vegetables used in the production of the infant products are likely to be from Spain and are unlikely to contain high levels of As because irrigation water is not polluted with As in Spain. Indeed, a study by Ferre-Huguet and others (2008) did not find any As in Spanish vegetables.

Table 3–Arsenic concentration (mg kg^{-1}) in Spanish pureed infant foods.

Infant food	As concentration (mg kg^{-1})
MEAT (chicken)	
Rice with chicken	0.055* ± 0.009c†
Carrot and rice in chicken broth	0.049 ± 0.007c
FISH (hake)	
Rice with hake	0.479 ± 0.012a
Purée of peas with rice and hake	0.225 ± 0.008b

*Values are the mean of 3 replicates; the ± values represent the standard errors.
†Values followed by the same letter, within a column, were not significantly different at $P < 0.001$.

Table 4–Formulation (%) of Spanish pureed infant foods under study.

Baby food	Formulation in label
Rice with hake	Cooking water, potatoes, hake (12%), rice (7%), onion, olive oil (1.2%), vegetable oils, celery, salt
Purée of peas with rice and hake	Cooking water, peas (20%), hake (9%), rice (7%), onion, olive oil (2%), lemon juice, salt
Rice with chicken	Cooking water, chicken (15%), rice (6%), carrots, corn starch, tomato, onion, olive oil (0.8%), vegetable oils, celery, salt
Carrot and rice in chicken broth	Chicken broth (45%), carrot (3.4%), chicken (10%), rice (5%), onion, corn starch, olive oil (0.5%), vegetable oils, parsley, salt, laurel

Twenty samples of rice marketed in Spain were obtained and t-As contents are summarized in Table 5. The mean value of t-As in all rice samples under analysis was 0.186 ± 0.015 mg kg^{-1} and values ranged from 0.102 to 0.351 mg kg^{-1}.

The main reason for including rice samples in this study was that this rice is quite often used in Spain for the preparation of homemade rice porridges, especially when the infant is older than 8 mo. Besides, at this age wholegrain rice can also be introduced.

In general, t-As contents in rice are usually 1 order of magnitude higher than in other cereals (Williams and others 2007; Sun and others 2008a, 2008b). The highest values of t-As were found in paddy rice with husk (mean of 0.216 ± 0.048 mg kg^{-1}), followed by brown rice (0.196 ± 0.044 mg kg^{-1}), and with the lowest values being found in white rice (0.173 ± 0.015 mg kg^{-1}). Sun and others (2008b) showed that high As contents accumulated in the bran layer of rice, reaching values of approximately 1 mg kg^{-1}; this conclusion supports the fact the t-As contents were higher in brown (wholegrain) rice compared to white (polished) rice.

Daily arsenic intake

The toxicology of As is independent of its source once it crosses the gut membrane. All indications are that the bioavailability of i-As from rice is high, in the order of 90% (Ackerman and others 2005). Food standards are out of step with drinking water regulations. EU and US drinking regulations are set at 0.01 mg L^{-1} for t-As and i-As, respectively, and both assume a daily consumption of 1 L of drinking water (CEU 1998; NRC 2001). These values equate to a predicted maximum As intake of 0.17 $\mu g\ d^{-1}\ kg^{-1}$ for a conservative body mass of 60 kg.

In this article the daily As intake will be discussed, according to the infant nutrition (main types of foods being consumed), at 3 different periods: (1st) 4 to 6 mo, (2nd) 6 to 8 mo, and (3rd) 8 to 12 mo. The daily intake of food for an infant at these 3 different periods are (Fenwick 2005): (1st) 37 g of infant rice (mean body weight, for girls and boys, of 6.65 kg), (2nd) 37 g of infant rice and 50 g of meat-based infant food (mean body weight of 7.55 kg), and (3rd) 37 g of infant rice, 80 g of meat-based infant food and 80 g of fish-based infant food (mean body weight of 8.30). The body weights considered were those of the 50th percentile. The mean intake for an infant in the 1st, 2nd, and 3rd age period is 0.317, 0.624, and 4.15 $\mu g\ d^{-1}\ kg^{-1}$, respectively. At mean exposure levels, infants are receiving more As in their diet than the maximum level assumed under both EU and US laws, if As consumption is expressed on a body weight basis. This calculation is based on the assumption that only one portion of infant rice, one portion of meat-based infant food, and one portion of fish-based infant food are consumed per day. In most cases, only one portion is given at the beginning of the period but the amount is increased to 2 servings at the end of the period. If additional servings are given, As intake is simply multiplied by the number of servings.

Table 5–Arsenic concentration (mg kg^{-1}) in rice marketed in Spain.

Rice type	As concentration (mg kg^{-1})
Paddy ($n = 4$)	0.216 ± 0.048
Paddy rice (Calasparra)	0.127* ± 0.001c†
Paddy rice (Valencia)	0.199 ± 0.027b
Paddy rice (Valencia)	0.188 ± 0.020b
Paddy rice (Valencia)	0.351 ± 0.022a
Brown ($n = 6$)	0.196 ± 0.044
Brown and organic rice (Spain)	0.338 ± 0.014a
Brown rice (Spain)	0.127 ± 0.010b
Brown rice (Spain)	0.333 ± 0.005a
Brown rice (Valencia)	0.113 ± 0.001b
Brown rice (Valencia)	0.126 ± 0.002b
Brown rice (Valencia)	0.137 ± 0.013b
White ($n = 11$)	0.154 ± 0.005
Spain ($n = 6$)	0.139 ± 0.010
White rice (Spain)	0.178 ± 0.011a
White rice (Spain)	0.137 ± 0.013c
White rice (Spain)	0.151 ± 0.009b
White rice (Valencia)	0.102 ± 0.002d
White rice (Valencia)	0.138 ± 0.004c
White rice (Valencia)	0.126 ± 0.009c
Thailand ($n = 2$)	0.184 ± 0.019
Aromatic Thai white rice	0.202 ± 0.008a
Aromatic Thai white rice	0.165 ± 0.008b
India ($n = 3$)	0.158 ± 0.019
Basmati Brajma white rice	0.157 ± 0.005b
West Bengal white rice	0.126 ± 0.009c
West Bengal white rice	0.192 ± 0.005a
Global mean	0.177 ± 0.016

*Values are the mean of 3 replicates; the ± values represent the standard errors.
†Values followed by the same letter, within the same type of rice, were not significantly different at $P < 0.001$.

Considering the speciation data by Meharg and others (2008b), which showed that approximately 53% of the t-As present in infant rice was present as i-As, the daily intakes of i-As determined in the present study will be 0.16 and 0.33 $\mu g\ d^{-1}\ kg^{-1}$ at the periods 4 to 6 and 6 to 8 mo of age, respectively. These figures represent that the daily intake of i-As at the 1st period was close to the maximum value assumed under EU/US legislations (0.17 $\mu g\ d^{-1}\ kg^{-1}$), while at the 2nd period it was almost double this legal threshold assumed for water. The intake of i-As during the 3rd period could be considered similar to that of the 2nd period, if it is considered that all As coming from the hake is under organic forms. According to Ciardullo and others (2008) more than 99% of the As found in fish muscle (0.40 to 0.95 mg kg^{-1} of fw), including hake, was present in the form of nontoxic arsenobetaine.

The CONTAM Panel of EFSA (2009) modelled the dose–response data from key epidemiologic studies and selected a benchmark response of 1% extra risk. The lowest $BMDL_{01}$ values were for lung cancer and this EFSA Panel concluded that the overall range of $BMDL_{01}$ values of 0.3 to 8.0 $\mu g\ kg^{-1}$ bw per day should be used instead of a single reference point in the risk characterization of i-As. The estimated dietary exposures to i-As of Spanish children were close or higher than those of adults but this does not necessarily indicate that children are at greater risk because the exposure estimates were within the range of $BMDL_{01}$ values and in general effects are due to long term exposures (EFSA 2009), although early-life exposure was associated with increased morbidity and mortality (Rahman and others 2009).

Finally, it is worth considering the homemade rice-based infant foods. It is necessary to remember that the mean t-As content in the rice used for the manufacturing of infant rice was estimated to be 0.077 mg kg^{-1}. If commercially available Spanish white rice (0.139 mg t-As kg^{-1}) would be used for preparing homemade rice porridges, the daily intake values reported before should be multiplied by a factor of 1.8 (0.139 compared to 0.077). If later (8 to 12 mo), brown rice would be used instead of white rice, the situation would be even worse because the concentration of t-As in this type of rice is higher, 0.196 mg kg^{-1} and a coefficient of 2.5 (0.196 / 0.077) should be applied to all values of daily intakes.

Conclusions

The finding of elevated As contents in infant rice and pureed infant foods commercialized in the Spanish market is of concern

and deserves special attention. Similarly to results previously reported for Swedish and UK infant products, the As contents in Spanish rice-based infant foods were also high. There are 3 main strategies to face this situation: (1) Questioning whether rice is suitable for infant foods and if the answer is no, removing rice form infant food formulations, (2) reducing the intake of rice through diversification of the diet such as including other cereals that do not contain high levels of As, or (3) trying to reduce the As content in rice intended for infant foods. Until the latter option comes into effect, some governments have chosen the first 2 options. For instance, in the UK, children younger than 4.5 y are advised against consuming rice milk because of concern for high As exposure. Similarly in Denmark, children below 10 kg are also advised against consuming rice milk. However, these 2 options are not satisfactory for certain groups of our society: (1) Infants with celiac disease, who have few other options than consuming rice flour; (2) rice farmers because these options seriously jeopardize their markets. Therefore, research needs to be carried out to identify approaches that significantly reduce the high As contents in rice. Current research is focused on: (1) screening of As levels in existing rice to identify varieties that have low As levels, (2) breeding rice to get rice cultivars with restricted As uptake and upward transport to the edible grain, and (3) modifying the current anaerobic growing practices in rice fields, moving towards more aerobic conditions, which will reduce As availability to rice plants but perhaps also their yields.

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PAPER

Essential and toxic elements in infant foods from Spain, UK, China and USA

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Spanish gluten-free rice, cereals with gluten, and pureed baby foods were analysed for essential macro-elements (Ca and Na), essential trace elements (Fe, Cu, Zn, Mn, Se, Cr, Co and Ni) and non-essential trace elements (As, Pb, Cd and Hg) using ICP-MS and AAS. Baby cereals were an excellent source of most of the essential elements (Ca, Fe, Cu, Mn and Zn). Sodium content was high in pureed foods to improve their flavour; fish products were also rich in Se. USA pure baby rice samples had the highest contents of all studied essential elements, showing a different nutrient pattern compared to those of other countries. Mineral fortification was not always properly stated in the labelling of infant foods. Complementary infant foods may also contain significant amounts of contaminants. The contents of Hg and Cd were low enough to guarantee the safety of these infant foods. However, it will be necessary to identify the source and reduce the levels of Pb, Cr and As in Spanish foods. Pure baby rice samples contained too much: Pb in Spain; As in UK; As, Cr and Ni in USA; and Cr and Cd in China.

Introduction

An infants' nutritional requirements can be completely fulfilled by breast milk or formula during the first six months of age, but after this time complementary feeding is necessary to boost energy and minerals intake.[1,2] For some minerals, fortification is required; for example Fe and Zn requirements may be difficult to meet from non-fortified complementary foods, especially in developing countries. The type, nature and extent to which complementary foods meet infants' nutritional needs are controversial, mainly because of factors such as nutrients stability and bioavailability.[3] It is important to mention that trace element analysis usually just gives the total content of trace elements or their species but does not consider bioavailability.[4] In view of all the above, complementary infant foods require special attention with respect to the occurrence of both essential and non-essential trace elements.

Several trace elements and minerals are essential for biological processes and play a vital role in normal growth and development.[2] Minerals are involved in many important functions in the body, *e.g.* enzymatic reactions, bone mineralization, hormones secretion, and protection of cells and biological membranes.[2,3] Infancy and early childhood are characterized by a very high growth rate and mineral and trace element requirements are critical during this period.[2,5] Nutritional imbalances at an early stage of life can have severe effects on infants' health in later life and links have been demonstrated with developmental delay, diabetes, obesity, hypertension and increased risk of heart disease.[3]

Most of the research on essential trace elements present in infant formula has focused only on a few elements, such as iron and zinc.[6] This means that scientific information on other essential elements is scarce or even missing. In this respect the Codex Stan 72-1981 indicates that guidance upper levels were

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Environmental impact

The diet of infants with celiac disease is mainly limited to rice-based foods. Baby cereals (with gluten) were richer in Ca, Fe, Cu, Mn, Zn, Cr and Ni than baby rice (gluten-free). Therefore, infants following a gluten-free diet will have lower daily intakes of most of the essential elements. In addition, the content of As in rice-based foods was elevated. It is important to identify the source of non-essential elements, especially As, in infant foods and reduce their contents. Different fortification strategies have been demonstrated. The US manufacturers fortified pure baby rice with most of the essential elements; this strategy is based on fulfilling the WHO/UNICEF recommended daily intakes. Other manufacturers only fortified their products with Fe and Zn.

established for most of the minerals and trace elements without sufficient information for a science-based risk assessment.[7]

On the other hand, any exposure to non-essential trace elements is undesirable. These non-essential and toxic trace elements may cause adverse health effects in infants even at low levels.[8] It has been reported that children are more susceptible to exposure to potentially toxic elements because of their greater intestinal absorption than adults, and lower threshold for adverse effects.[9] Infant foods may contain toxic minerals or trace elements mainly as a result of their natural occurrence in the raw materials/ingredients used in their formulation. For instance, it has been clearly stated that rice-based infant foods may contain high levels of total arsenic (t-As) and inorganic arsenic (i-As) as a consequence of the occurrence of this metalloid in the rice used to manufacture these products.[6,10–12]

Celiac disease is a digestive illness that interferes with the absorption of nutrients from food;[13] its only treatment is a gluten-free diet, excluding foods based on wheat, rye, and barley. In general, gluten-free infant rice is introduced in the infant's diet at month 4. However, no studies have dealt with the occurrence of essential and non-essential elements in infant foods specially designed for infants suffering from celiac disease. Consequently, the four objectives of this study were to compare: (i) the contents of essential (Ca, Na, Fe, Cu, Mn, Zn, Se, Cr, Ni and Co) and non-essential (As, Pb, Cd and Hg) elements in Spanish gluten-free infant rice and infant cereals with gluten, (ii) the contents of the same elements in Spanish infant rice/cereals with those of pureed infant foods (with fish or with meat), (iii) the daily intake of essential and non-essential elements, associated with rice/cereals and pureed foods, in infants between 4 and 12 months of age, and (iv) the contents of the same elements in pure infant rice from different countries: Spain, UK, China and USA.

Materials and methods

Chemicals

Trace element grade reagents were exclusively used in all analyses. Nitric acid (HNO_3) (70%) was obtained from Fluka Analytical (Steinheim, Germany), while hydrogen peroxide (H_2O_2) was from Fisher Scientific (Loughborough, UK). Monosodium arsenate (Na_2HAsO_4) and sodium arsenite ($NaAsO_2$) of reagent grade were purchased from Merck (Nottingham, UK).

Sample preparation

Spanish infant rice, infant cereals, pureed infant foods (with chicken or hake) and special infant foods were purchased (in triplicate) from pharmacies and national supermarket chains in the city of Alicante (Spain) during 2011 and were analysed for essential and trace element contents. The products under analysis were:

- 22 infant cereal samples (to be prepared using infant formula) from 7 commercial brands:
  - 13 gluten-free samples (rice and maize),
  - 9 samples of mixed cereals with gluten (wheat, barley, oat, rye, sorghum and millet); and,
- 14 samples of pureed infant foods:
  - 10 meat samples (all chicken),
  - 4 fish samples (all hake); and,
- 4 samples of special products: 2 samples of anti-diarrhoea rice mixtures, 1 sample of lactose-free cereals mixture, and 1 rice-based dessert.

In general, gluten-free infant rice is intended for consumption from 4 months of age while infant cereal samples (with gluten) are recommended for infants of 6 or more months of age.[14] All infant rice/cereals products were in powdered form while pureed infant foods were a mixture of cooking water, rice and other ingredients, mainly vegetables.

From the labelling of the Spanish products, it can be ascertained that foods were made either in Spain or the European Union, but no specific information on the source of the rice grain or other cereals was presented on the packaging.

Pure infant rice samples were imported in 2011 from Chinese and USA health shops. Samples from UK and Spain were obtained in 2011 from national supermarket chains in the cities of Aberdeen (Scotland) and Alicante (Spain), respectively. There were 14 samples from 11 Chinese manufacturers, 5 samples from 4 US manufacturers, 5 samples from 5 UK manufacturers and 7 samples from 7 Spanish manufacturers. Six of the Chinese samples had sugar added and even though the labelling did not specify its exact percentage it was estimated to range between 5 and 10%. These samples were considered as pure infant rice because no other cereal was used in their formulation.

Products were dried in an oven (Gallenkamp, Hotbox oven) at 80 °C until constant weights were reached; 24 h was required for infant rice and cereals, while 78 h was needed for infant foods. The solid samples were homogenized by grinding in a ball mill and stored in a desiccator in the dark until analysis.

Analysis of essential and non-essential trace elements

Samples were processed as previously described.[15] Briefly, approximately 0.2 g dried weight (dw) of infant products were weighed into 50 mL polypropylene digest tubes (Corning, NY), and 2 mL of concentrated nitric acid (HNO_3) were added. The mixture was left to imbibe overnight and then 2 mL of hydrogen peroxide (H_2O_2) were added and then samples were digested in a microwave oven (CEM Mars 5, CEM Corp., Matthews, NC). The temperature was raised, first to 55 °C, and held for 10 min, and then to 75 °C, and held for 10 min. Finally, the temperature was taken up to 95 °C and maintained for 30 min. Samples were cooled to room temperature and then the sample was diluted to a mass of 50 g with ultrapure deionized water (18 MΩ) obtained from a Milli-Q system (Millipore, Billerica, MA).

In each analytical batch, at least one reagent blank, one spike and one internationally certified reference material (CRM) were included to assess precision and accuracy for chemical analysis. The CRM used was rice flour NIST SRM 1568a.

A quadrupole ICP-MS 7500 (Agilent Technologies, Tokyo, Japan) was used to determine metals and trace elements concentration as described elsewhere.[15] A collision cell was used to negate argon chloride (ArCl) interferences. Samples were randomized prior to analysis. Standards were run after every 40 samples. Rhodium (10 μg L^{-1}) was added online as the internal standard.

Analysis of Ca, Na and Fe

These three elements were analysed in the previous sample digests by Atomic Absorption Spectroscopy (AAS) using a Perkin Elmer AAnalyst 100. The spectroscopic conditions recommended by Perkin Elmer were used for the analyses (Ca: 422.7 nm and slit 0.7 nm; Na: 589.0 nm and slit 0.7 nm; Fe: 248.3 nm and slit 0.2 nm). In each analytical batch at least one reagent blank, one spike and one CRM were included. Rice flour NIST SRM 1568a was used as the CRM.

Analysis of Hg

Samples for the Hg analysis were digested using the previously described protocol but without using H_2O_2. Mercury was analysed using a Perkin Elmer AAnalyst 300 coupled to a Flow Injection Analysis System FIAS 100. AA WinLab™ was used to process data. Spectroscopic conditions recommended by Perkin Elmer were used for the analysis (253.7 nm and slit 0.7 nm).

Estimation of daily intake

According to the World Health Organization (WHO), after 6 months of age it becomes increasingly difficult to breastfeed infants to meet their nutrient needs from human milk alone.[16] Thus, 6 months is the recommended age at which complementary foods should be introduced. However, and despite these guidelines, weaning occurs at an early age in most countries.[17] For example, Santamaria-Orleans *et al.* reported that Spanish infants started with weaning foods at 4.4 months of life.[18] Briefel *et al.* reported that in the period from 4–6 months US infants are introduced to infant cereals and pureed infant foods (mostly fruits and vegetables).[19]

In this article, the daily intake of minerals and trace elements will be discussed, according to the infant nutrition (main types of foods and amounts being consumed),[14] at four different ages: 4, 6, 8 and 12 months. The daily food intake for an infant at these four ages was: (i) *4 months*: 4 feeds of breast milk (140 mL per feed),[14,20] 20 g of gluten-free infant rice or infant cereals with gluten [mean body weight (bw), for girls and boys, of 6.65 kg according to Generalitat Valenciana];[21] (ii) *6 months*: 3 feeds of breast milk, 20 g of infant rice or infant cereals and 50 g of meat-based infant food (mean body weight of 7.55 kg); (iii) *8 months*: 2 feeds of breast milk, 30 g of gluten-free infant rice or infant cereals, 75 g of meat-based infant food and 75 g of fish-based infant food (mean body weight of 8.30 kg); and (iv) *12 months*: 2 feeds of breast milk, 30 g of infant rice or infant cereals, 100 g of meat-based infant food and 100 g of fish-based infant food (mean body weight of 9.30 kg). It has been assumed that infants are taking only breast milk (ideal scenario) and body weights considered were those of the 50th percentile.[21] The intake of minerals and trace elements from the water used to reconstitute infant rice/cereals was not considered due to differences from home to home and country to country.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) and Duncan's least-significant difference multi-comparison test to determine significant differences among samples (food type or country of origin). The statistical analyses were performed using SPPS 15.0 (SPSS Science, Chicago, Ill., U.S.A.).

Results and discussion

Quality assurance

The accuracy of the analytical methods was verified by analysing the CRM rice flour NIST SRM 1568a. The experimental results agreed with the certified reference values (Table 1), with experimental concentrations ranging between 64.2 and 99.5% of the certified values for Cd and As, respectively. Note that the digestion procedure was optimized around that of As, to compliment As speciation data published in Carbonell-Barrachina *et al.*,[12] in which the CRM recovery for As was 99.5%. The CRM NIST SRM 1568a contains low levels of some of the elements studied here and this may justify the low recovery of certain elements, for instance Cd. In order to verify the accuracy of the methods for those elements whose concentrations were not certified in the CRM, samples were spiked with the elements under study and the mean recovery for the spikes ranged between 77.0 and 118% for Se and Mn, respectively (Table 1).

Essential macro-elements and trace elements

Calcium contents were significantly ($p < 0.01$) higher in baby rice and cereals (mean of 1.42 g kg^{-1}) compared to pureed foods (mean of 0.16 g kg^{-1}) (Table 2). No significant differences were found among meat- and fish-based foods; however, baby cereals contained more Ca than baby rice, 1.67 g kg^{-1} compared to 1.17 g kg^{-1}. These experimental values agreed with data reported by Ljung *et al.*,[6] ranging from 0.016 to 1.67 g kg^{-1}, and were slightly lower than those reported by Melø *et al.*,[2] averaging 3.38 g kg^{-1}.

Differences were found among Ca contents in baby rice samples from different geographical origins, with USA having

Table 1 Quality assurance information, including limit of detection (LOD) and percentage of recovery of elements from spikes and certified reference materials (CRMs)

	Ca	Na	Fe	Cu	Mn	Zn	Se	Cr	Ni	Co	As	Pb	Cd	Hg
Sample	(g kg^{-1})		(mg kg^{-1})				(μg kg^{-1})				(μg kg^{-1})			
LOD (μg kg^{-1})	0.01	0.01	7	0.11	0.02	0.16	13	5	38	37	6	39	7	4
Spike recovery (%)	n.a.[a]	n.a.	n.a.	86.3	118	79.1	77.0	98.1	107	92.1	88.8	93.8	79.7	107
CRM (%)	85.5	75.6	75.8	79.5	96.1	70.6	93.2	n.a.[a]	n.a.	n.a.	99.5	n.a.	64.2	n.a.

[a] n.a. = not available.

Table 2 Concentrations of essential macro- and trace nutrient elements in commercial baby rice/cereals and baby foods

Sample	*n*		Ca (g kg^{-1})	Na (g kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Se (μg kg^{-1})	Cr (μg kg^{-1})	Ni (μg kg^{-1})	Co (μg kg^{-1})
Baby rice	13	Mean	1.17 ± 0.26[a]	0.08 ± 0.01c	47.7 ± 9.2a	1.08 ± 0.14bc	6.57 ± 0.95b	6.99 ± 0.72bc	52 ± 11b	118 ± 19a	155 ± 17bc	n.d.
		Median	1.03	0.08	48.6	1.18	6.64	7.41	50	126	139	n.d.
		Range	0.01–2.59	0.02–0.23	0–84.9	0.08–1.80	0.08–12.7	0.08–10.7	0–146	18–270	84–299	n.d.–84
Baby cereals	9	Mean	1.67 ± 0.25a	0.11 ± 0.03c	65.8 ± 9.3a	1.78 ± 0.32ab	13.2 ± 3.3a	8.36 ± 1.58b	52 ± 6b	222 ± 102a	308 ± 60a	n.d.
		Median	1.97	0.08	58.6	1.62	9.47	6.83	49	118	253	n.d.
		Range	0.01–2.48	0.01–0.20	23.0–117	0.02–3.08	0.02–28.9	0.02–14.9	23–79	17–978	n.d.[b]–579	n.d.
Meat baby food	10	Mean	0.16 ± 0.04b	1.03 ± 0.22ab	9.7 ± 3.9bc	0.23 ± 0.02c	0.81 ± 0.06c	2.78 ± 0.06bc	49 ± 14b	180 ± 30a	41 ± 5c	n.d.
		Median	0.14	1.04	2.4	0.24	0.81	2.81	31	138	39	n.d.
		Range	0.04–0.41	0.16–2.09	1.1–34.3	0.14–0.36	0.57–1.06	2.47–3.08	19–144	76–355	n.d.–73	n.d.
Fish baby food	4	Mean	0.16 ± 0.04b	0.86 ± 0.27b	2.9 ± 1.0c	0.31 ± 0.07c	1.14 ± 0.13bc	1.81 ± 0.03c	117 ± 25a	106 ± 9a	68 ± 14bc	n.d.
		Median	0.16	0.84	2.7	0.30	1.14	1.79	119	100	72	n.d.
		Range	0.06–0.27	0.19–1.54	0–6.6	0.12–0.51	0.78–1.52	1.72–1.91	50–181	85–139	n.d.–103	n.d.
Others	4	Mean	1.53 ± 0.32a	1.47 ± 0.32a	38.4 ± 16.5ab	2.61 ± 0.78a	2.61 ± 0.70bc	25.3 ± 6.8a	124 ± 30a	99 ± 36a	172 ± 63b	n.d.
		Median	1.71	1.63	34.6	3.36	2.75	31.0	137	65	119	n.d.
		Range	0.53–2.16	0.48–2.15	3.7–80.6	0.03–3.70	0.63–4.30	2.97–36.2	34–189	49–218	73–378	n.d.

[a] Values followed by the same letter, within the same column, were not significantly different at $p < 0.01$. [b] n.d. = not detected or below the limit of detection (LOD).

the highest content (10.3 g kg^{-1}), followed by Chinese samples (3.59 g kg^{-1}) and finally Spanish and UK samples (0.92 and 0.49 g kg^{-1}, respectively) (Table 3). These differences were probably related to fortification. Half of the Chinese samples indicated in their labelling that they were fortified with Ca; after grouping the fortified and non-fortified samples, the Ca contents were 4.1 and 2.9 g kg^{-1}, respectively. Only one of the five US samples reported the fortification in the labelling but the experimental Ca levels seemed to indicate that at least four of them were fortified with Ca.

The estimated daily intakes of Ca were not affected by the infant age or the presence of gluten in the infant diet and averaged 144 mg per day (Table 4). This value was very low compared with the 525 and 350 mg per day (Table 5) recommended by WHO/UNICEF for infants aged 0–11 and 12–23 months, respectively.[2,22] However, if the fortified pure baby rice is used for estimating the daily intake, this parameter will take values close to the recommended values; this fact implies that Ca fortification of baby rice and cereals seems necessary to fulfill the WHO/UNICEF requirements.

Sodium was ~0.1 g kg^{-1} in both baby rice and baby cereals, significantly ($p < 0.01$) increased in pureed foods, with a mean value (meat and fish samples) of 0.95 g kg^{-1} (Table 2). Sodium chloride (table salt) is used to enhance the flavour of food. Results found here agreed with previously reported data; for instance, Zand *et al.* found Na values ranging from 0.15 to 1.02 mg kg^{-1} in complementary infant food samples from UK.[3]

For pure baby rice, samples from USA had the highest Na content, followed by samples from China, Spain and UK (Table 3). The important difference found between the mean and median values of the USA product was due to one sample having a Na content as high as 1.11 g kg^{-1}.

The daily intake of Na increased with the age of the infant up to a value of ~190 mg per day at month 12 of age; however, the presence of gluten in the infant diet did not affect the Na intake (Table 4). According to WHO/UNICEF the recommended daily intake (RDI) of Na ranged between 280 and 500 mg per day at months 4 and 12 of age (Table 5);[2,22] estimated daily intakes in this study were below these recommended values. The WHO/UNICEF RDI values could be achieved only by using the US fortified samples of pure baby rice.

Iron contents were higher in baby cereals (mean of 65.8 mg kg^{-1}) than in baby rice (47.7 mg kg^{-1}) and significantly ($p < 0.01$) lower in pureed foods (6.3 mg kg^{-1}), especially in those based on fish (Table 2), in which the content was 2.9 mg kg^{-1}. Most of the baby rice and baby cereals were fortified with Fe, Zn and Ca. Results from Fe content were compared with the declared values from the manufacturer and even though there were some minor discrepancies, concentrations were within the minimum/maximum range of recommended values. As an example of this comparison, the analysed and declared Fe contents in Spanish baby cereals were 42.7 and 46.0 mg kg^{-1}; large differences were only found in the case of pureed meat baby foods, with analysed and declared values being 9.7 and 20.0 mg kg^{-1}.

Important differences in Fe fortification strategies are clearly shown in Table 3; with Fe contents being more than 27 times higher in samples from USA (588 mg kg^{-1}) compared to UK (21.7 mg kg^{-1}). High levels of Fe in infant foods have been

Table 3 Concentrations of essential macro- and trace nutrient elements (μg kg^{-1}) in samples of pure infant rice from China, USA, UK and Spain[a]

Country	*n*		Ca (g kg^{-1})	Na (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Se (μg kg^{-1})	Cr (μg kg^{-1})	Ni (μg kg^{-1})	Co (μg kg^{-1})
China	14	Mean	3.59 ± 0.61b[a]	145 ± 32b	74.4 ± 6.3b	2.07 ± 0.13a	16.9 ± 3.1ab	45.5 ± 3.7b	82 ± 13b	134 ± 5ab	190 ± 34bc	n.d.
		Median	2.91	107	75.0	2.12	13.5	40.6	71	131	204	n.d.
		Range	1.47–9.63	23–397	25.9–106	1.14–2.73	7.9–52–1	32.5–78.0	21–188	109–181	0–388	n.d.
USA	5	Mean	10.3 ± 2.9a	348 ± 182a	588 ± 34a	2.22 ± 0.14a	25.6 ± 3.4a	87.1 ± 25.2a	135 ± 26a	172 ± 32a	434 ± 130a	108 ± 48a
		Median	9.53	117	590	2.06	30.7	70.9	123	171	310	40
		Range	0.32–19.6	51–1107	485–707	2.01–2.83	15.0–32.9	13.7–179	47–225	52–257	234–1002	n.d.–308
UK	5	Mean	0.49 ± 0.28c	66 ± 8b	21.7 ± 3.9c	1.94 ± 0.12a	16.2 ± 2.9ab	18.3 ± 3.9c	51 ± 9b	96 ± 11b	50 ± 14c	n.d.
		Median	0.08	60	18.3	1.94	10.2	13.4	51	97	51	n.d.
		Range	0.03–2.62	25–105	8.3–51.0	1.31–2.71	7.6–33.1	8.4–53.5	n.d.[b]–111	52–198	0–106	n.d.
Spain	7	Mean	0.92 ± 0.36bc	84 ± 27b	42.7 ± 12.8bc	1.32 ± 0.14b	8.8 ± 0.9b	8.2 ± 0.6c	47 ± 18b	96 ± 24b	160 ± 15bc	n.d.
		Median	0.97	76	48.6	1.32	8.1	8.1	37	108	151	n.d.
		Range	0.01–2.28	0–225	0–81.8	0.69–1.80	6.6–12.7	5.5–10.7	n.d.–146	18–189	122–237	n.d.

[a] Values followed by the same letter, within the same column, were not significantly different at $p < 0.01$. [b] n.d. = not detected or below the limit of detection (LOD).

justified by low bioavailability as well as the concern for Fe deficiency in infants. However, Fe bioavailability from milk based products has increased in recent years mainly because of formula modification.[6,23]

Berglund *et al.* reported that in marginally low birth weight infants (<2.5 kg), a daily intake of 0.6 and 1.0 mg Fe per kg per day up to 6 months of age provided protection against Fe deficiency and Fe deficiency anaemia, respectively.[24] Estimated daily Fe intakes for Spanish infants ranged from 1.1 to 3.3 mg per day (Table 4). Consequently, values were close to these limits (1.5–2.5 mg per day), to negate Fe deficiency. However, daily Fe intakes could be an order of magnitude higher if pure baby rice from USA is considered. According to WHO/UNICEF the RDI of Fe should be 11 and 6 mg per day in the periods between 6–11 and 12–23 months of age (Table 5);[22] estimated daily Fe intakes in Spanish infants were below this recommended level. The WHO/UNICEF RDI values could be reached only by using the US fortified samples of pure baby rice.

Copper contents followed the trend: baby cereals > baby rice > pureed fish ≥ pureed meat, ranging from 0.23 to 1.78 mg kg^{-1} (Table 2). Copper contents were similar in samples of baby rice from China, USA and UK (mean of 2.08 mg kg^{-1}) and significantly lower in the Spanish samples (1.32 mg kg^{-1}) (Table 3). The daily intake of Cu averaged 150 and 166 μg per day in infants following gluten-free and gluten diets, respectively (Table 4). These values were lower than the recommended values of 300 and 400 μg per day for infants aged 0–11 and 12–23 months, respectively (Table 5).[2,22]

No Mn fortification was observed in this study. Manganese levels followed the order: baby cereals ≫ baby rice ≫ pureed fish > pureed meat, with mean values ranging from 0.81 to 13.2 mg kg^{-1} (Table 2). Pure baby rice samples from USA and Spain presented the highest and lowest Mn values of all samples under study, with mean values being 25.6 and 8.8 mg kg^{-1}, respectively (Table 3). The daily intakes of Mn increased with the infants' age, were higher in infants following a diet based on gluten-containing cereals and presented a maximum value of 592 μg per day (Table 4). There is a lack of scientific data on Mn requirements in infants and its potential toxicity, which is reflected in the wide range of Mn concentrations allowed in infant formula (7 to 670 μg L^{-1}) and the fact that no upper limit for Mn intake has been set for infants.[6,25]

In most cases infant foods are fortified with Zn; this was the case for most of the products evaluated in this study (Table 2). Zinc contents were significantly ($p < 0.01$) higher in both baby cereals and rice (mean of 7.7 mg kg^{-1}) compared to pureed foods (mean of 2.3 mg kg^{-1}); in addition, Zn contents were significantly ($p < 0.01$) higher in meat than in fish products.

Table 4 Daily intakes of essential macro- and trace nutrient elements in infant foods at 4, 6, 8 and 12 months of age

	Ca	Na	Fe	Cu	Mn	Zn	Se	Cr	Ni	Co
Age (month)	(mg per day)			(μg per day)						
Diet gluten-free										
4	163	1.6	1.12	162	133	588	8.3	2.08	3.10	n.a.[a]
6	136	53.1	1.57	138	173	615	9.0	11.4	5.15	n.a.
8	129	162	2.46	143	344	778	17.7	25.0	12.8	n.a.
12	137	191	2.78	156	393	893	21.8	32.1	15.6	n.a.
Diet with gluten										
4	169	2.2	1.48	171	234	595	8.2	3.91	5.43	n.a.
6	146	53.7	1.93	152	306	642	9.0	13.4	8.21	n.a.
8	144	162	3.00	164	543	819	17.7	28.1	17.4	n.a.
12	152	192	3.32	177	592	934	21.8	35.3	20.1	n.a.

[a] n.a. = not available because concentration was below the LOD.

Table 5 Recommended daily intake of essential macro- and trace nutrient elements and body weight for an infant at 4, 6, 8 and 12 months of age

		Ca[b]	Na[b]	Fe[b]	Cu[b]	Mn	Zn[b]	Se[b]
Age (month)	Body weight[a] (kg)	(mg per day)						
4	6.65	525	280	11	0.3	n.a.[c]	4.0	0.013
6	7.55	525	320	11	0.3	n.a.	5.0	0.010
8	8.30	525	320	11	0.3	n.a.	5.0	0.010
12	9.30	350	500	6	0.4	n.a.	6.5	0.015

[a] Generalitat Valenciana.[21] [b] WHO/UNICEF.[22] [c] n.a. = not available.

Even though only one out of the five US products studied indicated that pure baby rice was fortified with Zn, the Zn content in US samples (87.1 mg kg^{-1}) was about one order of magnitude higher than in Spanish rice (8.2 mg kg^{-1}) (Table 3). Eight out of the fourteen Chinese samples of baby rice were also fortified with Zn, and their mean content was 45.5 mg kg^{-1}. However, when samples were grouped as fortified and non-fortified, even slightly higher Zn contents were found in the non-fortified group, 49.4 mg kg^{-1}, compared to 42.5 mg kg^{-1} in the fortified products. This experimental finding might indicate that not all fortification is clearly indicated in the labelling of products.

Results from Zn content were compared with the declared values from the manufacturer and even though there were some minor discrepancies, concentrations were within the minimum/maximum range of recommended values. For instance, Chinese baby rice manufacturers declared that the Zn content was within the range of 25–70 mg kg^{-1} and the mean and median values experimentally found were 45.5 and 40.6 mg kg^{-1}, respectively.

The daily intake of Zn increased with the age of the infant and ranged from ~590 to 934 μg per day (Table 4). These values were below the recommended daily intakes given by WHO/UNICEF,[22] which ranged from 4000 to 6500 μg per day for infants of 4 and 12 months of age, respectively (Table 5). The WHO/UNICEF RDI values could be achieved only by using the US fortified samples of pure baby rice.

Contents of Se were independent of the cereal type (rice, wheat, *etc.*) and averaged ~50 μg kg^{-1} and significantly ($p < 0.01$) increased (117 μg kg^{-1}) when fish was included in the formulation of the pureed products (Table 2). Pure infant rice samples from USA had the highest Se content (135 μg kg^{-1}), followed by samples of China (82 μg kg^{-1}), UK and Spain (mean of 49 μg kg^{-1}) (Table 3); however, no information on Se supplementation was included in the labelling of the studied products. The estimated RDIs for infants 4, 6, 8, 12 months were approximately 8, 9, 18 and 22 μg per day, respectively (Table 4); these values were close to the RDA or even higher after month 8 of age (Table 5).

Chromium is an essential trace element but may create problems above certain concentrations.[9] Even though the highest contents of Cr were found in baby cereals and pureed meat samples, the most important fact was that all samples under study presented levels above 100 μg kg^{-1} (Table 2). The highest contents of Cr in pure baby rice were found in US (172 μg kg^{-1}) and Chinese (134 μg kg^{-1}) samples, followed by Spanish and UK products with mean values of 96 μg kg^{-1} (Table 3). Because of the scarcity of available data on metabolic responses to usual dietary Cr intakes, recommendations are established as Adequate Intakes (AIs) for different age/gender groups.[26] The AIs of Cr in infants from 0–6 and 7–12 months of age are 0.2 and 5.5 μg per day, respectively;[27] AIs for 0–6 months were based on the average content of Cr in human milk while the value for 7–12 months includes estimates from human milk and complementary foods. In the present study, the daily intake of Cr increased with the age of the infants, ranging from 2.08 μg per day up to 35.3 μg per day (Table 4). The experimental values for the estimated daily Cr intakes were always above the AI values, indicating that special precaution must be taken to reduce the Cr contents of infant foods.

The mean value of Ni in all the studied products was 149 μg kg^{-1}, and of special interest are the high levels detected in baby cereals, 308 μg kg^{-1} (Table 2). However, an important fact was that the content of Ni was relatively low in pureed foods, which are the basis of the diet after the 8th month of age. The country of origin had a very important effect on the Ni content in pure baby rice; USA samples showed the highest content (434 μg kg^{-1}), followed by samples from China and Spain with much lower contents (190 and 160 μg kg^{-1}, respectively), and finally UK samples with only 50 μg kg^{-1} (Table 3). The NRC indicated that it was not possible to establish Tolerable Upper Intake Levels (ULs) because of the lack of data on adverse effects in this age group and concern about the infant's ability to handle excess amounts of Ni.[27] The highest daily Ni intakes were those of infants consuming baby cereals (Table 4). For children from 1–3 years, the UL was 200 μg per day;[26] the experimental value estimated in this study for 1 year old infants was 20 μg per day, which is much lower than the UL value and seemed to indicate that no health problems are expected from the Ni found in infant foods.

Cobalt contents in all Spanish infant foods were below the LOD (Table 2) and measurable contents were only found in samples of pure baby rice from US, with mean and median values of 108 and 40 μg kg^{-1}, respectively (Table 3). The daily intake of Co by Spanish infants was considered zero (Table 4) because Co contents were below the LOD.

Non-essential trace elements

Inorganic As (i-As) has been classified by the International Agency for Research on Cancer as group 1 carcinogenic; this classification was based on the induction of primary skin cancer as well as the induction of lung and urinary bladder cancer.[28] Arsenic contents in Spanish infant foods ranged from 13 μg kg^{-1} in pureed meat foods up to 210 μg kg^{-1} in pureed fish samples

Table 6 Concentrations of non-essential trace elements (μg kg^{-1}) in commercial baby rice/cereals and baby foods

			As	Pb	Cd	Hg
Sample	*n*		(μg kg^{-1})			
Baby rice	13	Mean	126 ± 26b[a]	121 ± 39a	10 ± 6a	n.d.
		Median	84	84	n.d.	n.d.
		Range	46–315	n.d.[b]–493	n.d.–84	n.d.–21
Baby cereals	9	Mean	33 ± 6c	116 ± 37a	9 ± 2a	5 ± 2a
		Median	23	73	8	n.d.
		Range	15–65	n.d.–330	n.d.–23	n.d.–21
Meat baby food	10	Mean	13 ± 2c	n.d.	n.d.	n.d.
		Median	10	n.d.	n.d.	n.d.
		Range	9–26	n.d.–39	n.d.–9	n.d.
Fish baby food	4	Mean	210 ± 4a	n.d.	n.d.	8 ± 2a
		Median	210	n.d.	n.d.	7
		Range	198–220	n.d.	n.d.–12	n.d.–16
Others	4	Mean	15 ± 4c	78 ± 34a	n.d.	n.d.
		Median	12	62	n.d.	n.d.
		Range	8–29	n.d.–178	n.d.	n.d.

[a] Values followed by the same letter, within the same column, were not significantly different at $p < 0.01$. [b] n.d. = not detected or below the limit of detection (LOD).

(Table 6); however, Carbonell-Barrachina *et al.* proved that most of this As was present under cationic species, mainly arsenobetaine.[12] Not considering fish products, baby rice contained the highest content of As (126 μg kg^{-1}) and As significantly ($p < 0.01$) decreased in samples of baby cereals because the presence of As in cereals is about one order of magnitude lower than in rice mainly due to farming practices.[29] Samples of pure baby rice from UK and USA had significantly higher contents of As than Spanish and Chinese samples (Table 7); these differences were probably related to the geographical origin, rice cultivar, farming practices and/or processing protocols. Daily intakes of As were always higher in infants following gluten-free diets based on rice products, with values increasing with the infant age and being maximum at month 12 of age (2.82 μg kg^{-1} or 26.2 μg) (Table 8). If only inorganic As is considered in fish foods, the estimated daily intakes of As reached maximum values of 2.76 μg (0.30 μg per kg per day) and 5.55 μg (0.60 μg per kg per day) in infants following diets with and without gluten, respectively. The estimated dietary exposures to As of Spanish children ranged between 0.14 and 0.60 μg per kg per day; these values were below or within the overall range of $BMDL_{01}$ (0.3 to 8.0 μg per kg per day);[30] however, infants seem to be very susceptible to toxic effects of i-As.[31] More details on As speciation in Spanish infant foods and pure baby rice samples can be found in Carbonell-Barrachina *et al.*[12]

Lead, a well-known neurotoxic metal, was only found in concentrations above the limit of detection, LOD (39 μg kg^{-1}) in baby rice and cereals, with a mean value of 119 μg kg^{-1} (Table 6); the gluten factor did not affect the content of Pb in the Spanish products. The content of Pb in the Spanish infant baby rice was significantly ($p < 0.01$) higher (134 μg kg^{-1}) than those from other countries, whose contents were below the Pb LOD (Table 7). The daily intake of Pb increased up to month 8 and was constant until month 12 (Table 8), with maximum values being about 6.0 μg per day. A new guidance level for Pb cannot be established because the European CONTAM Panel considered that the provisional tolerable weekly intake (PTWI) for Pb in food is no longer appropriate as there is no threshold level for Pb under which there is no risk of adverse health effects in young children.[6,32] A significant effort should be made to identify the source of Pb and reduce its levels in Spanish rice-based infant foods.

Table 7 Concentrations of non-essential trace elements (μg kg^{-1}) in samples of pure infant rice from China, USA, UK and Spain

Country	*N*		As (μg kg^{-1})	Pb (μg kg^{-1})	Cd (μg kg^{-1})	Hg (μg kg^{-1})
China	14	Mean	135 ± 19b[a]	n.d.[b]	48 ± 13a	n.d.
		Median	105	n.d.	28	n.d.
		Range	70–353	n.d.–75	n.d.–138	n.d.–15
USA	5	Mean	253 ± 57a	n.d.	17 ± 2b	n.d.
		Median	213	n.d.	14	n.d.
		Range	164–496	n.d.	13–25	n.d.–6
UK	5	Mean	286 ± 33a	n.d.	n.d.	5 ± 1a
		Median	300	n.d.	n.d.	6
		Range	113–506	n.d.	n.d.–16	n.d.–10
Spain	7	Mean	180 ± 37ab	134 ± 71a	n.d.	5 ± 3a
		Median	145	47	n.d.	n.d.
		Range	80–315	n.d.–493	n.d.–22	n.d.–21

[a] Values followed by the same letter, within the same column, were not significantly different at $p < 0.01$. [b] n.d. = not detected or below the limit of detection (LOD).

Table 8 Daily mean intakes of non-essential trace elements (μg per day) in infant foods at 4, 6, 8 and 12 months of age

	As	Pb	Cd	Hg
Age (month)	(μg per day)			
Diet gluten—free				
4	2.86	2.70	0.23	n.a.[a]
6	2.03	2.63	0.23	n.a.
8	20.7	3.77	0.32	0.61
12	26.2	3.77	0.32	0.81
Diet with gluten				
4	0.92	2.32	0.19	0.09
6	1.56	2.53	0.21	0.10
8	17.9	3.62	0.29	0.75
12	23.5	3.62	0.29	0.95

[a] n.a. = not available because concentration was below the LOD.

Cadmium contents in Spanish infant foods ranged from trace levels up to a mean of ~10 μg kg^{-1} in baby rice and cereals (Table 6). However, the highest Cd contents were found in pure baby rice from China (48 μg kg^{-1}) and USA (17 μg kg^{-1}); Cd was below the LOD in Spanish and UK samples of pure baby rice (Table 7). Estimated daily intakes of Cd from the studied Spanish infant products averaged 0.03 μg kg^{-1} bw (Table 8); the EFSA recently lowered the PTWI from 7.0 to 2.5 μg kg^{-1} bw,[33] which can be converted into a tolerable daily intake of 0.36 μg per kg per day. These previous values indicated that no health problems should be expected from the Cd contained in the studied foods.

Mercury contents were close to or below the LOD in rice and cereal based infant foods (Table 6). Measurable Hg contents were found in fish pureed foods (mean of 8 μg kg^{-1} and a maximum value of 16 μg kg^{-1}) (Table 7). Consequently the Hg daily intake only was measurable (0.07–0.10 μg per kg per day) after the introduction of fish foods in the infant diet, this is from month 8 of age (Table 8), reaching a maximum value of 0.10 μg per kg per day.

Conclusions

Baby cereals presented the highest contents of most of the essential macro-elements and trace elements: Ca, Fe, Cu, Mn and Zn. Sodium content was significantly higher in pureed baby foods to improve the flavour of this type of products. Finally, Se was significantly higher in fish pureed foods. The estimated daily intakes of most of the essential minerals (Ca, Fe, Cu and Zn) were below the recommended values established by the WHO/UNICEF; this statement is especially evident in the case of Fe, probably because of the high WHO/UNICEF requirements established after considering the low bioavailability of this mineral in infant foods. On the other hand, the daily intake of Se seemed quite well adjusted to the WHO/UNICEF values. The significant differences among the estimated and WHO daily intakes of essential minerals and trace elements seemed to justify the strategy developed by the USA manufacturers of fortifying pure baby rice with Ca, Fe, Zn and perhaps Se. Baby cereals contained the highest contents of Cr and Ni, while As predominated in baby rice and similar contents of Pb were found in both baby rice and cereals. Cr was present also in significant contents in pureed baby foods. The contents of Co, Hg and Cd in Spanish infant foods were below or just above the limit of detection. Special precautions should be taken to reduce the contents of the following potentially toxic trace elements in different countries: Pb, Cr and As in Spain; As in UK; Cr, Ni and Co in USA; and Cd in China.

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Inorganic arsenic contents in rice-based infant foods from Spain, UK, China and USA

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ABSTRACT

Spanish gluten-free rice, cereals with gluten, and pureed baby foods were analysed for total (t-As) and inorganic As (i-As) using ICP-MS and HPLC–ICP-MS, respectively. Besides, pure infant rice from China, USA, UK and Spain were also analysed. The i-As contents were significantly higher in gluten-free rice than in cereals mixtures with gluten, placing infants with celiac disease at high risk. All rice-based products displayed a high i-As content, with values being above 60% of the t-As content and the remainder being dimethylarsinic acid (DMA). Approximately 77% of the pure infant rice samples showed contents below 150 μg kg^{-1} (Chinese limit). When daily intake of i-As by infants (4–12 months) was estimated and expressed on a bodyweight basis (μg d^{-1} kg^{-1}), it was higher in all infants aged 8–12 months than drinking water maximum exposures predicted for adults (assuming 1 L consumption per day for a 10 μg L^{-1} standard).

1. Introduction

Inorganic arsenic (i-As) has been classified by the International Agency for Research on Cancer (IARC, 2004) as group 1 carcinogenic; this classification was based on the induction of primary skin cancer as well as the induction of lung and urinary bladder cancer. Foods can also contain organic arsenic species (o-As), which are thought to have lower toxicities but with further study required to ascertain if this is correct (Mass et al., 2001; Styblo et al., 2000). It is generally considered that arsenobetaine, mainly derived from seafood (Cullen and Reimer, 1989; Edmonds and Francesconi, 1993), is relatively non-toxic compound that is excreted by humans without transformation (Navas-Acien et al., 2011; Sabbioni et al., 1991).

In 2009, the European Food Safety Authority (EFSA, 2009) Panel on Contaminants in the Food Chain assessed the risks to human health related to the presence of As in food. Its main conclusions were: (i) there is an urgent need for speciation data, (ii) i-As level in the diet should be reduced, (iii) children under three years of age are the most exposed to i-As, with their dietary exposure being 2–3 fold that of adults, and (iv) dietary exposure to i-As in infants is directly related to the intake of rice-based products. It was determined that the cereal-based products are the predominant route of exposure to i-As in the EU, with rice being the main contributor. Rice has higher grain As contents than other cereals (e.g. wheat and barley), as it is much more efficient in accumulating soil As (Williams et al., 2007b). Certain ethnic groups with heavy rice consumption may also be at risk. Recent human biomonitoring studies have clearly demonstrated increased exposure to i-As associated with rice consumption in the Bangladeshi population living in the UK (Cascio et al., 2011).

Several recent studies have shown that rice and rice-based infant products contain elevated contents of both t-As and i-As (Meharg et al., 2008b; Ljung et al., 2011). Vahter (2008, 2009) have provided evidence that children are particularly susceptible to some of the toxic effects of i-As. In fact, early-life exposure to fairly low levels of i-As in drinking water has associated with increased infant morbidity and mortality, as well as impaired child development during pregnancy (Wasserman et al., 2008; Rahman et al., 2009).

In general, pre-cooked, milled rice is a dominant carbohydrate source to weaning infants up to 1 year of age due to its material properties, tastelessness, low allergen potential and nutritional value, including the absence of gluten (Meharg et al., 2008b; Mennella et al., 2006). The dependence on rice for infants with food intolerance is even higher; for instance, rice is the basic cereal for infants with the celiac disease (Meharg et al., 2008a,b).

Celiac disease is a digestive illness that damages the small intestine and interferes with absorption of nutrients from food (NIDDK, 2008). The only treatment for celiac disease is a gluten-free diet, meaning not eating foods that contain wheat, rye, and barley.

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Despite these restrictions, people with celiac disease can eat a well-balanced diet with a variety of foods, including rice, maize, potato, soy, amaranth, quinoa, buckwheat, or bean flour instead of wheat flour (NIDDK, 2008). Celiac disease may start at any age, both during childhood and adolescence, and is also relatively common in adulthood (Rodrigo, 2006).

In a previous study Meharg et al. (2008b) studied the i-As content in 17 samples of pure baby rice from UK. In the present study a wider range of infant foods (infant rice, infant cereals, pureed meat and fish foods and special foods) from a different country (Spain) and a higher number of samples were evaluated ($n = 46$). Besides this is the first time that t-As and i-As contents in pure infant rice samples ($n = 31$) from different countries were compared (China, USA, UK, and Spain). Consequently, t-As and i-As contents were quantified in a total of 77 samples of infant foods, providing key speciation data for infant exposure to i-As.

Summarizing infant foods require special attention with respect to their i-As content. The four objectives of this study were to compare: (i) the contents of t-As and i-As in Spanish gluten-free infant rice and infant cereals with gluten, (ii) the contents of t-As and i-As in Spanish infant rice/cereals with those of infant foods (with fish or with meat), (iii) the daily intake of i-As (associated with rice/cereals and pureed foods) in infants between 4 and 12 months of age, and (iv) the contents of t-As and i-As in pure infant rice from different countries (China, USA, UK and Spain).

2. Materials and methods

2.1. Sample preparation

Spanish infant rice, infant cereals, pureed infant foods (with chicken or hake) and special infant foods were purchased (in triplicate) in pharmacies and national supermarket chains in the city of Alicante (Spain) during 2011 and were analysed for t-As and i-As contents. The products under analysis were:

- 22 infant cereals samples (to be prepared using infant formula) and from 7 commercial brands:
  - 13 gluten-free samples (rice and maize),
  - 9 samples of mixed cereals with gluten (wheat, barley, oat, rye, sorghum and millet); and,
- 19 samples of pureed infant foods:
  - 11 meat samples (all chicken),
  - 8 fish samples (5 hake, 1 bass and 2 sole); and,
- 5 samples of special products: 2 samples of anti-diarrhoea rice mixtures, 1 sample of lactose-free cereals mixture, and 2 rice-based desserts.

All infant rice/cereals products were in powdered form while pureed infant foods were a mixture of cooking water, rice and other ingredients, mainly vegetables. From the labelling of the Spanish products, it can be ascertained that foods were made either in Spain or the European Union, but no specific information on the source of the rice grain or other cereals was presented on the packaging.

Pure infant rice samples were imported in 2011 from Chinese and USA health shops. Samples from UK and Spain were obtained in 2011 from national supermarket chains in the cities of Aberdeen (Scotland) and Alicante (Spain), respectively. There were 14 samples from 11 Chinese manufacturers, 5 samples from 4 US manufacturers, 5 samples from 5 UK manufacturers and 7 samples from 7 Spanish manufacturers. Six of the Chinese samples had sugar added ranging from 5 to 10%; these samples were considered as pure infant rice because no other cereal was used in their formulation.

Products were dried in an oven (Gallenkamp, Loughborough, UK) at 80 °C until constant weights were reached; 24 h was required for infant rice and cereals, while 78 h was needed for infant foods. The solid samples were homogenized by grinding in a ball mill and stored in a desiccator in the dark until analysis.

2.2. Total arsenic

Samples were processed as described in a published methodology (Williams et al., 2007b). Trace element grade reagents were exclusively used in all analysis. Briefly, approximately 0.2 g dried weight (dw) of infant products were weighed into 50 mL polypropylene digest tubes (Corning, NY), and 2 mL of concentrated nitric acid (HNO_3) were added. The mixture was left to imbibe overnight and then 2 mL of hydrogen peroxide (H_2O_2) were added and then samples were digested in a microwave oven (CEM Mars 5, CEM Corp., Matthews, NC). The temperature was raised, first to 55 °C, and held for 10 min, and then at 75 °C, and held for 10 min. Finally, the temperature was taken up to 95 °C and maintained for 30 min. Samples were cooled to room temperature and then the sample was diluted up to 50 g with ultrapure deionized water obtained from a Milli-Q system (Millipore, Billerica, MA).

In each analytical batch, at least one reagent blank, one spike (50 μL of 10 mg As L^{-1}) and one internationally certified reference material (CRM) were included to assess precision and accuracy for chemical analysis. The CRM, rice flour NIST SRM 1568a was used.

A quadrupole ICP-MS 7500 (Agilent Technologies, Santa Clara, CA, USA) was used to determine t-As concentration as described elsewhere (Williams et al., 2007b). A collision cell was used to negate argon chloride (ArCl) interferences. Samples were randomized prior to analysis. Standards were run after every 40 samples. Concentrations were determined using five-point calibrations (from 0.1 to 10.0 μg L^{-1}) calculated from a multi-element standard (Claritas PPT®). Rhodium (10 μg L^{-1}) was added online post-column as the internal standard.

Mean As recovery from the rice CRM was 289 ± 37 μg kg^{-1} (all data are expressed as mean ± standard error) compared to a certified value of 290 ± 30 μg kg^{-1}. Spike recovery was 97 ± 3%.

2.3. Arsenic speciation

Speciation extraction procedures followed those of (Zhu et al., 2008a,b). Around 0.2 g of rice-based infant products was accurately weighed out into a 50 mL polypropylene digest tube and 10 mL of 1% HNO_3 were added to steep overnight. Then samples were extracted in a microwave oven using the conditions previously described for t-As. Samples were cooled to room temperature and approximately 2 mL samples were centrifuged at 12,000 g for 15 min. The supernatant was filtered through 0.45 μm filter (Millipore) and 900 μL were mixed with 100 μL of H_2O_2 and left overnight at 4 °C. Then samples were kept at 4 °C until analysis (<1 day of cold storage). Quality controls of CRM and blanks were run with each extract batch.

Arsenic speciation was quantified by HPLC–ICP-MS using HPLC (HP1100, Agilent Technologies) coupled to the ICP-MS as described elsewhere (Sun et al., 2008a,b). Chromatographic separation consisted of a precolumn (11.2 mm, 12–20 μm) (Hamilton, Reno, NV, USA) and a PRP-X100 10-μm anion-exchange column (150 × 4.1 mm) (Hamilton). The mobile phase consisted of 6.66 mM ammonium hydrophosphate ($NH_4H_2PO_4$) and 6.66 mM ammonium nitrate (NH_4NO_3), adjusted to pH 6.2 using ammonia. Rhodium (10 μg L^{-1}) was added online post-column as the internal standard. Retention time for the As species was determined using a species mix comprising standards of 50 μg L^{-1} arsenite, arsenate, DMA, MMA and arsenobetaine. DMA standards (0.5–25.0 μg L^{-1}) were used to calibrate the instrument.

Recovery of t-As (sum of all As species) in NIST rice flour CRM was 277 ± 4 μg kg^{-1} compared to a certified value of 290 ± 30 μg kg^{-1}. No rice CRM certified speciation is available; however results, (i-As 95 ± 5 μg kg^{-1} and o-As 182 ± 3 μg kg^{-1}) were in good agreement with those previously reported by Raab et al. (2009) i-As 99 μg kg^{-1} and o-As 185 μg kg^{-1}.

3. Results and discussion

3.1. Arsenic and arsenic speciation in Spanish infant products

t-As contents in Spanish infant products ranged from 13 ± 2 to 619 ± 278 μg kg^{-1} fresh weight (fw) in meat- and fish-based infant foods, respectively (Table 1). However, these data could be misleading because of the high contents of cationic As species, such as arsenobetaine, in the fish products (Sharma and Sohn, 2009). In this particular study, cationic As accounted for 94.8% of the t-As present in the fish infant foods. In general, i-As ranged from 7 ± 1 to 69 ± 8 μg kg^{-1} in fish-based infant foods and gluten-free infant rice, respectively, with an overall mean of 29 ± 5 μg kg^{-1}.

Gluten-free infant rice (13 products) was investigated: 7 samples had rice content above 80%, 1 had a rice content of ≈60% and 5 samples had contents below 45%. The first 7 samples can be considered as pure infant rice because no other cereal was used while in the last 6 samples other gluten-free cereals, such as maize and tapioca, were also used. Gluten-free rice samples had the second highest t-As content (126 ± 26 μg kg^{-1}), after the food that included fish, but the highest content of i-As (69 ± 8 μg kg^{-1}).

Both the t-As and i-As contents significantly decreased in the next two groups of samples under analysis, 9 samples of infant cereals with gluten (wheat, oat, barley, rye, and sorghum) and 11 samples meat-based infant foods, all from chicken. The t-As contents were 33 ± 6 and 13 ± 2 μg kg^{-1}, while the contents of i-As were 26 ± 5 and 8 ± 1 μg kg^{-1}, respectively.

Table 1
Total arsenic and arsenic species concentrations (μg kg^{-1} fresh weight) in samples of Spanish commercial infant rice and infant food.

Sample	*n*		t-As (μg kg^{-1})	∑ As species (μg kg^{-1})	i-As (μg kg^{-1})	DMA (μg kg^{-1})	i-As (%)	DMA (%)
Gluten-free Infant Rice	13	Mean	126 ± 26	127 ± 26	69 ± 8	56 ± 18	64 ± 5	35 ± 4
		max.	315	324	121	198	89	62
		min.	46	43	29	10	36	11
Infant cereals (with gluten)	9	Mean	33 ± 6	27 ± 6	26 ± 5	nd	98 ± 1	nd
		max.	65	55	49	5	100	10
		min.	15	10	10	nd	90	nd
Infant Food (meat)	11	Mean	13 ± 2	12 ± 2	8 ± 1	nd	74 ± 5	26 ± 5
		max.	26	24	11	14	91	58
		min.	9	8	7	nd	42	9
Infant Food (fish)	8	Mean	619 ± 278	552 ± 239	7 ± 1	5 ± 1	nd	nd
		max.	2310	2009	11	11	5	nd
		min.	159	151	nd	nd	nd	nd
Others	5	Mean	13 ± 4	14 ± 5	12 ± 4	2 ± 1	88 ± 6	13 ± 6
		max.	29	32	27	5	100	29
		min.	6	5	nd	nd	71	nd
LOQ (μg kg^{-1})			6	5	5	5		
CRM (%)			92.4	97.5	95.9	na		

nd = below LOQ [limit of quantification determined as three times the standard deviation of the blanks, limit of detection (LOD), multiplied by the proper dilution factor]; na = not available.

The group of fish-based infant foods consisted of 8 samples of infant foods with fish content ranging from 10 to 20%. This group had the highest t-As content (619 ± 278 μg kg^{-1}) but the lowest i-As content (7 ± 1 μg kg^{-1}). Most of the t-As present in fish is under non-toxic organic species, o-As, with arsenobetaine being the major form of As in marine fish and most other seafood (*9, 29*). Samples containing hake and bass had similar t-As contents, 194 and 276 μg kg^{-1}, respectively; however, samples from sole had significantly higher contents (1583 μg kg^{-1}).

The final group consisted of 5 samples of special foods including mixtures of cereals specially prepared to control diarrhoea problems, lactose-free rice mixture, and rice desserts. These samples had one of the lowest t-As content (13 ± 41 μg kg^{-1}) but an important percentage being i-As, 12 ± 4 μg kg^{-1} (88%).

i-As and DMA accounted for more than 90% of the t-As present in all rice-based products under study, with i-As and DMA representing 65.0 and 18.1% of the t-As, respectively. With the exception of the fish infant foods, the percentage of i-As in all other infant products was above 60% with a mean value of 78.1% and reaching almost 100% in infant cereals with gluten (mean of 98%). In several recent studies rice and rice-based infant foods often contains elevated concentrations of As, most of which is in the most toxic inorganic form (Meharg et al., 2008a,b; Signes-Pastor et al., 2009; Sun et al., 2009).

Fig. 1A shows a positive correlation ($R^2 = 0.8504$) between the i-As content and the rice percentage, with i-As increasing as the rice percentage increases. From the data represented in Fig. 1B is possible to classify the products under study in different groups. The first group includes gluten-free infant rice, infant cereals with gluten, chicken-based infant foods and other infant products and it was characterised by a linear relationship ($R^2 = 0.8840$) between t-As and rice percentage. Finally, there were three groups of samples clearly out of this linear relationship. The first one consisted of hake- and bass-based infant foods, which are characterized by high t-As (most of it presumably coming from the hake and being under cationic forms) and low rice percentage. Similarly, the second group consisted of sole-based products and were characterised by extremely high t-As contents and very low rice percentage. The third set consisted of three samples of pure infant rice with very high t-As contents; each one of these products is commercialized by a different company, meaning that there are 3 companies using rice with very elevated t-As contents.

With the exception of the fish-based samples, there was a initial linear correlation between t-As and i-As, with i-As contents

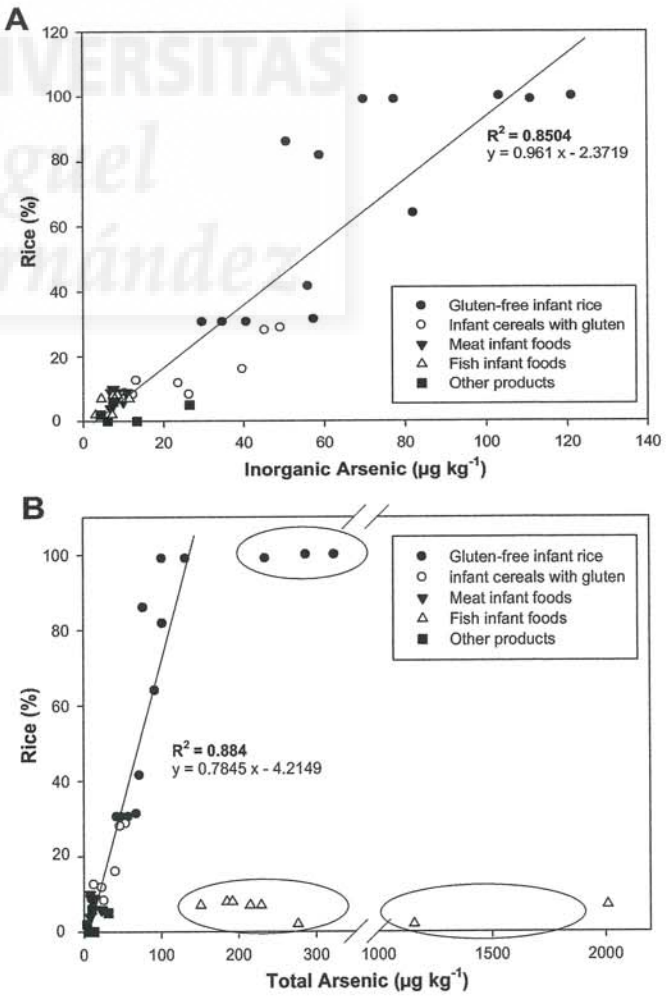

Fig. 1. Relationship between inorganic (A) or total arsenic (B) contents and rice percentage in samples of Spanish infant rice and infant food.

increasing linearly up to ~150 μg kg^{-1} of t-As and then plateauing at an i-As content of ~110 μg kg^{-1} (Fig. 2).

3.2. Daily arsenic intake from Spanish infant products

The toxicology of As is independent of source once it crosses the gut membrane. All indications are that the bioavailability of i-As from rice is high, in the order of 90% (Ackerman et al., 2005; Juhasz et al., 2006). Therefore, the i-As from rice-based foodstuffs will be as toxic to infants as those coming from other sources, such as drinking water.

Food standards are out of step with drinking water regulations. EU and US drinking regulations are set at 10 μg L^{-1} for t-As and i-As, respectively, and both assume a daily consumption of 1 L of drinking water (Council of the European Union, 1998; NRC, 2001). These values equate to a predicted maximum daily As intake of 0.17 μg d^{-1} kg^{-1} for a conservative body mass of 60 kg.

According to the World Health Organization (2009) after 6 months of age, it becomes increasingly difficult to breastfeed infants to meet their nutrient needs from human milk alone. Thus, 6 months is the recommended appropriate age at which to introduce complementary foods. However and despite these guidelines, weaning occurs at an early age in most countries (Synnot et al., 2007); for example, Santamaria-Orleans et al. (2005) reported that Spanish infants started with weaning foods at 4.4 months of life. Briefel et al. (2004) reported that in the period from 4 to 6 months US infants are introduced to infant cereals and pureed infant foods (mostly fruits and vegetables), with a mean daily consumption of 187 g. During the period of 6–8 months, the consumption of infant cereals reached its highest values.

In this article, the daily As intake will be discussed, according to the infant nutrition (main types of foods being consumed), at four different ages: 4, 6, 8 and 12 months. The daily food intake for a infant at these four periods were: (i) 4 months: 4 feeds of breast milk [140 mL $feed^{-1}$ (Sievers et al., 2002)], 20 g of gluten-free infant rice [mean body weight, for girls and boys, of 6.65 kg according to Generalitat Valenciana (2011)]; (ii) 6 months: 3 feeds of breast milk, 20 g of infant rice and 50 g of meat-based infant food (mean body weight of 7.55 kg); (iii) 8 months: 2 feeds of breast milk, 30 g of gluten-free infant rice or infant cereals with gluten, 75 g of meat-based infant food and 75 g of fish-based infant food (mean body weight of 8.30 kg); and (iv) 12 months: 2 feeds of breast milk, 30 g of infant rice or infant cereals, 100 g of meat-based infant food and 100 g of fish-based infant food (mean body weight of 9.30 kg). It has been assumed that infants are taking only breast milk (ideal scenario), the water used to reconstitute infant rice/cereals is arsenic-free (which is the normal situation in most of the European Union countries) and body weights considered were those of the 50th percentile.

Considering all the above information on daily food intake, body weight and results on i-As contents in the different infant foods under study, the mean intake for a 4, 6, 8 or 12 month infant, with no food related illnesses and, therefore, being able to eat cereals with gluten, are 0.05, 0.16, 0.25 and 0.26 μg d^{-1} kg^{-1}, respectively. However, if the infants suffer from the celiac disease and must consume only gluten-free products (rice), the daily intakes are significantly increased up to 0.26, 0.27, 0.41 and 0.40 μg d^{-1} kg^{-1} for the same ages.

At mean exposure all infants above 4 months of age consuming gluten-free infant rice products are receiving more As in their diet than the maximum level, for an average weight adult, assumed under both EU and US laws for adults (0.17 μg d^{-1} kg^{-1}), if i-As consumption is expressed on a body weight basis (Fig. 3A). For infants consuming cereals with gluten, their daily intake of i-As was above the EU and US legislations after 8 months of age. Data showed that the daily intake of i-As (μg kg^{-1} d^{-1}) increased from month 4 to 8 and was constant up to 12 months. The energy needed for the

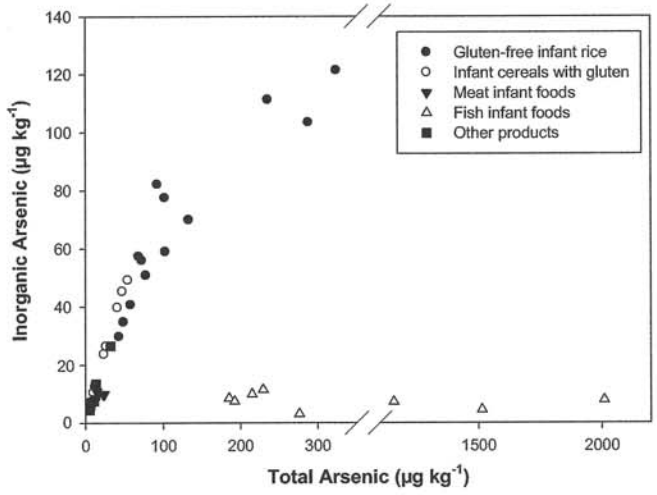

Fig. 2. Relationship between inorganic arsenic and total arsenic in samples of Spanish infant rice and infant food.

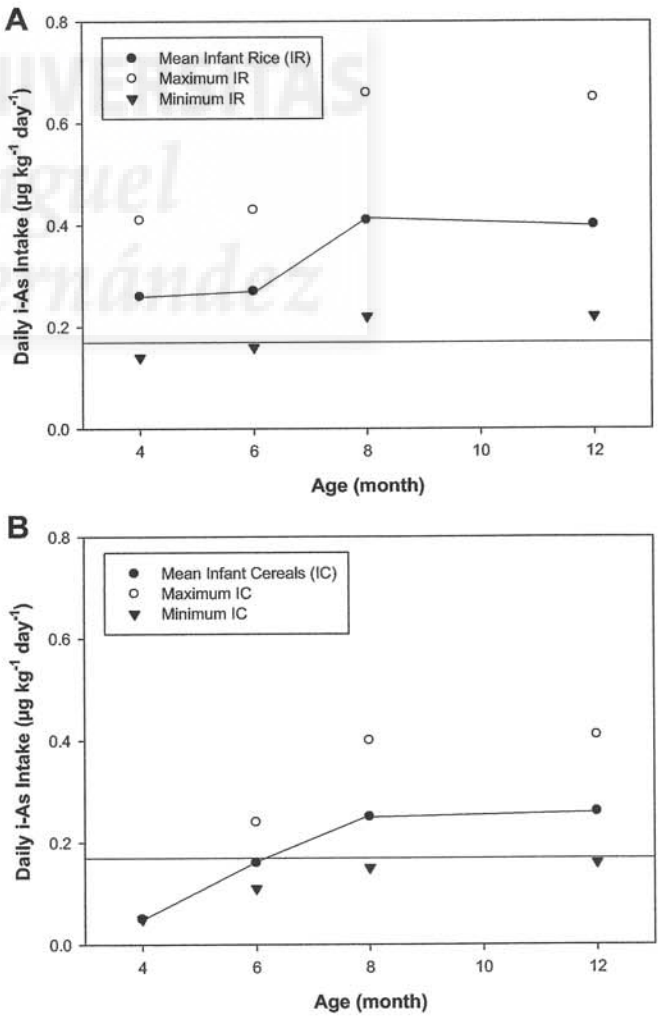

Fig. 3. Daily intake of i-As (μg day^{-1}) for Spanish infants (4, 6, 8 and 12-months old) consuming either gluten-free infant rice (A) or infant cereals with gluten (B).

period 12–23 months will be the same, about 550 kcal d^{-1}, with the food intake taking the similar values but at the same time the body weight will increase from 9.3 to 12 kg. Consequently, the daily intake of i-As (μg d^{-1} kg^{-1}) will decrease from 0.40 down to 0.31. It can be stated that infants have the highest daily intake of i-As during the period from the 8 to 12 months (Fig. 3A).

All calculations were based on the assumption that only one portion of infant rice, one portion of meat-based infant food and one portion of fish-based infant food are consumed per day. In most cases, only one portion is given at the beginning of the period but the amount is increased to 2 servings at the end of the period. Besides, infant foods are presented in jars of 200–250 g, which is somehow promoting parents to use the content in one or two servings. If additional servings are given, i-As intake must be simply multiplied by the number of servings.

The CONTAM Panel of EFSA (2009) modelled the dose–response data from key epidemiologic studies and selected a benchmark response of 1% extra risk. The lowest $BMDL_{01}$ values were for lung cancer and this EFSA Panel concluded that the overall range of $BMDL_{01}$ values of 0.3–8.0 μg kg^{-1} bw per day should be used instead of a single reference point in the risk characterization of i-As. The estimated dietary exposures to i-As of Spanish children were close or higher than those of adults but this does not necessarily indicate that children are at greater risk because the exposure estimates were within the range of $BMDL_{01}$ values and in general effects are due to long term exposures (EFSA, 2009), although infants seem to be very susceptible to toxic effects of i-As (Rahman et al., 2009).

Finally, it must be remembered that it is not just infant cereals/rice (with or without gluten) and rice-based infant foods (based on meat or fish) that are of concern. A wide range of other rice products are fed to infants, such as formula, crackers, biscuits, crisped and puffed rice cereals, pasta, noodles, puddings, plain polished and whole grain rice, etc. are made primarily with, or are formulated with, rice (Meharg et al., 2008a,b) and these have shown to be high in i-As. The WHO (2009) recommends that the infant diet can be complemented with 1–2 snacks per day. Finally, if water containing i-As is used to reconstitute infant rice/cereals it could be an important factor; although high levels of i-As in drinking water is not the normal scenario in countries from the European Union, including Spain.

3.3. *Arsenic and arsenic speciation in pure infant rice from different countries*

i-As and DMA are the dominant As species in rice grain (Williams et al., 2006, 2007a). Similarly i-As and DMA represented 66 ± 3% and 33 ± 3% (means of all products under study) of the t-As present in all infant rice samples studied (Table 2). Therefore, pure infant rice reflected grain As speciation. DMA percentages were higher in samples from USA and Spain, while i-As percentages were higher in the Chinese and UK samples.

The contents of t-As followed the order USA ≈ UK > Spain > China, however the sequence for i-As contents was UK > USA ≥ China > Spain. These differences could be due to different rice sourcing (differences in rice cultivars, farming conditions, processing conditions). In general, the highest values of both t-As and i-As were observed in samples from organic brown rice, with the number of brown rice samples in a particular group/country significantly affecting the reported sequences.

Results for i-As from the present study (Table 2) ranged from 10 to 267 μg kg^{-1}, with a mean of 117 ± 9 μg kg^{-1}, and were comparable to those previously reported by Meharg et al. (2008b) for pure infant rice samples from the UK. These authors studied 17 samples from 3 British manufacturers obtained at different supermarkets in the city of Aberdeen and reported i-As levels ranging from 60 to 160, with a median of 110 μg kg^{-1}. However, Ljung et al. (2011) reported lower values of t-As (30 μg kg^{-1}) in samples of pure infant rice from Sweden.

Three Chinese samples out of 14, 2 US samples out of 5 and 2 UK samples out of 5 were above the maximum threshold for i-As content established in the Chinese legislation, 150 μg i-As kg^{-1} (USDA, 2006), while all samples from Spain were below this maximum level. It is important to remark that most of the samples with i-As contents above the Chinese threshold were organically cultivated (6 out of 7) and were marketed as brown rice (4 out of 7), with high As content coming from rice bran (Sun et al., 2008a,b). In summary 77% of the samples under study (total number of samples 31) could be legally marketed in China and elsewhere, while Meharg et al. (2008b) found that only 65% of their baby rice ($n = 17$) was below this threshold. Several are the factors that could be behind these differences: the rice cultivar (Norton et al., 2009a,b), the geographical origin (Meharg et al., 2009) and the type and conditions of rice processing (Signes et al., 2008); however, no clear indication of the rice cultivar or geographical origin of the rice used was included in the labelling of the products. It is important that information on rice cultivar and geographical origin is included in the labelling of all rice-based infant foods. Also, it is 3 years since Meharg et al. (Meharg et al., 2008a,b) published their findings and infant food manufacturers are aware of this issue and have taken action to counteract high i-As content, especially in the light of the EFSA (2009) which again re-emphasised concerns regarding infant rice. Food manufacturers are starting to request As analyses to rice suppliers.

To assess the variability of t-As and i-As contents in samples from the same manufacturer samples of pure infant rice from two manufacturers were obtained at different locations, with samples belonging to different batches. The results showed significant differences for both manufacturers. The values for the t-As contents for manufacturers A and B were 286, 301, 327 and 353 μg kg^{-1}

Table 2
Total arsenic and arsenic species concentrations (μg kg^{-1} fresh weight) in samples of pure infant rice from China, USA, UK and Spain.

Country	*N*		t-As (μg kg^{-1})	∑ As species (μg kg^{-1})	i-As (μg kg^{-1})	DMA (μg kg^{-1})	i-As (%)	DMA (%)
China	14	Mean	135 ± 19	148 ± 16	114 ± 15	33 ± 3	76 ± 2	23 ± 2
		max.	353	290	247	63	88	35
		min.	70	73	52	21	62	12
USA	5	Mean	253 ± 62	260 ± 66	125 ± 14	127 ± 54	55 ± 8	43 ± 8
		max.	496	515	159	334	81	65
		min.	164	134	93	35	31	18
UK	5	Mean	237 ± 49	248 ± 52	162 ± 29	83 ± 46	71 ± 9	29 ± 9
		max.	394	415	267	265	84	64
		min.	137	135	107	28	34	15
Spain	7	Mean	181 ± 36	181 ± 38	85 ± 10	93 ± 28	53 ± 5	45 ± 5
		max.	315	288	111	178	75	62
		min.	36	38	10	25	5	5

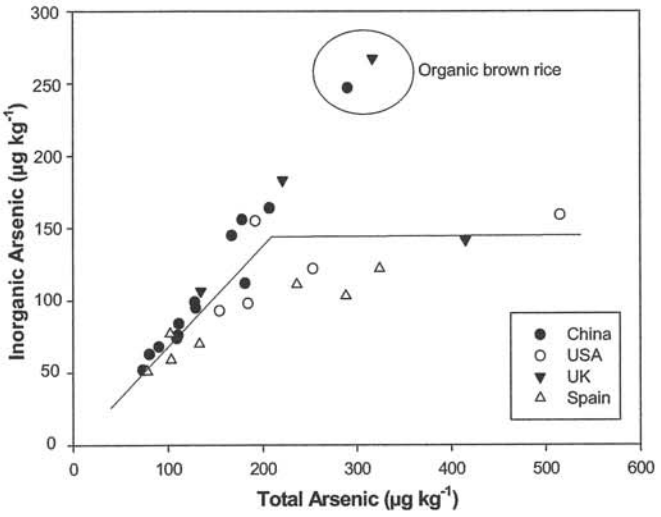

Fig. 4. Relationship between inorganic or total arsenic contents in samples of pure infant rice from China, USA, UK and Spain.

(mean of 317 μg kg^{-1}) and 381, 385 and 479 μg kg^{-1} (mean of 415 μg kg^{-1}), respectively. This variability could be related, among other factors, to different rice batches and/or suppliers, which could result in different rice cultivar and/or different geographical origin.

In general, there was a linear correlation between t-As and i-As in pure infant rice from different geographical origins, with i-As contents increasing linearly up to ~200 μg kg^{-1} of t-As and then plateauing at an i-As content of ~150 μg kg^{-1} (Fig. 4); only two samples (one from China and one from UK) of organic brown rice showed significantly higher i-As contents than this maximum. This pattern was similar to the one previously described in this manuscript for Spanish infant products (Fig. 2) and to that reported by Meharg et al. (2008b) for UK pure baby rice.

4. Conclusions

The contents of i-As was higher in Spanish gluten-free products based on rice than in similar products prepared using mixtures of other cereals with gluten (wheat, barley, oat, etc.), with mean contents being 69 and 26 μg kg^{-1}, respectively. The contents of i-As were much lower in pureed infant foods compared to rice and/or cereals products, however, the content of t-As in fish foods was significantly higher than in any other food but due to the elevated content (~95%) of non-toxic cationic compounds, mainly arsenobetaine. The maximum daily intake of i-As was estimated in the period from 8 to 12 months of age, with 0.41 and 0.26 μg d^{-1} kg^{-1} for infants consuming products without or with gluten, respectively. Pure infant rice samples from Spain presented lower i-As content compared to samples from other countries, China, USA and UK. The products with the highest contents of both t-As and i-As were those manufactured using organic brown rice, which has nowadays a huge demand among consumers caring for natural and/or ecological products. The finding of elevated contents of i-As in infant products and consequently elevated intakes of i-As in infants older than 4 months is of concern and deserves further attention. Infants with celiac disease, who are forced to consume gluten-free products, with high percentages of rice, are at serious risk due to the most elevated intakes of i-As. There is a fundamental need to reduce the i-As of rice used in baby products as i-As is a class one, non-threshold and carcinogen. Therefore, the main conclusion of this study is that there are groups of infants, e.g. with celiac disease, which are highly exposed to toxic i-As but the good news is that solutions can be found, at least theoretically; some potential options that deserve further investigation are: (i) screening of As levels in existing rice to identify varieties that have low i-As contents; (ii) breeding rice to get rice cultivars with restricted As uptake and upward transport to the edible grain; (iii) modifying the current anaerobic growing practices (flooding of fields) for rice, moving towards more aerobic conditions, which will reduce As availability to rice plants; (iv) reducing the rice content of baby foods; and/or (v) sourcing rice from low arsenic regions.

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Chapter 7

OCCURRENCE OF INORGANIC ARSENIC IN RICE-BASED INFANT FOODS: SOIL-RICE-INFANT RELATIONSHIPS

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ABSTRACT

About 100 million rural people in Asia (mainly Bangladesh and India) are exposed to arsenic-polluted drinking water and agricultural products in what it is considered the biggest mass poisoning case in the human history. This problem that seemed so distant from the European countries is perhaps closer than expected due to the high consumption of rice-based foods by babies and infants. It is well demonstrated that elevated contents of inorganic arsenic (iAs) are found in infant products, such as rice milk and baby rice, being marketed in European countries. Workable solutions to limit arsenic in paddy rice by breeding rice cultivars with low arsenic accumulation are being sought but still are not fully implemented. Meanwhile, simple recommendations for processing and cooking rice as preliminary unit operations in the manufacturing of infant foods will significantly help in reducing arsenic exposure in European infants. For instance, avoiding the use of organic brown rice (high concentrations of arsenic are found in rice bran) and cooking rice using high volumes of arsenic-free water may be easy and cheap ways of reducing arsenic exposure in infants.

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INTRODUCTION

The word arsenic (As) has made its way through history on the toxic properties of a number of its compounds and the strength of its killing properties [1]. Fortunately there are great differences in the toxicity of different compounds, and the species that are most commonly found in soils, sediments, plants, and edible vegetal products are not the most toxic. The inorganic arsenic forms (iAs) are more toxic, both acute and chronic, than the organic ones (oAs). Furthermore, iAs is non-threshold, class 1 carcinogen; this classification was based on the induction of primary skin cancer, as well as the induction of lung and urinary bladder cancer. Consequently, it is very important to produce As speciation data and not only data on total As (tAs) in foods, including infant products. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a Provisional Tolerable Weekly Intake (PTWI) for iAs of 0.015 mg/kg of bodyweight/week in 1988. EFSA has recently concluded that this PTWI is no longer appropriate as data had shown that iAs causes cancer of the lung and urinary bladder in addition to skin, and that a range of adverse health effects had been reported at exposures lower than those reviewed by the JECFA [2].

A proposed model of a local soil arsenic system is shown in Figure 1. In this cycle arsenic pesticides and soil parental materials are the main inputs [3]. The most important translocation factors are adsorption by soil (1), precipitation as insoluble materials (2), oxidation/reduction reactions (3), leaching or runoff (4), volatilization (5) after biomethylation (6), and uptake by vegetation and incorporation into the food chain (7). The most obvious pathway linking contaminated soils and man is food chain (Figure 2), but potable surface and ground waters, respired and ingested dust and tobacco fumes may also play significant roles. Food chain relationships are critical as contaminants tend to become more concentrated as they move up the food-chain (e.g. soil $\Rightarrow$ plant $\Rightarrow$ herbivore $\Rightarrow$ carnivore). The most likely dietary sources of arsenic, with the exception of fish and shellfish [4,5] are crops and vegetables grown in contaminated soil [6-8]. Some additional arsenic may be found in milk and meat products derived from animals grazed on contaminated land [9]. However, the peculiarity of rice farming, flooding soils for periods of time long enough to drastically reduce their redox potential will make a huge difference compared to other cereals [10]. In anaerobic paddy rice soil systems arsenic is more mobile than in aerobic wheat soils. Transfer of arsenic from soil to grain was one order of magnitude greater in rice than in wheat or barley mainly due to high shoot/soil ratios for rice, ~0.8, compared to 0.2 and 0.1 for barley and wheat, respectively [10].

The adsorption and retention of arsenic by soils control its persistence, activity, movement, transformation, and ecological effects. Arsenic adsorption is related to the pH, chemical and physical properties (e.g. amount of sesquioxides, clay, and exchangeable Ca^{2+} and Mg^{2+}), and cation exchange capacity of soils, and to the amount of arsenic in the soils. The influence of redox on arsenic solubility in soils was found [11-14] to be governed by (i) the dissolution of Fe-oxyhydroxides and concurrent release of coprecipitated arsenate, and (ii) reduction of arsenate to arsenite.

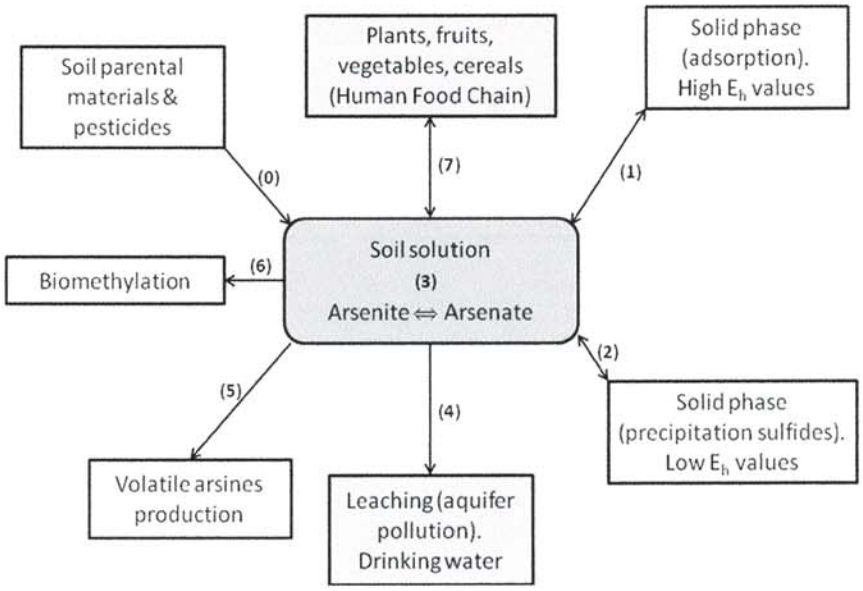

Figure 1. Arsenic cycle in the soil system.

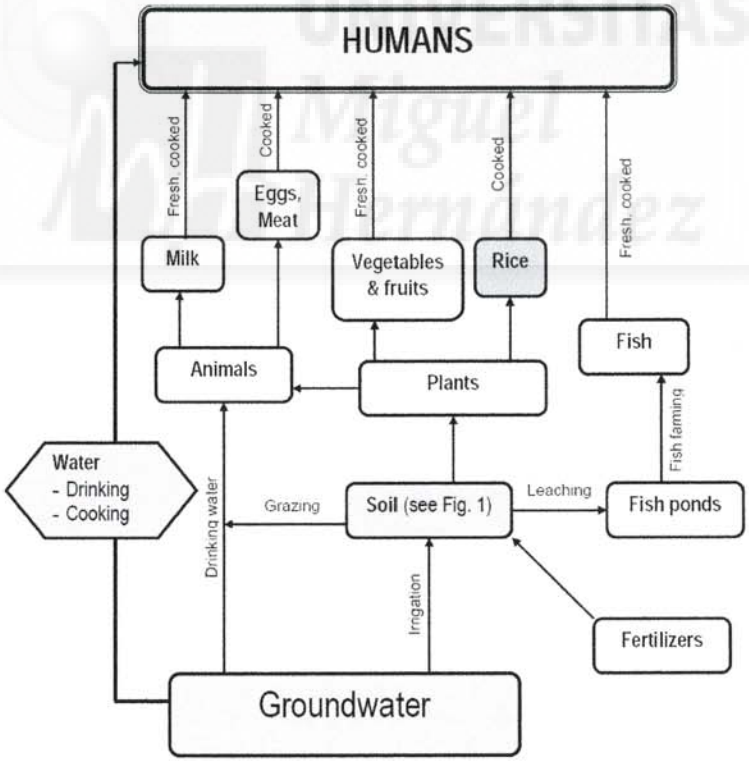

Figure 2. Possible pathways of arsenic ingestion in rural populations of India and Bangladesh [8].

In soils of rice-producing areas of the USA elevated arsenic concentrations are most commonly associated with MAA, a herbicide widely used for post-emergence weed control in cotton [15,16] as a direct spray. Methylarsonic acid residues can cause severe damage to succeeding rotational crops, such as rice as was the case in fields of Louisiana [15,16]. On the other hand, in Asia (Bangladesh and India) most of the arsenic found in rice grain is present under inorganic species because the main source of arsenic is the groundwater. In world-wide rice-producing areas, arsenic is considered the probable cause for straighthead, a physiological disease of flooded rice that results in blank florets, distorted palea and lemma and, in extreme cases, failure of panicles to form [15-17]. The affected panicles are erect rather than bent and have few filled florets [18]. Consequently, there is a clear relationship between rice and arsenic; relationship that is exclusive of rice and does not apply to other cereal, such as wheat or corn.

WHY THIS ASSOCIATION BETWEEN RICE AND ARSENIC?

Arsenicals were widely used in agriculture as pesticides or plant defoliants for many years. Now the use of both inorganic and organic arsenicals is legally forbidden or it is reduced as much as possible. However, a legacy of contaminated orchard soils has been left behind due to the extensive use of inorganic forms in the past; this is of great importance because residues from the application of these compounds can produce phytotoxic effects long after application has ceased. Besides, it is also important to highlight that the most popular fertilizers N-P-K will incorporate trace amounts of arsenic to agricultural soils as contaminants due to the high chemical similarity between phosphorus and arsenic.

Soils have been referred as heavy metals and trace elements "sinks"; however, what they really do is to convert a short-term problem, through adsorption onto clays and Fe and Mn oxides or precipitation of insoluble sulfides under oxidizing or reducing redox conditions, respectively, in a long-term risk. The biogeochemical conditions of soils containing trace amounts of arsenic could someday change and the most toxic arsenic species could become more available. Therefore, precaution must always be taken when working with arsenic-polluted-soils even if the sink mechanism of soils and sediments seems to be solving current pollution hazards.

This precaution must be even higher in the case of paddy rice fields because of their very specific farming conditions; flooding the soils will almost simultaneously solubilize ferric iron oxides, which are the main compounds responsible for the arsenic adsorption to soils, and will reduce arsenate [As(V)] to the most toxic species arsenite [As(III)]. As mentioned previously, the reducing conditions under which rice is grown will induce the reduction and subsequent dissolution of the ferric iron oxides and hydroxides to soluble ferrous iron. Later pentavalent arsenate will be reduced to the more mobile and toxic trivalent arsenite (Figure 3). An anomalously high soil concentration due to contamination generally implies greater availability to plants. But, the bioavailability of arsenic is controlled by a large number of factors, including the source and form of the element, soil pH, redox and drainage conditions as well as the type and amount of organic matter present [19].

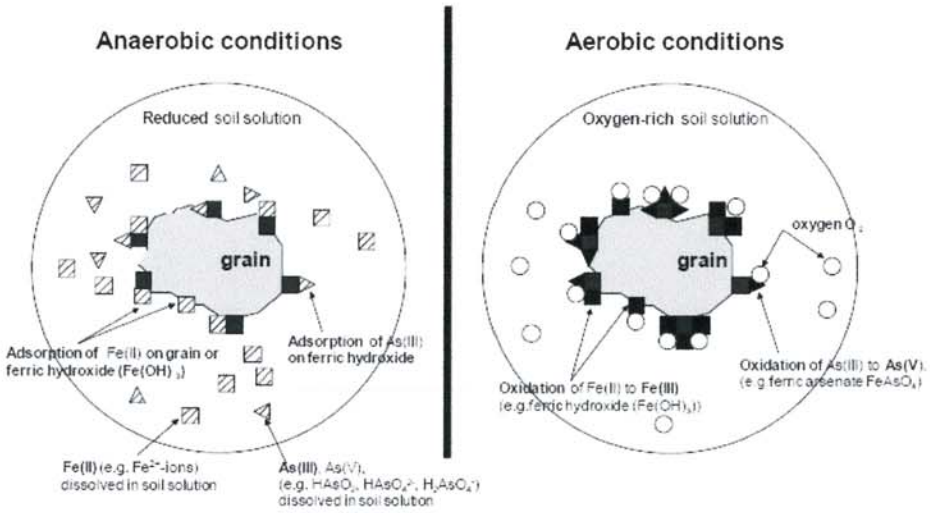

Figure 3. Effect of soil redox potential on the geochemistry of arsenic on soil particles.

PARTICULARITIES OF RICE PLANTS

In general the highest residues of arsenic are found in roots, with intermediate contents being found in green-vegetables, with edible seeds and fruits containing the lowest levels of arsenic. In this way, arsenic contents tend to be restricted during the transport within the plant system. Consequently, arsenic contents in cereals, including rice, are expected to be low. Williams et al. [10] demonstrated that this statement is not true for rice. These authors reported that in rice the median shoot/soil transfer factors were nearly 50 times higher than those in wheat and barley. However, median grain/shoot transfer factors for wheat and barley were 4 times higher than those of rice. Additionally, several authors have clearly demonstrated that methylated oAs are rapidly moved from the roots into shoot tissues in different plants, including turnip [20], tomato [6] and beans [21]. The differences in these transfer factors among rice, wheat and barley are probably due to differences in arsenic speciation and dynamics in anaerobic rice soils compared to aerobic soils fro barley and wheat. In summary, transfer of arsenic from soil to grain was an order of magnitude greater in rice than in wheat and barley, despite lower rates of shoot-to-grain transfer in rice.

ARSENIC POLLUTION — A GLOBAL ISSUE

Contamination of groundwater with arsenic and the impact of this contamination on humans have been reported in 23 countries, but the magnitude of this problem is especially severe in Bangladesh and India [7]. As a consequence of long periods of exposure to high levels of arsenic intake from drinking water and cooked rice, people suffer from damage to the skin, kidneys, brain, heart, and circulation; bladder and lung cancers are the major killers [8]. About 100 million rural people in Asia are exposed to arsenic-polluted drinking water

and agricultural items in what is considered the worst mass poisoning event in the human history.

This problem that seemed until a couple of years very distant from people living in the European Union (EU) is now closer than expected. The main exposure route to iAs in the EU is dietary [2], and it is of particular importance for young infants because of their high food consumption per body mass. The national exposures to iAs from food and drinking water in the European Union have been estimated to range between 0.1 and 0.6 μg/kg body weight (bw) per day for average consumers [2]. The main food subclasses contributing to the iAs exposure were cereal grains and cereal based products, food for special dietary uses, bottled water, coffee and beer, rice grains and rice based products, fish and vegetables. That early-life exposure to iAs via baby rice may increase the risk for adverse health effects was first reported by Meharg et al. [22]. Their main conclusion was that when baby iAs intake from rice products was considered, median consumption (0.2 μg/kg/day) was higher than drinking water maximum exposures predicted for adults in these regions when water intake was expressed on a bodyweight basis. The need for legislation on As in baby food in order to prevent excessive As exposure in infants consuming rice-based products has been highlighted [23].

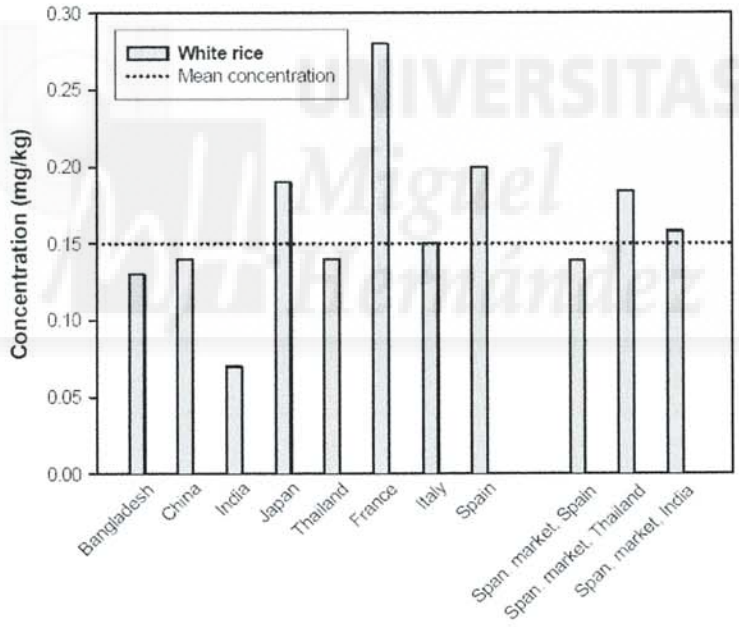

Figure 4. Total arsenic contents in white rice produced and marketed in different countries [24,52].

High As accumulation in rice occurs due to paddy cultivation, i.e. anaerobic conditions, where soil As is highly available for plant uptake [8]. European rice has high tAs and iAs contents as depicted in Figure 4, in which the highest contents were found in rice samples from France, Italy and Spain together with samples of Japan. Besides, Burló et al. [24] has shown that samples from different geographical origin are being marketed worldwide. As an example these authors have reported arsenic contents in rice samples from Spain, Thailand and India marketed in Spain.

Recent studies showed high iAs in rice products marketed worldwide. For instance, Signes-Pastor et al. [25] studied As speciation in Japanese rice drinks and condiments sold in the UK. These authors concluded that a) rice based products displayed higher iAs contents than those from barley and millet; b) most of the tAs in the rice products was iAs (63-83 %), and c) high consumers of Japanese products could be at serious risk.

EXPOSURE TO ARSENIC WITHIN THE EUROPEAN UNION

By modeling the dose-response data from key epidemiological studies and selecting a benchmark response of 1% extra risk, a range of benchmark dose lower confidence limit ($BMDL_{01}$) values between 0.3 and 8 μg/kg b.w. per day were identified for cancers of the lung, skin and bladder, as well as skin lesions [2].

The EFSA [2] identified the food subclasses of cereal grains and cereal based products to be the dominant pathway of exposure to "iAs" in the EU (Figure 5). It must be noted that within this food category, due to its high tAs amount, rice grain is one of the major contributors to the iAs forms (Figure 6). Thus, rice and rice-based products drastically contribute to iAs and are the dominant pathway of iAs exposure in the EU. Other contributors to iAs exposure are cereal and cereal products, food for special dietary uses, coffee and beer, fish and fish products, and other vegetables and vegetable products [2]. As rice-based products are often used in weaning foods for infants, exposure of infants to As is of great importance and its assessment is a priority.

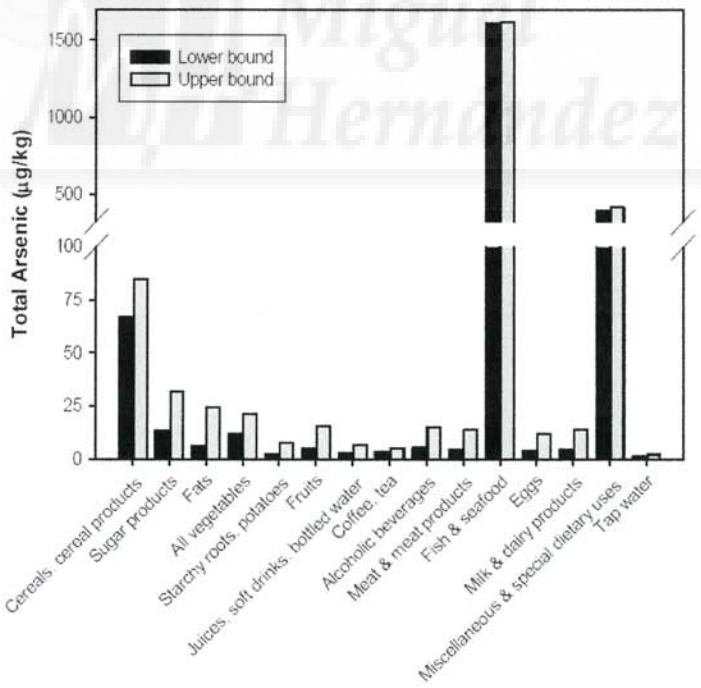

Figure 5. Total arsenic contents in the main food categories of European consumers [2].

Carbonell-Barrachina et al. [26] have estimated the intake of iAs in Spanish infants of 4, 6, 8 and 12 months of age with the celiac disease (force to consume mainly rice-based products) and control infants at mean values of 0.26, 0.27, 0.40 and 0.41 μg/kg/day and 0.05, 0.16, 0.25 and 0.26 μg/kg/day, respectively. These results clearly show that infants with celiac disease are at high risk regarding exposure to inorganic arsenic.

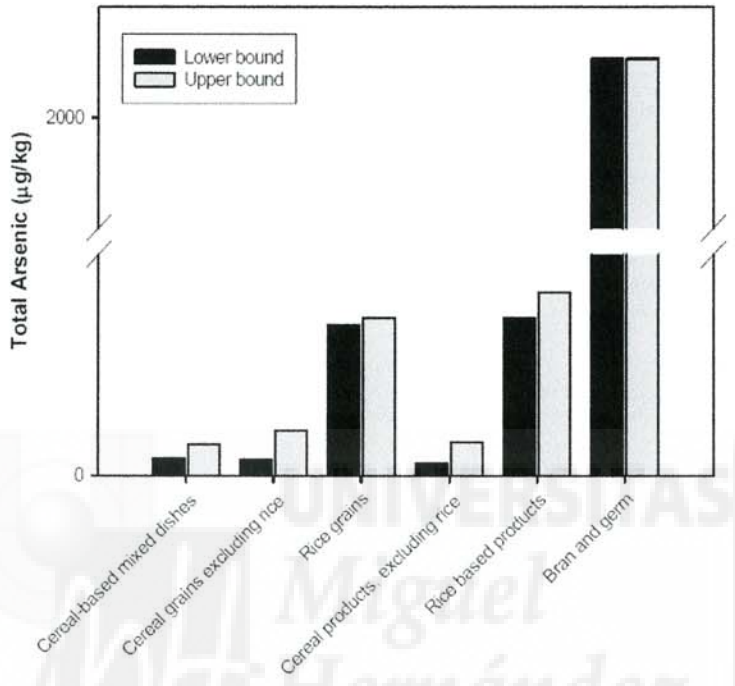

Figure 6. Total arsenic contents in products from the different subcategories within the food category "cereal and cereal products" [2].

ARSENIC IN RICE-BASED INFANT PRODUCTS MARKETED IN THE EUROPEAN UNION

Most children are weaned using, initially, pure rice porridge, a precooked, dried and milled product [22,27]; milled rice is a dominant carbohydrate source to weaning babies up to 1 year of age due to its blandness, material properties, low allergenic and nutritional value. As the child develops, this porridge is used for the basis of more complex meals, by mixing it initially (from 6 months) with puréed fruits or vegetables and later (from 8 months) with meat (mainly chicken) and fish (mainly hake), either home-made mixtures with baby rice or pre-prepared commercial products. Rice biscuits are used during teething. Many common food items eaten by infants are also rice-based such as cereals and biscuits [22,27]. This dependence on rice is exacerbated in infants with food intolerances. Rice milk is often used as an animal milk substitute for those with milk intolerance [27]; while rice based products are the staple for those with gluten intolerances [28]. Children from families that follow typical Southeast Asian rice based diets will have further enhanced exposures. Even though there is

no data for infants, the Bangladeshi community living in the UK has a *ca.* 30-fold higher rice consumption than white Caucasians; this trend seems to be quite realistic also for infants and will imply a very important exposition to rice and thus to iAs in this population [27].

Recent studies have proved that high iAs are also present in rice products commercialised in the EU, for instance Japanese rice drinks and condiments [25]. *Rice milk*, an alternative to cow milk for lactose intolerance sufferers, has been recently analysed in UK [28]. Results proved that all samples analysed in a supermarket survey would fail the EU limit (10 μg tAs L^{-1}), with up to 3 times this concentration recorded, while out of the subset that had As species determined, 80 % had iAs levels above 10 μg L^{-1}. Later Meharg et al. [22] and Carbonell-Barrachina et al. [26] studied the contents of iAs in *baby rice* and *baby cereals* from UK and Spain. The iAs contents were significantly higher in gluten-free rice than in cereals mixtures with gluten, placing infant with celiac disease at high risk. All rice-based products displayed a high iAs content, with values being >60% of the total As content and the remainder being dimethylarsinic acid (DMA). A clear positive correlation was found between the iAs content and the rice percentage in Spanish infant foods, with iAs increasing as the rice percentage increased (Figure 7). Finally, it is important to highlight that not just pure and supplemented infant rice are of concern, because a wide range of other rice based products that are fed to babies, such as crackers, biscuits, crisped and puffed rice cereals, pasta, noodles, puddings, plain polished and whole grain rice are made primarily with, or are formulated with, rice.

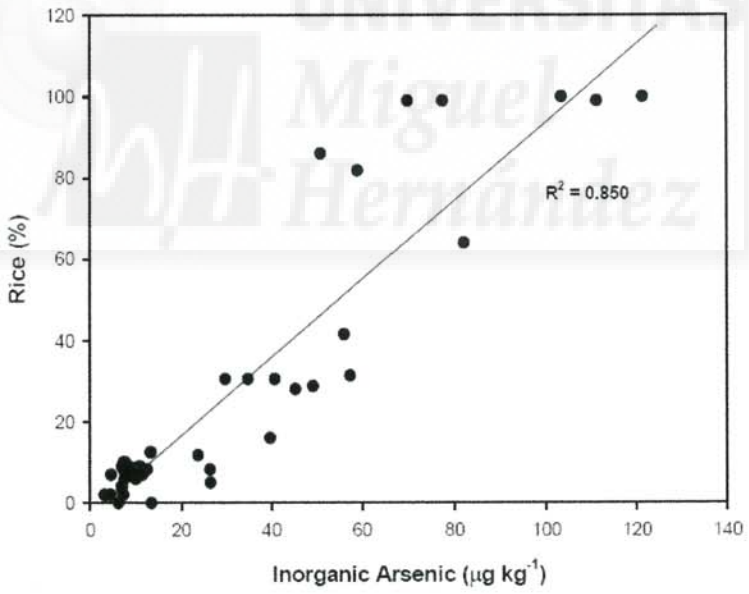

Figure 7. Relationship between inorganic arsenic contents and rice percentage in Spanish infant foods [26].

Because of high food consumption rates per body mass, children are at very higher risk of exposure of dietary contaminants, including As, than adults from the EU [2,22,27]. The UK Food Standards Agency (UK FSA) has already issued advice that children under 4.5 years do not take rice milk [29], as typical consumption patterns would lead to iAs intakes exceeding

WHO Permissible Maximum Tolerable Daily Intakes (PMTDI). EU baby rice itself is elevated in arsenic and leads to a high dietary exposure [22]. Similarly, rice based products pose a risk to young children due to high iAs content [28,30]. The EFSA arsenic review concluded that iAs levels in EU diets are at a level consistent with the possibility of risk to consumers, that rice was one of the most important sources of iAs, and that young children had the highest exposures with respect to the general population.

Very recently Ljung et al. [31] studied the contents of essential (Ca, Mg, Fe, Zn, Cu, Mn, Mo and Se) and toxic (As, Cd, Sb, Pb and U) elements in infant formula from Sweden and reported elevated tAs concentrations in rice-based products. These authors concluded that contents of toxic elements such as As, Cd and Pb have to be kept at an absolute minimum in food products intended for infant consumption.

STRATEGIES TO REDUCE ARSENIC OCCURRENCE IN RICE-BASED INFANT FOODS

The most important conclusion of the Scientific Opinion on Arsenic in Food of the EFSA [2] was that the dietary exposure to iAs should be reduced. The two obvious approaches to reduce infant exposure to As are: (i) to use rice grain from areas or cultivars with low grain As, and (ii) to switch from rice to other grain crops, such as oat, barley, maize and wheat as cereal carbohydrate/protein source. The second approach will imply the bankrupt of all rice-based business and will be almost impossible to implement. However, other options also exist and deserve consideration. These options include (i) modifying the current agronomic practices of rice growing to reduce As availability to plants, (ii) making pre-treatments to rice before its use as a food ingredient, (iii) optimizing the conditions of unit operations involving contact of rice with water and the time and temperature of thermal treatments during rice-based infant food manufacture. Consequently in the next sections different practical approaches to reduce the content of As in rice-based products will be discussed. These approaches will be classified in two categories: (i) those that can be implemented during rice farming and (ii) those that can be implemented during rice processing at the facilities of food companies.

BEFORE AND DURING RICE FARMING

The As content in rice is related to environmental and genetic factors [32,33], with soil As (concentration and species) having a dominant effect [10]. It has been demonstrated that different growing regions from one country can produce rice with differing As contents, for example the As content in rice from Cadiz and Seville regions of Spain is lower than in rice from other areas surveyed to date within Spain (Valencia, Delta del Ebro and Calasparra) [10,24,34]. Therefore, the first option to reduce As content in rice is to screen As levels in existing rice to identify varieties that have low As levels.

French rice grain is amongst the highest globally surveyed to date. This may be due to specific cultivars employed in the Camargue, or it may be due soil availability and/or management practice. Also, it seems that addition of organic matter, or organic rich soils,

may lead to enhanced As methylation, which may be desirable as organic arsenic (oAs) species are less toxic than inorganic ones.

Studies by Norton et al. [33] have clearly shown that arsenic uptake, transport and accumulation in the edible rice grain are affected by cultivar. These authors studied the agronomic behavior of 76 cultivars from Bangladesh and reported stable genetic differences in As accumulation. Later Norton et al. [32] cultivated 13 rice cultivars with low arsenic accumulation under different climatic conditions at two sites in three countries, Bangladesh, China and India. These authors concluded that breeding low grain As cultivars that will have consistently low grain tAs and iAs contents over multiple environments using traditional breeding approaches may be difficult, although four cultivars with interesting potential were identified and had low grain As across all field sites.

Finally, it must be mentioned that aerobic rice cultivation is now starting to be considered worldwide with the aim of increasing efficiency in the management of nitrogen fertilizers and mainly water, resource that for different reasons is getting more and more deficient worldwide. Compared to continuous flooding, aerobic management lowers As availability to plant uptake and consequently As assimilation by rice plants and the content of As in the edible grain [35]. However, this practice opens new issues related to the adaptation capability of traditional European cultivars to the increased soil oxygen availability and, therefore, to their future quantitative and qualitative performances with respect to grain yield and quality. Soil redox potential strongly affects microbial activity in the rhizosphere, the biogeochemical cycles of nutrients, the morpho-physiological features of roots, the availability of toxic elements (e.g., Cd, As) and, in the end, to alter plants productivity and the quality of final products [36].

On the other hand, aerobic conditions can affect the availability of other toxic elements, such as Cd. Arao et al. [35] studied the effects of water management in rice paddy on As and Cd contents in Japanese rice, concluding that flooding increased As concentrations in rice grains, whereas aerobic treatment increased the concentration of Cd. Consequently, As and Cd have opposite behaviors in the soil-plant system. When a paddy field is flooded and the soil is under reducing conditions, any Cd in the soil combines with sulfur to form CdS, which has a low solubility in water, while the dissolution of Fe-oxyhydroxides and the concurrent release of the co-precipated As will increase As availability to rice plants [14]. However, when the field is drained and the soil is under aerobic conditions, CdS is converted into $CdSO_4$, which is soluble in water, while As will be readily absorbed and/or co-precipitated onto hydrated iron oxides, mainly as arsenate. This means that the solubility of Cd and As change depending on the redox potential (Eh) of the soil. So, an equilibrium between soluble levels of As and Cd should be reached in paddy fields, otherwise rice grain will be either polluted with As or Cd, but polluted.

Summarizing, although it is desirable to reduce the iAs content of rice, alteration of agronomic practices should not detrimentally impact of micronutrients of nutritionally importance (.e.g., Fe and Zn), of vitamins, or of nutraceutic metabolites already scarce in rice grains [37] or increase the contents of other toxic elements such as Cd.

AFTER RICE HAS ENTERED THE FOOD INDUSTRY

Changes to the tAs content and to As species might take place during the manufacturing and/or preparation of food for human consumption. Various processes may cause a considerable increase or decrease in the As concentrations in food commodities and thus in the actual dietary exposure to As. For example, traditional washing and soaking of rice may significantly reduce the As levels. Changes in tAs content of the food can occur due (i) to losses (solubilization) to the cooking medium or preservation solution or (ii) to losses (volatilization) due to intense and long heat treatments. Additionally, toxic As species can be converted to other arsenicals or *vice versa* during food preparation.

Pre-Processing

Rice can be pre-treated before its incorporation into the manufacturing process of rice-based infant foods. Some of the external layers of the rice grain (Figure 8) contain significant contents of As and their removal will reduce the exposure to iAs of consumers of rice-based products.

Initially Signes et al. [38] compared the two rice dehusking processes (removal of the external hull or husk of the rice grain) currently in use in India, wet (soaking and boiling of rice and mechanical hulling, leading to parboiled rice) and dry (mechanical hulling, leading to atab rice). The dry method was recommended if As-free water was not available; however, soaking and light boiling resulted in lower As concentrations if non-polluted water was used. Therefore, the use of high volumes of water for washing and boiling the rice could be good ways of easily and significantly reducing the As content of rice, before starting the production of rice flour for rice-based infant foods.

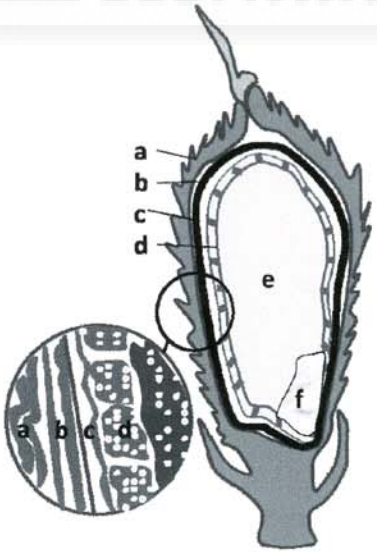

Figure 8. Morphology of the rice grain (a: hull; b: bran; c: polish; d: aleurone layer; e: starchy endosperm; f: embryo).

Simultaneously, Torres-Escribano et al. [39] reported a higher iAs concentration in brown rice, also known as whole-grain rice, compared to white rice (Figure 9), which might indicate that part of the As is attached to components of the bran. Sun et al. [30] also reported that iAs in rice bran and its products are an order of magnitude higher than in bulk grain, reaching concentrations of ~ 1000 µg/kg d.w. Besides, the layer of bran can be of different color, brown, reddish or even black. Norton et al. [33] found that Bangladeshi landraces with red bran had significantly more grain As than the cultivars with brown bran.

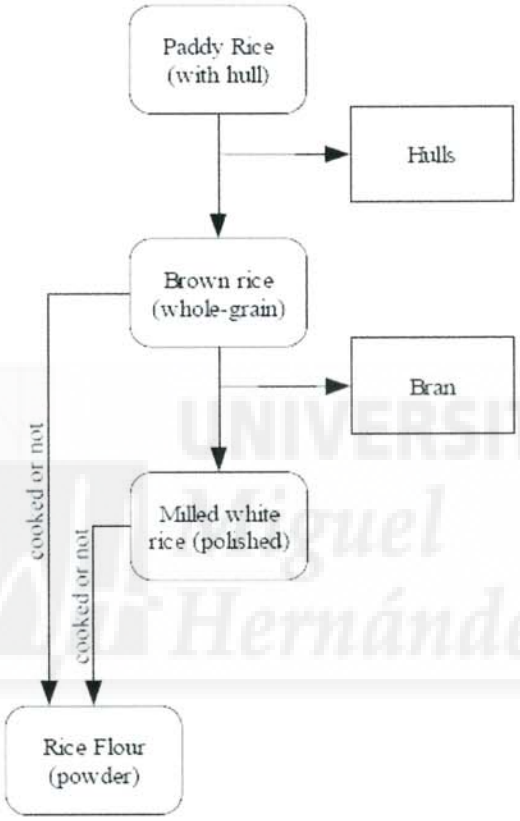

Figure 9. Pre-processing of paddy rice.

Summarizing, polishing brown rice to obtain white rice may lead to a substantial decrease in the contents of both tAs and iAs.

Later, brown or white rice can be cooked before entering the final manufacturing of rice-based products. The three most common methods of cooking rice in Asia are known as: a) traditional, b) intermediate and c) contemporary. In the *traditional method*, rice is washed until washings become clear, the washings are discarded and rice is boiled in excess water until cooked; finally, the remaining water is discarded. Rice cooked following the *intermediate method* is washed as above but is boiled with less water until no water is left to discard. Finally, in the *contemporary method* unwashed rice is boiled with low water volume until no water is left to discard [40]. Signes *et al.* [41] and Signes-Pastor *et al.* [7] simulated these three cooking methods in their facilities and recommended the use of the traditional

method (using high volumes of water for the washing and cooking steps); this method significantly reduced the content of tAs from a maximum of 387 to 258 μg/kg. Similar conclusions were previously reached by *Sengupta et al.* [40], who cooked rice using low-As water (<3 μg As L^{-1}) using traditional and modern methods and found that the traditional method (wash until clear; cook with rice:water ratio of 1:6; discard excess water) removed up to 57% of the As from the initial rice. Approximately half of the As was lost in the wash water and the other hand in the discard water.

At the same time, Signes et al. [42] simulated cooking of rice with different levels of As (>50 μg/L) species in the cooking water and concluded that As concentration in cooked rice was always higher than that in the raw rice and ranged from 227 to 1642 μg/kg. Mondal and Polya [43] reported values of As in cooked rice of 170 μg/kg (during two household surveys in West Bengal, Nadia district), Smith et al. [44] of 350 μg/kg for a Bangladesh household survey, Bae et al. [45] of 270 μg/kg for a Bangladesh on site survey, Rahman et al. [46] of 320 μg/kg for a Bangladesh field survey and Roychowdhury et al. [47] of 370 μg/kg for a West Bengal household survey. Perhaps, the lower t-As concentrations reported in these later studies compared to the laboratory study by Signes et al. [42] were due to the use of cooking water with low-As contents or even free-As water.

In addition, Signes et al. [42] showed that the As speciation was not significantly affected by the cooking process, probably because the temperature reached during rice cooking, ~ 100ºC, was lower than required for promoting species exchanges. Van Elteren and Slejokovek [48] studied the effect of high temperatures on aqueous standards of various species of As and concluded that temperatures above 150ºC were required to found significant changes in As speciation. A later study carried out by Devesa et al. [49] agreed with this statement and concluded that these high temperatures can be attained in some cooking treatments in which the surface of the food is in direct contact with the heat source (grilling, frying or baking) and reaches temperatures close to 250ºC; similar results were found by Hanaoka et al. [50] and Torres-Escribano et al. [39].

Finally, Raab et al. [51] systematically investigated tAs and iAs in different rice types basmati, long-grain, polished (white) and wholegrain (brown) that had undergone various types 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) during cooking, as well as steaming, were investigated. Rinse washing was effective at removing about 10 % of the tAs and iAs from basmati rice, but was less effective for other rice types. While steaming reduced tAs and iAs rice content, it did not do so consistently across all rice types investigated. High volume water cooking effectively removed both tAs and iAs by 35 % and 45 %, respectively, in the long-grain and basmati rice, compared to uncooked (raw) rice. This study indicates that rinse washing and a high volume of non-contaminated cooking water are effective in reducing the As content of cooked rice, specifically the inorganic.

During Processing

The diagram on Figure 10 shows a general procedure for the manufacturing of a typical cereal-based baby food. As can be seen the only unit operation involving a heat treatment is "drum drying". The temperature reach in the surface of the drum during this drying process

could be as high as that reported for grilling and therefore could significantly affect the content and speciation of As in the rice flour. However, the options to reduce the As content in cooked rice when entering the processing line are very limited.

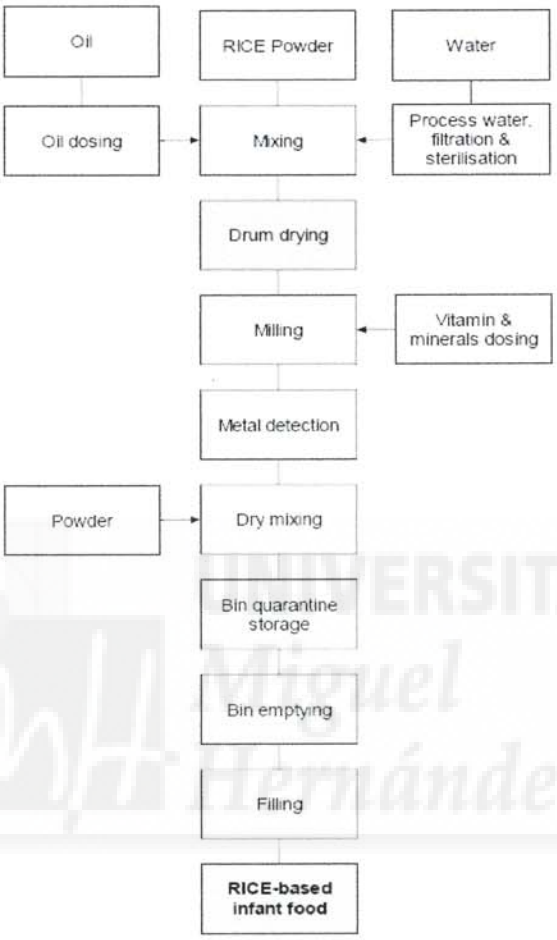

Figure 10. General manufacturing process of a rice based infant foods.

In summary, the effects of food processing on the concentrations of As in rice-based infant foods depends mainly on (i) proper selection of the raw ingredients (from low As accumulating cultivars and from geographical areas with low soil As contents) and (ii) proper pre-processing (removal of husk and/or bran layers, washing, rinsing and cooking with high volumes of water) of rice grain before entering the main manufacturing line.

ACKNOWLEDGMENTS

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4. RESULTADOS Y DISCUSIÓN

4-RESULTADOS Y DISCUSIÓN

Dada la prioridad por las autoridades de la UE en el control de metales pesados en la alimentación infantil, en esta Tesis Doctoral se ha realizado un estudio sobre la situación actual. Hemos recogido los datos necesarios para una primera evaluación, en función de los objetivos marcados.

Se ha determinado la concentración de cuatro metales pesados (As, Pb, Cd y Hg) en alimentos infantiles de 4 zonas geográficas diferentes (China, USA, UK y España). Se estudiaron en total 40 muestras que se clasificaron en 5 grupos:

1. Cereales sin gluten, se trata de un preparado liofilizado a base de arroz y maíz.
2. Cereales con gluten, preparado liofilizado a base de cereales entre ellos, trigo, centeno, avena, sorgo, cebada, arroz y maíz.
3. Potitos de carne, producto derivado de la trituración de un preparado a base de carne combinado con verduras y arroz.
4. Potitos de pescado, producto derivado de la trituración de un preparado a base de pescado combinado con verduras y arroz.
5. Otros, clasificación realizada a diversos productos comercializados en esta etapa infantil como leche sin lactosa y galletas.

4.1 Elementos traza metálicos en alimentos infantiles

En el artículo "*Essential and toxic elements in infant foods from Spain, UK, China and USA*" publicado en *Journal of Environmental Monitoring* (2012), se presentó los valores de As, Pb, Cd, Hg total que fueron determinados en los diferentes grupos de alimentos, representados en la **Figura 5**. Cabe destacar que el arsénico es el contaminante que aparece con mayor frecuencia, de hecho todos los productos infantiles contenían dicho metal en cantidades mensurables. El segundo contaminante que presentó una mayor presencia, no sólo en frecuencia sino también en concentración, fue el plomo; sobre todo en los productos a base de cereales. El resto de contaminantes (Cd y Hg) presentaron valores muy bajos e incluso no detectables en algunas muestras.

La contaminación por plomo en los alimentos supuso un riesgo importante hasta 1970, año en el que se prohibió su uso en el combustible (Larse *et al.,* 2002). La entrada de este contaminante en las plantas es vía foliar, de ahí que una alta concentración se acumule

en hortalizas de hoja y cereales (EFSA, 2012). Como se observa en la **Figura 5**, el plomo está presente en todos los productos a base de cereales, presentando valores similares entre los cereales sin gluten y con gluten, de forma general se establece una contaminación en cereales sin haber una distinción entre los mismos. Otros productos presentaron un menor contenido en Pb por ser mezcla de cereales con otro tipo de productos como frutas, de ahí que la media se reduzca respecto al resto de productos a base de cereales.

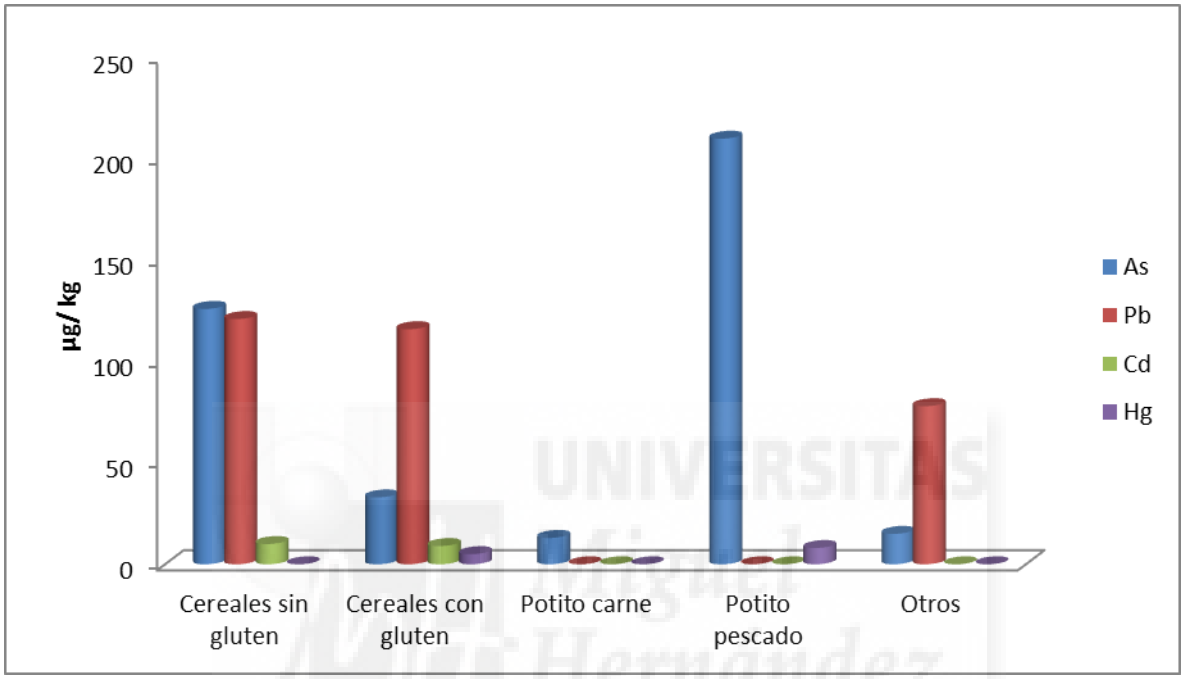

Figura 5. Representación gráfica de los valores medios de As, Pb, Cd, Hg total expresados en µg/kg en alimentos infantiles comercializados en diferentes países.

Si nos centramos en los valores de arsénico mostrados en la **Figura 5**, observamos que las concentraciones más altas se presentan en los productos a base de pescado seguido de los cereales sin gluten. Además, se aprecia la variabilidad en la concentración de As en los diferentes productos a base de cereales, lo que indica que su presencia puede ser asociada de forma directa a un tipo de producto.

El arsénico inorgánico presenta una toxicidad mayor que la del plomo, teniendo en cuenta los valores de ingesta semanal tolerable, 15 frente a 25 µg/(kg peso x semana), respectivamente. Los valores presentados en la **Figura 5** hacen referencia al As-t; conviene estudiar las especies arsenicales de los productos analizados.

4.2 Especiación del arsénico

Como se recalca en el capítulo " *Occurrence of inorganic arsenic in rice-based infant foods: soil-rice-infant relationships*" publicado en el *libro Arsenic: sources, environmental impact, toxicity and human health a medical geology perspective*, cabe destacar que la especies arsenicales inorgánicas (As-i) presentan mayor toxicidad que las especies orgánicas (As-o). El As-o se elimina fácilmente a través de la orina, con valores alrededor de 70 % en 24 h (Domínguez Carmona, 2009); además, el As-i fue considerado como agente cancerígeno del grupo I por la IARC (1987). Conociendo estas diferencias en la toxicidad de las especies arsenicales, resulta interesante considerar la especiación en este tipo de productos. La **Tabla 5** presenta los datos publicados en el artículo "*Inorganic arsenic contents in rice-based infant foods from Spain, UK, China and USA*" en la revista *Environmental Pollution* (2012). Se determinó el contenido en As-i y dimetilarsénico (ADMA) de los productos analizados. El DMA es el producto de transformación primario en las reacciones de metilación de especies inorgánicas, llevadas a cabo por enzimas presentes en microorganismos.

Muestra	n		As-t	As especies	As-i	ADMA	As-i	ADMA
			(µg /kg)				(%)	
Cereales sin gluten	13	Media	126 ±26	127 ±26	69 ± 8	56 ± 18	64 ± 5	35 ± 4
		Mediana	84	92	59	25	69	31
		Rango	46-315	43-324	29-121	10-198	36-89	11-62
Cereales con gluten	9	Media	33 ± 6	27 ± 6	26 ± 5	Nd	98 ± 1	nd
		Mediana	23	24	24	0	100	0
		Rango	15-65	10-55	10-49	nd-5	90-100	nd-10
Potito carne	11	Media	13 ± 2	12 ± 2	8 ± 1	Nd	74 ± 5	26 ± 5
		Mediana	10	9	7	2	81	19
		Rango	9-26	8-24	7-11	nd-14	42-91	9-58
Potito pescado	8	Media	619 ±278	552 ±239	7 ±1	5 ±1	nd	nd
		Mediana	220	222	7	5	3	nd
		Rango	159-2310	151-2009	nd-11	nd-11	nd-5	nd-nd
Otros	5	Media	13 ± 4	14 ± 5	12 ± 4	2 ± 1	88 ± 6	13 ± 6
		Mediana	11	9,8	9,8	0,5	91,5	8,5
		Rango	6-29	5-32	nd-27	nd-5	71-100	nd-29
LOQ (µg/ kg)			6	5	5	5		
CRM (%)			92,4	97,5	95,9	Na		

nd= por debajo del LQ [límite de cuantificación determinado como tres veces por debajo de la desviación estándar de los blancos , límite de detección (LD), multiplicado por el factor de dilución apropiado];

na= no disponible.

Tabla 5. Valores de especiación del arsénico (µg/kg; %) en productos infantiles comercializados en diferentes países.

Teniendo en cuenta la mediana de As-t en los productos a base de cereales sin gluten y potitos de pescado, 84 µg/kg y 220 µg/kg respectivamente, y la concentración de As-i 69 µg/kg y 3 µg/kg respectivamente; un 64±5 % del As-t se encuentra en formas inorgánicas en los productos a base de cereales sin gluten frente a un porcentaje muy bajo en los productos a base de pescado.

El alto contenido en As-o en los productos a base de pescado se debe a la vía de entrada del arsénico en la cadena trófica. La entrada de As-o se produce por el consumo de algas, las cuales poseen las enzimas capaces de transformar las formas inorgánicas a orgánicas. Como resultado de este tipo de transformación se producen formas complejas de arsénico como arsenobetaína, arsenoazúcares (predominante en algas) y arsenolípidos (OMS, 1998). La concentración de ADMA que se ha encontrado en pescado fue baja, el 98 % del arsénico presente en el pescado se encuentra en sus formas orgánicas, principalmente en forma de arsenobetaína (ATSDR, 2007).

Cabe resaltar el alto porcentaje de As-i en los productos de origen vegetal; principalmente en: cereales con gluten, productos a base carne (alrededor 15% carne) y otros productos, superior al 88% frente al 60% presente en cereales sin gluten (principalmente arroz).

Por otro lado, cabe incidir en las diferencias encontradas en la concentración tanto de arsénico como de sus especies arsenicales en los productos de un mismo grupo, con un rango de 46-315 µg As-t/kg en cereales sin gluten y 159-2310 µg As-t/kg en potitos de pescado, siendo necesario esclarecer dichas variaciones, las cuales se exponen posteriormente.

Otro dato que llama la atención, es que en el resto de productos analizados, el porcentaje correspondiente a las formas inorgánicas supera el 60 %. En la **Figura 6** representamos la relación entre el As-i frente al As-t y se observó que todos los productos tiene un comportamiento similar, sobre todo en los productos a

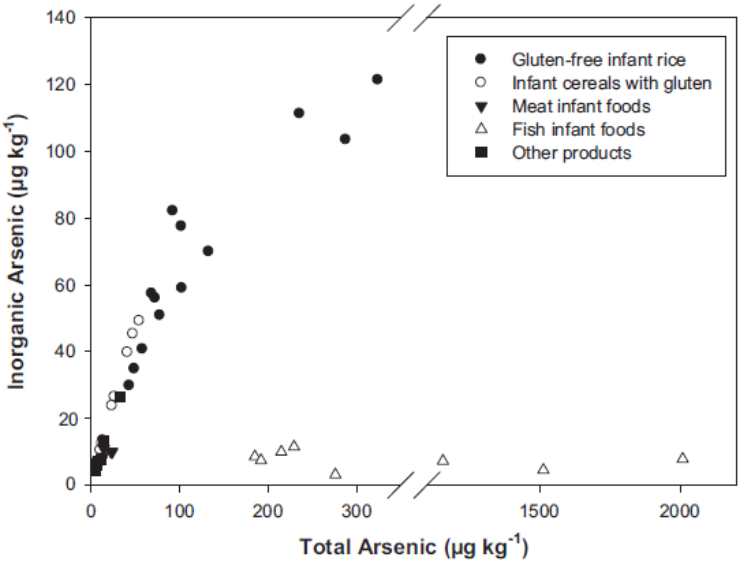

Figura 6. Gráfica que relaciona el contenido en As-i frente al As-t en los productos infantiles analizados.

base de cereales. La única excepción fueron los productos a base de pescado en los que el As-i fue constante. Teniendo en cuenta que el factor común en los productos a base de cereales es que contiene arroz y maíz, representamos **la Figura 7** en la cual se observó la relación entre el As-i/cantidad arroz en la fórmula (%). Se obtuvo una regresión lineal (R^2) de 0,85, por lo que se estableció de forma clara una relación entre el contenido de arroz y el contenido de As-i. Por tanto, el arroz fue la principal fuente de As-i en los alimentos infantiles.

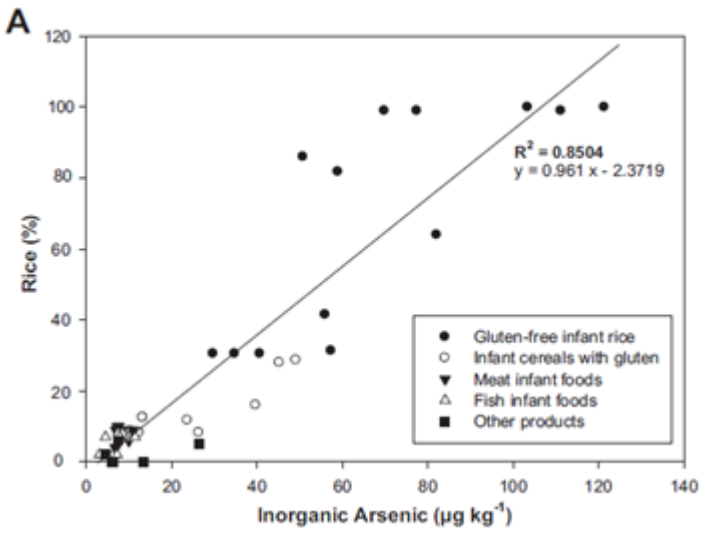

Figura 7. Gráfica que relaciona el contenido en As-i frente a la concentración de arroz en los productos infantiles analizados.

4.3 Contenido de arsénico en arroz

Durante muchos años el arsénico ha sido aplicado en la producción agrícola en forma de productos fitosanitarios (Carbonell A.A *et al.*, 1995), lo que ha producido la contaminación de suelos; además hay acuíferos contaminados de forma natural por dicho metal (Marin *et al.*, 1993). La peculiaridad que hace que el arroz sea el cereal que más arsénico acumule es la condición de inundación del cultivo, la cual aumenta la disponibilidad del arsénico para ser absorbido, como se expone en el capítulo *"Occurrence of inorganic arsenic in rice-based infant foods: soil-rice-infant relationships*" publicado en el libro *Arsenic: sources, environmental impact, toxicity and human health a medical geology perspective*. Las condiciones de anaerobiosis, así como una mayor disolución del óxido ferroso, encargado de capturar el As-i tanto en sus formas pentavalentes (V) como trivalentes (III), son los factores claves que conllevan a aumentar la disponibilidad del mismo.

En la **Tabla 6** se muestran los valores de As-t encontrados en arroces de diferente origen y tipo, presentados en el artículo "*Arsenic contents in Spanish infant rice, pureed infant foods and rice"* en la revista *Journal of Food Science* (2012). Las diferencias de As-t observadas en arroz de origen español, tailandés e indio; presentan valores medios de 0,139, 0,184 y 0,158 mg/kg respectivamente. Estos valores confirman la problemática de la

contaminación por As en ciertas zonas geográficas (**Imagen 12**). Los cultivos del Oeste de Bengala han sido regados durante mucho tiempo con aguas contaminadas por As (Meharg *et al.*, 2003).

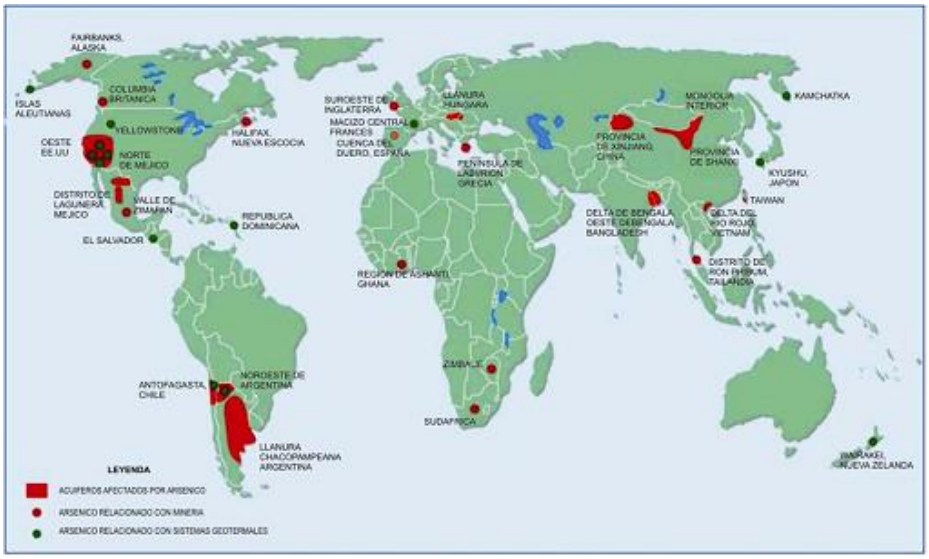

Imagen 12. Mapa zonas contaminadas por arsénico

Por otro lado, vemos que las mayores concentraciones de arsénico se presentan en el arroz con cascarilla e integral con valores medios; 0,216 y 0,196 mg As-t/kg, respectivamente, frente a un 0,154 mg As-t /kg en arroz blanco. Esta diferencia supone que el contenido medio de As-t en la cascara es de alrededor un 28 %, por lo que se corrobora los datos bibliográficos que apuntan que la cascarilla acumula una cantidad importante de arsénico (Sun *et al.* 2008a).

Las diferencias encontradas en la concentración de As-t en el arroz con cascarilla; 0,188-0,351 mg As-t/kg, se deben a variables específicas de cada cultivo, como puede ser la distancia al foco contaminante o los métodos de procesado. Un estudio realizado por Signes *et al.* (2008b) comparó dos procesos de descascarillado de arroz: (i) húmedo, el descascarillado se produce tras una cocción del arroz y (ii) seco, descascarillado mecánico, observando diferencias significativas en el contenido de As-t entre ambos procesos. Estudios realizados por Torres-Escribano *et al.*, (2008), mostraron que a temperaturas elevadas se producen cambios en las especies arsenicales. Por tanto, no sólo el procesado del arroz interviene en las variaciones de arsénico, sino también el posterior proceso de elaboración del producto a base de arroz. Además, si el agua utilizada en la cocción presenta As puede

pasar al arroz llegando a aumentar la concentración de éste en un 10 % e incluso hasta un 35 % (Alam *et al.*, 2005).

Tipo arroz	As concentración (mg/kg)
Arroz con cascara (n = 4)	0,216±0,048
Arroz con cascara (Calasparra)	0,127∗ ±0,001c†
Arroz con cascara (Valencia)	0,199±0,027b
Arroz con cascara (Valencia)	0,188±0,020b
Arroz con cascara (Valencia)	0,351±0,022ª
Integral (n = 6)	0,196±0,044
Integral y arroz orgánico (España)	0,338±0,014ª
Integral arroz (España)	0,127±0,010b
Integral arroz (España)	0,333±0,005ª
Integral arroz (Valencia)	0,113±0,001b
Integral arroz (Valencia)	0,126±0,002b
Integral arroz (Valencia)	0,137±0,013b
Blanco (n = 11)	0,154±0,005
España (n = 6)	0,139±0,010
Blanco arroz (España)	0,178±0,011ª
Blanco arroz (España)	0,137±0,013c
Blanco arroz (España)	0,151±0,009b
Blanco arroz (Valencia)	0,102±0,002d
Blanco arroz (Valencia)	0,138±0,004c
Blanco arroz (Valencia)	0,126±0,009c
Tailandia (n = 2)	0,184±0,019
Aromático Thai blanco arroz	0,202±0,008ª
Aromático Thai blanco arroz	0,165±0,008b
India (n = 3)	0,158±0,019
Basmati Brajma blanco arroz	0,157±0,005b
Oeste Bengala blanco arroz	0,126±0,009c
Oeste Bengala blanco arroz	0,192±0,005ª
Media global	0,177±0,016

* Valor de la media de 3 repeticiones; ± valor representa el error estándar

† La misma letra dentro de una columna indica que no eran significativamente diferentes con un $p<0,001$

Tabla 6. Contenido de As-t (mg/kg) en arroces de diferente origen y tipo

En base a estas variables, en el artículo "*Arsenic contents in Spanish infant rice, pureed infant foods and rice*" en la revista *Journal of Food Science* (2012) se muestran los resultados del análisis de As-t en cereales sin gluten y cereales con gluten en 5 marcas diferentes, comercializadas en España (**Figura 8**). Aparte de observarse claramente la relación entre el contenido en As-t y cantidad en arroz (porcentaje mayor en los cereales sin gluten), se pudo observar que el factor "origen de la materia prima" también juega un papel importante en el grado de contaminación por As.

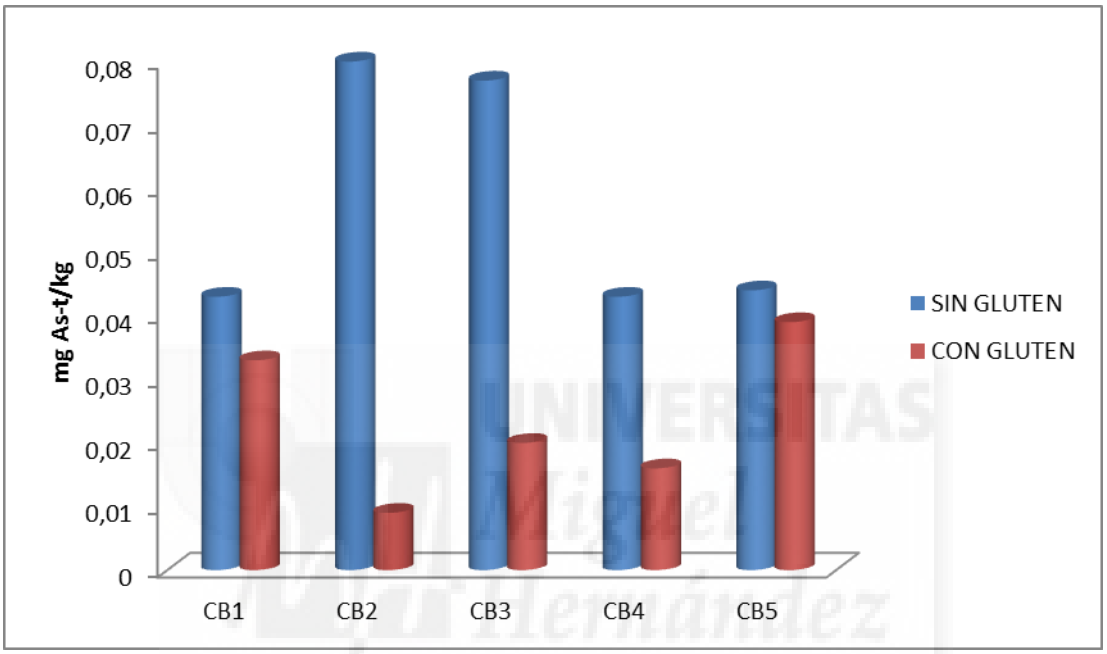

Figura 8. Representación valores de As-t (mg/kg) en productos a base de cereales sin gluten y con gluten en 5 marcas diferentes comercializados en España

La diferencia en el contenido de As-t entre los productos sin gluten y con gluten de la marca CB1 y CB5 es bastante baja en relación al resto de marcas; supone 0,01 y 0,005 mg/kg respectivamente. Esta variación es un indicio de que el arroz utilizado en la producción de los cereales sin gluten no es del mismo origen al utilizado en la producción de cereales con gluten, teniendo en cuenta la concentración de arroz en las formulas (**Tabla 8**).

	Formulación etiqueta	
Papillas de cereales	**Sin gluten**	**8 Cereales**
CB1	Harina (96 %): hidrolizado de arroz y maíz, arroz y maíz	Harina (trigo, cebada, centeno, maíz, sorgo, arroz, avena y mijo)
CB2	Harina de cereales de dextrina (56 %): arroz y maíz	Harina de cereales dextrina (70 %): trigo, arroz, cebada, centeno, maíz, mijo, sorgo y avena
CB3	La harina de los cereales parcialmente dextrina (94 %): arroz, maíz y tapioca	Harina de cereales parciales dextrina (96 %): trigo, maíz, arroz, cebada, avena, centeno, sorgo y el mijo
CB4	Harina de hidrolizado (96 %): arroz, maíz y tapioca	Harina de hidrolizado (94 %): trigo, maíz, arroz, avena, cebada, centeno, el sorgo y el mijo
CB5	Cereales (61 %): harina de arroz y harina de maíz	Harina de cereales (66 %): trigo, cebada, centeno, maíz, arroz, avena, sorgo y el mijo

CB= marca comercial

Tabla 8. Información del etiquetado de los productos a base de cereales sin gluten y con gluten comercializados en España.

Dada la importancia del origen del arroz en el contenido de As, en la **Tabla 9** se presentan los datos en función de la procedencia de los alimentos infantiles analizados, presentados en el artículo publicado en *Journal of Environmental Monitoring* (2012). Los mayores valores de As-t encontrados aparece en el siguiente orden decreciente USA > UK > España > China, con valores; 253±62 µg/kg > 237±49 µg/kg > 181±36 µg/kg > 135±19 µg/kg, respectivamente. Dicha ordenación, corresponde con las zonas afectadas de contaminación por arsénico en agua subterránea, excepto China; sin embargo no es de extrañar conociendo la reciente reglamentación sobre el arsénico impuesta en este tipo de productos con un límite máximo de 150 µg/kg (Heikens, 2006), lo que supuso una retirada del mercado del 35 % de productos infantiles.

Por otro lado, los productos de USA a pesar de tener los mayores valores de As-t, el porcentaje correspondiente al As-i fue del 55 %; algo similar ocurrió con la especiación en los productos españoles. Mientras, los valores para el As-i en los productos de UK y China llegaron a superar el 70 %. Para entender estos resultados se deben tener en cuenta el histórico de la presencia del mismo en el suelo; en el caso de EEUU durante mucho tiempo se utilizaba el As-o en un herbicida aplicado contra las malezas en el cultivo del algodón. Ello

supuso un riesgo significativo, teniendo en cuenta la rotación de cultivos entre algodón-arroz puesto que el suelo quedó con un alta carga de arsénico en forma de ADMA (Marin *et al.,* 1993); este hecho sucedió ampliamente en regiones sureñas tales como Louisiana, como se expone en el capítulo "*Occurrence of inorganic arsenic in rice-based infant foods: soil-rice-infant relationships*" publicado en el libro *Arsenic: sources, environmental impact, toxicity and human health a medical geology perspective*.

Muestra	N		As-t	As especies	As-i	DMA	As-i	DMA
			(µg /kg)				(%)	
China		Media	135 ±19	148±16	114±15	33±3	76±2	23±2
	14	Mediana	105	129	97	29	76	25
		Rango	70-353	73-290	52-247	21-63	62-88	12-35
USA		Media	253±62	260±66	125±14	127±54	55±8	43±8
	5	Mediana	213	192	122	86	53	47
		Rango	164-496	134-515	93-159	35-334	31-81	18-65
UK		Media	237± 49	248±52	162±29	83±46	71±9	29± 9
	5	Mediana	188	221	142	38	79	21
		Rango	137-394	135-415	107-267	28-265	34-84	15-64
España		Media	181±36	181±38	85± 10	93±28	53±5	45±5
	7	Mediana	145	133	77	62	53	45
		Rango	36-315	38-288	10-111	25-178	5-75	5-62

Tabla 9. Valores de especiación de arsénico (mg/kg; %) en función de la procedencia de los productos infantiles analizados.

4.4 Exposición de contaminantes a través de la ingesta de alimentos infantiles

Con los datos obtenidos un aspecto importante era evaluar la exposición dietética a metales pesados en ese grupo poblacional. En la **Figura 9** se representa una estimación de la evolución de la exposición al As-i expresado en µg/ (kg peso x día), con una tendencia creciente que corresponde con la introducción de nuevos alimentos. Dicho modelo representa un alto porcentaje de la población infantil (dieta con gluten), sin tener en cuenta casos aislados en los que las indicaciones alimentarias cambian por situaciones especiales, como un lactante bajo peso o episodios gastrointestinales puntuales en los cuales se ve aumentado el consumo de arroz. Y las situaciones especiales, como una intolerancia al gluten (dieta sin gluten), la celiaquía supone una dependencia al consumo de arroz y maíz en sustitución al resto de cereales durante toda la vida. Si comparamos la exposición al As-i en ambos casos en cada etapa (4, 6, 8, 12 meses) supone 0,05, 0,16, 0,25, 0,25 µg/(kg corporal x día) frente al 0,26, 0,27, 0,4, 0,4 µg/(kg corporal x día) en los casos especiales. Esta situación experimental implica una exposición de As-i del doble, convirtiéndose la población celiaca un grupo poblacional de alto riesgo de exposición al arsénico. En los dos supuestos evaluados nos encontramos dentro del rango marcado como RfD para el As-i, situado entre 0,1-0,8 µg/(kg peso x día).

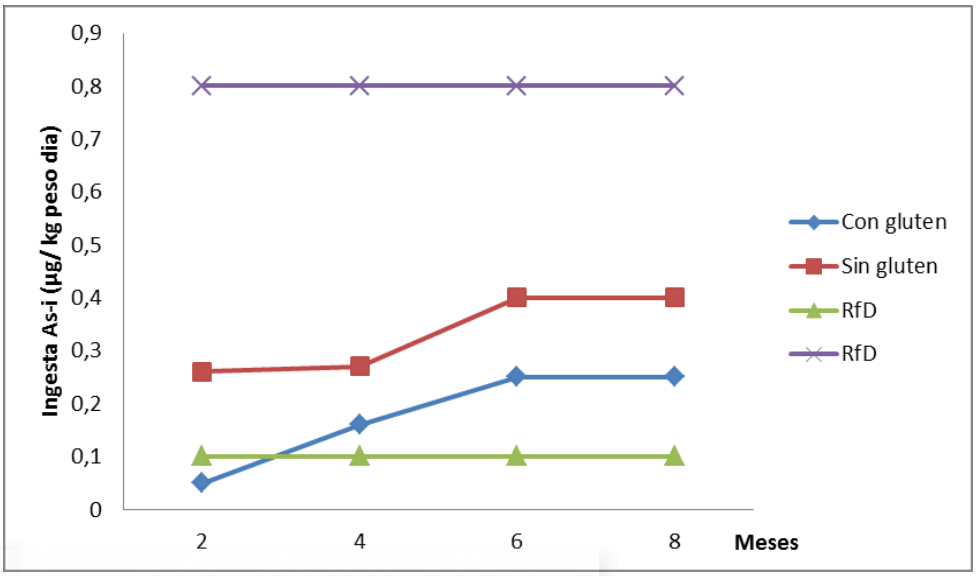

Figura 9. Evolución de la exposición al As-i (µg/peso corporal día) en lactantes menores de 12 meses con el consumo de los alimentos infantiles analizados

Cabe resaltar que el caso evaluado, supuso una situación crítica en la que el arroz aparece en todas las tomas. A partir de los 8 meses se observa el punto más alto de exposición. Estos valores tan altos se deben: (1) por un lado, a que hay dos tomas con una fuente protéica animal (potito carne y potito pescado) y (2) a un aumento en la cantidad de dichas tomas, que continua hasta el año de vida.

Según las evaluaciones emitidas por la EFSA desde 2004 sobre el riesgo que supone en lactantes la exposición a metales pesados, se estimó la exposición dietética de los mismo según los datos recogidos en el artículo "*Essential anda toxic elements in infant foods from Spain, UK, China and USA*" publicado en *Journal of Environmental Monitoring* (2012) y siguiendo las mismas indicaciones que en el modelo anterior. En la **Tabla 10** se presentan los valores de exposición dietética diarios en dos grupos poblacionales; (a) dieta sin gluten, en casos de celiaquía y (b) dieta tolerante al gluten. En el caso del grupo con una dieta sin gluten, supuso una mayor exposición a contaminantes como el As, Pb y Cd. Por otro lado, conforme se van introduciendo nuevos alimentos se observó en ambos grupos un aumento de la exposición a metales pesados y metaloides As > Pb > Cd > Hg, en orden decreciente de exposición. En cambio las diferencias encontradas en la exposición entre caso de dieta sin gluten y con gluten es bastante menor en el caso del Pb, Cd y Hg, en comparación con la exposición al As.

	As	**Pb**	**Cd**	**Hg**
Edad (meses)		**(µg/(kg peso x día))**		
Dieta sin gluten				
4	0,43	0,40	0,03	n.a.[a]
6	0,26	0,35	0,03	n.a.
8	2,5	0,45	0,40	0,07
12	2,82	0,40	0,03	0,08
Dieta con gluten				
4	0, 13	0.34	0,02	0,01
6	0,20	0.33	0,02	0,01
8	2,16	0.44	0,03	0,09
12	2,52	0.39	0,03	0,10

[a] n.a.= no disponible

Tabla 10. Exposicion de metales pesados por el consumo de los productos infantiles analizados en lactantes menos de 12 meses.

4.5 Evaluación de la exposición de contaminantes a través de la ingesta alimentos infantiles

Existen diferentes parámetros para evaluar la exposición de contaminantes; sin embargo el ISTP es el valor de referencia utilizado por la EFSA en sus últimas publicaciones sobre la evaluación de exposición a metales pesados en la población Europea:

- BMDLx: se trata de índice de Benchmark, el cual hace referencia a la cantidad de sustancia o contaminante, expresado en µg/kg peso corporal día, que aumenta en X % un determinado efecto sobre la salud. El mismo se utiliza para evaluar la toxicidad y se tiene en cuenta para marcar los límites que se exponen a continuación. En el comunicado de la EFSA (2004) se mencionó un valor de 0,3-0,8 µg As-i/(kg peso corporal x día) para el riesgo de cáncer.
- RfD (dosis de referencia): en este sentido la EFSA solo menciona una RfD para el metil-mercurio y arsénico inorgánico; 1,6 µg/(kg peso x día) y 0,1-0,8 µg/(kg peso x día), respectivamente.
- ISTP (Ingesta Semanal Tolerable Provisional), referente que la EFSA tiene en cuenta para sus evaluaciones:
  - ✓ Arsénico inorgánico, 15 µg/(kg peso x semana) (FAO/OMS 1989) lo que supone de forma diaria 2,1 µg/kg peso. En el comunicado de la EFSA se concluye que el mismo no es apropiado y requiere de una revisión.
  - ✓ Plomo, 25 µg/(kg peso x semana) (FAO/OMS 1983) lo que supone de forma diaria 3,5 µg/kg peso. En el comunicado de la EFSA se concluye el dicho valor es alto y se debe realizar una revisión del mismo.
  - ✓ Cadmio, 7 µg/(kg peso x semana) (FAO/OMS 1993) lo que supone de forma diaria 1 µg/kg peso. Por el momento, dicho valor es adecuado en la evaluación según la EFSA
  - ✓ Mercurio, 5 µg/(kg peso x semana) (FAO/OMS 1978) lo que supone de forma diaria 0,7 µg/kg. Por el momento, dicho valor es adecuado en la evaluación según la EFSA

La **Figura 9** muestra que la exposición al As-i expresada en µg/(kg peso x día) se encuentra dentro del rango de RfD para el As-i. Sin embargo; en todas las etapas se supera el límite inferior de 0,1 µg/(kg peso x día) en la dieta sin gluten. Por otro lado, también se

supera el límite inferior del $BDML_{01}$de 0,3 µg As-i/(kg peso x día), para el cáncer de pulmón, piel y vejiga, a partir de los 6 meses.

Las conclusiones llevadas a cabo por la EFSA para el As-i y Pb se debe a los recientes estudios sobre BDML y a la relación ingesta/peso en niños, sobre todo menores de 3 años. Por otro lado, teniendo en cuenta los valores encontrados en los alimentos infantiles y el Reglamento CE 141/2006 sobre el contenido máximo de diversos contaminantes químicos, cero residuos, así como las aportaciones de la EFSA (2004), los productos analizados aportan una cantidad importante de As, por tanto es imprescindible encontrar vías de reducción del mismo en las materias primas.

Otro aspecto a tener en cuenta en la evaluación de la exposición dietética de estos contaminantes es considerar la biodisponibilidad y absorción intestinal, la cual se ve influenciada por multitud de parámetros. Por otro lado, la capacidad de eliminación del organismo determina el grado de exposición así como el efecto y aparición de síntomas frente a la exposición.

La biodisponibilidad del As-i a partir del arroz es alta, del orden del 90 % (Ackerman *et al.*, 2005); sin embargo los datos para alimentos infantiles a base de arroz deben ser estudiados. Diversos estudios apuntan que el selenio aumenta la metilación del arsénico inorgánico, lo que implica una rápida eliminación a través de la orina (Verret *et al.* 2005; Son *et al.* 2008).

En el caso del plomo la absorción intestinal varía dentro del intervalo 40-60 %. Diversos estudios han afirmado que la absorción del Pb es mayor en niños que en adultos (Alexander *et al.,* 1974; Ziegler *et al.,* 1978; Heard & Chamberlain, 1982; James *et al.,* 1985; Rabinowitz *et al.,* 1980). Por otro lado, intervienen otros factores; (1) el estado nutricional del hierro; Barany *et al.* (2005) mostraron que un estado nutricional de hierro correcto reducía la absorción intestinal de plomo, y/o (2) la ingesta simultánea del calcio reduce de forma considerable la absorción de plomo en el intestino (Mahaffey *et al.,* 1986;. Ziegler *et al.,* 1978).

En cuanto a los datos de absorción intestinal del cadmio apuntan a valores bajos de 5-10 %. Esta situación esto se debe a que el Cd queda retenido en la mucosa intestinal por la

unión a las metalotioneinas. El cinc reduce la absorción del cadmio aumentando la capacidad de retención de la mucosa intestinal (Foulkes & McMullen 1986).

La biodisponibilidad del mercurio depende de la forma en la que se presente; las formas inorgánicas alcanzan valores entorno 80-90 %. Por otro lado, el selenio es capaz de neutralizar el mercurio, así como otros metales pesados en el organismo (Zeng *et al.,* 2005).

Como vemos, los minerales esenciales juegan un papel importante en el comportamiento del estos metales pesados y metaloides en el organismo; por tanto se convierte indispensable evaluar el contenido de los mismos en los alimentos infantiles.

La **Tabla 11** muestra los contenidos en minerales esenciales (Ca, Na, Fe, Cu, Mn, Zn, Se, Cr, Ni y Co). De forma general se observaron mayores valores de estos micronutrientes en los productos a base de cereales y otros productos analizados.

Muestra	N		Ca (g /kg)	Na (mg /kg)	Fe (mg /kg)	Cu (mg /kg)	Mn (mg /kg)	Zn (mg/ kg)	Se (µg /kg)	Cr (µg/ kg)	Ni (µg/ kg)	Co (µg/ kg)
Cereales sin gluten		Media	1,17± 0,26a[a]	0,08± 0,01c	47,7± 9,2c	1,08± 0,14bc	6,57± 0,95b	6,99± 0,72bc	52± 11b	118± 19ª	155± 17bc	n.d[b]
	13	Mediana	1,03	0,08	48,6	1,18	6,64	7,41	50	126	139	n.d
		Rando	0,01-2,59	0,02-0,23	0-84,9	0,08-1,80	0,08-12,7	0,08-10,7	0-146	18-270	84-299	n.d-84
Cereales con gluten		Media	1,67± 0,25ª	0,11± 0,03c	65,8± 9,3a	1,78± 0,32ab	13,2± 3,3a	8,36± 1,58b	52± 6b	222± 102ª	308± 60a	n.d
	9	Mediana	1,97	0,08	58,6	1,62	9,47	6,83	49	118	253	n.d
		Rango	0,01-2,48	0,01-0,20	23,0-117	0,02-3,08	0,02-28,9	0,02-14,9	23-79	17-978	n.d-579	n.d
Potito carne		Media	0,16± 0,04b	1,03± 0,22ab	9,7± 3,9bc	0,23± 0,02c	0,81± 0,06c	2,78± 0,06bc	49± 14b	180± 30ª	41± 5c	n.d
	10	Mediana	0,14	1,04	2,4	0,24	0,81	2,81	31	138	39	n.d
		Rango	0,04-0,41	0,16-2,09	1,1-34,3	0,14-0,36	0,57-1,06	2,47-3,08	19-144	76-355	n.d-73	n.d
Potito pescado		Media	0,16± 0,04b	0,86± 0,27b	2,9± 1,0c	0,31± 0,07c	1,14± 0,13bc	1,81±0,03c	117± 25a	106± 9ª	68± 14bc	n.d
	4	Mediana	0,16	0,84	2,7	0,30	1,14	1,79	119	100	72	n.d
		Rango	0,06-0,27	0,19-1,54	0-6,6	0,12-0,51	0,78-1,52	1,72-1,91	50-181	85-139	n.d-103	n.d
Otros		Media	1,53± 0,32ª	1,47± 0,32ª	38,4± 16,5ab	2,61± 0,78a	2,61± 0,70bc	25,3± 6,8a	124± 30a	99± 36ª	172± 63b	n.d
	4	Mediana	1,71	1,63	34,6	3,36	2,75	31,0	137	65	119	n.d
		Rango	0,53-2,16	0,48-2,15	3,7-80,6	0,03-3,70	0,63-4,30	2,97-36,2	34-189	49-218	73-378	n.d

[a] La misma letra dentro de una columna indica que no eran significativamente diferentes con un p<0,001

[b] n.d.= no detectado por debajo del límite de detección (LD)

Tabla 11. Valores de micronutrientes en los productos infantiles analizados de diferentes países.

El calcio se encontró de forma mayoritaria en cereales con gluten, otros productos y cereales sin gluten con valores 1,67±0,25, 1,53±0,32 y 1,17±0,26 g/kg no presentando

diferencias significativas entre los tres grupos. Si tenemos en cuenta que en la **Figura 5,** los mayores valores de plomo detectados fueron en estos productos, se podría deducir que la absorción del mismo se verá reducida por la presencia de este nutriente esencial.

En cuanto al cinc, los mayores valores se observaron también en los productos a base de cereales con gluten y cereales sin gluten con valores 8,36±1,58 y 6,99±0,72 mg/kg respectivamente. El dato que llama la atención es el de "otros productos analizados", en los cuales se encontraron valores tan altos como 25,3±6,8 mg/kg; siendo significativamente diferentes al resto de muestras.

El selenio es el único mineral con un efecto sobre los cuatro elementos toxicos de estudio, entre ellos aumenta la eliminación del As-i por facilitar su metilación en el organismo. Los productos que más selenio presentaron fueron "otros productos analizados" con un valor medio de 124±30 µg/kg, aunque no presentaron diferencias significativas con los potitos a base de pescado que presentaron un valor medio de 117±25 µg/kg.

En cuanto a los valores de hierro, también se observaron los valores más altos en los productos a base de cereales sin gluten 47,7±9,2 mg/kg y con gluten 65,8±9.3 mg/kg, así como en "otros productos analizados" que presentaron una concentración media de 38,4±16 mg/kg. Sin embargo, para relacionar el efecto del hierro con la absorción del plomo conviene determinar si el consumo de los productos analizados cubre las necesidades en cada etapa.

5. CONCLUSIONES

5-CONCLUSIONES

1. El arsénico y plomo son los contaminantes con mayor presencia en los alimentos infantiles; siendo el arsénico el elemento traza que presenta los valores más altos en los alimentos analizados.
2. En los productos a base de cereales más del 60 % del As-t se encuentra en la forma más toxica (As-i).
3. El alto contenido en As en los productos a base de cereales se relaciona de forma directa con el arroz.
4. La variabilidad del contenido de arsénico en arroz viene determinada por el origen y procesado del mismo. Por tanto, la vía de reducción de la exposición al arsénico es una rigurosa y selectiva elección de materias primas en la producción de alimentos infantiles a base de arroz.
5. La celiaquía supone una dependencia de por vida al arroz, convirtiéndose en un grupo poblacional de riesgo, principalmente por la exposición al arsénico. Es necesario realizar un estudio en profundidad sobre la exposición en diferentes etapas de la vida de este grupo poblacional, teniendo en cuenta los productos comercializados de forma específica para este grupo en diferentes mercados mundiales.
6. El contenido en minerales influye en la absorción, metabolismo y eliminación de metales pesados y metaloides, reduciendo el efecto producido por los mismos. Se debe por tanto, tener en cuenta el contenido de los mismos en la formulación de los alimentos infantiles, como medio de reducción de los efectos tóxicos de dicho metales y metaloides en el organismo. Para ello conviene realizar más estudios sobre biodisponibilidad y comportamiento en dicho productos así como en este grupo poblaciones.
7. El selenio es el único mineral con un efecto protector en los cuatro metales y metaloides a estudio. Afortunadamente, la concentración del mismo en los productos analizados es alta. Sin embargo, para establecer las relaciones directas sobre los cuatro elementos tóxicos y en definitiva establecer el riesgo de exposición, se debe realizar estudios tanto *in vitro* como *in vivo* en este grupo poblacional.

6. BIBLIOGRAFÍA

6-BIBLIOGRAFÍA

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