Universidad de Granada

Facultad de Medicina

Departamento de Medicina Legal, Toxicología

y Antropología Física

DESARROLLO NEUROPSICOLÓGICO Y TRASTORNOS DE LA CONDUCTA EN POBLACIÓN INFANTIL POR EXPOSICIÓN A METALES PESADOS

Memoria presentada por

MIGUEL RODRÍGUEZ BARRANCO

para optar al grado de Doctor por la Universidad de Granada

Granada, Febrero 2015

Editorial: Universidad de Granada. Tesis Doctorales
ISBN: 978-84-9125-067-8
URI: http://hdl.handle.net/10481/39989

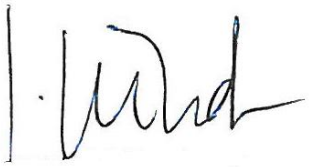
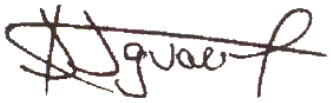
Universidad de Granada

Departamento de Medicina Legal, Toxicología y Antropología Física
Facultad de Medicina

El doctorando D. Miguel Rodríguez Barranco y los directores de la tesis Dña. Marina Lacasaña Navarro, D. Fernando Gil Hernández y D. Juan Alguacil Ojeda, garantizamos, al firmar esta tesis doctoral, que el trabajo ha sido realizado por el doctorando bajo la dirección de los directores de la tesis y hasta donde nuestro conocimiento alcanza, en la realización del trabajo, se han respetado los derechos de otros autores a ser citados, cuando se han utilizado sus resultados o publicaciones.

Granada a 03 de noviembre de 2014.

Director/es de la Tesis

Fdo.: Marina Lacasaña Fdo.: Fernando Gil Fdo.: Juan Alguacil

Doctorando

Fdo.: Miguel Rodríguez Barranco

Esta tesis doctoral ha sido desarrollada en el marco del proyecto de investigación financiado por la Consejería de Salud de la Junta de Andalucía titulado "Exposición a metales pesados y arsénico y su asociación con el desarrollo neuroconductual y trastornos de la conducta en población infantil residente en Huelva" (Exp: PI0755/2010), cuya investigadora principal es la Dra. Marina Lacasaña Navarro.

Parte de los resultados de esta investigación han sido publicados en tres artículos científicos en revistas indexadas con factor de impacto, los cuales constituyen esta Memoria de Tesis Doctoral como compendio de publicaciones.

La referencia completa y datos bibliométricos de las publicaciones se detallan a continuación:

Rodríguez-Barranco M, Lacasaña M, Aguilar-Garduño C, Alguacil J, Gil F, González-Alzaga B, Rojas-García A. Association of arsenic, cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: a systematic review and meta-analysis. Sci Total Environ. 2013 Jun 1;454-455:562-77. Review. PMID: 23570911
Factor de impacto 2013 (JCR Science Edition): 3.163.
Posición 40 de 215 (Q1, D2). Campo de conocimiento: *Environmental Sciences*

Rodríguez-Barranco M, Lacasaña M, Gil F, Lorca A, Alguacil J, Rohlman DS, González-Alzaga B, Molina-Villaba I, Mendoza R, Aguilar-Garduño C. Cadmium exposure and neuropsychological development in school children in southwestern Spain. Environ Res. 2014 Jul 18;134C:66-73. doi: 10.1016/j.envres.2014.06.026. PMID: 25046814

Factor de impacto 2013 (JCR Science Edition): 3.951.
Posición 15 de 160 (Q1, D1). Campo de conocimiento: *Public, Environmental & Occupational Health*

Rodríguez-Barranco M, Gil F, Hernández A, Alguacil J, Lorca A, Mendoza R, Molina-Villaba I, González-Alzaga B, Aguilar-Garduño C, Rohlman DS, Lacasaña M. Postnatal arsenic exposure and attention impairment in Spanish school children. CORTEX. 2014 [in press]

Factor de impacto 2013 (JCR Science Edition): 6.042.
Posición 4 de 49 (Q1, D1). Campo de conocimiento: *Behavioral Sciences*

AGRADECIMIENTOS

Cuando se llega a la meta de un largo y tortuoso camino es inevitable echar la vista atrás y recordar a todas aquellas personas que han estado cerca durante ese trayecto y han contribuido de una u otra forma a llevar a buen fin la realización de este proyecto académico y personal. Es por ello que quiero mostrar mi más sincero agradecimiento a las personas que me han acompañado en esta aventura:

En primer lugar a Marina Lacasaña, no sólo por su excelente labor como coordinadora de los proyectos en los que he participado y como directora de esta Tesis, sino también por el apoyo personal y la motivación que me ha ofrecido en todo momento para animarme a continuar. Ella es la principal responsable de que iniciara esta andadura y la que más ha contribuido a su desarrollo y culminación, así que quiero darle las gracias especialmente.

A Fernando Gil y Juan Alguacil, co-directores de esta Tesis, por haber puesto al servicio de esta investigación su enorme experiencia y conocimiento en sus respectivos campos profesionales, lo cual ha enriquecido sin duda la calidad de este trabajo.

A Andrés Lorca, Ramón Mendoza, Beatriz González, Clemente Aguilar y Antonio Hernández, por sus valiosas aportaciones a los artículos científicos que componen esta Tesis, y por el excelente trabajo realizado en el proyecto de investigación en el que se enmarca.

A Diane S. Rohlman por la cesión del software informático y el asesoramiento para la aplicación de las pruebas del BARS, que han resultado imprescindibles para llevar a cabo esta investigación, así como por su aportación en las publicaciones.

A mis compañeros y compañeras de la Escuela Andaluza de Salud Pública (EASP) que me han ayudado con el tortuoso proceso burocrático, y a la Dirección de la EASP por su apoyo financiero.

A mis padres, por el gran esfuerzo que os supuso ayudarme a cumplir mis sueños y alcanzar mis metas, apoyándome y alentándome a continuar en todo momento, y por todos los valores que me habéis legado, de los que me siento muy orgulloso.

A mis hermanos, Daniel y Cristina, porque a pesar de la distancia os siento cerca y sé que compartís conmigo mis fracasos y mis éxitos. Os quiero.

Y un especial agradecimiento a mi esposa, Rosa, que me ha apoyado emocionalmente en todo este camino, soportando mis altibajos, sufriendo pacientemente mis ausencias por las interminables jornadas de trabajo, animándome en las momentos más difíciles y confiando plenamente en mí. Simplemente por estar ahí, a mi lado,… gracias.

Y por último, como no agradecer a los niños y niñas participantes en el estudio, a sus familias y al personal docente de los centros educativos colaboradores, sin los cuales esta investigación nunca hubiera sido posible.

Muchas gracias a todos.

A mi hija Elsa

"El problema con el mundo es que los estúpidos están seguros de todo y los inteligentes están llenos de dudas".

Bertrand Russell
Filósofo y Matemático

ÍNDICE

ABREVIATURAS

ATSDR*: Agency for Toxic Substances and Disease Registry*

BARS: *Behavioral Assessment and Research System*

CBCL*: Child Behaviour Checklist*

CIE: Cociente Intelectual Ejecutivo

CIT: Cociente Intelectual Total

CIV: Cociente Intelectual Verbal

CPT: *Continuous Performance Test*

FFQ: *Food Frequency Questionnaire*

IC 95%: intervalo de confianza al 95%

IMC: Índice De Masa Corporal

ISI: *Inter-Stimulus Interval*

MDI*: Mental Development Index*

MG: media geométrica

RTT: *Reaction Time Test*

SAT: *Selective Attention Test*

STROBE*: Strengthening the Reporting of Observational Studies in Epidemiology Checklist*

TDAH: Trastorno de Déficit de Atención e Hiperactividad

TRF: *Teacher's Report Form*

WHO: *World Health Organization*

WISC-IV: *Wechsler Intelligence Scale for Children, version IV*

RESUMEN

Durante las últimas décadas ha aumentado la preocupación acerca del riesgo para la salud que representa la exposición a metales pesados debido a su potencial efecto neurotóxico. Los niños y niñas son especialmente vulnerables frente a la exposición a tóxicos ambientales ya que presentan diferencias muy marcadas con respecto a los adultos en relación a su exposición.

Entre todos los metales pesados, el plomo (Pb) y el mercurio (Hg) han sido los más estudiados y sus efectos tóxicos los más difundidos. Sin embargo la evidencia de los efectos tóxicos de otros metales como el cadmio (Cd) o el manganeso (Mn) y metaloides como el arsénico (As) han sido menos evaluados en humanos, y la evidencia de sus efectos neurotóxicos deriva principalmente de estudios en animales.

En Andalucía, hasta el momento, prácticamente no se han realizado estudios epidemiológicos que evalúen el potencial efecto nocivo de la exposición a metales pesados sobre la salud de la población infantil. Sólo un estudio hasta la fecha ha evaluado la asociación entre la exposición a mercurio total y la función cognitiva en población infantil de Granada.

Los objetivos de este estudio fueron, por un lado, recopilar y sintetizar la evidencia científica publicada hasta la fecha acerca del efecto de la exposición a cadmio, manganeso y arsénico sobre el desarrollo neuropsicológico y trastornos de la conducta en niños y niñas. Por otro lado, se pretendía evaluar, a través en una investigación original, el efecto de la exposición a cadmio y arsénico en el desarrollo neuropsicológico y trastornos de la conducta en niños y niñas entre 6 y 9 años residentes en municipios colindantes a la Ría de Huelva.

La Ría de Huelva es un complejo sistema de canales de drenaje que separan varias zonas de las marismas que pertenece a un área caracterizada durante décadas por una alta actividad industrial y minera. La Ría de Huelva tiene altos niveles de elementos metálicos como Zn, Cu, Pb, As, Hg y Cd a partir de tres fuentes principales de contaminación: los vertidos industriales de más de cuarenta industrias químicas cercanas; las aguas residuales urbanas de la ciudad de Huelva; y las aportaciones fluviales de los ríos Odiel y Tinto, que presentan aguas ácidas y altos niveles de elementos metálicos.

Los resultados de esta investigación muestran una asociación negativa entre la exposición posnatal a Cd con el desarrollo

neuropsicológico y entre la exposición posnatal a As con deterioro de la función de atención, incluso a niveles de exposición por debajo de los límites considerados de riesgo. Estos déficits durante la etapa escolar pueden afectar a las interacciones con los compañeros y profesores, así como ser determinantes en la trayectoria de resultados escolares. Por otra parte, el deterioro de la atención está relacionado directamente con un mayor riesgo de desarrollar trastornos neuroconductuales tales como trastorno de déficit de atención e hiperactividad (TDHA).

Estos resultados proporcionan evidencia adicional sobre el efecto neurotóxico de la exposición postnatal a dosis bajas a Cd y As en la población infantil, y apoyan las diferencias de género en la neurotoxicidad de ciertos metales que se sugieren en otros estudios.

Sin embargo, debido a la escasa evidencia científica disponible hasta la fecha, se necesitan más investigaciones que permitan confirmar los posibles efectos neurotóxicos de estos elementos metálicos sobre la población infantil.

CAPÍTULO I

INTRODUCCIÓN

I. INTRODUCCIÓN

I.1. Metales pesados y exposición infantil

Los efectos neurológicos agudos y crónicos asociados con la exposición laboral a diferentes neurotóxicos como los metales pesados han sido estudiados en numerosas investigaciones. Sin embargo, en la actualidad el problema más inquietante desde el punto de vista de la salud pública lo representa la exposición a bajas dosis de mezclas de contaminantes en poblaciones no laboralmente expuestas, especialmente mujeres embarazadas y niños/as que residen en zonas industriales, a través de la exposición por la inhalación de contaminantes procedentes de inmisiones industriales, así como por el consumo de agua y alimentos contaminados.

Durante las últimas décadas ha aumentado enormemente la preocupación acerca del riesgo para la salud que representa la exposición a metales pesados tales como el Pb, el Hg, el Cd y el Mn, así como a otros elementos como el As, debido a su potencial efecto neurotóxico[1] y a su capacidad acumulativa en los órganos diana[2]. De las más de 1000 sustancias químicas de las que se conoce que tienen efectos neurotóxicos

en experimentos con animales, el Pb, el metilmercurio (Me-Hg) y el As pertenecen a los 12 compuestos de los que se ha demostrado que producen trastornos en el neurodesarrollo en humanos y disfunción cerebral subclínica[3]. Grandjean y Landrigan sugieren que la exposición continuada a estos compuestos neurotóxicos podría estar creando una "epidemia silenciosa" en la sociedad moderna, siendo la responsable de un descenso subclínico permanente en el cociente intelectual, que tendría como consecuencia un mayor fracaso escolar, una disminución de la productividad económica y un mayor riesgo de comportamientos criminales y antisociales[3,4]. El carácter global de esta pandemia implicaría un impacto enorme sobre la salud pública. Sólo en EE.UU. se estima que se pierden 23.285.000 puntos de cociente intelectual total (CIT) al año debido a la exposición ambiental a Pb y Me-Hg en la población infantil menor de 5 años de edad[5].

Los niños y niñas son especialmente susceptibles frente a la exposición a tóxicos ambientales ya que presentan diferencias muy marcadas con respecto a los adultos en relación a su exposición[6]. En primer lugar, los niños se caracterizan por presentar mecanismos de desintoxicación inmaduros, por lo que son más sensibles que los adultos a

los efectos de estas sustancias. Su especial vulnerabilidad a este tipo de riesgo está también relacionada con otras características: físicas (elevada relación superficie-volumen, etapas críticas de crecimiento y desarrollo), alimenticias (beben más volumen de agua y comen más alimentos por unidad de peso corporal que los adultos) y conductuales (contacto directo con el suelo y otras superficies, facilidad para llevar todo tipo de objetos a la boca, etc.), así como por su expectativa de vida. Este hecho ha convertido a la población infantil en un grupo prioritario de estudio en relación a la exposición a contaminantes ambientales[7].

El prolongado calendario de la maduración neurológica implica un amplio periodo de vulnerabilidad biológica que comienza en el primer mes post-concepcional y continua a través de la gestación, la infancia y la adolescencia. El sistema nervioso central consta de diferentes áreas responsables de campos funcionales específicos (p.ej. control motor, función sensorial, inteligencia, etc.). Estas áreas se desarrollan de forma secuencial pero interdependiente, de manera que la interferencia en cualquiera de las fases o procesos de su maduración puede afectar a etapas posteriores del desarrollo[8]. Los dominios cognitivos que pueden verse afectados por la acción de los metales pesados son numerosos y

dependen de cada compuesto específico, si bien algunos de esos dominios tienen en común como potenciales inhibidores a muchos de los metales pesados como potenciales inhibidores: déficits auditivos, déficits visuales, déficits motores, déficits de la memoria y comportamientos externalizantes[9].

Los niños se encuentran expuestos a metales pesados desde el periodo fetal, por la propia exposición de la madre durante el embarazo, así como por la movilización de los distintos compuestos tóxicos desde los tejidos maternos durante el embarazo, y en etapas posteriores como la lactancia, a través de la leche materna. Durante la infancia y pre-adolescencia la exposición continúa a través de la ingesta de agua y alimentos, la inhalación y/o la absorción dérmica.

Entre todos los metales pesados, el Pb y el Hg han sido los más estudiados y sus efectos tóxicos los más difundidos[10,11]. Sin embargo la evidencia de los efectos tóxicos de otros metales como el Cd o el Mn y metaloides como el As han sido menos evaluados en humanos[1,12], y la evidencia de sus efectos neurotóxicos deriva principalmente de estudios en animales[13-16].

I.3. Cadmio

El cadmio (Cd) es un elemento presente en la naturaleza con concentraciones que varían entre 0,1 y 5 ppm, que suele aparecer asociado al zinc (Zn) y, en menor medida, al Pb y al cobre (Cu). No obstante, está considerado como el séptimo elemento potencialmente más peligroso para la salud humana en el medio ambiente, y ocupa el tercer puesto en esa clasificación dentro de los metales pesados después del Pb y el Hg[17].

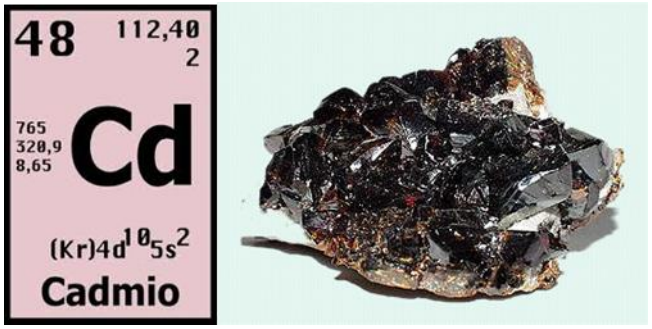


Figura 1. Aspecto y propiedades químicas y físicas del cadmio.

Más del 80% de la producción de Cd se destina a la fabricación de baterías de níquel-cadmio, aunque también se utiliza como pigmento para plásticos y en la industria cerámica y del cristal. Se estima que la producción mundial de Cd en el año 2010 ascendió a 22.000 toneladas, si

bien las reservas mundiales de este metal alcanzaron las 660.000 toneladas. Los mayores productores mundiales de Cd son China y la República de Corea, que generan entre ambas el 40% del total[18].

Las principales fuentes de exposición a Cd en niños son los alimentos, el humo del tabaco y el polvo doméstico. La placenta actúa como una barrera para el Cd y muy pequeña cantidad (<10%) se transfiere al feto[19] y la leche humana[20]. Por lo tanto, el feto y el recién nacido estarían protegidos contra el Cd. Sin embargo, la exposición al Cd se inicia a una edad muy temprana a través de las tres fuentes anteriormente citadas[21]. Si bien el Cd en la sangre revela la exposición más reciente, el Cd en la orina refleja la carga corporal y es un indicador de la exposición acumulada a largo plazo [22].

La exposición a Cd puede alcanzar niveles altos en las zonas cercanas a las industrias emisoras[23,24], excediendo los valores ambientales de seguridad recomendados por la Organización Mundial de la Salud[25,26].

Tanto los experimentos llevados a cabo en animales, como estudios epidemiológicos, han demostrado que el Cd es tóxico para los pulmones, los riñones, el hígado, el sistema digestivo, el tejido óseo y las gónadas,

puede causar cáncer, y resulta neurotóxico[21]. Aunque hay evidencia de los efectos neurotóxicos del Cd procedentes de estudios experimentales en animales[13,27,28], pocos estudios han evaluado el efecto de la exposición al Cd en el desarrollo neuropsicológico de los niños, y sus resultados son en su mayoría poco concluyentes[12].

Aun así, algunos estudios han observado asociación significativa entre la exposición prenatal a Cd y el desarrollo neuropsicológico en la infancia. En un estudio de cohorte prospectiva se encontraron menores puntuaciones en el cociente intelectual total (CIT) y en el cociente intelectual ejecutivo (CIE) a los 4 años de edad en los niños que tuvieron niveles más altos de Cd en la sangre del cordón umbilical en el momento del nacimiento[29]. En otro estudio se observó un descenso significativo en el CIT a los 5 años de edad de -0.8 puntos al duplicar los niveles de Cd en la orina materna durante el embarazo, siendo esta asociación más fuerte en las niñas que en niños[30].

Sin embargo existen menos evidencias de la asociación entre la exposición postnatal a Cd y el neurodesarrollo infantil. La mayoría de los estudios que han evaluado la exposición postnatal a Cd no han observado una asociación significativa con el desarrollo neuropsicológico en

niños[21,31,32], mientras que solo un estudio realizado en Estados Unidos observó una asociación significativa entre la exposición posnatal a este compuesto y problemas de aprendizaje en niños entre 6 y 15 años[33].

Respecto a la relación con trastornos de la conducta, en un estudio transversal desarrollado en China, se observaron mayores problemas de sociabilidad y atención asociados a niveles más altos de Cd en pelo de niños entre 7 y 16 años[24]. Sin embargo, en otro estudio caso-control apareado desarrollado en los Emiratos Árabes Unidos no se encontró asociación significativa entre la exposición a Cd y TDAH en niños de 5 a 15 años[34].

I.4. Manganeso

El manganeso (Mn) es un elemento muy común en el medioambiente, siendo el quinto metal pesado y el duodécimo elemento más abundante de la tierra, y está presente en la naturaleza en sus formas inorgánicas y orgánicas. En la industria es enormemente empleado en la producción de hierro y acero y en los procesos de fundición

(ferromangánica). Además, el óxido de Mn es ampliamente empleado como fertilizante y en la industria de la cerámica[35].

La mayor parte del Mn en sangre se une a los eritrocitos[36]. Es un nutriente esencial para el organismo, interviene en la formación de tejidos y en la formación de hueso, así como en el metabolismo lipídico y de carbohidratos y está implicado en el sistema inmune, habiéndose relacionado con la prevención del cáncer. No obstante, dependiendo de la ruta y la dosis de exposición, se acumula en el organismo, especialmente en el cerebro, ocasionando daños neurológicos[16,35].

Figura 2. Aspecto y propiedades químicas y físicas del manganeso.

El Mn está presente en la cadena alimenticia en todos los alimentos y en el agua de consumo, usualmente en concentraciones inferiores a 5 mg/Kg, y es detectable en prácticamente todas las muestras de partículas

en suspensión en el aire[37]. Asimismo, el humo del tabaco también presenta pequeñas concentraciones de Mn, por lo que podría ser una fuente de exposición a este metal, sobre todo para niños que residan en viviendas de fumadores[35]. Las recomendaciones de la Organización Mundial de la Salud establecen un nivel máximo de Mn en agua potable de 400 μg/l[26] y según la *Agency for Toxic Substances and Disease Registry* (ATSDR), los niveles tolerables de Mn en sangre oscilan entre 4 y 15 μg/l en adultos[35].

La mayoría de los artículos que han evaluado efectos sobre el neurodesarrollo por exposición a Mn han observado una asociación negativa con el CIT y, en casi todos los casos, con el dominio verbal. Estas asociaciones han sido detectadas tanto en neonatos y niños de hasta 12 meses, como en la infancia más avanzada[12].

Algunos estudios han observado déficits en el *Mental Development Index* (MDI), problemas de memoria y menor habilidad manual asociados a exposiciones más altas de Mn en neonatos y niños menores de 1 año[38,39]. Otros estudios han demostrado una asociación negativa entre la exposición a Mn y la inteligencia en niños de entre 6 y 13 años[32,40-45].

En relación a trastornos de la conducta, varios estudios han observado un mayor riesgo de TDAH asociado a la exposición a Mn[34,46,47], así como mayor riesgo de comportamientos internalizantes y externalizantes[48,49].

I.5. Arsénico

El arsénico (As) es un metaloide que se encuentra presente en el medio ambiente a concentraciones bajas de modo natural, siendo el elemento quincuagésimo segundo en abundancia de la corteza terrestre, en la que se encuentra en un promedio de 2 ppm. Concentraciones más elevadas pueden hallarse en zonas con actividad volcánica o depósitos geológicos de minerales de azufre. No obstante, existe una fuente de emisión de As más importante de origen antropogénico, procedente de actividades tales como la minería, la fundición, el uso de plaguicidas, el uso de conservantes para madera, la combustión de carbón o la incineración de residuos[50]. La ATSDR señala al As como la primera de las 275 sustancias potencialmente más peligrosas para la salud humana presentes en el medio ambiente, en base a su abundancia, su toxicidad y

por los potenciales niveles de exposición que puede alcanzar en humanos[17].

Se estima que la producción mundial de As ascendió a las 54,500 toneladas en el año 2010, siendo China el principal productor con casi la mitad de la producción, seguido de Chile con un 21% del total. Las reservas mundiales de este compuesto se estima que son 20 veces la producción mundial en un año[18].

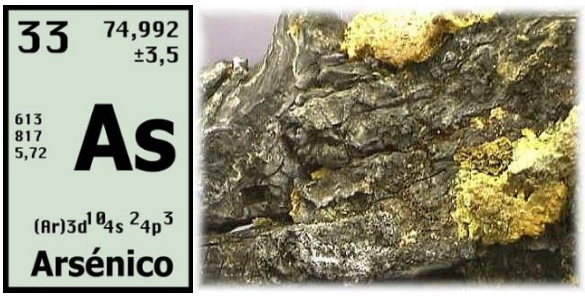


Figura 3. Aspecto y propiedades químicas y físicas del manganeso.

El As se categoriza como orgánico e inorgánico, dependiendo de la existencia o no de un enlace de carbono, y puede encontrarse en uno de sus tres estados de oxidación, -3, +3 y +5, siendo la forma trivalente la de mayor toxicidad. Las formas inorgánicas del arsénico son, en general, más

tóxicas que las orgánicas, siendo las responsables de la mayoría de casos de intoxicación por As en humanos[51].

No obstante, aunque los compuestos orgánicos de As se consideran menos tóxicos que los inorgánicos, algunas formas orgánicas como el monometil arsénico (MMA), el dimetil arsénico (DMA) han demostrado tener efectos negativos sobre la salud en animales de experimentación[51] y se ha llegado a observar asociación entre estos compuestos y el neurodesarrollo infantil[52].

Las principales vías de exposición a As inorgánico son la ingestión de agua, así como la inhalación de aire y polvo contaminados, mientras que las formas orgánicas del As se encuentran principalmente en pescados y mariscos en forma de arsenobetaina y arsenocolina, siendo generalmente atóxicas[50]. Tras la ingestión, tanto las formas orgánicas como las inorgánicas son absorbidas al torrente sanguíneo desde el tracto gastrointestinal aproximadamente en un 60-90%[53]. En el proceso metabólico del As primero la forma inorgánica pentavalente (arseniato) cambia a la forma trivalente (arsenito), y ésta sufre un proceso de metilación en el hígado dando lugar a las formas orgánicas MMA y DMA[54].

Tanto las formas orgánicas resultantes como las formas inorgánicas no metiladas son expulsadas a través de la orina.

Las consecuencias sobre la salud de la exposición a As incluyen efectos respiratorios, gastrointestinales, hematológicos, hepáticos, renales, dérmicos, neurológicos e inmunológicos, además de tener efectos perjudiciales sobre el sistema nervioso central y el desarrollo cognitivo en los niños[52]. Según las recomendaciones de la ATSDR, los niveles normales de As total en humanos no deberían superar los 100 μg/l en orina y 1 μg/g en pelo[51].

Cada vez hay más evidencia de que la exposición al As tiene un efecto negativo sobre la función intelectual de los niños. Varios estudios han demostrado una relación inversa entre la exposición al As y el CIT[32,44,55-60], el CIV o la comprensión verbal[32,44,55-57,61], el CIE[45,57,59], la función motora[62], así como la memoria[32,44,63]. En un reciente meta-análisis se ha estimado que un aumento del 50% en los niveles de As en el agua potable se asocia con una disminución significativa de -0,6 puntos en el CIT y -0,3 puntos en el CIE en niños entre 5-15 años[12].

Por el contrario, pocos estudios han evaluado la relación entre la exposición a As y los trastornos de la conducta, y sus resultados son poco concluyentes[12]. En un reciente estudio caso-control apareado desarrollado en los Emiratos Árabes Unidos, los autores no encontraron diferencias significativas en los niveles de As en sangre entre casos diagnosticados de TDAH y controles sanos entre 5 y 15 años de edad[34]. En otros dos estudios transversales desarrollados en Bangladesh y México no se observaron diferencias significativas entre el As en orina y los trastornos de conducta en niños de 6 a 11 años[48,64]. Sin embargo, existen evidencias de un efecto negativo en el comportamiento por la exposición laboral a altas dosis de As en población adulta[53,65], y estudios en animales han demostrado que la exposición a As inorgánico durante el desarrollo se asocia con problemas de conducta[66,67].

I.6. La Ría de Huelva

La Ría de Huelva, es un ecosistema natural situado en el suroeste de Andalucía, en la provincia de Huelva, que abarca una extensión aproximada de 150 km^2.

Está constituida por un complejo sistema de canales de drenaje que separan varias zonas de las marismas. Este ecosistema es controlado por el régimen de mareas y por la afluencia de los ríos Tinto y Odiel, así como por medio de dos canales que intercambian agua directamente con el mar abierto. La Ría de Huelva tiene altos niveles de elementos metálicos como Zn, Cu, Pb, As, Hg y Cd a partir de tres fuentes principales de contaminación: los vertidos industriales de más de cuarenta industrias químicas cercanas, las aguas residuales urbanas de la ciudad de Huelva y las aportaciones fluviales de los ríos Odiel y Tinto, que tienen aguas ácidas y altos niveles de elementos metálicos[23].

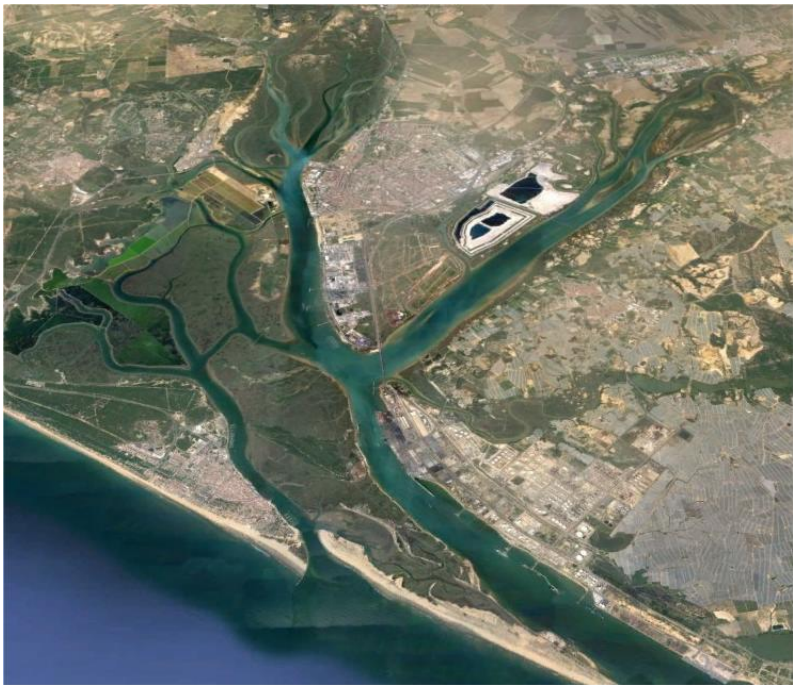


Figura 4. Vista aérea de la zona de la Ría de Huelva.

El área que abarca la Ría de Huelva es considerada una de las regiones industriales más importantes de Europa, y también una de las regiones fluviales más contaminadas del mundo debido a la actividad industrial (que incluye, por ejemplo, una planta de fertilizantes, una fundición de cobre, una papelera, una fábrica de cemento, plantas termoeléctricas, plantas petroquímicas, y diferentes industrias químicas) y a la antigua actividad minera de la zona[68].

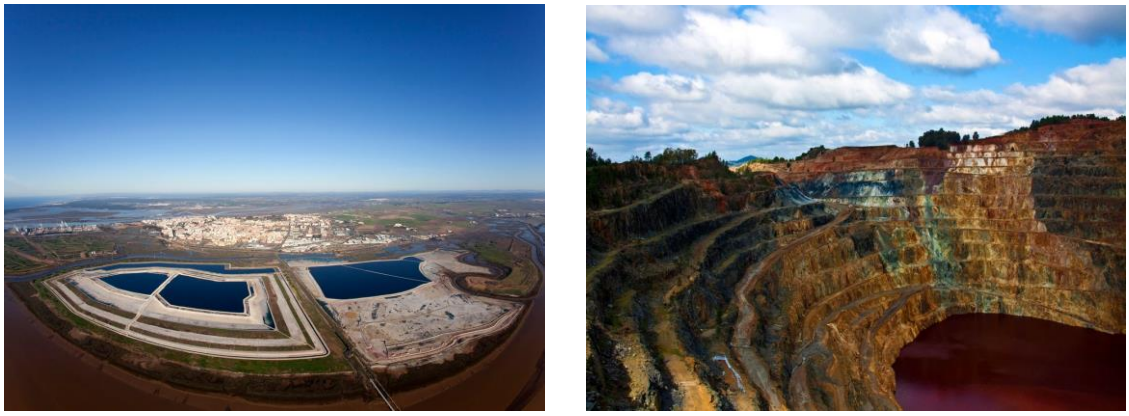


Figura 5. Balsas de residuos industriales de fosfoyesos y explotación minera en la zona de la Ría de Huelva.

La presencia de metales pesados y metaloides, ha sido cuantificada en la zona, y se ha descrito una correlación significativa entre los niveles de Pb y Hg en los sedimentos y en distintas especies de moluscos consumidas habitualmente por la población local, así como la presencia en

el aire de mayores niveles de partículas (PM_{10}) que contienen As y Pb en Huelva en comparación con otras ciudades de España[68]. Los niveles de Cd en las emisiones industriales medidos en la proximidad de la fábrica de fundición de cobre en la zona de la Ría de Huelva fueron 6,71 ng/m^3 en agosto de 2005, por encima del umbral de seguridad recomendado por la Organización Mundial de la Salud[25] de 5 ng/m^3, aunque la concentración media diaria en el aire del centro de la ciudad entre 2001 y 2008 fue inferior a 1 ng/m^3 [(69)].

Figura 6. Algunas de las instalaciones industriales presentes en la zona de estudio.

Los niveles ambientales de As medidos en sedimentos de la Ría de Huelva fueron de media 179 μg/g en 2006, variando entre 36 y 405 μg/g[23]. Los valores ambientales promedio entre 2001 y 2008 medidos en aire en

la proximidad de la fábrica de fundición de cobre fueron superiores al límite fijado por la Directiva Europea 2004/107/EC, que lo establece en 6 ng/m^3 desde enero de 2013, y los picos de concentración llegaron a alcanzar hasta los 326 ng/m^3 [69].

Así, la Ría de Huelva representa una zona de especial interés y preocupación desde el punto de vista de la salud pública por el potencial efecto negativo sobre la salud de estas exposiciones ambientales.

I.7. Justificación

En Andalucía, hasta el momento, prácticamente no se han realizado estudios epidemiológicos que evalúen el potencial efecto nocivo de la exposición a metales pesados sobre la salud de la población infantil. Sólo un estudio hasta la fecha ha evaluado la asociación entre los niveles de Hg total en pelo y la función cognitiva en población infantil de Granada[70]. Los únicos datos que se han reportado acerca de la exposición a metales pesados en población andaluza corresponden a los niveles de As presentes en orina, y de algunos otros metales pesados como Cd, cromo (Cr), Cu y níquel (Ni) de una muestra de adultos[68], y en una muestra de niños

residentes en la Ría de Huelva y en las capitales de provincia[71]. Los resultados de estos estudios muestran que la población adulta residente en Huelva presentaba, por ejemplo, niveles de As en orina más elevados que la población de otras capitales andaluzas en las que no existe la contaminación industrial que afecta a la Ría de Huelva.

En la presente memoria de Tesis Doctoral se pretende ahondar en el conocimiento de los potenciales efectos neurotóxicos de los metales pesados y metaloides con mayor presencia en la zona de estudio, que podrían afectar al desarrollo neuropsicológico y neuroconductual de la población infantil, por ser especialmente vulnerable a las exposiciones a tóxicos ambientales.

CAPÍTULOS II y III

HIPÓTESIS Y OBJETIVOS

II. HIPÓTESIS

La exposición postnatal a Cd, Mn y As tiene efectos neurotóxicos que afectan el desarrollo neuropsicológico infantil y aumenta el riesgo de desarrollar trastornos de la conducta.

III. OBJETIVOS

III.1. Objetivos generales

1. Recopilar y sintetizar la evidencia científica publicada hasta la fecha acerca del efecto de la exposición pre y postnatal a Cd, Mn y As sobre el desarrollo neuropsicológico y trastornos de la conducta en niños y niñas.

2. Evaluar el efecto de la exposición posnatal aguda y crónica a Cd, Mn y As en el desarrollo neuropsicológico y trastornos de la conducta en niños y niñas entre 6 y 9 años residentes en Huelva y municipios colindantes a la Ría de Huelva.

III.2. Objetivos específicos

1. Desarrollar una revisión sistemática de la literatura científica para recopilar y sintetizar los resultados de los estudios publicados hasta la fecha que relacionan la exposición pre y postnatal a Cd, Mn y As con desarrollo neuropsicológico y trastornos de la conducta en niños y niñas. Cuando la información disponible lo permita, se realizará también un meta-análisis de los resultados de los estudios individuales para obtener una estimación combinada del tamaño del efecto.

2. Describir la exposición posnatal aguda y crónica a metales pesados (Cd, Pb, Hg, Mn) y As en población infantil de 6 a 9 años a través de las concentraciones de estos en orina y pelo.

3. Evaluar la asociación entre las concentraciones de metales pesados (Cd, Pb, Hg, Mn) y As, y el desarrollo neuropsicológico y los trastornos de la conducta de la población infantil.

4. Evaluar las diferencias de género en la asociación entre la exposición aguda y crónica a metales pesados (Cd, Pb, Hg, Mn) y As, y los resultados de las pruebas de desarrollo neuropsicológico y trastornos de la conducta de los niños.

CAPÍTULO IV

METODOLOGÍA

IV. METODOLOGÍA

IV.1. Métodos del objetivo general 1

IV.1.1. Estrategia de búsqueda

Se realizó una búsqueda bibliográfica en las bases de datos médicas online PubMed, EMBASE, CINHAL, Lilacs y REPIDISCA, considerando como límites de búsqueda la fecha de publicación desde enero del 2000 hasta marzo del 2012, estudios en humanos y escritos en inglés, español, francés o italiano. La sintaxis de búsqueda empleada fue la siguiente:

(child* OR infant* OR school* OR postnatal OR prenatal OR post-natal OR pre-natal OR fetal OR pregnan*) AND (neurodevelopment* OR behavior OR behaviour OR mental OR intelligence OR cognitive OR "attention deficit disorder with hyperactivity" OR ADHD) AND ("cadmium"[MeSH Terms] OR "arsenic"[MeSH Terms] OR "manganese"[MeSH Terms])

IV.1.2. Criterios de inclusión

Se incluyeron en la revisión aquellos artículos que cumplían los siguientes criterios: (a) artículos originales; (b) evaluación de exposición

pre o post natal a arsénico (As), cadmio (Cd) o manganeso (Mn); (c) población de estudio hasta 16 años de edad; (d) estudio de los efectos sobre el desarrollo neuropsicológico o trastornos de la conducta derivados de la exposición a metales (Cd y Mn) y arsénico (As), incluyendo:

1) Desarrollo neuropsicológico: cociente intelectual o grado de desarrollo en las áreas motora, de comunicación, cognitiva, de atención y/o de memoria.

2) Trastornos de la conducta: TDAH, problemas de conducta oposicionista y desafiante, comportamientos internalizantes (ansiedad/depresión, antisociabilidad, etc.) y comportamientos externalizantes (romper reglas, agresividad, etc.).

IV.1.3. Criterios de exclusión

Se excluyeron los artículos basados en estudios de casos o series de casos, diseños ecológicos, revisiones bibliográficas y aquellos que evaluaron la exposición a As, Cd y Mn exclusivamente de manera indirecta a través de cuestionarios (consumo de tabaco de los padres, dieta de la madre durante el embarazo, dieta del niño, etc.).

IV.1.4. Evaluación de la calidad metodológica de los artículos

A falta de un instrumento validado actualmente para evaluar la calidad metodológica de estudios con diseño observacional, y dado que todos salvo uno de los artículos incluidos en la presente revisión presentaban diseños de este tipo, se utilizó el *Strengthening the Reporting of Observational Studies in Epidemiology Checklist* (STROBE)[72] para valorar la calidad metodológica de los estudios. Esta herramienta fue inicialmente desarrollada para evaluar la claridad en la comunicación de resultados de investigaciones con diseño observacional, y ha sido usada en recientes revisiones sistemáticas para evaluar la calidad metodológica de estudios observacionales[73,74].

De los 22 ítems que componen la lista STROBE se seleccionaron los 9 correspondientes al apartado de "*Methods*", que valoran diferentes aspectos de la metodología de un estudio observacional. Tras la evaluación, se realizó una clasificación de la calidad metodológica de la siguiente forma: los artículos que cumplían entre 0-3 ítems de los 9 se consideraron con calidad metodológica baja, entre 4-6 media y entre 7-9 alta.

La evaluación de la calidad metodológica de cada artículo se realizó por dos evaluadores independientes y las discrepancias fueron resueltas por un tercer evaluador.

IV.1.5. Meta-análisis

Se realizó un meta-análisis de los resultados publicados en los diferentes estudios con el objetivo de dar una estimación global y resumida de la magnitud del efecto de la exposición a As y Mn sobre el neurodesarrollo infantil. El meta-análisis se restringió a los estudios que evaluaron el cociente intelectual total (CIT), cociente intelectual verbal (CIV) y el cociente intelectual ejecutivo (CIE) mediante alguna de las versiones de la escala Wechsler de inteligencia y que utilizaron técnicas de regresión lineal para estimar el efecto.

No obstante, el hecho de que los artículos presentaran los resultados con diferentes enfoques analíticos, requirió un esfuerzo previo para homogeneizar la magnitud del efecto observada en cada estudio. Para solventar el inconveniente de las diferentes transformaciones en la variable independiente (logaritmo natural, logaritmo en base 10 o

ninguna), se recalculó cada efecto para expresarlo según un cambio relativo en la variable de exposición.

Con más detalle, en un modelo de regresión lineal donde la variable de exposición está transformada mediante logaritmo natural, $c \cdot \beta$ es el cambio en la variable respuesta por el cambio de c unidades logarítmicas en la variable de exposición (es decir, cuando $\ln(X_1)-\ln(X_0)=c$). Por otro lado, un cambio relativo en la variable de exposición original X es de la forma $X_1/X_0=k$, donde k representa el número de veces que aumenta X. Tomando logaritmos en esta expresión y aplicando sus propiedades se obtiene $\ln(X_1)-\ln(X_0)=\ln(k)$. Por tanto, enlazando con lo anterior, $\ln(k) \cdot \beta$ representa el cambio absoluto en la variable respuesta por un cambio relativo de k veces en la variable original. Análogamente, si la transformación es en base 10, $\log_{10}(k) \cdot \beta$ representa el cambio en la respuesta para el mismo cambio relativo k en la exposición. En el caso de variables no transformadas, se optó por considerar el cambio absoluto que se produciría en la variable respuesta por un cambio relativo igual a k en la media de la distribución de la variable de exposición, es decir, el efecto se calculó como $(k-1) \cdot E(X) \cdot \beta$.

De esta forma se consiguió expresar el efecto en todos los estudios para un mismo cambio relativo en la exposición. En el análisis en particular de esta investigación se optó por considerar *k*=1.5, lo que equivale a estudiar la variación absoluta en la respuesta cuando la exposición aumenta un 50%.

La heterogeneidad de los estudios se evaluó mediante el test de DerSimonian & Laird y el valor del coeficiente I^2, que representa el porcentaje de la variabilidad total atribuible a la heterogeneidad[75].

Para realizar el meta-análisis se empleó el paquete estadístico Stata 11 (StataCorp. LP, 2009, TX).

IV.2. Métodos del objetivo general 2

IV.2.1. Diseño y población de estudio

Se desarrolló un estudio transversal entre enero y marzo de 2012, en la zona de influencia de la Ría de Huelva, región en el suroeste de España (Huelva, Andalucía). Se seleccionaron aleatoriamente 13 escuelas de un total de 38 escuelas públicas de siete municipios colindantes con la zona de la Ría de Huelva (Aljaraque, Huelva, Palos de la Frontera, Punta Umbría, San Juan del Puerto, Tharsis y Valdelamusa).

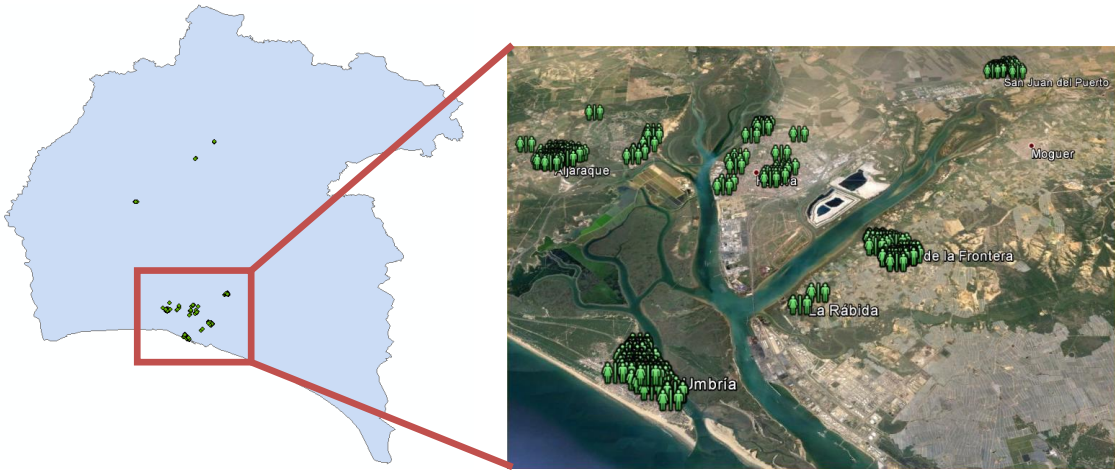


Figura 7. Distribución geográfica de la muestra participante.

Un total de 2.199 padres de los niños matriculados en las escuelas seleccionadas fueron invitados a participar en el estudio, y de ellos 315 aceptaron participar. Un subconjunto de 203 padres entre los que se negaron a participar completó un breve cuestionario de no participación.

Finalmente, una muestra de 261 niños de 6-9 años fue seleccionada mediante muestreo aleatorio simple entre los que cumplieron los criterios de inclusión y cuyos padres dieron su consentimiento informado firmado. Los criterios de inclusión contemplaron la residencia ininterrumpida en el área de estudio durante al menos un año, y que al menos uno de los progenitores o tutor hablara español con fluidez. Entre los criterios de exclusión se establecieron la existencia de antecedentes de problemas pre y perinatales, diabetes, trastornos neurológicos, trauma cerebral, cirugía bajo anestesia general, y enfermedad hepática o renal. Ninguno de los niños seleccionados tuvo que ser excluido del estudio por alguno de estos motivos.

El tamaño de muestra de 261 niños se calculó *a priori* para proporcionar una potencia del 80% a un nivel de significación del 0.05, a fin de detectar una disminución de - 4 puntos en el cociente intelectual

total (CIT) de la escala WISC por duplicar la exposición a Pb, basándose en los resultados observados por Tong et al. (1996)[76].

IV.2.2. Recogida de datos

IV.2.2.1. Toma, transporte y almacenamiento de muestras biológicas

El día antes de la evaluación neuropsicológica, se proporcionó a los niños un recipiente de polipropileno para recoger una muestra de orina de primera hora de la mañana. Antes de ser utilizado, todo el material de polipropileno fue limpiado por inmersión en una solución de HNO_3 al 10% (v/v) durante 24 h. Finalmente fueron enjuagados con varios lavados de agua Milli-Q® y se secaron.

Las muestras de orina fueron recogidas por el equipo de campo en las escuelas al comienzo de las clases al día siguiente. Las muestras fueron transportadas refrigeradas a -4° C hasta el laboratorio, y se almacenaron a -20° C hasta su análisis. El mismo día se recogió un mínimo de 100 mg de pelo del cuero cabelludo por personal previamente entrenado. Las muestras de pelo se cortaron de la parte posterior de la cabeza lo más

cerca posible al cuero cabelludo y se transportaron y almacenaron en bolsas de plástico completamente selladas hasta su análisis. El mismo día de la toma de muestra los niños fueron pesados y medidos para calcular el índice de masa corporal (IMC).

IV.2.2.2. Determinación de metales y metaloides

En el marco de esta investigación se analizaron las muestras biológicas para determinar las concentraciones de cuatro metales y un metaloide: manganeso (Mn), plomo (Pb), mercurio (Hg), cadmio (Cd) y arsénico (As).

Los niveles de Cd, Mn, Pb, Hg y As en las muestras de orina y pelo se determinaron mediante un espectrómetro de absorción atómica Perkin-Elmer Analyst 800 (Perkin Elmer, Norwalk, EE.UU.) equipado con cámara de grafito y muestreador automático AS-800, así como con corrector de fondo Zeeman y tubos de grafito con plataforma de L'vov integrada. Igualmente se empleó un sistema analítico de inyección de flujo (FIAS) acoplado a una generación de hidruros para la determinación de As y Hg. Se emplearon los modificadores de matriz apropiados para los elementos

metálicos estudiados[77,78]. Las muestras de referencia para el análisis de orina (ref. 201205) fueron suministradas por Seronorm (Billingstad, Noruega) y el material de referencia NIES No. 5 para el análisis del pelo se obtuvo del Instituto Nacional de Estudios Ambientales, Agencia Japonesa para el Medio Ambiente.

Las muestras de pelo se mantuvieron en recipientes de polipropileno, previamente lavados con ácido nítrico diluido. A continuación, se lavaron por enjuague abundante con agua Milli-Q y se lavó por ultrasonidos en una solución de etanol (Merck, Darmstadt, Alemania) y de nuevo con agua Milli-Q. El pelo limpio se secó durante la noche a temperatura ambiente. Las muestras de pelo se digirieron tras la adición de 1 ml de HNO_3 (Merck), 0.5 ml de HCl (Merck), 2 ml de H_2O_2 (Merck) y 2 ml de H_2O durante 30 min en un horno microondas Multiwave 3000 (Anton Parr, Graz, Austria)[78].

Siguiendo las recomendaciones de la IUPAC (*International Union of Pure and Applied Chemistry*), los procedimientos analíticos desarrollados para la determinación de compuestos de metales en orina y pelo fueron previamente validados y publicados por miembros de nuestro grupo de investigación[77,78], incluyendo el límite de detección (LOD) y cuantificación

(LOQ), rango lineal, precisión (mínimo, media y reproducibilidad), recuperación y la masa característica, entre otros parámetros. Los límites de detección para los metales estudiados se muestran en la tabla 1.

Tabla 1. Límite de detección de los metales analizados		
Elemento	***Orina***	***Pelo***
Arsénico	0,030 μg/l	0,0033 μg/g
Cadmio	0,030 μg/l	0,0033 μg/g
Manganeso	0,120 μg/l	0,0132 μg/g
Mercurio	0,002 μg/l	0,0002 μg/g
Plomo	0,830 μg/l	0,0913 μg/g

IV.2.2.3. Evaluación del desarrollo neuropsicológico y trastorno de atención

Se administró de forma individual la escala de inteligencia de **Wechsler para niños - cuarta edición (WISC-IV)**[79] para evaluar la función intelectual de éstos. Esta escala proporciona medidas de la capacidad intelectual general mediante el CIT, y para cuatro dominios cognitivos específicos:

- Comprensión verbal: que consta de los pruebas de semejanzas, vocabulario, comprensión e información.

- Razonamiento perceptivo: diseño con cubos, conceptos con dibujos, matrices y figuras incompletas.

- Memoria de trabajo: retención de dígitos, sucesión de números y letras y aritmética.

- Velocidad de procesamiento: claves, búsqueda de símbolos y registros.

En el caso de que cualquiera de las pruebas no pudiera completarse, fue reemplazada con un prueba equivalente según las directrices del manual[79]. Esta circunstancia sólo se produjo en cinco casos.

Adicionalmente, se aplicaron tres pruebas del **Sistema de Investigación y Evaluación del Comportamiento (BARS)**[80] para evaluar la función cognitiva atencional:

- Prueba de tiempo de reacción (RTT): en esta prueba se presenta un estímulo (un cuadrado) en la pantalla y se le pide al sujeto que presione un botón con su mano dominante lo más rápido posible

para que desaparezca. Se presentan un total de 50 ensayos. El intervalo inter-estímulo (ISI) es variable (entre 1 y 3 segundos), y, si el sujeto no emite la respuesta después de 3 segundos, desaparece de la pantalla la presentación del estímulo. Esta tarea está diseñada para medir la velocidad de respuesta a un estímulo visual[81]. Las medidas obtenidas en esta prueba son la latencia de respuesta en milisegundos y la latencia de respuesta excluyendo los ensayos con latencias superiores a 1000 milisegundos.

- Prueba de ejecución continua (CPT): mide la atención, y consiste en que los sujetos presionen un botón con su mano dominante tan rápido como les sea posible cuando se presenta en la pantalla un estímulo objetivo (círculo cerrado) precedido por un estímulo señal (signo más). Durante la prueba aparecen también una serie de distractores (flechas hacia abajo, triángulos, estrellas y hexágonos). Se presentaron un total de 300 estímulos, con un intervalo fijo de presentación de 0,05 segundos y un ISI de 1 segundo. A partir de esta prueba se calculó el porcentaje de falsas alarmas, el porcentaje de omisiones y la latencia de respuesta en milisegundos.

- Prueba de atención selectiva (SAT): en esta prueba se presentan dos cajas equidistantes en la pantalla, uno a la derecha de la pantalla y la otra a la izquierda. Al sujeto se le instruye para pulsar un botón cada vez que un pequeño punto aparezca dentro de la caja (con su mano derecha si aparece a la derecha y con su mano izquierda si aparece a la izquierda) y hacer caso omiso de los puntos que aparezcan fuera de las cajas. La tarea tiene una duración total de 600 segundos, donde el 80% de las presentaciones están dentro de la caja y el 20% lo están fuera. El ISI comienza inicialmente en 2 segundos y se actualiza en base a la respuesta de los participantes, disminuyendo en 0,1 segundos en el caso de acierto, y aumentando en 0,1 segundos en el caso de fallo. Esta tarea evalúa la atención selectiva y la atención o concentración sostenida[81]. A partir de esta prueba se calculó el porcentaje de falsas alarmas, el porcentaje de omisiones y la latencia de respuesta en milisegundos.

Figura 7. Aplicación de la prueba BARS.

Se aplicó además una prueba basada en realidad virtual para la **evaluación de la capacidad de atención (AULA)**[82]. AULA se basa en el paradigma de otras pruebas de ejecución continua, pero se realiza en un entorno de realidad virtual que se visualiza por medio de un conjunto especial de gafas de realidad virtual con sensores de movimiento. El escenario es similar a un aula de la escuela secundaria o primaria, y la perspectiva (es decir, lo que el participante ve) sitúa al niño sentado en uno de los pupitres mirando a la pizarra. AULA fue desarrollado con el objetivo de ser utilizado como una ayuda para el diagnóstico clínico del TDAH, basado en dos modelos de atención derivados del estudio de TDAH[83,84]. AULA presenta un conjunto de estímulos visuales y auditivos, en

presencia o ausencia de distractores, y está compuesto por dos ejercicios principales:

- Tarea No-X: en esta prueba los sujetos deben presionar el botón cuando no ven o escuchan el estímulo diana (por ejemplo, cuando no oyen o ven en la pizarra "manzana"). Esta tarea genera una sobre-estimulación que deriva en respuestas rápidas, inexactas e inadecuadas, por lo que proporciona una medida de la impulsividad.

- Tarea X: en este caso los sujetos deben presionar el botón cuando ven o escuchan el estímulo diana (por ejemplo cuando oyen o ven en la pizarra "siete"). Esta tarea conduce a una hipo-activación y por lo tanto tiende a reducir la velocidad y dar respuestas variables e ineficientes, por lo que proporciona una medida de inatención.

Para cada tarea, se obtienen varias medidas:

a) Errores de omisión: esta medida está relacionada con la atención selectiva y focalizada, y se produce cuando el niño no pulsa el botón cuando debería hacerlo.

b) Errores de comisión: esta medida está relacionada con la falta de control motora o falta de inhibición de la respuesta, y se produce cuando el niño presiona el botón cuando no debería de hacerlo.

c) Tiempo de reacción: es el tiempo necesario para responder a un estímulo, y tiende a ser mayor en las personas con déficit de atención, ya que tienden a procesar la información más lentamente.

d) Actividad motora: gracias a los sensores de movimiento colocados en el dispositivo de realidad virtual, los movimientos de la cabeza del usuario son capturados para registrar su frecuencia y relevancia (es decir, si los movimientos son necesarios o innecesarios).

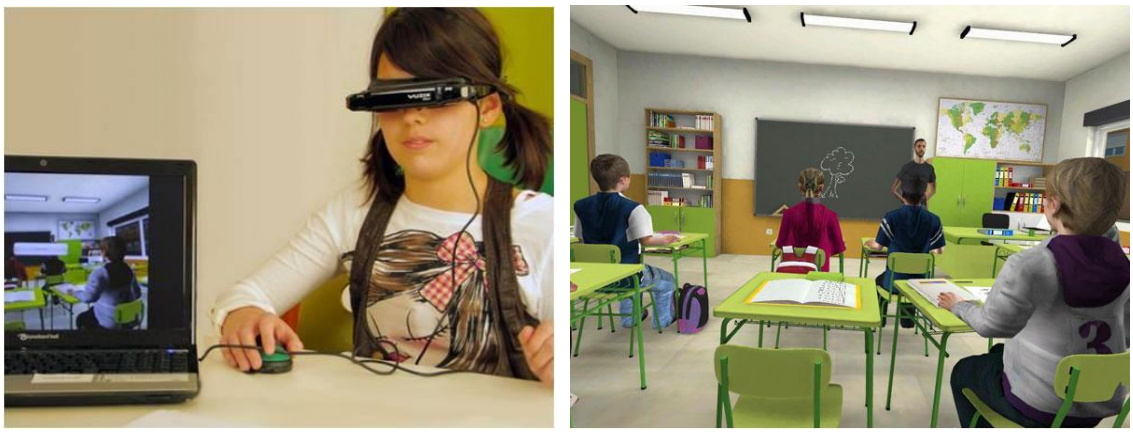


Figura 8. Aplicación de la prueba AULA en un entorno de realidad virtual.

Estas medidas se calculan tanto para la tarea No-X como para la tarea X, y se expresan como cantidades tipificadas que oscilan entre 20 y 80 puntos. Una puntuación alta significa peores resultados en la tarea y, por lo tanto, más deterioro de la atención.

El equipo de evaluación neuropsicológica lo componían un coordinador (neuropsicólogo) y tres psicólogas previamente entrenadas. Aparte de unas pocas excepciones, todas las pruebas se realizaron en el mismo día, con un descanso de 45 minutos entre la escala de la inteligencia y las pruebas informatizadas. Las pruebas de inteligencia fueron administradas por dos psicólogas diferentes y los tests informatizados por una tercera. La escala de inteligencia de cada niño (WISC-IV) fue administrada por la misma psicóloga, siguiendo los estándares descritos en el manual de administración, lo que garantiza la unidad de criterios para la recolección de los datos[79].

Además de las pruebas aplicadas directamente a los niños, se emplearon dos escalas que evalúan características del comportamiento a través de cuestionarios: el ***Child Behavior Checklist*** **(CBCL)**[85] y el ***Teacher's Report Form*** **(TRF)**[86].

El CBCL es una escala de 120 ítems que permite evaluar problemas emocionales y del comportamiento de los niños entre 6 y 18 años. En ella se recogen datos de una variedad de actitudes y comportamientos que los padres o tutores legales puntúan como 0 – Nunca; 1 – Algunas veces cierto; 2 – Cierto muy a menudo. La información recogida se refiere a los últimos 6 meses. Las puntuaciones a las distintas preguntas son combinadas para crear medidas o *scores* que reflejan posibles problemas emocionales y de conducta, como son: ansiedad/depresión, aislamiento, quejas somáticas, problemas sociales, problemas de pensamiento, problemas de atención, conductas de ruptura de normas y conductas agresivas.

Las puntuaciones directas de cada escala se obtienen mediante la sumatoria de las puntuaciones 0-1-2 otorgadas a cada ítem, y se transforman en puntuaciones T en función del sexo y la edad. Las puntuaciones T pueden clasificarse de acuerdo a unos puntos de corte establecidos que delimitan el rango normal de variación y el rango patológico. Cifras superiores a 70 puntos (correspondiente al percentil 98) se consideran en el rango clínico patológico, y entre 65 y 69 puntos (percentil 93) se situarían en el rango subclínico.

El TRF, por su parte, es un formulario que rellenan los profesores o educadores que tienen contacto habitual con los niños. Al igual que en el CBCL, se recoge información de diversos aspectos del comportamiento y actitudes de los niños y se combina la información en *scores* que permiten identificar 6 síndromes distintos: problemas afectivos, de ansiedad, somáticos, trastorno de déficit de atención e hiperactividad (TDAH), problemas de negativismo desafiante y problemas de conducta. Las puntuaciones directas son igualmente transformadas en puntuaciones T que pueden clasificarse en el rango normal, subclínico y clínico del síndrome en función de los percentiles 93 y 98.

IV.2.2.4. Información recogida a través de cuestionarios

Dos cuestionarios (uno autoadministrado y otro mediante entrevista telefónica) fueron completados por la madre, padre o tutor para obtener información sobre las características demográficas y socioeconómicas, exposiciones ambientales y domésticas, la historia ocupacional de los padres, información sobre el parto, antropometría al nacer y lactancia , asi como el tiempo diario que los niños dedicaban a

diversas actividades (dormir, jugar, hacer deporte, con el ordenador o la consola y estudiar).

Se determinó la exposición ocupacional materna y paterna a metales pesados y arsénico durante el embarazo y después del nacimiento a través de la matriz de empleo-exposición para la investigación y vigilancia de la salud y seguridad ocupacional en los trabajadores de España (MatEmESp)[87]. Para lograr esto, se codificó la historia ocupacional según la Clasificación Nacional de Ocupaciones del Instituto Nacional de Estadística, y se vinculó con la matriz de exposición laboral. A partir de esta matriz se pudo evaluar la exposición a As, Cd, Cr, Fe, Ni y Pb.

La dieta de los niños se evaluó mediante un cuestionario validado semi-cuantitativo de frecuencia de consumo de alimentos (FFQ)[88]. A partir del FFQ se calculó el número de porciones o raciones por semana de cada grupo de alimentos (lácteos; huevos, carnes y pescados; verduras y legumbres; frutas; pan, cereales y similares; y aceites, grasas y dulces).

IV.2.2.5. Cociente intelectual de la madre y calidad del contexto familiar

Una psicóloga visitó cada familia para cuantificar el cociente intelectual de la madre mediante la prueba breve de inteligencia de Kaufman[89], y para evaluar la calidad del contexto familiar usando una versión adaptada de la escala Etxadi-Gangoiti[90,91]. En este estudio la escala Etxadi-Gangoiti fue adaptada para ser administrada a niños de 6 a 10 años de edad; el coeficiente de fiabilidad alpha de Cronbach para la escala adaptada fue de 0,72. La escala Etxadi-Gangoiti es una breve escala para evaluar la calidad del contexto familiar que incluye ítems actualizados del *Home Observation for Measurement of the Environment Inventory* (HOME)[92] y la *Developmental History Scale*[93].

IV.2.5. Análisis estadístico

Las concentraciones de compuestos en las muestras de orina y pelo se describieron mediante medias geométricas e intervalos de confianza al 95% (IC95%). A los valores de metales o As por debajo del límite de detección se les asignó el LOD dividido por la raíz cuadrada de 2. Las puntuaciones de las pruebas de desarrollo neuropsicológico y atención

fueron descritas por medio de la media aritmética y la desviación estándar. El IMC se calculó como el peso (kg) dividido por la altura (m) al cuadrado, y se clasificó en cuatro grupos de acuerdo con las tablas de crecimiento según sexo y edad referidas a la población española[94]: bajo peso (percentil 3 o inferior), normal (P3 a P85), sobrepeso (P85 a P95) y obesidad (P95 o superior). Se calculó el promedio de horas por día dedicado a diversas actividades ponderando el número de horas dedicadas a la actividad en los días entre semana y en fin de semana.

Las diferencias entre niños y niñas se evaluaron mediante la prueba de Mann-Whitney para las variables de exposición (con distribución no normal según la prueba de Shapiro-Wilk) y con la prueba de *t* de Student para las variables respuesta (con distribución normal según la prueba de Shapiro-Wilk). Las diferencias entre participantes y no participantes en las características demográficas y socioeconómicas fueron contrastadas por medio de la prueba del Chi-cuadrado. Se utilizaron las pruebas de Mann-Whitney o Kruskal-Wallis para evaluar diferencias en los niveles de exposición entre grupos cuando la variable categórica presentaba dos o más de dos categorías respectivamente.

Se utilizaron modelos de regresión lineal multivariante para evaluar el efecto neurotóxico de la exposición a los elementos estudiados, tomando como variables dependientes los resultados de las pruebas neuropsicológicas y de atención, y como variables independientes la concentración de los metales y As en orina y pelo. Las variables de exposición fueron transformadas mediante logaritmo en base 2 con el fin de suavizar sus distribuciones asimétricas. Por lo tanto, los coeficientes de regresión se interpretan como el cambio en la variable dependiente asociado con duplicar los niveles del compuesto (o un aumento del 100%).

En los modelos de regresión multivariante se controló por potenciales confusores, considerando inicialmente como candidatos el sexo y la edad del niño; el índice de masa corporal (IMC); la edad, índice de inteligencia, educación y ocupación de la madre; la educación y ocupación del padre; ingresos mensuales familiares; el área de residencia (urbana, metropolitana o rural); cercanía de la vivienda a áreas mineras, industriales o campos de cultivo; la exposición ocupacional materna y paterna a metales pesados durante el embarazo y después del nacimiento; la situación familiar (vive con los padres biológicos u otra situación); edad gestacional, peso, altura y circunferencia de la cabeza al

nacer; alimentación con leche materna; consumo de alcohol y tabaco durante el embarazo; presencia de mascotas en el hogar; frecuencia de limpieza; consumo de tabaco en el hogar; agua habitual de consumo; frecuencia de consumo de alimentos; promedio de horas diarias dedicadas a dormir, juegos activos, deporte, ordenador o consolas y a estudiar; evaluador del WISC-IV; y la escala de evaluación de la calidad del contexto familiar.

Los modelos multivariantes fueron construidos en tres pasos: en un primer paso, se introdujeron como covariables fijas el sexo, la edad y nivel de creatinina en orina; en un segundo, se incluyeron las variables asociadas a la exposición o el resultado ($p < 0,20$ en el análisis bivariado) en los modelos como potenciales factores de confusión; en una tercera etapa, se desarrolló un procedimiento hacia atrás (*backward*) con el criterio de $p > 0,10$ en el test de Wald para eliminar del modelo las covariables no relevantes.

Adicionalmente, se incluyeron en los modelos finales términos de interacción multiplicativa entre los niveles de exposición y el sexo a fin de evaluar el posible papel del sexo como modificador del efecto sobre el

neurodesarrollo y problemas de atención. El efecto para cada sexo se derivó de estos modelos de interacción.

Todos los análisis estadísticos fueron desarrollados con el paquete de software estadístico Stata 11 (Stata Corp. LP 2009, TX).

CAPÍTULO V

PRINCIPALES RESULTADOS

V. PRINCIPALES RESULTADOS

V.1. **Publicación A:** revisión sistemática y meta-análisis

Los resultados de la revisión sistemática y el meta-análisis asociado han sido publicados en el artículo científico nº 1 de esta memoria de Tesis Doctoral[12].

De los 156 artículos derivados inicialmente de la búsqueda bibliográfica, 41 cumplían finalmente los criterios de inclusión: 18 evaluaron los efectos del As, 6 los del Cd y 17 los del Mn, sobre el neurodesarrollo y trastornos de la conducta.

La mayoría de los artículos tenían un diseño epidemiológico transversal (78% para evaluar efectos neurotóxicos del As, 50% del Cd y 76% del Mn). La medida de exposición se realizó mayoritariamente mediante la determinación de biomarcadores de exposición en orina y agua de bebida en el caso del As, y en pelo y sangre para el Cd y Mn. El 76% de los estudios evaluó el efecto de estos compuestos en el neurodesarrollo y el 24% sobre trastornos de la conducta.

La mayoría de estudios que evaluaron exposición a As (13 de 15) y de los que evaluaron exposición a Mn (9 de 12) encontraron un efecto negativo significativo sobre el desarrollo neuropsicológico infantil, casi todos ellos relacionado con la exposición posnatal. En contraste, ninguno de los estudios que evaluaron exposición posnatal a Cd observaron un efecto significativo sobre el neurodesarrollo, mientras que el único estudio que evaluó exposición prenatal encontró asociación con la capacidad intelectual. Los resultados del meta-análisis sugieren que un aumento del 50% en los niveles de As en orina del niño provocaría una disminución de -0.42 puntos (IC95%: -0.89 ; 0.05; p=0.077) en el CIT de los niños entre los 5 y los 15 años de edad. Esta disminución se observó igualmente en el CIV con -0.32 puntos (p=0.083), pero no fue significativa para el CIE (theta=-0.08; p=0.330). Asimismo, un aumento del 50% en los niveles de As en el agua de consumo habitual de los niños provocaría una disminución significativa (p=0.044) de -0.57 (IC95%: -1.12 ; -0.01) en el CIT en ese mismo rango de edad. En el CIE la reducción alcanzaría los -0.33 puntos (p=0.083), mientras que en el CIV no se observó una disminución estadísticamente significativa (theta=-0.07; p=0.429).

En relación al Mn, el efecto combinado indica que por cada aumento del 50% en los niveles de Mn en pelo, se produce una disminución significativa ($p<0.001$) de -0.72 puntos (IC95%: -1.08 ; -0.35) en el CIT de los niños entre los 6 y los 13 años de edad. Esta disminución fue superior en el CIV, ascendiendo a -1.17 puntos ($p=0.002$), y alcanzó los -0.44 puntos en el CIE ($p=0.036$).

Ninguno de los 3 estudios que evaluaron la asociación entre la exposición posnatal a As total y trastornos de la conducta encontraron un efecto significativo. En el caso del Cd sólo uno de los dos estudios publicados hasta la fecha habían observado relación entre la exposición posnatal a Cd con problemas de atención y problemas de sociabilidad. Por el contrario, todos los estudios (5 en total) que evaluaron exposición posnatal a Mn encontraron un efecto sobre trastornos de la conducta, incluyendo comportamientos internalizantes y externalizantes, trastorno disruptivo, hiperactividad y TDAH.

V.2. Publicación B: exposición a Cd y desarrollo neuropsicológico

En el artículo nº 2 de la memoria de esta Tesis Doctoral se han publicado los resultados de la asociación entre la exposición actual a Cd y el desarrollo neuropsicológico del estudio transversal desarrollado por el equipo de investigación en el área de la Ría de Huelva[95].

Los resultados mostraron una asociación negativa significativa entre los niveles de Cd en orina y las puntuaciones del WISC-IV, después de ajustar por posibles factores de confusión. En la muestra total, duplicar los niveles de Cd en orina de los niños se asoció con 1,2 puntos menos en el CIT (IC95%: -2,49 ; 0,03), que afectaba en mayor medida a la comprensión verbal (β= -1,8; IC95%: -3,2 ; -0,4). En cambio, no se observaron asociaciones significativas entre los niveles de Cd en orina y las medidas obtenidas a partir del BARS. Los niveles de Cd en pelo no se asociaron con ninguno de los resultados del WISC-IV o del BARS.

No obstante, esta asociación significativa inversa entre los niveles de Cd en orina y las puntuaciones del WISC-IV se observó entre los niños, pero no entre las niñas. En los niños, el CIT disminuyó dos puntos (IC95%: -3,8 ; - 0,4) al duplicar los niveles de Cd en orina, mientras que en las niñas

dicha asociación no fue estadísticamente significativa (IC95%: - 2,1 ; 1,0). Por otra parte, los niños tuvieron peores puntuaciones en todos los dominios medidos por WISC-IV por el aumento de los niveles de Cd en orina, mientras que en las niñas no fueron observados estos efectos. Sólo el tamaño del efecto sobre la comprensión verbal fue similar en ambos sexos, siendo estadísticamente significativo en los niños y casi alcanzó la significación estadística en las niñas. Los dominios más afectadas en los niños por la exposición a Cd fueron la comprensión verbal (β = -2,0; IC95%: - 4,0 ; - 0,1) y el razonamiento perceptivo (β = - 1,8; IC95%: - 3,7 ; 0,1), observándose en este último caso la asociación cerca de la significación estadística (p = 0,06).

V.3. Publicación C: exposición a As y problemas de atención

En el artículo nº 3 de la memoria de esta Tesis Doctoral se han publicado los resultados de la asociación entre la exposición a As y problemas de atención del estudio transversal desarrollado por el equipo de investigación en el área de la Ría de Huelva[96].

Los resultados de este análisis mostraron una asociación significativa entre los niveles As en orina y medidas de atención derivadas de las pruebas BARS y AULA. Tras ajustar por posibles factores de confusión, la duplicación de los niveles de As en orina se asoció con un exceso en la latencia de respuesta de 3,6 ms (IC95%: 0,4 ; 6,8) en el SAT. Además, el mismo cambio en la exposición se asoció con aumentos del 12,3 ms (IC95%: 3,5 ; 21,1) en la latencia de RTT, que se reducen a 5,5 ms (IC95%: 1,3 ; 9,8), cuando se excluían los ensayos con latencias mayores de 1000 ms. En relación con la prueba de AULA, una duplicación de los niveles de As en orina se asoció con más errores de omisión tanto en la tarea X, que refleja la impulsividad, (β = 0,6; IC95%: 0,1 ; 1,1) como en la tarea No-X, que refleja falta de atención, (β = 0,5; IC95%: 0,03 ; 1,0).

Los resultados del CBCL y el TRF para evaluar problemas de atención y TDAH respectivamente no fueron significativos, ni utilizando la escala como variable continua ni en función de los puntos de corte de criterio diagnóstico.

No se obtuvieron resultados significativos en los términos de interacción entre As en orina y sexo para ninguno de los resultados evaluados, por lo que este efecto puede considerarse similar en niños y en niñas.

CAPÍTULO VI

DISCUSIÓN

VI. DISCUSIÓN

La exposición a contaminantes ambientales se ha convertido en un problema de salud pública acuciante en nuestra sociedad actual. Cada vez estamos expuestos a un mayor número de tóxicos, con una mayor intensidad y durante un mayor tiempo a lo largo de nuestras vidas. Estas exposiciones crónicas comienzan desde antes del nacimiento y continúan de forma acumulativa hasta el final de la vida. Las autoridades sanitarias y los organismos de vigilancia epidemiológica se esfuerzan en proponer niveles de referencia por encima de los cuales se considera que existen efectos negativos sobre la salud pública. Sin embargo, estos niveles de referencia han demostrado no ser en determinados casos niveles de seguridad, ya que se han encontrado efectos negativos sobre la salud humana por debajo de estos límites.

Determinar los efectos a largo plazo sobre la salud de la exposición continuada a tóxicos ambientales a dosis bajas es sumamente complejo de abordar. Es necesario desarrollar estudios de seguimiento durante años y sobre un gran número de sujetos para observar estos posibles efectos, lo que los hace altamente costosos. Sin embargo, es necesario que los

organismos encargados de velar por la salud pública hagan todos los esfuerzos necesarios para dar respuesta a una creciente preocupación de la población sobre el potencial riesgo sobre su salud de la contaminación ambiental. Además de los estudios epidemiológicos, los programas de biomonitorización humana destacan actualmente como las herramientas más efectivas para monitorizar la exposición de la población a todo tipo de contaminantes ambientales y evaluar sus principales determinantes.

Los estudios epidemiológicos desarrollados sobre grupos de población vulnerables, como los niños, las mujeres embarazadas o los ancianos, son de especial relevancia dado que los efectos sobre la salud pueden resultar en estos casos especialmente importantes e irreversibles.

En el desarrollo de la revisión sistemática y el meta-análisis llevados a cabo en esta investigación hemos encontrado evidencia del efecto neurotóxico de metales pesados como el Mn y metaloides como el As que afectarían al desarrollo cognitivo de los niños y a su capacidad intelectual. Sin embargo pocos estudios habían abordado el efecto neurotóxico del Cd en población infantil. En relación a los trastornos de la conducta la evidencia era aún más escasa respecto al Cd y el As, en contraste con un

número significativo de estudios que habían observado asociación de la exposición a Mn con problemas conductuales[12].

La investigación llevada a cabo sobre la población infantil de nuestro estudio residente en las proximidades de la zona industrial de la Ría de Huelva demostró un efecto adverso de la exposición a Cd sobre el desarrollo neuropsicológico, aportando evidencia adicional al potencial efecto neurotóxico de este metal pesado sobre la población infantil[95]. La exposición a As también resultó significativamente asociada con problemas de atención en los niños de la zona de estudio, cuando tan sólo un estudio hasta la fecha había observado una relación con trastornos de la conducta[64].

En ambos casos, los niveles de exposición a los que se observó este efecto adverso estuvieron por debajo de aquellos considerados de riesgo. La Comisión para la Biomonitorización Humana de la Agencia Federal Alemana de Medioambiente establece los límites por encima de los cuales existe riesgo para la salud de población infantil en 15 μg/l y 2 μg/l de As y Cd en orina respectivamente[97]. Ningún participante en el estudio excedió estos valores de referencia, por lo tanto el efecto sobre el neurodesarrollo psicológico y trastornos de la conducta observados en este estudio se

producen a niveles de exposición teóricamente considerados como seguros.

Algunos estudios han apuntado a diferencias de género o sexo en los efectos sobre la salud de los tóxicos ambientales, y se sabe que ciertos metales pueden actuar de manera diferente en niños y niñas debido a la diferencia en el patrón de la exposición, el metabolismo o la susceptibilidad individual[98,99]. Los efectos neurotóxicos del Pb y el Hg parecen ser más pronunciados en los niños que en las niñas, pero no todos los estudios epidemiológicos encuentran este resultado[100]. Un hallazgo común también parece ser un mayor riesgo de déficit neuropsicológico en niñas que en niños por la exposición a As, si bien pocos estudios han evaluado de forma sistemática estas diferencias de género[101]. Por el contrario, no existen evidencias suficientes que sugieran una modificación del efecto relacionada con el género para otros metales pesados tales como el Mn o el Cd.

En la presente investigación se observó un efecto negativo de la exposición a Cd sobre el desarrollo neuropsicológico que afectaba más a los niños que a las niñas. Por el contrario, en el análisis que se desarrolló para evaluar la exposición a As no se observaron diferencias significativas

entre sexos en el efecto sobre problemas de atención, por lo que esta relación se producía con la misma intensidad en niños y niñas.

Existen evidencias biológicas de que tanto la exposición a Cd como a As pueden alterar el desarrollo neurológico en la corta infancia, influyendo sobre las capacidades cognitivas y el comportamiento. Los experimentos con animales han demostrado que el Cd afecta el metabolismo del cerebro, inhibiendo las enzimas que contienen grupos sulfhidrilo. Por lo tanto, la exposición crónica al Cd tiene un efecto depresor sobre los niveles de varios neurotransmisores tales como la noradrenalina, la serotonina y la acetilcolina[102,103]. Los estudios en animales también han confirmado que el Cd puede atravesar la barrera hematoencefálica[104], lo que refuerza la hipótesis de que la barrera hematoencefálica no impide que el Cd llegue al cerebro durante las etapas precoces del desarrollo de los niños[105]. Esta evidencia sugiere que el Cd alcanza el sistema nervioso central directamente, provocando así un efecto neurotóxico en el desarrollo del niño[21,106]. Otros estudios han demostrado el papel de Cd como disruptor endocrino, que actúa sobre los estrógenos, hormonas tiroideas y de crecimiento, todas ellas con un importante papel en el desarrollo del cerebro[107,108]. Esta interacción entre el Cd con las diversas

hormonas podría también explicar las diferencias entre sexos observadas en este estudio[99].

En relación al As hay poca evidencia sobre el mecanismo mediante el cual puede afectar el desarrollo neuroconductual en los niños. Uno de los posibles mecanismos involucrados se relaciona con un aumento del estrés oxidativo, lo que provoca daños en el ADN. Aunque el As no participa directamente en las reacciones redox, puede inducir el estrés oxidativo a través del agotamiento de glutatión reducido (GSH) y la inhibición de enzimas que contienen grupos tiol. Además, el As incide sobre la regulación de varios genes antioxidantes, incluyendo la superóxido dismutasa (SOD) y la tiorredoxina (Trx-1), lo que sugiere mecanismos compensatorios para superar los efectos de subproductos oxidativos de la toxicidad del As sobre las enzimas que contienen tiol relacionados con el metabolismo del arsénico[109]. Por otro lado, los animales experimentales tratados con As han mostrado alteraciones en la función del hipocampo, la morfología y de señalización, lo que lleva a un comportamiento cognitivo alterado[101]. Tanto las exposiciones a As durante el desarrollo como en adultos han demostrado un déficit en la neurogénesis adulta en el hipocampo a través de una reducción de la

proliferación de las células progenitoras neurales y del número de neuronas maduras[110]. El As también afecta a los receptores de NMDA en el hipocampo, que desempeñan un papel esencial en la plasticidad sináptica. El deterioro resultante de las neuronas del hipocampo puede tener un impacto en el aprendizaje y la memoria a largo plazo, y conduce a trastornos neuroconductuales y disfunciones cognitivas[14,15]. Otras alteraciones en el cerebro por la neurotoxicidad del As incluyen alteración de glutamatérgico, colinérgico y señalización monoaminérgica y la alteración de la señalización del receptor de glucocorticoides, responsable de la desregulación del eje hipotálamo-hipófisis-suprarrenal[101].

Esta investigación presenta algunas limitaciones. La principal es que, al tratarse de un estudio transversal, no permite establecer causalidad. Sin embargo la dirección más plausible de la asociación es la secuencia exposición – efecto, ya que es poco creíble que los niños con menor cociente intelectual modifiquen voluntariamente su exposición, más aun cuando estas exposiciones son en su mayor porcentaje involuntarias. Además, una sola medición de la carga corporal actual no permite evaluar las exposiciones pasadas. Una mejor comprensión de la relación exposición-efecto puede obtenerse a través de estudios prospectivos

siguiendo los niños desde el periodo fetal hasta la infancia y realizando mediciones de los niveles de metales y los resultados de las pruebas de neurodesarrollo a intervalos regulares de tiempo.

A su vez, uno de los puntos fuertes de este estudio es que todos los niños seleccionados proporcionan la muestra de orina, y las pruebas neuropsicológicas directas fueron completadas por entre el 95% y el 99% de los sujetos, por lo que se puede descartar un posible sesgo de no respuesta que influyese en los resultados observados.

En resumen, nuestros resultados muestran una asociación negativa entre la exposición posnatal a Cd con el desarrollo neuropsicológico y entre la exposición posnatal a As con deterioro de la función de atención, que podrían tener consecuencias importantes en la vida posterior de los niños. Estos déficits durante la etapa escolar pueden afectar a las interacciones con los compañeros y profesores, así como ser determinantes en la trayectoria de resultados escolares. Por otra parte, el deterioro de la atención está relacionado directamente con un mayor riesgo de desarrollar trastornos neuroconductuales tales como TDAH.

Nuestros resultados proporcionan evidencia adicional sobre el efecto neurotóxico de la exposición postnatal a dosis bajas a Cd y As en la población infantil, y apoyan las diferencias de género en la neurotoxicidad de ciertos metales que se sugieren en otros estudios.

Sin embargo, debido a la escasa evidencia científica disponible hasta la fecha, se necesitan más investigaciones que permitan confirmar los posibles efectos neurotóxicos de estos elementos metálicos sobre la población infantil.

CAPÍTULO VII

CONCLUSIONES

VII. CONCLUSIONES

1. Existe evidencia en la literatura científica reciente que establece una asociación negativa entre la exposición posnatal a Mn con el desarrollo neuropsicológico y los trastornos de la conducta en población infantil.

2. La exposición posnatal a Cd se asoció con un déficit en el desarrollo neuropsicológico de la población infantil residente en el área de influencia de la Ría de Huelva, siendo este efecto de mayor intensidad en los niños que en las niñas.

3. La exposición posnatal a As se asoció con déficits de atención en la población infantil residente en el área de influencia de la Ría de Huelva, afectando con la misma intensidad a los niños y a las niñas.

CAPÍTULO VIII

BIBLIOGRAFÍA

VIII. BIBLIOGRAFÍA

1. Counter SA, Buchanan LH. Mercury exposure in children: a review. Toxicol Appl Pharmacol. 2004;198:209-30.

2. Gil F, Pla A. Biomarkers as biological indicators of xenobiotic exposure. J Appl Toxicol. 2001;21:245-55.

3. Grandjean P, Landrigan PJ. Neurobehavioural effects of developmental toxicity. Lancet Neurol. 2014;13:330-8.

4. Grandjean P, Landrigan PJ. Developmental neurotoxicity of industrial chemicals. Lancet. 2006;368:2167-78.

5. Bellinger DC. Comparing the population neurodevelopmental burdens associated with children's exposures to environmental chemicals and other risk factors. Neurotoxicology. 2012;33:641-3.

6. Landrigan PJ, Kimmel CA, Correa A, Eskenazi B. Children's health and the environment: public health issues and challenges for risk assessment. Environ Health Perspect. 2004;112:257-65.

7. Au WW. Susceptibility of children to environmental toxic substances. Int J Hyg Environ Health. 2002;205:501-3.

8. Rice D, Barone S, Jr. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect. 2000;108 Suppl 3:511-33.

9. Riccio C, Sullivan J, Cohen M. Neuropsychological assessment and intervention for childhood and adolescent disorders. Hoboken, New Jersey: John Wiley & Sons, Inc.; 2010.

10. Jakubowski M. Low-level environmental lead exposure and intellectual impairment in children--the current concepts of risk assessment. Int J Occup Med Environ Health. 2011;24:1-7.

11. Schoeman K, Bend JR, Hill J, Nash K, Koren G. Defining a lowest observable adverse effect hair concentrations of mercury for

neurodevelopmental effects of prenatal methylmercury exposure through maternal fish consumption: a systematic review. Ther Drug Monit. 2009;31:670-82.

12. Rodriguez-Barranco M, Lacasana M, Aguilar-Garduno C, Alguacil J, Gil F, Gonzalez-Alzaga B, et al. Association of arsenic, cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: a systematic review and meta-analysis. Sci Total Environ. 2013;454-455:562-77.

13. Shagirtha K, Muthumani M, Prabu SM. Melatonin abrogates cadmium induced oxidative stress related neurotoxicity in rats. Eur Rev Med Pharmacol Sci. 2011;15:1039-50.

14. Luo JH, Qiu ZQ, Shu WQ, Zhang YY, Zhang L, Chen JA. Effects of arsenic exposure from drinking water on spatial memory, ultra-structures and NMDAR gene expression of hippocampus in rats. Toxicol Lett. 2009;184:121-5.

15. Kruger K, Straub H, Hirner AV, Hippler J, Binding N, Musshoff U. Effects of monomethylarsonic and monomethylarsonous acid on evoked synaptic potentials in hippocampal slices of adult and young rats. Toxicol Appl Pharmacol. 2009;236:115-23.

16. Aschner M, Guilarte TR, Schneider JS, Zheng W. Manganese: recent advances in understanding its transport and neurotoxicity. Toxicol Appl Pharmacol. 2007;221:131-47.

17. ATSDR. Agency for Toxic Substances and Disease Registry, 2011. The Priority List of Hazardous Substances, 2011: Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services.; 2011 [cited 2014 Apr 14]. Available from: http://www.atsdr.cdc.gov/spl/.

18. U.S. Geological Survey, Mineral commodity summaries 2011. Reston, Virginia: U.S. Geological Survey; 2011.

19. Osman K, Akesson A, Berglund M, Bremme K, Schutz A, Ask K, et al. Toxic and essential elements in placentas of Swedish women. Clin Biochem. 2000;33:131-8.

20. Hallen IP, Jorhem L, Lagerkvist BJ, Oskarsson A. Lead and cadmium levels in human milk and blood. Sci Total Environ. 1995;166:149-55.

21. Cao Y, Chen A, Radcliffe J, Dietrich KN, Jones RL, Caldwell K, et al. Postnatal cadmium exposure, neurodevelopment, and blood pressure in children at 2, 5, and 7 years of age. Environ Health Perspect. 2009;117:1580-6.

22. Klotz K, Weistenhofer W, Drexler H. Determination of cadmium in biological samples. Met Ions Life Sci. 2013;11:85-98.

23. Blasco J, Gomes T, García-Barrera T, Rodríguez-Romero A, Gonzalez-Rey M, Morán-Roldán F, et al. Trace metal concentrations in sediments from the southwest of the Iberian Peninsula. Scientia Marina. 2010;74:99-106.

24. Bao QS, Lu CY, Song H, Wang M, Ling W, Chen WQ, et al. Behavioural development of school-aged children who live around a multi-metal sulphide mine in Guangdong province, China: a cross-sectional study. BMC Public Health. 2009;9:217.

25. WHO. Air quality guidelines for Europe, 2nd edition. Copenhagen: World Health Organization Regional Office for Europe; 2000 [cited 2014 Jul 14]. Available from: http://www.euro.who.int/__data/assets/pdf_file/0005/74732/E71922.pdf .

26. WHO. Guidelines for drinking-water quality. 3rd edition. Geneva, Switzerland: World Health Organization Regional Office for Europe; 2004 [cited 2014 July 14]. Available from: http://www.who.int/water_sanitation_health/dwq/gdwq3rev/en/.

27. Jin T, Lu J, Nordberg M. Toxicokinetics and biochemistry of cadmium with special emphasis on the role of metallothionein. Neurotoxicology. 1998;19:529-35.

28. ATSDR. Agency for Toxic Substances and Disease Registry, 2012. Toxicological Profile for Cadmium. Atlanta, GA: Department of Health and Human Services, Public Health Services.; 2012.

29. Tian LL, Zhao YC, Wang XC, Gu JL, Sun ZJ, Zhang YL, et al. Effects of gestational cadmium exposure on pregnancy outcome and development in the offspring at age 4.5 years. Biol Trace Elem Res. 2009;132:51-9.

30. Kippler M, Tofail F, Hamadani JD, Gardner RM, Grantham-McGregor SM, Bottai M, et al. Early-life cadmium exposure and child development in 5-year-old girls and boys: a cohort study in rural Bangladesh. Environ Health Perspect. 2012;120:1462-8.

31. Torrente M, Colomina MT, Domingo JL. Metal concentrations in hair and cognitive assessment in an adolescent population. Biol Trace Elem Res. 2005;104:215-21.

32. Wright RO, Amarasiriwardena C, Woolf AD, Jim R, Bellinger DC. Neuropsychological correlates of hair arsenic, manganese, and cadmium levels in school-age children residing near a hazardous waste site. Neurotoxicology. 2006;27:210-6.

33. Ciesielski T, Weuve J, Bellinger DC, Schwartz J, Lanphear B, Wright RO. Cadmium exposure and neurodevelopmental outcomes in U.S. children. Environ Health Perspect. 2012;120:758-63.

34. Yousef S, Adem A, Zoubeidi T, Kosanovic M, Mabrouk AA, Eapen V. Attention deficit hyperactivity disorder and environmental toxic metal exposure in the United Arab Emirates. J Trop Pediatr. 2011;57:457-60.

35. ATSDR. Agency for Toxic Substances and Disease Registry, 2008. Toxicological Profile for Manganese. Atlanta, GA: Department of Health and Human Services, Public Health Services.; 2008.

36. Gil F, Gisbert-Calabuig JA. Intoxicaciones por otros metales. Capítulo 70. En: Gisbert-Calabuig Medicina Legal y Toxicología, Villanueva E, 6ª ed. Barcelona: Masson SA; 2004.

37. WHO. World Health Organization. Manganese. Environmental health criteria 17 Geneva, Switzerland: World Health Organization; 1981 [cited 2014 Jul 18]. Available from: http://www.inchem.org/documents/ehc/ehc/ehc017.htm.

38. Takser L, Mergler D, Hellier G, Sahuquillo J, Huel G. Manganese, monoamine metabolite levels at birth, and child psychomotor development. Neurotoxicology. 2003;24:667-74.

39. Claus Henn B, Ettinger AS, Schwartz J, Tellez-Rojo MM, Lamadrid-Figueroa H, Hernandez-Avila M, et al. Early postnatal blood manganese levels and children's neurodevelopment. Epidemiology. 2010;21:433-9.

40. Bouchard MF, Sauve S, Barbeau B, Legrand M, Brodeur ME, Bouffard T, et al. Intellectual impairment in school-age children exposed to manganese from drinking water. Environ Health Perspect. 2011;119:138-43.

41. Menezes-Filho JA, Novaes Cde O, Moreira JC, Sarcinelli PN, Mergler D. Elevated manganese and cognitive performance in school-aged children and their mothers. Environ Res. 2011;111:156-63.

42. Riojas-Rodriguez H, Solis-Vivanco R, Schilmann A, Montes S, Rodriguez S, Rios C, et al. Intellectual function in Mexican children living in a mining area and environmentally exposed to manganese. Environ Health Perspect. 2010;118:1465-70.

43. Kim Y, Kim BN, Hong YC, Shin MS, Yoo HJ, Kim JW, et al. Co-exposure to environmental lead and manganese affects the intelligence of school-aged children. Neurotoxicology. 2009;30:564-71.

44. Wasserman GA, Liu X, Parvez F, Factor-Litvak P, Ahsan H, Levy D, et al. Arsenic and manganese exposure and children's intellectual function. Neurotoxicology. 2011;32:450-7.

45. Wasserman GA, Liu X, Parvez F, Ahsan H, Levy D, Factor-Litvak P, et al. Water manganese exposure and children's intellectual function in Araihazar, Bangladesh. Environ Health Perspect. 2006;114:124-9.

46. Farias AC, Cunha A, Benko CR, McCracken JT, Costa MT, Farias LG, et al. Manganese in children with attention-deficit/hyperactivity disorder: relationship with methylphenidate exposure. J Child Adolesc Psychopharmacol. 2010;20:113-8.

47. Ericson JE, Crinella FM, Clarke-Stewart KA, Allhusen VD, Chan T, Robertson RT. Prenatal manganese levels linked to childhood behavioral disinhibition. Neurotoxicol Teratol. 2007;29:181-7.

48. Khan K, Factor-Litvak P, Wasserman GA, Liu X, Ahmed E, Parvez F, et al. Manganese exposure from drinking water and children's classroom behavior in Bangladesh. Environ Health Perspect. 2011;119:1501-6.

49. Bouchard M, Laforest F, Vandelac L, Bellinger D, Mergler D. Hair manganese and hyperactive behaviors: pilot study of school-age children exposed through tap water. Environ Health Perspect. 2007;115:122-7.

50. Orloff K, Mistry K, Metcalf S. Biomonitoring for environmental exposures to arsenic. J Toxicol Environ Health B Crit Rev. 2009;12:509-24.

51. ATSDR. Agency for Toxic Substances and Disease Registry, 2007. Toxicological Profile for Arsenic. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services; 2007.

52. Rosado JL, Ronquillo D, Kordas K, Rojas O, Alatorre J, Lopez P, et al. Arsenic exposure and cognitive performance in Mexican schoolchildren. Environ Health Perspect. 2007;115:1371-5.

53. Hall AH. Chronic arsenic poisoning. Toxicol Lett. 2002;128:69-72.

54. Healy SM, Wildfang E, Zakharyan RA, Aposhian HV. Diversity of inorganic arsenite biotransformation. Biol Trace Elem Res. 1999;68:249-66.

55. Calderon J, Navarro ME, Jimenez-Capdeville ME, Santos-Diaz MA, Golden A, Rodriguez-Leyva I, et al. Exposure to arsenic and lead and neuropsychological development in Mexican children. Environ Res. 2001;85:69-76.

56. Hamadani JD, Grantham-McGregor SM, Tofail F, Nermell B, Fangstrom B, Huda SN, et al. Pre- and postnatal arsenic exposure and child development at 18 months of age: a cohort study in rural Bangladesh. Int J Epidemiol. 2010;39:1206-16.

57. Rocha-Amador D, Navarro ME, Carrizales L, Morales R, Calderon J. Decreased intelligence in children and exposure to fluoride and arsenic in drinking water. Cad Saude Publica. 2007;23 Suppl 4:S579-87.

58. Wang SX, Wang ZH, Cheng XT, Li J, Sang ZP, Zhang XD, et al. Arsenic and fluoride exposure in drinking water: children's IQ and growth in Shanyin county, Shanxi province, China. Environ Health Perspect. 2007;115:643-7.

59. Wasserman GA, Liu X, Parvez F, Ahsan H, Factor-Litvak P, Kline J, et al. Water arsenic exposure and intellectual function in 6-year-old children in Araihazar, Bangladesh. Environ Health Perspect. 2007;115:285-9.

60. Wasserman GA, Liu X, Parvez F, Ahsan H, Factor-Litvak P, van Geen A, et al. Water arsenic exposure and children's intellectual function in Araihazar, Bangladesh. Environ Health Perspect. 2004;112:1329-33.

61. von Ehrenstein OS, Poddar S, Yuan Y, Mazumder DG, Eskenazi B, Basu A, et al. Children's intellectual function in relation to arsenic exposure. Epidemiology. 2007;18:44-51.

62. Parvez F, Wasserman GA, Factor-Litvak P, Liu X, Slavkovich V, Siddique AB, et al. Arsenic exposure and motor function among children in Bangladesh. Environ Health Perspect. 2011;119:1665-70.

63. Tsai SY, Chou HY, The HW, Chen CM, Chen CJ. The effects of chronic arsenic exposure from drinking water on the neurobehavioral development in adolescence. Neurotoxicology. 2003;24:747-53.

64. Roy A, Kordas K, Lopez P, Rosado JL, Cebrian ME, Vargas GG, et al. Association between arsenic exposure and behavior among first-graders from Torreon, Mexico. Environ Res. 2011;111:670-6.

65. Bolla-Wilson K, Bleecker ML. Neuropsychological impairment following inorganic arsenic exposure. J Occup Med. 1987;29:500-3.

66. Rodriguez VM, Carrizales L, Mendoza MS, Fajardo OR, Giordano M. Effects of sodium arsenite exposure on development and behavior in the rat. Neurotoxicol Teratol. 2002;24:743-50.

67. Xi S, Sun W, Wang F, Jin Y, Sun G. Transplacental and early life exposure to inorganic arsenic affected development and behavior in offspring rats. Arch Toxicol. 2009;83:549-56.

68. Aguilera I, Daponte A, Gil F, Hernandez AF, Godoy P, Pla A, et al. Biomonitoring of urinary metals in a population living in the vicinity of industrial sources: a comparison with the general population of Andalusia, Spain. Sci Total Environ. 2008;407:669-78.

69. Sanchez de la Campa AM, de la Rosa J, Gonzalez-Castanedo Y, Fernandez-Camacho R, Alastuey A, Querol X, et al. Levels and chemical composition of PM in a city near a large Cu-smelter in Spain. J Environ Monit. 2011;13:1276-87.

70. Freire C, Ramos R, Lopez-Espinosa MJ, Diez S, Vioque J, Ballester F, et al. Hair mercury levels, fish consumption, and cognitive development in preschool children from Granada, Spain. Environ Res. 2010;110:96-104.

71. Aguilera I, Daponte A, Gil F, Hernandez AF, Godoy P, Pla A, et al. Urinary levels of arsenic and heavy metals in children and adolescents living in the industrialised area of Ria of Huelva (SW Spain). Environ Int. 2010;36:563-9.

72. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol. 2008;61:344-9.

73. Olmos M, Antelo M, Vazquez H, Smecuol E, Maurino E, Bai JC. Systematic review and meta-analysis of observational studies on the prevalence of fractures in coeliac disease. Dig Liver Dis. 2008;40:46-53.

74. Ricci-Cabello I, Ruiz-Perez I, Olry de Labry-Lima A, Marquez-Calderon S. Do social inequalities exist in terms of the prevention, diagnosis, treatment, control and monitoring of diabetes? A systematic review. Health Soc Care Community. 2010;18:572-87.

75. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327:557-60.

76. Tong S, Baghurst P, McMichael A, Sawyer M, Mudge J. Lifetime exposure to environmental lead and children's intelligence at 11-13 years: the Port Pirie cohort study. BMJ. 1996;312:1569-75.

77. Gil F, Capitan-Vallvey LF, De Santiago E, Ballesta J, Pla A, Hernandez AF, et al. Heavy metal concentrations in the general population of Andalusia, South of Spain: a comparison with the population within the area of influence of Aznalcollar mine spill (SW Spain). Sci Total Environ. 2006;372:49-57.

78. Olmedo P, Pla A, Hernandez AF, Lopez-Guarnido O, Rodrigo L, Gil F. Validation of a method to quantify chromium, cadmium, manganese, nickel and lead in human whole blood, urine, saliva and hair samples by electrothermal atomic absorption spectrometry. Anal Chim Acta. 2010;659:60-7.

79. Wechsler D. WISC-IV administration and scoring manual. San Antonio, TX: Harcourt Assessment; 2003.

80. Rohlman DS, Gimenes LS, Eckerman DA, Kang SK, Farahat FM, Anger WK. Development of the Behavioral Assessment and Research System (BARS) to detect and characterize neurotoxicity in humans. Neurotoxicology. 2003;24:523-31.

81. Lezak M, Howieson D, Bigler E, Tranel D. Neuropsychological Assessment. New York: Oxford University Press; 2004.

82. Iriarte Y, Diaz-Orueta U, Cueto E, Irazustabarrena P, Banterla F, Climent G. AULA--Advanced Virtual Reality Tool for the Assessment of Attention: Normative Study in Spain. J Atten Disord. 2012.

83. Barkley RA. Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. Psychol Bull. 1997;121:65-94.

84. Sergeant J, Oosterlaan J, Van der Meere J. Information processing and energetic factors in attention-deficit/hyperactivity disorder. In C. Herbert & A. E. Hogan (Eds.), Handbook of disruptive behavior disorders. Dordrecht, Netherlands: Kluwer; 1999.

85. Achenbach TM, Rescorla LA. Manual for the ASEBA School-Age Forms & Profiles. Burlington, VT: University of Vermont, Research Center for Children, Youth and Families; 2001.

86. Achenbach TM. Manual for the Teacher's Report Form and 1991 profile. Burlington, VT: Department of Psychiatry, University of Vermont; 1991.

87. Garcia AM, Gonzalez-Galarzo MC, Kauppinen T, Delclos GL, Benavides FG. A job-exposure matrix for research and surveillance of occupational health and safety in Spanish workers: MatEmESp. Am J Ind Med. 2013;56:1226-38.

88. Vioque J, Navarrete-Munoz EM, Gimenez-Monzo D, Garcia-de-la-Hera M, Granado F, Young IS, et al. Reproducibility and validity of a food frequency questionnaire among pregnant women in a Mediterranean area. Nutr J. 2013;12:26.

89. Kaufman A, Kaufman N. Kaufman Brief Intelligence Test Manual: Circle Pines, MN: American Guidance Service; 1990.

90. Arranz Freijo EB, Olabarrieta Artetxe F, Manzano Fernández A, Galende Pérez N. Etxadi–Gangoiti scale: a proposal to evaluate the family contexts of two-year-old children†. Early Child Development and Care. 2014;184:933-48.

91. Velasco D, Sanchez de Miguel M, Egurza M, Arranz E, Aranbarri A, Fano E, et al. Family context assessment in a public health study. Gac Sanit. 2014;28:356-62.

92. Caldwell BM, Bradley RH. Home Observation for Measurement of Environment: Administration manual. Little Rock, AR: University of Arkansas; 2003.

93. Pettit GS, Bates JE, Dodge KA. Supportive parenting, Ecological Context, and Children's Adjustment: A seven-Year Longitudianl Study. Child development. 1997;68:908-23.

94. Sobradillo B, Aguirre A, Aresti U, Bilbao A, Fernández-Ramos C, Lizárraga AL, H., et al. Curvas y tablas de crecimiento (Estudios

Longitudinal y Transversal). Bilbao, Spain: Fundación Faustino Orbegozo; 2004.

95. Rodriguez-Barranco M, Lacasana M, Gil F, Lorca A, Alguacil J, Rohlman DS, et al. Cadmium exposure and neuropsychological development in school children in southwestern Spain. Environ Res. 2014;134C:66-73.

96. Rodríguez-Barranco M, Gil F, Hernández A, Alguacil J, Lorca A, Mendoza R, et al. Postnatal arsenic exposure and attention impairment in Spanish school children. CORTEX. 2014;[in press].

97. Schulz C, Wilhelm M, Heudorf U, Kolossa-Gehring M. Reprint of "Update of the reference and HBM values derived by the German Human Biomonitoring Commission". Int J Hyg Environ Health. 2012;215:150-8.

98. Mergler D. Neurotoxic exposures and effects: gender and sex matter! Hanninen Lecture 2011. Neurotoxicology. 2012;33:644-51.

99. Vahter M, Akesson A, Liden C, Ceccatelli S, Berglund M. Gender differences in the disposition and toxicity of metals. Environ Res. 2007;104:85-95.

100. Llop S, Lopez-Espinosa MJ, Rebagliato M, Ballester F. Gender differences in the neurotoxicity of metals in children. Toxicology. 2013;311:3-12.

101. Tyler CR, Allan AM. The Effects of Arsenic Exposure on Neurological and Cognitive Dysfunction in Human and Rodent Studies: A Review. Curr Environ Health Rep. 2014;1:132-47.

102. Singhal RL, Merali Z, Hrdina PD. Aspects of the biochemical toxicology of cadmium. Fed Proc. 1976;35:75-80.

103. Stowe HD, Wilson M, Goyer RA. Clinical and morphologic effects of oral cadmium toxicity in rabbits. Arch Pathol. 1972;94:389-405.

104. Andersson H, Petersson-Grawe K, Lindqvist E, Luthman J, Oskarsson A, Olson L. Low-level cadmium exposure of lactating rats causes alterations in brain serotonin levels in the offspring. Neurotoxicol Teratol. 1997;19:105-15.

105. Provias JP, Ackerley CA, Smith C, Becker LE. Cadmium encephalopathy: a report with elemental analysis and pathological findings. Acta Neuropathol. 1994;88:583-6.

106. Petersson Grawe K, Teiling-Gardlund A, Jalkesten E, Oskarsson A. Increased spontaneous motor activity in offspring after maternal cadmium exposure during lactation. Environ Toxicol Pharmacol. 2004;17:35-43.

107. Schantz SL, Widholm JJ. Cognitive effects of endocrine-disrupting chemicals in animals. Environ Health Perspect. 2001;109:1197-206.

108. Takiguchi M, Yoshihara S. New aspects of cadmium as endocrine disruptor. Environ Sci. 2006;13:107-16.

109. Rodriguez VM, Limon-Pacheco JH, Carrizales L, Mendoza-Trejo MS, Giordano M. Chronic exposure to low levels of inorganic arsenic causes alterations in locomotor activity and in the expression of dopaminergic and antioxidant systems in the albino rat. Neurotoxicol Teratol. 2010;32:640-7.

110. Liu S, Piao F, Sun X, Bai L, Peng Y, Zhong Y, et al. Arsenic-induced inhibition of hippocampal neurogenesis and its reversibility. Neurotoxicology. 2012;33:1033-9.

CAPÍTULO IX

PUBLICACIONES

Association of arsenic, cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: a systematic review and meta-analysis

Autores: Rodríguez-Barranco M, Lacasaña M, Aguilar-Garduño C, Alguacil J, Gil F, González-Alzaga B, Rojas-García A

Sci Total Environ. 2013 Jun 1;454-455:562-77. Review

Science of the Total Environment 454–455 (2013) 562–577

Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Review

Association of arsenic, cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: A systematic review and meta-analysis

Miguel Rodríguez-Barranco [a], Marina Lacasaña [a,b,*], Clemente Aguilar-Garduño [b,c], Juan Alguacil [b,d], Fernando Gil [e], Beatriz González-Alzaga [a], Antonio Rojas-García [b]

[a] Andalusian School of Public Health (EASP), Granada, Spain
[b] CIBER of Epidemiology and Public Health (CIBERESP), Madrid, Spain
[c] Centre Superior d'Investigació en Salut Pública, Conselleria de Sanitat, Valencia, Spain
[d] Department of Environmental Biology and Public Health, University of Huelva, Huelva, Spain
[e] Department of Legal Medicine and Toxicology, University of Granada, Granada, Spain

HIGHLIGHTS

- We evaluated the association between As, Cd and Mn with neurodevelopment in children.
- A 50% increase in As levels is associated with a 0.4 decrease in the IQ of children.
- A 50% increase in Mn levels is associated with a 0.7 decrease in the IQ of children.
- There is evidence of association between Mn exposure with attention deficit disorder with hyperactivity.

ARTICLE INFO

Article history:
Received 10 December 2012
Received in revised form 12 March 2013
Accepted 13 March 2013
Available online 9 April 2013

Keywords:
Neurodevelopment
Attention deficit disorder with hyperactivity
Arsenic
Cadmium
Manganese
Children

ABSTRACT

The aim of this study was to analyse the scientific evidence published to date on the potential effects on neurodevelopment and behavioural disorders in children exposed to arsenic, cadmium and manganese and to quantify the magnitude of the effect on neurodevelopment by pooling the results of the different studies.

We conducted a systematic review of original articles from January 2000 until March 2012, that evaluate the effects on neurodevelopment and behavioural disorders due to pre or post natal exposure to arsenic, cadmium and manganese in children up to 16 years of age. We also conducted a meta-analysis assessing the effects of exposure to arsenic and manganese on neurodevelopment.

Forty-one articles that evaluated the effects of metallic elements on neurodevelopment and behavioural disorders met the inclusion criteria: 18 examined arsenic, 6 cadmium and 17 manganese. Most studies evaluating exposure to arsenic (13 of 18) and manganese (14 of 17) reported a significant negative effect on neurodevelopment and behavioural disorders. Only two studies that evaluated exposure to cadmium found an association with neurodevelopmental or behavioural disorders. The results of our meta-analysis suggest that a 50% increase of arsenic levels in urine would be associated with a 0.4 decrease in the intelligence quotient (IQ) of children aged 5–15 years. Moreover a 50% increase of manganese levels in hair would be associated with a decrease of 0.7 points in the IQ of children aged 6–13 years.

There is evidence that relates arsenic and manganese exposure with neurodevelopmental problems in children, but there is little information on cadmium exposure. Few studies have evaluated behavioural disorders due to exposure to these compounds, and manganese is the only one for which there is more evidence of the existence of association with attention deficit disorder with hyperactivity.

Contents

* Corresponding author at: Andalusian School of Public Health (EASP), Campus Universitario de Cartuja, c/Cuesta del Observatorio 4, 18080 Granada, Spain. Tel.: +34 958 027400.
E-mail address: marina.lacasana.easp@juntadeandalucia.es (M. Lacasaña).

0048-9697/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scitotenv.2013.03.047

1. Introduction

Acute and chronic neurological effects associated with occupational exposure to different neurotoxicants such as metallic trace elements have been the subject of much research. However, currently, the most concerning problem from a public health point of view is exposure to low doses of pollutant mixtures among populations in non-occupational settings, especially pregnant women and children living in industrial areas. They are exposed by inhaling pollutants from industrial emissions, and by eating and drinking polluted food and water.

Over the last few decades there has been an exponential increase in concern about the health risks of exposure to metallic trace elements such as lead, mercury, cadmium, manganese and arsenic, because of their potential neurotoxic effect (Counter and Buchanan, 2004) and their accumulative capacity in target organs (Gil and Pla, 2001). More than 1000 chemical substances are known to have neurotoxic effects in experimental animals. Of these, lead, methylmercury and arsenic are three of the five substances that have been shown to cause neurodevelopmental disorders in humans and subclinical brain dysfunction. Grandjean and Landrigan (2006) suggested that continued exposure to these neurotoxic compounds could be creating a "silent pandemic" in modern society, being responsible for a subclinical, permanent decrease in IQ, leading to increased school failure, diminished economic productivity and increased risk of criminal and antisocial behaviour. The global nature of this pandemic could have a huge impact on public health. Bellinger (2012) estimated that 23,285,000 Full-Scale IQ points are lost due to environmental exposure to lead and methylmercury only in the U.S. population of children less than 5 years old.

Children are particularly susceptible to environmental toxic exposure as they present striking differences versus adults in terms of exposure (Landrigan et al., 2004). First, children are characterised by their immature detoxification mechanisms, which makes them more susceptible than adults to the effects of these substances. Their heightened vulnerability to this type of risk is also particularly related to physical aspects (high surface area: volume ratio, critical growth and development stages), food (children drink more water and eat more food per unit of body weight than adults) and behaviour (direct contact with the ground and other surfaces, tendency to put everything into their mouths, etc.). This situation has made children a prioritised target study group for exposure to environmental pollutants (Au, 2002).

The time to neurological maturity means an extensive period of biological vulnerability that starts in the first month post-conception and continues through gestation, childhood and adolescence. The central nervous system consists of different areas that are responsible for specific functional domains (e.g. motor control, sensory function, intelligence, etc.). These areas develop in a sequential order but they are interdependent, and so interference during any maturing phase or process can affect later stages of development (Rice and Barone, 2000). Many domains can be affected by the action of metallic trace elements, depending on the compound involved, although some domains are inhibited by all the elements mentioned above, such as auditory, visual system, motor and memory deficits, and externalising behaviour (Riccio et al., 2010).

Children are exposed to metallic trace elements through the mother's exposure and the mobilisation of various toxic compounds from maternal tissues during pregnancy, and at later stages through breast feeding. During childhood and pre-adolescence, exposure continues through food and water intake, inhalation and/or dermal absorption.

Toxic effects of lead and mercury have been widely evaluated in epidemiological studies (Jakubowski, 2011; Schoeman et al., 2009). However, evidence of toxic effects of other metallic trace elements such as arsenic, cadmium and manganese have been less evaluated in humans (Counter and Buchanan, 2004), and the evidence of their neurotoxic effects derives from experimental studies in animals (Shagirtha et al., 2011; Luo et al., 2009; Krüger et al., 2009; Aschner et al., 2007).

The aim of this systematic review is to examine the scientific evidence published to date on potential effects on neurodevelopment and behavioural disorders in children exposed to arsenic, cadmium and manganese, which have been subject to less study than lead and mercury, but also have potential neurotoxic effects in children. We also aim to quantify the magnitude of the effect on neurodevelopment, by means of Full Scale IQ and verbal and performance domains, pooling the results of the different studies.

2. Methods

2.1. Search strategy

We carried out a literature search in the online medical databases PubMed, EMBASE, ISI Web of Knowledge, CINHAL, Lilacs and REPIDISCA, using the following search limiters: publication date from January 2000 to March 2012, studies in humans and written in English, Spanish, French or Italian. We used the search syntax:

(child*OR infant*OR school*OR postnatal OR prenatal OR post − natal OR pre − natal OR fetal OR pregnan*) AND (neurodevelopment*OR behavior OR behaviour OR mental OR intelligence OR cognitive OR "attention deficit disorder with hyperactivity"OR ADHD) AND ("cadmium" [MeSHTerms] OR "arsenic" [MeSH Terms] OR"manganese"[MeSH Terms]).

2.2. Inclusion criteria

In our review we included studies that met the following criteria: (a) original articles; (b) assessment of pre- or post-natal exposure to arsenic (As), cadmium (Cd) or manganese (Mn) through a biomarker of exposure or environmental sample of exposure; (c) study population up to 16 years of age; (d) study of neurodevelopment or behavioural disorders derived from exposure to metallic trace elements (As, Cd and Mn), including:

1) Neurodevelopment: intelligence quotient (IQ) or degree of development in motor, communication, cognitive, attention and/or memory fields.
2) Behavioural disorders: attention deficit hyperactivity disorder (ADHD), oppositional defiant problems, internalising behaviours (anxious/depressed, withdrawn/depressed, somatic complaints), externalising behaviours (rule-breaking behaviour, aggressive behaviour).

2.3. Exclusion criteria

We excluded articles based on case studies or case series, ecological designs, literature reviews and those that only evaluated exposure to arsenic, cadmium and manganese indirectly through questionnaires (parents' smoking habits, mother's diet during pregnancy, child's diet, etc.).

2.4. Assessment of methodological quality of the articles

In the current absence of a validated instrument to assess the methodological quality of studies with an observational design, and in view of the fact that all but one of the studies reviewed had this type of design, we used the checklist in the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement (von Elm et al., 2008) to assess the methodological quality of the studies. This tool was initially developed to assess clarity in communicating research results in observational studies, and it has been used in recent systematic reviews to assess the methodological quality of observational studies (Olmos et al., 2008; Scales and Dahm, 2008; Ricci-Cabello et al., 2010).

Of the 22 items that make up the checklist, the 9 that related to the methods section were selected, which assess the different aspects of methodology in an observational study (Appendix B). After performing the assessment, the methodological quality was classified as follows: articles that met 0–3 of the 9 items were considered as having low methodological quality, 4–6 items as medium and 7–9 items as high methodological quality.

2.5. Meta-analysis

We performed a meta-analysis of the results reported by different studies in order to make an overall estimate and summary of the magnitude of the effect of arsenic and manganese exposure on children's neurodevelopment. The meta-analysis was restricted to studies that evaluated Full Scale IQ, Verbal IQ and Performance IQ using any version of the Wechsler scale and linear regression techniques to estimate the effect. There were too few studies assessing cadmium exposure to be able to perform a meta-analysis on this compound.

Furthermore, the fact that the articles reported results using different analytical approaches meant that some pre-processing was needed to homogenise the magnitude of effect observed in each study. To overcome the obstacle of the different transformations used for the independent variable (natural log, log base 10 or none), we recalculated each effect to express it as a relative change in the exposure variable.

More specifically, in a linear regression model where the exposure variable is transformed by natural logarithm, $c \cdot \beta$ is the change in the response variable based on the change in c log units in the exposure variable (i.e., when $\ln(X_1) - \ln(X_0) = c$). Furthermore, a relative change in the original exposure variable X is denoted as $X_1/X_0 = k$, where k is the number of times that X increases. Taking the logarithms in this expression and applying their properties, the following is obtained: $\ln(X_1) - \ln(X_0) = \ln(k)$. Therefore, it follows that $\ln(k) \cdot \beta$ represents the absolute change in the response variable based on a relative change that is k times the original variable. Similarly, if base 10 is used for the transformation, $\log_{10}(k) \cdot \beta$ represents the change in the response for the aforementioned k relative change in exposure. For untransformed variables, we decided to consider the absolute change that would occur in the response variable based on a relative change equal to k in the mean of the distribution of the exposure variable. In short, the effect was calculated as $(k - 1) \cdot E(X) \cdot \beta$.

Table 1
Characteristics of articles that met the inclusion criteria (number and percentage).

	Arsenic $n = 18$	Cadmium $n = 6$	Manganese $n = 17$
Type of study design			
Cross-sectional	14 (77.8)	3 (50.0)	13 (76.4)
Case–control	1 (5.6)	1 (16.7)	2 (11.8)
Cohort	3 (16.6)	1 (16.7)	2 (11.8)
Randomised clinical trial	0 (0)	1 (16.7)	0 (0)
Exposure measure[a]			
Drinking water	9 (50.0)	1 (16.7)	4 (23.5)
Child urine	13 (72.2)	0 (0)	0 (0)
Child blood	3 (16.6)	2 (33.3)	11 (64.7)
Child hair	1 (5.6)	3 (50.0)	7 (41.2)
Child tooth	0 (0)	0 (0)	1 (5.9)
Child nails	1 (5.6)	0 (0)	0 (0)
Maternal urine	2 (11.1)	0 (0)	0 (0)
Maternal blood	0 (0)	1 (16.7)	0 (0)
Cord blood	0 (0)	1 (16.7)	1 (5.9)
Placenta	0 (0)	1 (16.7)	1 (5.9)
Soil	0 (0)	1 (16.7)	0 (0)
Analytical technique[b]			
AAS	9 (50.0)	1 (16.7)	6 (35.3)
AFS	1 (5.6)	0 (0.0)	0 (0.0)
ICP-MS	8 (44.4)	5 (83.3)	11 (64.7)
Effect measure			
Neurodevelopment	15 (83.4)	4 (66.7)	12 (70.6)
Behavioural disorders	3 (16.6)	2 (33.3)	5 (29.4)

[a] Totals exceed 100% because a study can measure several biomarkers.
[b] AAS: atomic absorption spectrophotometry; AFS: atomic fluorescence spectrometry; ICP-MS: inductively coupled plasma mass spectrometry.

Table 2
Characteristics of studies assessing exposure to arsenic.

Location	First author & year	Age	Sample size	Study design	Confounders accounted for	Exposure measure	Type of arsenic	Mean ± SD (range)	Psychological test	Observed effect	MQ
Neurodevelopment											
Pakistan	Abbas et al. (2012)	8–15 years	Not reported	Cross-sectional	None	Water, Urine	T-As	Not reported	RPM	AsW↓ Full-scale score	Low
Bangladesh	Hamadani et al. (2011)	5 years	2260	Longitudinal cohort	Age, HOME, father's education, mother's BMI and IQ, assets, housing, number of children in the household, gestational age, birth length, concurrent HAZ and testers	Maternal urine at pregnancy Child urine	In-As T-As	84 μg/l maternal urine[a] (26–415)[d] 51 μg/l child urine[a] (20–238)[d]	WPPSI–III	AsU↓ Full-scale & verbal IQ in girls, but not in boys	High
Bangladesh	Parvez et al. (2011)	8–11 years	304	Cross-sectional	Sex, school attendance, head circumference, mother's intelligence, plasma ferritin, blood Pb, and selenium	Water, Blood, Urine, Nails	T-As	43.3 ± 73.6 μg/l Water 4.8 ± 3.2 μg/l Blood 78.0 ± 72.1 μg/l Urine 5.9 ± 6.3 μg/g Nails	BOT-2	AsW, AsB, AsU, AsN↓ Motor function	High
Bangladesh	Wasserman et al. (2011)	8–11 years	299	Cross-sectional	Maternal intelligence, maternal age, school months, head circumference, plasma ferritin and blood manganese	Blood	T-As	4.81 ± 3.22 μg/l	WISC-IV	AsB↓ Full-scale score, verbal comprehension & working memory	High
Bangladesh	Hamadani et al. (2010)	18 months	2112	Longitudinal cohort	Age, sex, assets, housing, mother's education, mother's BMI, gestational age, number of children in the household, birth length, head circumference and 18-month WHZ	Maternal urine at pregnancy Child Urine	In-As T-As	96 μg/l maternal urine[a] (46–219)[b] 35 μg/l child urine[a] (18–80)[b]	BSID-II	Non-significant effect	High
Bangladesh	Tofail et al. (2009)	7 months	1799	Longitudinal cohort	Age, sex, mothers' and fathers' education, housing, assets, income, mothers' BMI and parity, birth length, head circumference, gestational age and length at 7 months	Maternal urine at pregnancy	In-As	84 μg/l[a] (42–230)[b]	BSID-II	Non-significant effect	High
Mexico	Rocha-Amador et al. (2007)	6–10 years	132	Cross-sectional	Pb blood, socioeconomic status, mother's education, height-for-age z-score and transferrin saturation	Water, Urine	T-As	194 ± 1.3 μg/l water[c] 116 ± 2.2 μg/g crea[c]	WISC-RM	AsW↓ Full-scale, performance & verbal IQ AsU↓ Full-scale IQ	High
Mexico	Rosado et al. (2007)	6–8 years	602	Cross-sectional	Age, sex, mother's school level, Hb, Pb and for Pb × AsU interaction	Urine	In-As, MMA, DMA	58.1 ± 33.2 μg/l[c]	WISC-RM NLS CAT	AsU↓ Digit span subscale, letter sequencing, visual search	High

(continued on next page)

Table 2 (*continued*)

Location	First author & year	Age	Sample size	Study design	Confounders accounted for	Exposure measure	Type of arsenic	Mean ± SD (range)	Psychological test	Observed effect	MQ
India	von Ehrenstein et al. (2007)	5–15 years	351	Cross-sectional	Age, sex, maternal and paternal education, father's occupation, number of rooms in the house, type of house building material, BMI, and mother's age	Water, Urine	In-As	147 ± 322 μg/l Water (1–2480) 78 ± 61 μg/l Urine (2–375)	WISC-III CRT SBIS	AsU↓ Vocabulary test, object assembly test & picture completion test	High
China	Wang et al. (2007)	8–12 years	720	Cross-sectional	None	Water, Urine	T-As	190 ± 183 μg/l Water 73 ± 3 μg/l Urine	CRT-RC2	AsU↓ Full-scale IQ	High
Bangladesh	Wasserman et al. (2007)	6 years	301	Cross-sectional in a follow-up cohort	Maternal education, maternal intelligence, home stimulation, school attendance, height, head circumference, water Mn, blood Pb	Water, Urine	T-As	120 ± 134 μg/l Water 110.7 ± 132.8 μg/l Urine	WPPSI–III	AsW↓ Full-scale & performance IQ & processing speed	High
USA	Wright et al. (2006)	11–13 years	31	Cross-sectional	Sex, maternal education	Hair	T-As	0.018 ± 0.014 μg/g (0.001–0.055)	WASI CVLT-C WRAML	AsH↓ Full-scale & verbal IQ & memory test	Low
Bangladesh	Wasserman et al. (2004)	10 years	201	Cross-sectional in a follow-up cohort	Maternal education, maternal intelligence, house type, tv access, height and head circumference	Water, Urine	T-As	118 ± 145 μg/l Water 116.6 ± 148.8 μg/l Urine	WISC-III	AsW↓ Full-scale & performance IQ	High
Taiwan	Tsai et al. (2003)	13–14 years	109	Cross-sectional	Sex, education	Water	T-As	184.99 ± 225.29 μg/l	NES2-T	AsW↓ Pattern memory & switching attention	High
Mexico	Calderón et al. (2001)	6–9 years	41	Cross-sectional	Sex, age, socioeconomic status and parent's education	Urine	T-As	62.9 ± 0.03 μg/g crea (27.5–186.2)	WISC-RM	AsU↓ Full-scale & verbal IQ	Medium
Behavioural disorders											
Bangladesh	Khan et al. (2011)	8–11 years	201	Cross-sectional	Sex, maternal education, arm circumference, and log-transformed BMI	Water, Blood, Urine	T-As	43.7 ± 67.0 μg/l Water 5.1 ± 3.3 μg/l Blood 81.2 ± 75.2 μg/l Urine	CBCL	Non-significant effect	High
Mexico	Roy et al. (2011)	6–7 years	526	Cross-sectional	Age, sex, maternal education, family socioeconomic status, ownership of home, crowding at home, Hb and blood Pb	Urine	T-As, MMA, DMA	52.5 μg/l[a]	CPRS-R CTRS-R	Non-significant effect	High
United Arab Emirates	Yousef et al. (2011)	5–15 years	18/74	Matched case–control	Age and gender	Blood	T-As	Not reported	CTRS-CPRS CBCL DSM-IV	Non-significant effect	Medium

[a] Median.
[b] Interquartile range.
[c] Geometric mean.
[d] 10th and 90th percentiles; *AsH*: arsenic in hair; *AsN*: arsenic in nails; *AsS*: arsenic in soil; *AsU*: arsenic in urine; *AsW*: arsenic in drinking water; *BMI*: body mass index; *DMA*: dimethylarsinic acid; *HAZ*: height-for-age z-score; *Hb*: haemoglobin; *In-As*: inorganic arsenic; *IQ*: intelligence quotient; *MMA*: monomethylarsonic acid; *MQ*: methodological quality; *Pb*: lead; *T-As*: total arsenic; *WHZ*: weight for height z score.

Thus, we were able to express the effect for the same relative change in exposure in all studies. In this analysis specifically, we decided to define k as 1.5, which is the equivalent to studying the absolute variation in the response when exposure is increased by 50%.

We assessed study heterogeneity by means of the DerSimonian and Laird test and the I^2 coefficient value (Higgins et al., 2003), representing the percentage of total variability attributable to heterogeneity.

We used the statistical package Stata 11 (StataCorp. LP, 2009, TX) to perform the meta-analysis.

3. Results

Of the 156 articles that were initially identified in the literature search, 41 met the inclusion criteria: 18, 6 and 17 articles evaluated the effects of arsenic, cadmium and manganese, respectively, on neurodevelopment and behavioural disorders.

Most articles had a cross-sectional epidemiological design (78%, 50% and 76% evaluated the neurotoxic effects of arsenic, cadmium and manganese, respectively). Exposure was mainly measured by determining exposure biomarkers in urine and drinking water (in the case of arsenic) and in hair and blood (cadmium and manganese). In 24 of the 41 articles included in this review (59%), metallic trace elements were measured by inductively coupled plasma mass spectrometry (ICP-MS), in 16 articles (39%) by atomic absorption spectrophotometric (AAS) and only one study used atomic fluorescence spectrometry (AFS). On the other hand, 76% of the studies evaluated the effect of these compounds on neurodevelopment and 24% on behavioural disorders (Table 1).

The main instruments used to evaluate neurodevelopment in children were the different versions of the Wechsler intelligence scale (KEDI-WISC, WASI, WISC-III, WISC-IV, WISC-R, WISC-RM, WPPSI-III, WPPSI-R) for children aged between 5 and 15, and the Bayley scale (BSID-II) for children aged 0 to 3. This type of test was used in 22 of the 31 articles that evaluated the effect of these metallic elements on children's neurodevelopment. In general, Conner's Parent and Teacher Rating Scales (CPRS-R and CTRS-R) and the Child Behaviour Checklist (CBCL) were used to evaluate behavioural disorders.

Most of the studies included in the review were of high methodological quality. 17%, 15% and 68% were categorised as having low, medium and high methodological quality, respectively.

Tables 2–4 show the most relevant characteristics of the studies included in the systematic review.

3.1. Arsenic

3.1.1. Overview

Arsenic (As) is a metalloid that is naturally present in low concentrations in the environment. It ranks 52nd in abundance in the earth's crust, with an average value of 2 μg/g. Higher concentrations can be found in areas of volcanic activity and geological sulphur deposits. However, a more significant source of arsenic emission are anthropogenic activities such as mining, smelting, pesticides, wood preservatives, coal combustion and waste incineration (Orloff et al., 2009). The Agency for Toxic Substances and Disease Registry (ATSDR) classifies arsenic as number one on its list of 275 substances present in the environment that pose the most significant potential threat to human health, gauged by abundance, toxicity and potential for exposure in humans (ATSDR, 2011).

It is estimated that world production of arsenic amounted to 54,500 tonnes in 2010. China was the main producer, accounting for about half the production, followed by Chile with 21% of the total. World reserves of this compound are estimated to be 20 times the world annual production (U.S. Geological Survey, 2011).

Arsenic is categorised as organic or inorganic, depending on the presence or absence of a carbon bond, and may be found in one of three oxidation states, −3, +3 and +5, the trivalent form being the most toxic. The inorganic forms of arsenic are generally more toxic than the organic forms, and are responsible for most cases of arsenic poisoning in humans (ATSDR, 2007).

However, although the organic arsenic compounds are considered to be less toxic than the inorganic forms, some of the former, such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), have been shown to have deleterious effects in experimental animal health, including neurological effects (ATSDR, 2007), although only one study in humans has observed an association between these compounds and children's neurodevelopment (Rosado et al., 2007).

The main routes of exposure to inorganic arsenic are ingestion of drinking water and inhalation of polluted air and dust, the former being the most important route in the case of millions of children in countries with high levels of arsenic in water, such as Argentina, Chile, Mexico, China, Hungary, India, Bangladesh and Vietnam (Smedley and Kinniburgh, 2002). Organic arsenic, in turn, is mainly found in fish and seafood in the form of arsenobetaine and arsenocholine (Orloff et al., 2009). After ingestion, about 60–90% of organic and inorganic forms alike are absorbed into the bloodstream from the gastrointestinal tract (Hall, 2002). During metabolism, the inorganic pentavalent form of arsenic (arsenate) first changes to the trivalent form (arsenite), and the latter then undergoes methylation in the liver, yielding the organic forms MMA and DMA (Healy et al., 1999). Both the resulting organic forms and the inorganic unmethylated forms are excreted through the urine.

The health consequences of arsenic exposure include respiratory, gastrointestinal, haematological, hepatic, renal, skin, neurological and immunological effects, as well as damaging effects on the central nervous system and cognitive development in children (Argos et al., 2010; Rosado et al., 2007).

3.1.2. Reference values

According to ATSDR recommendations (ATSDR, 2000, 2007), normal levels of total arsenic in children should not exceed 50 μg/l in urine and 1 μg/g in hair. All studies except Hamadani et al. (2010) found mean levels in urine above this threshold value. In the only study that measured arsenic in hair (Wright et al., 2006), the mean levels were considerably lower than the reference value of 1 μg/g (Table 2). However, studies that measured arsenic in drinking water detected mean levels between 4 and 20 times higher than the reference value of 10 μg/l that is recommended by the World Health Organization (WHO, 2004). These studies were conducted in Mexico (Rocha-Amador et al., 2007), India (von Ehrenstein et al., 2007), China (Wang et al., 2007), Bangladesh (Parvez et al., 2011; Khan et al., 2011; Wasserman et al., 2004, 2007) and Taiwan (Tsai et al., 2003).

3.1.3. Effects on neurodevelopment

In 13 of the 15 articles studied, we found that arsenic exposure had a significant negative effect on neurodevelopment in children aged between 5 and 15 years. In most studies, this deleterious effect affected Full Scale IQ. More specifically, a deficit was found in verbal and performance domains, with memory being affected to a lesser extent (Table 2).

Rocha-Amador et al. (2007) observed a decrease in Full Scale IQ in children aged 6–10 years when total arsenic levels were increased in urine and drinking water. Abbas et al. (2012) and Wang et al. (2007) obtained a similar result when levels were increased in drinking water and urine, respectively, in children aged 8–15 years. Wasserman et al. observed the same association with total arsenic levels in water in two studies conducted in 2004 and 2007 in children aged 6 and 10 years, respectively. In a later study conducted in children aged 8–11 years, they observed a decrease in Full Scale IQ score, verbal comprehension and working memory, associated with increased levels of

Table 3
Characteristics of studies assessing exposure to cadmium.

Location	First author & year	Age	Sample size	Study design	Confounders accounted for	Exposure measure	Mean ± SD (range)	Psychological test	Observed effect	MQ
Neurodevelopment										
USA	Cao et al. (2009)	2, 5, 7 years	675	Randomised clinical trial	Treatment group, age, caregiver's IQ, clinic centre, single parent, language, race, sex, parent's employment, parent's education and blood Pb level	Blood	Placebo pretreatment 0.21 μg/l[b] Placebo post treatment 0.20 μg/l[b] Treatment pretreatment 0.21 μg/l[b] Treatment post treatment 0.21 μg/l[b]	BSID-II WPPSI-R WISC-III NEPSY CPRS-R CVLT-C WLPB-R BASC	Non-significant effect	High
China	Tian et al. (2009)	4.5 years	106	Prospective cohort	Cord blood Pb, maternal age, height, weight, gestational weeks, maternal education, method of delivery, breast feeding, nursery school age, tobacco exposure and family income	Placenta, maternal blood, cord blood	0.15 μg/g placenta[a] 1.80 μg/l maternal blood[a] 0.60 μg/l cord blood[a]	WPPSI-R	CdCB↓ Full-scale & performance IQ	High
USA	Wright et al. (2006)	11–13 years	32	Cross-sectional	Sex, maternal education	Hair	0.058 ± 0.058 μg/g (0.016–0.293)	WASI CVLT-C WRAML	Non-significant effect	Low
Spain	Torrente et al. (2005)	12–14 years	100	Cross-sectional	Age and socioeconomic status	Hair	ND μg/g (<0.03–0.26)	Cognitive test	Non-significant effect	Low
Behavioural disorders										
United Arab Emirates	Yousef et al. (2011)	5–15 years	18/74	Matched case–control	Age and gender	Blood	Not reported	CTRS CPRS CBCL DSM-IV	Non-significant effect	Medium
China	Bao et al. (2009)	7–16 years	549	Cross-sectional	Sex, age, family incoming, farther education and mother education	Hair, Water, Soil	0.10 μg/g hair 7.09 μg/l water 0.528 μg/g soil	CBCL	CdH↑ Withdrawn, social problems & attention problems	High

CdCB: cadmium in cord blood; *CdH*: cadmium in hair; *CdU*: cadmium in urine; *IQ*: intelligence quotient; *MQ*: methodological quality; *ND*: not detected; *Pb*: lead.
[a] Median.
[b] Geometric mean.

Table 4
Characteristics of studies assessing exposure to manganese.

Location	First author & year	Age	Sample size	Study design	Confounders accounted for	Exposure measure	Mean ± SD (Range)	Psychological Test	Observed effect	MQ
Neurodevelopment										
Canada	Bouchard et al. (2011)	6–13 years	362	Cross-sectional	Maternal education, family income, home stimulation score and family structure, age, sex, IQ testing session, source of water and Fe	Hair, Water	0.7 μg/g hair[a] (0.1–21.0) 30.8 μg/l water[a] (0.1–2700)	WASI	MnH↓ Full-scale & verbal IQ MnW↓ Full-scale, verbal & performance IQ	High
Brazil	Menezes-Filho et al. (2011)	6–12 years	83	Cross-sectional	Maternal education and nutritional status	Hair, Blood	5.8 ± 11.5 μg/g hair[b] (0.10–86.7) 8.2 ± 3.6 μg/l Blood (2.7–23.4)	WISC-III	MnH↓ Full-scale & verbal IQ	High
Bangladesh	Parvez et al. (2011)	8–11 years	304	Cross-sectional	Sex, school attendance, head circumference, mother's intelligence, plasma ferritin, and blood Pb, and selenium	Water, Blood	725.5 ± 730.5 μg/l Water 14.7 ± 3.7 μg/l Blood	BOT-2	Non-significant effect	High
Bangladesh	Wasserman et al. (2011)	8–11 years	299	Cross-sectional	Maternal intelligence, maternal age, school months, head circumference, plasma ferritin and blood arsenic	Blood	14.78 ± 3.72 μg/l	WISC-IV	MnB↓ Full-scale score, working memory & Perceptual Reasoning	High
Mexico	Claus Henn et al. (2010)	12–24 months	448	Longitudinal cohort	Sex, gestational age, Pb, Hb, maternal IQ and maternal education	Blood	24.3 ± 4.5 μg/l	BSID-II	MnB ↓ Mental Development Index	High
Mexico	Riojas-Rodríguez et al. (2010)	7–11 years	79	Cross-sectional	Age, sex, maternal education, Pb and Hb	Hair, Blood	12.1 μg/g hair[b] (4.2–48.0) 9.7 μg/l blood[b] (5.5–18.0)	WISC-R	MnH↓ Full-scale & verbal IQ	High
Korea	Kim et al. (2009)	8–11 years	261	Cross-sectional	Age, gender, parents education, family income, maternal smoking during pregnancy, birth weight of the child, mother's age at the time of birth and indirect smoking status	Blood	14.3 ± 3.8 μg/l (5.30–29.02)	KEDI-WISC	MnB↓ Full-scale & verbal IQ	High
USA	Wasserman et al. (2006)	10 years	142	Cross-sectional in a follow-up cohort	Maternal education and intelligence, house type, family ownership of a television, child height and head circumference, water As and blood Pb	Water, Blood	795 ± 755 μg/l Water 12.8 ± 3.2 μg/l Blood	WISC-III	MnW↓ Full-scale, verbal & performance IQ	High

(continued on next page)

Table 4 (*continued*)

Location	First author & year	Age	Sample size	Study design	Confounders accounted for	Exposure measure	Mean ± SD (Range)	Psychological Test	Observed effect	MQ
USA	Wright et al. (2006)	11–13 years	32	Cross-sectional	Sex and maternal education	Hair	0.47 ± 0.46 μg/g (0.09–2.15)	WASI CVLT-C WRAML	MnH↓ Full-scale & verbal IQ & memory test	Low
Spain	Torrente et al. (2005)	12–14 years	100	Cross-sectional	Age and socioeconomic status	Hair	0.18 ± 0.28 μg/g (0.0–1.97)	Cognitive test	Non-significant effect	Low
France	Takser et al. (2003)	Neonates	247	Prospective	Gender and maternal education	Hair, Cord blood, Placenta	0.75 μg/g hair[b] 38.5 μg/l cord blood[b] 0.10 μg/g placenta[b]	B-L scales McCarthy	MnCB↓ Attention, non-verbal memory & hand skill	Medium
Malaysia	Zaleha et al. (2003)	7–12 years	25	Cross-sectional	None	Blood	1.41 ± 0.76 μg/l (0.40–3.40)	TONI-2	Non-significant effect	Low
Behavioural disorders										
Bangladesh	Khan et al. (2011)	8–11 years	201	Cross-sectional	Sex, maternal education, arm circumference, and log-transformed BMI	Water, Blood	889.2 ± 783.7 μg/l Water 15.1 ± 3.9 μg/l Blood	CBCL	MnW↑ Total, internalising & externalising scores	High
United Arab Emirates	Yousef et al. (2011)	5–15 years	18/74	Matched case–control	Age and gender	Blood	Not reported	CTRS CPRS CBCL DSM-IV	MnB↑ ADHD	Medium
Brazil	Farias et al. (2010)	7–15 years	106/35	Case–control	None	Blood	Cases: 4.5 ± 3.6 μg/l Controls: 3.5 ± 2.2 μg/l	SNAP-IV CBCL DSM-IV	MnB↑ ADHD	Medium
Canada	Bouchard et al. (2007)	6–15 years	46	Cross-sectional	Age, sex, family income	Hair	5.1 ± 4.3 μg/g (0.28–20.0)	CTRS-R CPRS-R	MnH↑ Oppositional & hyperactivity subscales	High
USA	Ericson et al. (2007)	11–13 years	27	Cross-sectional in a follow-up cohort	Pb	Tooth	Not reported	DBDS CBCL	MnT↑ Disruptive disorder & ADHD	High

ADHD: attention deficit and hyperactivity disorder; *As*: arsenic; *Hb*: haemoglobin; *IQ*: intelligence quotient; *MnB*: manganese in blood; *MnCB*: manganese in cord blood; *MnH*: manganese in hair; *MnT*: manganese in tooth; *MnW*: manganese in drinking water; *MQ*: methodological quality; *Pb*: lead.

[a] Median.

[b] Geometric mean.

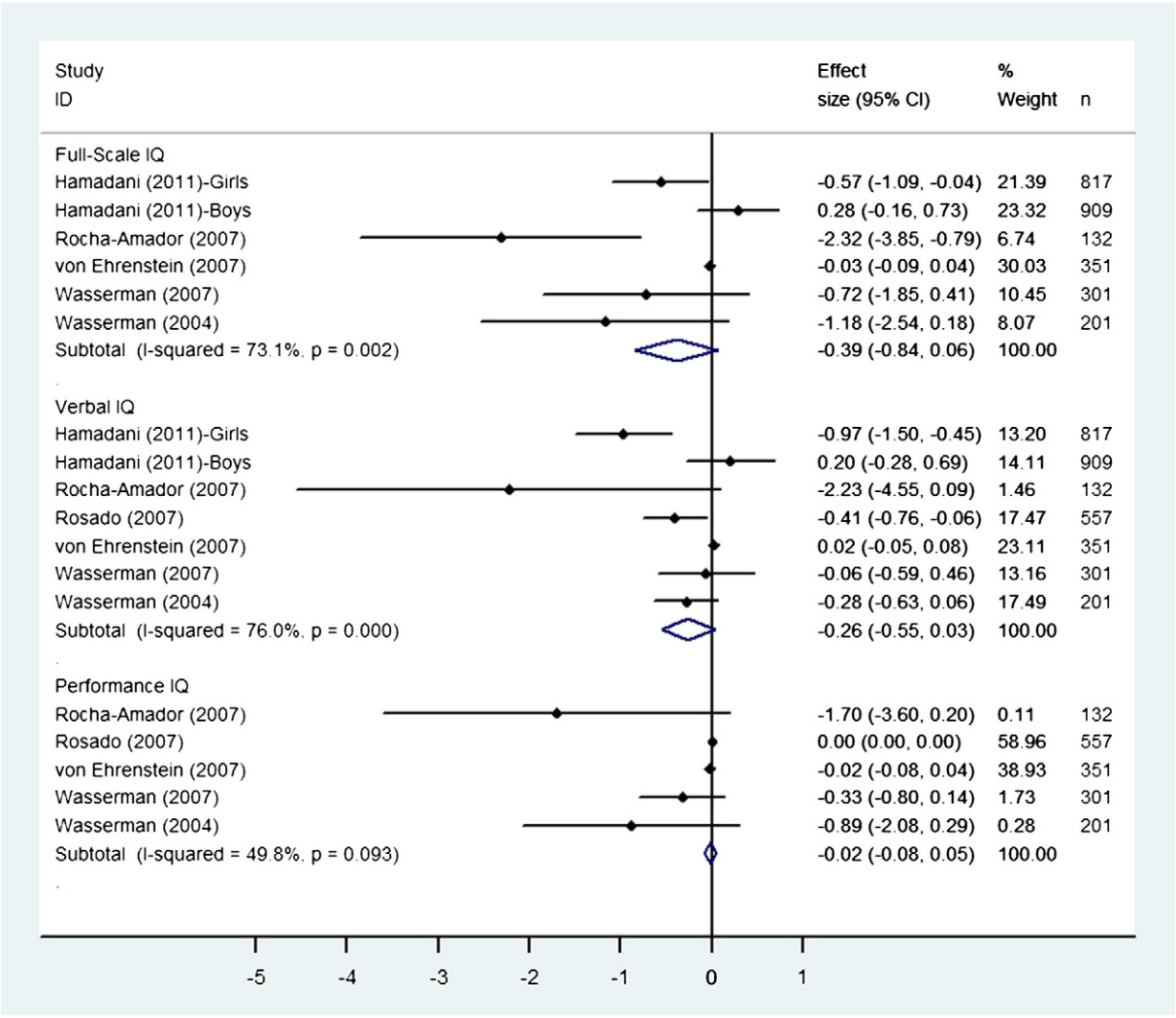


Fig. 1. Forest plot of effect size on intellectual quotient (IQ) by a 50% increment in urine As levels.

total arsenic in blood (Wasserman et al., 2011). Calderón et al. (2001) observed a negative effect of total arsenic in urine on Full Scale and Verbal IQ in children aged 6–9 years, while Hamadani et al. (2011) detected the same effect in 5-year-old girls. However, the finding was not statistically significant in boys. Wright et al. (2006) also observed a negative effect on Full-scale and Verbal IQ when they studied total arsenic levels in hair among children aged 11–13 years (Table 2).

Parvez et al. (2011) observed negative associations between total arsenic levels in urine, water, blood and nails, and motor function in children aged 8–11 years, while Tsai et al. (2003) showed an association between increased levels of total arsenic in drinking water, and memory and attention problems in children aged 13 and 14. In the study by Rosado et al. (2007) a negative effect was observed from arsenic levels in urine of children aged 6–8 years on digit span, letter sequencing and visual search subscales; a stronger association was found with organic forms of arsenic (DMA and MMA) than with inorganic forms (arsenate and arsenite). Furthermore, in the study by von Ehrenstein et al. (2007) a negative association was found between inorganic arsenic in urine, and vocabulary test, object assembly test and picture completion test scores in children aged 5–15 years. In two articles that assessed prenatal exposure in the same cohort of pregnant women (Tofail et al., 2009; Hamadani et al., 2010), no significant association was found with neurodevelopment in the first months of life, despite this study having the largest sample size. The study estimated prenatal exposure through inorganic arsenic levels in maternal urine at weeks 8 and 30 of gestation; neurodevelopment was assessed using BSID-II at 7 and 18 months of age, respectively (Table 2).

3.1.4. Results of the meta-analysis

We conducted separate meta-analyses on the results of the articles that assessed arsenic exposure in urine (six articles) and drinking water (four articles). In the studies by Rosado et al. (2007) and von Ehrenstein et al. (2007) we considered the Digit Span subscale score for the verbal domain and the Coding subscale for the performance domain, since Verbal and Performance IQ scores were not explicitly reported. Hamadani et al. (2011) reported separate results for boys and girls, and so we included the two effects independently in our meta-analysis.

Results were highly heterogeneous in both cases (73% for Full Scale IQ, 76% for Verbal IQ and 50% for Performance IQ), and so we decided to apply a random effects model to combine the results. However, this heterogeneity was not discordant, since all studies except the one by Hamadani et al. (2011) in boys and the one by von Ehrenstein et al. (2007), suggested a negative effect on IQ. The

combined magnitude of effect suggests that a 50% increase in arsenic levels in urine causes a decrease of −0.39 point (95% CI: −0.84; 0.06; $p = 0.090$) in the Full Scale IQ of children aged 5–15 years. A decrease of −0.26 point ($p = 0.081$) was also observed in the verbal IQ, whereas it was not significant in the performance IQ (theta = −0.02; $p = 0.586$) (Fig. 1).

Furthermore, a 50% increase in arsenic levels in children's regular drinking water would cause a significant decrease ($p = 0.052$) of −0.56 point (95% CI: −1.13; 0.01) in the Full Scale IQ for the same age range. In the performance IQ, the decrease would be −0.33 point ($p = 0.050$), whereas no statistically significant decrease was observed in the verbal IQ (theta = −0.06; $p = 0.394$) (Fig. 2).

3.1.5. Effects on behavioural disorders

None of the three studies that assessed arsenic exposure and its relation with children's behavioural conduct (Khan et al., 2011; Roy et al., 2011; Yousef et al., 2011) observed a significant effect. The first study measured total arsenic in water, blood and urine, the second measured it in urine and the third in blood.

3.2. Cadmium

3.2.1. Overview

Cadmium (Cd) is a scarce element in nature with concentrations ranging from 0.1 to 5 μg/g. It is usually found combined with zinc, and to a lesser extent with lead and copper. However, it is rated seventh in the ATSDR list of elements posing the most significant potential threat to human health in the environment, and it ranks third in the metallic trace elements subdivision of the same list, behind lead and mercury (ATSDR, 2011).

Over 80% of cadmium production goes to manufacturing nickel–cadmium batteries, although it is also used as a pigment for plastics and in the ceramics and glass industry. It is estimated that world production of cadmium amounted to 22,000 tonnes in 2010, while world reserves of this metal stood at 660,000 tonnes. The world's largest producers of cadmium are China and the Republic of Korea, which together account for 40% of total production (U.S. Geological Survey, 2011).

The main sources of cadmium exposure in children are food, cigarette smoke and household dust. Both animal experiments and epidemiological studies alike have confirmed that cadmium is toxic to lung, kidney, liver, digestive system, bone tissue and gonads. It can cause cancer and it is also neurotoxic (Cao et al., 2009).

3.2.2. Reference values

In 2007, the American Conference of Governmental Industrial Hygienists stated a maximum recommended value in humans of 5 μg/l of cadmium in blood (ACGIH, 2007). None of the reviewed studies that measured the concentration of cadmium in blood (Cao et al., 2009; Tian et al., 2009) in children, maternal blood or umbilical cord blood, exceeded these reference values (Table 3). The article by Yousef et al. (2011) did not state the mean level of cadmium in blood.

We did not find any recommendations regarding reference values of cadmium in hair, which was the exposure biomarker used in 3 of the 6 reviewed studies.

The study by Bao et al. (2009) conducted in China measured cadmium levels in drinking water, and it found a concentration that was double the reference value of 3 μg/l stated in the Guidelines for drinking-water quality published by the World Health Organization (WHO, 2004).

3.2.3. Effects on neurodevelopment

Only one of the four studies that evaluated the effects of cadmium exposure on neurodevelopment showed a significant negative effect (Table 3). The study by Tian et al. (2009) in a prospective cohort found lower Full-Score IQ and Performance IQ at 4 years of age in children who had higher levels of cadmium in cord blood at birth.

3.2.4. Effects on behavioural disorders

With regard to the assessment of behavioural disorders, the cross-sectional study by Bao et al. (2009), conducted in China, found a higher frequency of withdrawal, social problems and attention problems associated with higher levels of cadmium in hair in children aged 7–16 years. Yousef et al. (2011) did not find any significant association between cadmium exposure and ADHD (Table 3).

3.3. Manganese

3.3.1. Overview

Manganese (Mn) is a very common element in the environment; it is the fifth most abundant metallic trace element and the twelfth most abundant element in the earth's crust and is present in nature in inorganic and organic forms. In industry it is widely used in iron and steel production and foundry processes (iron and manganese casting). Also, manganese is commonly used as an agrochemical and in the ceramics industry. In blood, most manganese binds to erythrocytes (Gil and Gisbert-Calabuig, 2004). It is an essential nutrient for the body; it plays a part in tissue and bone formation as well as in fat and carbohydrate metabolism. It is also involved in the immune system and has been associated with cancer prevention. However, depending on the exposure route and dose, it accumulates in the body, especially in the brain, and causes neurological damage due to its accumulation in the central nervous system (Aschner et al., 2007; ATSDR, 2008).

Manganese is present in the food chain in all food and drinking water, usually at levels below 5 mg/kg. It is detectable in almost all samples of particles suspended in the air (WHO, 1981).

Furthermore, cigarette smoke also has low levels of Mn, which could make it a source of manganese exposure, particularly for children who live in households where there are smokers (ATSDR, 2008).

3.3.2. Reference values

Two studies conducted in Bangladesh and another in the USA that measured manganese levels in drinking water (Parvez et al., 2011; Khan et al., 2011; Wasserman et al., 2006) found that levels were well above the World Health Organization recommendation of 400 μg/l (WHO, 2004). In the study conducted in Canada (Bouchard et al., 2011) values were much lower that the reference limit (31 μg/l) (Table 4).

According to the Agency for Toxic Substances and Disease Registry, "normal" levels for manganese in blood range from 4 to 14 μg/l (ATSDR, 2008). Two studies that we reviewed found levels that exceeded the upper limit of normal, one in blood of children in Mexico (Claus Henn et al., 2010 with 24.3 μg/l), and another in cord blood in France (Takser et al., 2003 with 38.5 μg/l) (Table 4).

We did not find any reference values for normal levels of manganese in hair, which was the exposure measure used in 7 of the 17 studies included in our review.

3.3.3. Effects on neurodevelopment

Most articles that evaluated the effects of manganese exposure on neurodevelopment found a negative association with Full Score IQ and, in almost all cases, with the verbal domain (Table 4). These associations were detected both in newborn and 12-month-old infants and also in children aged 6–13 years.

Claus Henn et al. (2010) detected deficits in the Mental Development Index (MDI) associated with higher and lower levels of manganese in blood at 12 months of age in a cohort of children, but this association was not maintained in the MDI at 18 and 24 months of age. In another longitudinal study, Takser et al. (2003) observed attention, memory

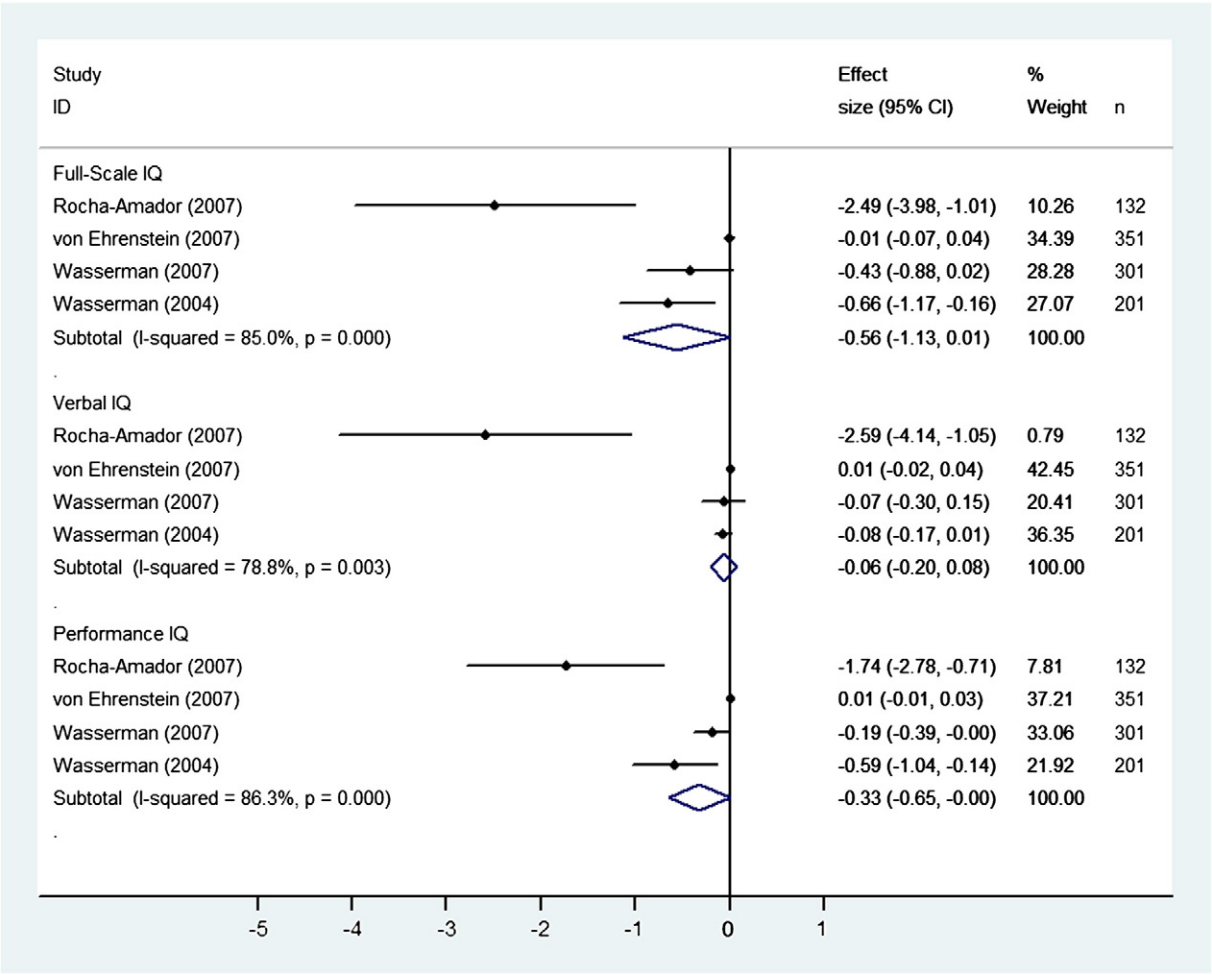


Fig. 2. Forest plot of effect size on intellectual quotient (IQ) by a 50% increment in water As levels.

and hand skill problems in neonates, associated with manganese levels in cord blood.

Bouchard et al. (2011), Menezes-Filho et al. (2011), Riojas-Rodríguez et al. (2010) and Wright et al. (2006) found a negative association between manganese levels in hair of children aged 6–13 years and Full Scale IQ and Verbal IQ. These were all cross-sectional studies. Wasserman et al. (2011) and Kim et al. (2009) reported the same finding when they studied manganese levels in blood in children aged 8–11 years.

Wasserman et al. (2006) and Bouchard et al. (2011) found that children who drank water with higher concentrations of manganese obtained lower scores on the Full Scale, Verbal and Performance IQ. Only 3 out of the 12 studies (Parvez et al., 2011; Torrente et al., 2005; Zaleha et al., 2003) did not find a significant association between manganese exposure and effects on neurodevelopment (Table 4).

3.3.4. Results of the meta-analysis

In the meta-analysis we included the results of articles that evaluated manganese exposure through levels in children's hair, because in this case we had sufficient data to apply these statistical techniques. We included four articles that evaluated the association between manganese levels in hair and Full Scale IQ, Verbal IQ and Performance IQ through linear regression using the Wechsler scale.

Both the DerSimonian and Laird test and the I^2 coefficient showed absence of heterogeneity among the studies (<0.1%). The combined effect shows that for each 50% increase in manganese levels in hair, there is a significant decrease ($p < 0.001$) of −0.70 point (95% CI: −1.07; −0.34) in the Full Scale IQ of children aged 6–13 years (Fig. 3). This decrease was greater in the Verbal IQ, reaching −1.26 points ($p = 0.008$), and was −0.42 point in the Performance IQ ($p = 0.039$).

3.3.5. Effects on behavioural disorders

With regard to behavioural disorders, all reviewed articles showed a positive association between manganese exposure and behavioural disorders in children aged between 5 and 15 years (Table 4). Three of the five studies (Yousef et al., 2011; Farias et al., 2010; Ericson et al., 2007) found a higher risk of attention deficit hyperactive disorder (ADHD) associated with manganese exposure, measured through levels in blood (in the first two studies) or in teeth (in the third). Khan et al. (2011) observed higher scores on internalising and externalising behaviour associated with higher levels of manganese in the regular drinking water of children aged 8–11, while Bouchard et al. (2007) found a similar result associated with oppositional and hyperactivity subscale scores of Conner's scales when they measured manganese levels in hair of children aged 6–15 (Table 4).

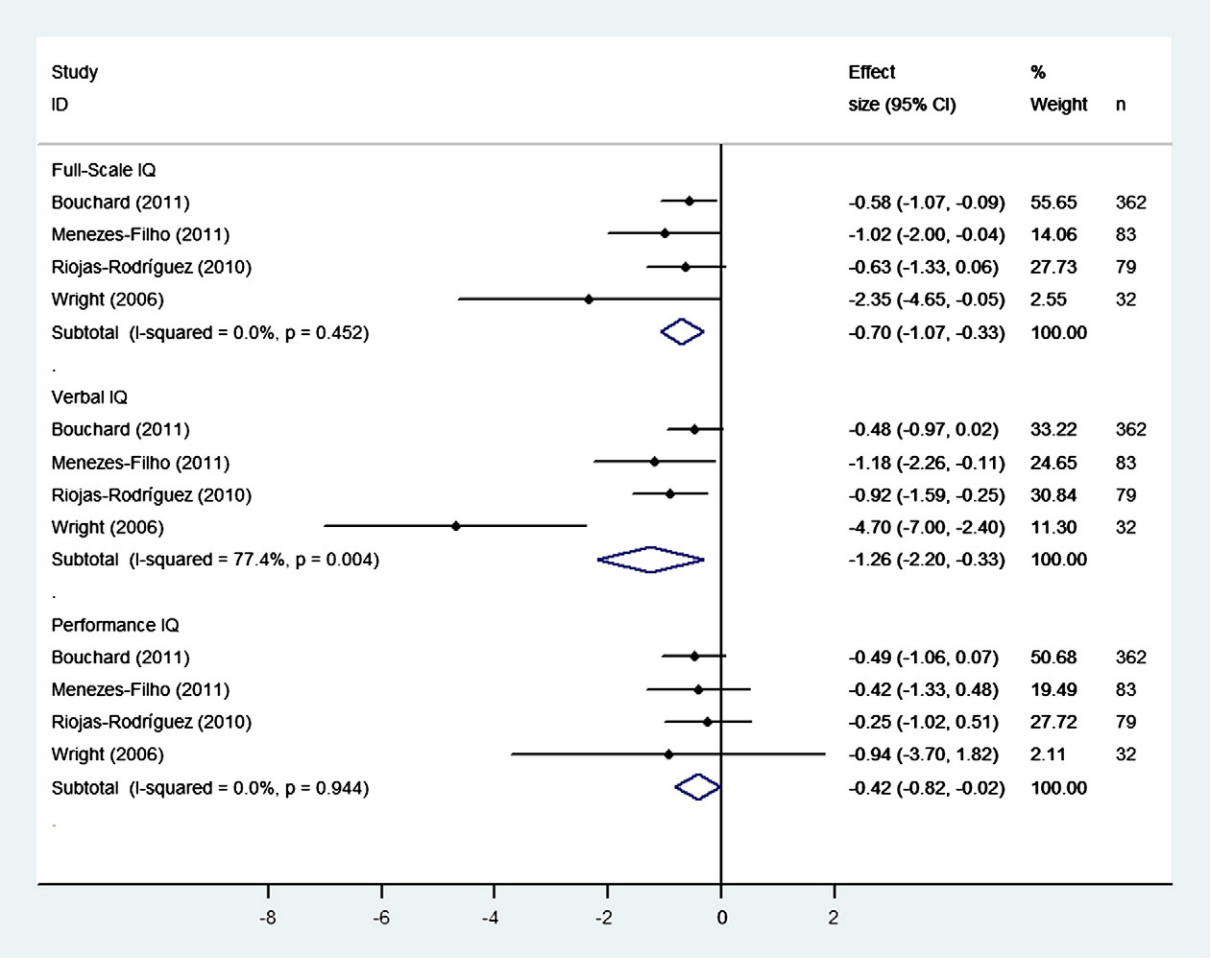


Fig. 3. Forest plot of effect size on intellectual quotient (IQ) by a 50% increment in hair Mn levels.

4. Discussion and conclusions

The results of this review show that there is evidence in the recent scientific literature that relates arsenic and manganese exposure with neurodevelopmental problems in children, but there is little information on cadmium exposure. Few studies have evaluated behavioural disorders due to exposure to these compounds, and manganese is the only one of the three metallic elements studied in this review for which there is more evidence of the existence of association with ADHD.

After pooling and synthesising the results of the studies published to date, it appears that for every 50% increase in arsenic levels (either in urine or in regular drinking water) there could be approximately a 0.5 decrease in the IQ of children aged 5–15 years. The results for magnitude of effect in verbal and performance domains depended on whether arsenic exposure was measured in urine or drinking water, but both measurements suggest a similar trend. The only study that evaluated prenatal exposure to arsenic did not find an association with neurodevelopment in the first months of life, despite this study having the largest sample size. This effect on IQ was observed at levels ranging from 51 to 117 μg/l in urine and from 118 to 194 μg/l in water. All studies included in this meta-analysis found mean arsenic levels in urine above the reference value of 50 μg/l (which is the "safety limit" recommended by ATSDR), and levels detected in drinking water were 10 to 20 times above the limit recommended by the WHO. This shows that these results were obtained in areas with high environmental levels of arsenic (Bangladesh, Mexico and India), and therefore it may not be possible to extrapolate the effects to populations with low levels of exposure.

One of the main mechanisms of neurotoxicity from arsenic that might explain these findings is related to increased oxidative stress, which causes DNA damage (Singh et al., 2011). It has been shown that exposure to arsenic and its metabolites affects NMDA receptors in the hippocampus, which play an essential role in synaptic plasticity, learning and memory. This may lead to neurobehavioural disorders and cognitive dysfunctions (Luo et al., 2009; Krüger et al., 2009). Recent studies have also pointed out the role of oxidative stress associated with exposure to arsenic and other metallic trace elements as a cause of neuronal insult in certain pathologies such as autism (Kern and Jones, 2006).

In relation to manganese, the result of our meta-analysis suggests that a 50% increase in levels in hair would be associated with a decrease of 0.7 point in the IQ of children aged 6–13 years. This effect is observed both in the performance and verbal domains, being more marked in the latter. Although only two studies evaluated prenatal exposure to manganese, in both cases negative effects were also found in neurodevelopment in the first months of life. The mean levels of manganese at which this effect was detected in neurodevelopment varied between 0.5 and 12 μg/g in hair. Although we did not find any reference values for manganese levels in hair, mean levels in water and blood reported in these studies

were well below the limits recommended by the WHO and ATSDR, which suggests that this effect could even occur at low levels of exposure.

Claus Henn et al. (2010) described a relation between manganese with mental development as an inverted U-shaped curve in children aged 12 months. Since all studies included in the meta-analysis summarised the relation in a linear or log-linear function, we do not know if this complex relation persists in the age range used in these studies (6–13 years). If this were the case, the results of the individual studies and the result of the meta-analysis alike would underestimate the effect of the decreasing part of the inverted U described by Claus Henn et al. (2010), that is, from a certain threshold of exposure, which is impossible to determine in this study.

It is known that the central nervous system is the first target of manganese toxicity. Although it is also known that manganese is toxic to cells and can impair transport systems, enzyme activity and receptor functions, the way in which manganese is neurotoxic has not yet been clearly established. Most research on manganese neurotoxic mechanisms have focused on studying dopaminergic system disturbances, but there is evidence to suggest action on other neurotransmitters, including GABA and glutamate in the basal ganglia and other brain regions (ATSDR, 2008; Aschner et al., 2007; Fitsanakis et al., 2006).

Few studies published to date have evaluated neurodevelopmental problems and behavioural disorders due to cadmium exposure. Of all the studies included in our review, an association was only observed in two conducted in China, which found significantly higher levels than other studies, suggesting that these effects may not be observable at low levels of exposure.

Animal experiments have shown that cadmium affects brain metabolism, inhibiting sulfhydryl-containing enzymes. Therefore, chronic exposure to cadmium has a depressant effect on the levels of various neurotransmitters such as norepinephrine, serotonin and acetylcholine (Singhal et al., 1976; Stowe et al., 1972). Animal studies have also shown that cadmium can cross the blood–brain barrier (Andersson et al., 1997), and this strengthens the hypothesis that the blood–brain barrier does not prevent cadmium from reaching the brain during early development stages in children (Provias et al., 1994). These evidences suggest that cadmium reaches the central nervous system directly, which would cause a neurotoxic effect in child development, and have an impact on neurodevelopment (Cao et al., 2009; Petersson-Grawe et al., 2004).

One of the main limitations of a meta-analysis is the possible existence of publication bias. We used Begg's and Egger's tests to quantify bias, and did not obtain conclusive results to suggest the existence of publication bias in any of the three meta-analyses. Furthermore, although there was generally high heterogeneity among study results, it was not discordant in any cases, and so we were able to control it by using appropriate statistical techniques.

All the studies included in the meta-analysis except one were classified as having a high methodological quality according to the procedure described in the methodology. They all had a suitable design and methodology for the research purposes and they investigated potential confounders to avoid the presence of bias as far as possible, which would have influenced results. For these reasons we believe that the results obtained in these studies, and therefore those obtained in our meta-analysis, are reliable.

Although there is apparently clear evidence of an association between exposure to arsenic and manganese during childhood and neurodevelopmental problems, it is necessary to delve further into the effects of prenatal exposure to these compounds, as there is little knowledge of this type of exposure. The small number of studies that have evaluated neurodevelopmental problems due to cadmium exposure makes it impossible to draw clear conclusions regarding this compound. More research is needed to further study the potential effects of exposure to arsenic and cadmium on attention deficit hyperactivity disorder and other behavioural disorders in children.

Finally, only two articles out of the 41 included in this review presented results stratified by sex; all the others treated this variable as a confounder in the statistical analysis. It should be noted that none of these articles made an analysis of gender differences in order to disaggregate social differences from merely biological characteristics. This would identify differences in exposure patterns of children associated with differences in the way that they interact with the environment. This approach should provide a better understanding of the mechanisms and pathways underlying the complex relationship between exposure and effect, and provide information to implement preventive intervention strategies (Mergler, 2012; Clougherty, 2010).

Conflict of interest

The authors declare that they do not have conflicts of interest.

Acknowledgements

We thank the Regional Health Council of Andalusia (Spain) for financially supporting this project (project number: PI0755/2010).

Appendix A

Abbreviations of the scales used to assess neurodevelopmental and behavioural disorders:

B-L scales Brunet–Lézine scales, 1993
BASC Behavioural Assessment System for Children rating scale, 1992
BOT-2 Bruininks–Oseretsky test, version 2, 2005
BSID Bayley Scales of Infant Development, Spanish version, 1977
BSID-II Bayley Scales of Infant Development—II, 1993
CAT Cognitive Abilities Test (Detterman, 1988)
CBCL Child Behaviour Checklist, 1991
CPRS Conners' Parent Rating Scaled, 1973
CPRS-R Conners' Parent Rating Scale—Revised, 1997
CRT Combined Raven's Test, 1983
CRT-RC2 Combined Raven's Test—The Rural in China methods, 1983
CTRS Conners' Teachers Rating Scale, 1973
CTRS-R Conners' Teachers Rating Scale—Revised, 2000
CVLT-C California Verbal Learning Test—Children, 1994
DBDS Disruptive Behavior Disorders Scale, 1992
DSM-IV Diagnostic and Statistical Manual of Mental Disorders, 1994
KEDI-WISC Korean Educational Development Institute—Wechsler Intelligence Scales, 1986
McCarthy McCarthy scales, 1976
NEPSY Developmental Neuropsychological Assessment, 1999
NES2-T Neurobehavioral Evaluation System 2, Taiwanese version, 1996
NLS Number and letter sequencing test, 1992
RPM Raven's Progressive Matrices, 1956
SBIS Stanford–Binet Intelligence Scale third revision, 1973
SNAP-IV Swanson, Nolan and Pelham, version IV, 1995
TONI-2 Test of Nonverbal Intelligence (second edition), 1990
WASI Wechsler Abbreviated Scale of Intelligence, 1999
WISC-III Wechsler Intelligence Scale for Children, version III, 1991
WISC-IV Wechsler Intelligence Scale for Children, version IV, 2003
WISC-R Wechsler Intelligence Scale for Children—Revised, 1983
WISC-RM Wechsler Intelligence Scale for Children—Revised Mexican Version, 1993
WLPB-R Woodcock Language Proficiency Battery—Revised, 1991
WPPSI-III Wechsler Preschool and Primary Scale of Intelligence, 3rd edition, 2002
WPPSI-R Wechsler Preschool and Primary Scales of Intelligence—Revised, 1989
WRAML Wide Range Assessment of Memory and Learning, 1990

Appendix B

Methods section of STROBE checklist

Methods	Item no.	Recommendation
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case–control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants (*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case–control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	*(a)* Describe all statistical methods, including those used to control for confounding *(b)* Describe any methods used to examine subgroups and interactions *(c)* Explain how missing data were addressed (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case–control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy *(e)* Describe any sensitivity analyses

References

Abbas S, Qureshi EMA, Ahmad F, Vehra S, Khan AU. An assessment of relationship between arsenic in drinking water, health status and intellectual functioning of children in district Kasur. Pak J Nutr 2012;11:150–3.

ACGIH. American conference of governmental industrial hygienists, 2007. Cadmium. Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists; 2007. p. 17.

Andersson H, Petersson-Grawe K, Lindqvist E, Luthman J, Oskarsson A, Olson L. Low-level cadmium exposure of lactating rats causes alterations in brain serotonin levels in the offspring. Neurotoxicol Teratol 1997;19:105–15.

Argos M, Kalra T, Rathouz PJ, Chen Y, Pierce B, Parvez F, et al. Arsenic exposure from drinking water, and all-cause and chronic-disease mortalities in Bangladesh (HEALS): a prospective cohort study. Lancet 2010;376:252–8.

Aschner M, Guilarte TR, Schneider JS, Zheng W. Manganese: recent advances in understanding its transport and neurotoxicity. Toxicol Appl Pharmacol 2007;221:131–47.

ATSDR. Agency for Toxic Substances and Disease Registry, 2000. Medical management guidelines for acute chemical exposures, vol. III. Atlanta: US Department of Health and Human Services 2000.

ATSDR. Agency for Toxic Substances and Disease Registry, 2007. Toxicological profile for arsenic. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services 2007.

ATSDR. Agency for Toxic Substances and Disease Registry, 2007. Toxicological profile for manganese. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services 2008.

ATSDR. Agency for Toxic Substances and Disease Registry, 2011. The priority list of hazardous substances, 2011. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services 2011 [(http://www.atsdr.cdc.gov/spl/). Accessed 10 January 2012].

Au WW. Susceptibility of children to environmental toxic substances. Int J Hyg Environ Health 2002;205:501–3.

Bao QS, Lu CY, Song H, Wang M, Ling W, Chen WQ, et al. Behavioural development of school-aged children who live around a multi-metalsulphide mine in Guangdong province, China: a cross-sectional study. BMC Public Health 2009;9:217.

Bellinger DC. Comparing the population neurodevelopmental burdens associated with children's exposures to environmental chemicals and other risk factors. Neurotoxicology 2012;33:641–3.

Bouchard M, Laforest F, Vandelac L, Bellinger D, Mergler D. Hair manganese and hyperactive behaviors: pilot study of school-age children exposed through tap water. Environ Health Perspect 2007;115:122–7.

Bouchard MF, Sauvé S, Barbeau B, Legrand M, Brodeur MÈ, Bouffard T, et al. Intellectual impairment in school-age children exposed to manganese from drinking water. Environ Health Perspect 2011;119:138–43.

Calderón J, Navarro ME, Jimenez-Capdeville ME, Santos-Diaz MA, Golden A, Rodriguez-Leyva I, et al. Exposure to arsenic and lead and neuropsychological development in Mexican children. Environ Res 2001;85:69–76.

Cao Y, Chen A, Radcliffe J, Dietrich KN, Jones RL, Caldwell K, et al. Postnatal cadmium exposure, neurodevelopment, and blood pressure in children at 2, 5, and 7 years of age. Environ Health Perspect 2009;117:1580–6.

Claus Henn B, Ettinger AS, Schwartz J, Téllez-Rojo MM, Lamadrid-Figueroa H, Hernández-Avila M, et al. Early postnatal blood manganese levels and children's neurodevelopment. Epidemiology 2010;21:433–9.

Clougherty JE. A growing role for gender analysis in air pollution epidemiology. Environ Health Perspect 2010;118:167–76.

Counter SA, Buchanan LH. Mercury exposure in children: a review. Toxicol Appl Pharmacol 2004;198:209–30.

Ericson JE, Crinella FM, Clarke-Stewart KA, Allhusen VD, Chan T, Robertson RT. Prenatal manganese levels linked to childhood behavioural disinhibition. Neurotoxicol Teratol 2007;29:181–7.

Farias AC, Cunha A, Benko CR, McCracken JT, Costa MT, Farias LG, et al. Manganese in children with attention-deficit/hyperactivity disorder: relationship with methylphenidate exposure. J Child Adolesc Psychopharmacol 2010;20:113–8.

Fitsanakis VA, Au C, Erikson KM, Aschner M. The effects of manganese on glutamate, dopamine and γ-aminobutyric acid regulation. Neurochem Int 2006;48:426–33.

Gil F, Gisbert-Calabuig JA. Intoxicaciones por otros metales. Capítulo 70. In: Villanueva E, editor. Gisbert-Calabuig Medicina Legal y Toxicología6ª ed. ; 2004. p. 964–80. [Masson SA, Barcelona (ISBN 84-458-1415-X)].

Gil F, Pla A. Biomarkers as biological indicators of xenobiotic exposure. J Appl Toxicol 2001;21:245–55 Review article.

Grandjean P, Landrigan PJ. Developmental neurotoxicity of industrial chemicals. Lancet 2006;368:2167–78 [Review].

Hall AH. Chronic arsenic poisoning. Toxicol Lett 2002;128:69–72.
International Programme on Chemical Safety. Arsenic and arsenic compounds. Environmental health criteria, 224. Geneva: World Health Organization 2001.

Hamadani JD, Grantham-McGregor SM, Tofail F, Nermell B, Fängström B, Huda SN, et al. Pre- and postnatal arsenic exposure and child development at 18 months of age: a cohort study in rural Bangladesh. Int J Epidemiol 2010;39:1206–16.

Hamadani JD, Tofail F, Nermell B, Gardner R, Shiraji S, Bottai M, et al. Critical windows of exposure for arsenic-associated impairment of cognitive function in pre-school girls and boys: a population-based cohort study. Int J Epidemiol 2011;40:1593–604.

Healy SM, Wildfang E, Zakharyan RA, Aposhian HV. Diversity of inorganic arsenite biotransformation. Biol Trace Elem Res 1999;68:249–66.

Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557–60.

Jakubowski M. Low-level environmental lead exposure and intellectual impairment in children—the current concepts of risk assessment. Int J Occup Med Environ Health 2011;24:1–7.

Kern JK, Jones AM. Evidence of toxicity, oxidative stress, and neuronal insult in autism. J Toxicol Environ Health B Crit Rev 2006;9:485–99.

Khan K, Factor-Litvak P, Wasserman GA, Liu X, Ahmed E, Parvez F, et al. Manganese exposure from drinking water and children's classroom behavior in Bangladesh. Environ Health Perspect 2011;119:1501–6.

Kim Y, Kim BN, Hong YC, Shin MS, Yoo HJ, Kim JW, et al. Co-exposure to environmental lead and manganese affects the intelligence of school-aged children. Neurotoxicology 2009;30:564–71.
Krüger K, Straub H, Hirner AV, Hippler J, Binding N, Musshoff U. Effects of monomethylarsonic and monomethylarsonous acid on evoked synaptic potentials in hippocampal slices of adult and young rats. Toxicol Appl Pharmacol 2009;236:115–23.
Landrigan PJ, Kimmel CA, Correa A, Eskenazi B. Children's health and the environment: public health issues and challenges for risk assessment. Environ Health Perspect 2004;112:257–65.
Luo JH, Qiu ZQ, Shu WQ, Zhang YY, Zhang L, Chen JA. Effects of arsenic exposure from drinking water on spatial memory, ultra-structures and NMDAR gene expression of hippocampus in rats. Toxicol Lett 2009;184:121–5.
Menezes-Filho JA, NovaesCde O, Moreira JC, Sarcinelli PN, Mergler D. Elevated manganese and cognitive performance in school-aged children and their mothers. Environ Res 2011;111:156–63.
Mergler D. Neurotoxic exposures and effects: gender and sex matter! Hänninen lecture 2011. Neurotoxicology 2012;33:644–51.
Olmos M, Antelo M, Vazquez H, Smecuol E, Maurino E, Bai JC. Systematic review and meta-analysis of observational studies on the prevalence of fractures in coeliac disease. Dig Liver Dis 2008;40:46–53.
Orloff K, Mistry K, Metcalf S. Biomonitoring for environmental exposures to arsenic. J Toxicol Environ Health B 2009;12:509–24.
Parvez F, Wasserman GA, Factor-Litvak P, Liu X, Slavkovich V, Siddique AB, et al. Arsenic exposure and motor function among children in Bangladesh. Environ Health Perspect 2011;119:1665–70.
Petersson-Grawe K, Teiling-Gardlund A, Jalkesten E, Oskarsson A. Increased spontaneous motor activity in offspring after maternal cadmium exposure during lactation. Environ Toxicol Pharmacol 2004;17:35–43.
Provias JP, Ackerley CA, Smith C, Becker LE. Cadmium encephalopathy: a report with elemental analysis and pathological findings. Acta Neuropathol 1994;88:583–6.
Ricci-Cabello I, Ruiz-Perez I, Olry de Labry-Lima A, Marquez-Calderon S. Do social inequalities exist in terms of the prevention, diagnosis, treatment, control and monitoring of diabetes? A systematic review. Health Soc Care Community 2010;18:572–87.
Riccio C, Sullivan J, Cohen M. Neuropsychological assessment and intervention for childhood and adolescent disorders. Hoboken, New Jersey: John Wiley & Sons, Inc. 2010.
Rice D, Barone Jr S. Critical periods of vulnerability for the developing nervous system. Evidence from humans and animals models. Environ Health Perspect 2000;108(Suppl. 3):511–33.
Riojas-Rodríguez H, Solís-Vivanco R, Schilmann A, Montes S, Rodríguez S, Ríos C, et al. Intellectual function in Mexican children living in a mining area and environmentally exposed to manganese. Environ Health Perspect 2010;118:1465–70.
Rocha-Amador D, Navarro ME, Carrizales L, Morales R, Calderón J. Decreased intelligence in children and exposure to fluoride and arsenic in drinking water. Cad Saude Publica 2007;23(Suppl. 4):S579–87.
Rosado JL, Ronquillo D, Kordas K, Rojas O, Alatorre J, Lopez P, et al. Arsenic exposure and cognitive performance in Mexican schoolchildren. Environ Health Perspect 2007;115:1371–5.
Roy A, Kordas K, Lopez P, Rosado JL, Cebrian ME, Vargas GG, et al. Association between arsenic exposure and behavior among first-graders from Torreón, Mexico. Environ Res 2011;111:670–6.
Scales Jr CD, Dahm P. The critical use of population-based medical databases for prostate cancer research. Curr Opin Urol 2008;18:320–5.
Schoeman K, Bend JR, Hill J, Nash K, Koren G. Defining a lowest observable adverse effect hair concentrations of mercury for neurodevelopmental effects of prenatal methylmercury exposure through maternal fish consumption: a systematic review. Ther Drug Monit 2009;31:670–82.
Shagirtha K, Muthumani M, Prabu SM. Melatonin abrogates cadmium induced oxidative stress related neurotoxicity in rats. Eur Rev Med Pharmacol Sci 2011;15:1039–50.
Singh AP, Goel RK, Kaur T. Mechanisms pertaining to arsenic toxicity. Toxicol Int 2011;18:87–93.
Singhal RL, Merali Z, Hrdina PD. Aspects of the biochemical toxicology of cadmium. Fed Proc 1976;35:75–80.
Smedley, Kinniburgh. A review of the source, behaviour and distribution of arsenic in natural waters. Appl Geochem 2002;17:517–68.
Stowe HD, Wilson M, Goyer RA. Clinical and morphologic effects of oral cadmium toxicity in rabbits. Arch Pathol 1972;94:389–405.
Takser L, Mergler D, Hellier G, Sahuquillo J, Huel G. Manganese, monoamine metabolite levels at birth, and child psychomotor development. Neurotoxicology 2003;24:667–74.
Tian LL, Zhao YC, Wang XC, Gu JL, Sun ZJ, Zhang YL, et al. Effects of gestational cadmium exposure on pregnancy outcome and development in the offspring at age 4.5 years. Biol Trace Elem Res 2009;132:51–9.
Tofail F, Vahter M, Hamadani JD, Nermell B, Huda SN, Yunus M, et al. Effect of arsenic exposure during pregnancy on infant development at 7 months in rural Matlab, Bangladesh. Environ Health Perspect 2009;117:288–93.
Torrente M, Colomina MT, Domingo JL. Metal concentrations in hair and cognitive assessment in an adolescent population. Biol Trace Elem Res 2005;104:215–21.
Tsai SY, Chou HY, The HW, Chen CM, Chen CJ. The effects of chronic arsenic exposure from drinking water on the neurobehavioral development in adolescence. Neurotoxicology 2003;24:747–53.
U.S. Geological Survey. Mineral commodity summaries 2011. U.S. Geological Survey 2011 [198 pp.].
von Ehrenstein OS, Poddar S, Yuan Y, Mazumder DG, Eskenazi B, Basu A, et al. Children's intellectual function in relation to arsenic exposure. Epidemiology 2007;18:44–51.
von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. STROBE initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol 2008;61:344–9.
Wang SX, Wang ZH, Cheng XT, Li J, Sang ZP, Zhang XD, et al. Arsenic and fluoride exposure in drinking water: children's IQ and growth in Shanyin county, Shanxi province, China. Environ Health Perspect 2007;115:643–7.
Wasserman GA, Liu X, Parvez F, Ahsan H, Factor-Litvak P, van Geen A, et al. Water arsenic exposure and children's intellectual function in Araihazar, Bangladesh. Environ Health Perspect 2004;112:1329–33.
Wasserman GA, Liu X, Parvez F, Ahsan H, Levy D, Factor-Litvak P, et al. Water manganese exposure and children's intellectual function in Araihazar, Bangladesh. Environ Health Perspect 2006;114:124–9.
Wasserman GA, Liu X, Parvez F, Ahsan H, Factor-Litvak P, Kline J, et al. Water arsenic exposure and intellectual function in 6-year-old children in Araihazar, Bangladesh. Environ Health Perspect 2007;115:285–9.
Wasserman GA, Liu X, Parvez F, Factor-Litvak P, Ahsan H, Levy D, et al. Arsenic and manganese exposure and children's intellectual function. Neurotoxicology 2011;32:450–7.
WHO, World Health Organization. Manganese. Environmental health criteria, 17 1981 [Geneva, Switzerland: (http://www.inchem.org/documents/ehc/ehc/ehc017.htm) Accessed 24 February 2012].
WHO, World Health Organization. Guidelines for drinking-water quality 3rd ed. 2004 [Geneva, Switzerland: (http://www.who.int/water_sanitation_health/dwq/gdwq3/en/). Accessed 21 February 2012].
Wright RO, Amarasiriwardena C, Woolf AD, Jim R, Bellinger DC. Neuropsychological correlates of hair arsenic, manganese, and cadmium levels in school-age children residing near a hazardous waste site. Neurotoxicology 2006;27:210–6.
Yousef S, Adem A, Zoubeidi T, Kosanovic M, Mabrouk AA, Eapen V. Attention deficit hyperactivity disorder and environmental toxic metal exposure in the United Arab Emirates. J Trop Pediatr 2011;57:457–60.
Zaleha MI, Hayaati AR, Rizam AR, Jamaludin M, Osman A. Micronutrients and its correlation with mental performance among schoolchildren in Bario, Sarawak: a preliminary study. Med J Malaysia 2003;58:309–19.

Cadmium exposure and neuropsychological development in school children in southwestern Spain

Rodríguez-Barranco M, Lacasaña M, Gil F, Lorca A, Alguacil J, Rohlman DS, González-Alzaga B, Molina-Villaba I, Mendoza R, Aguilar-Garduño C

Environ Res. 2014 Jul 18;134C:66-73

Environmental Research 134 (2014) 66–73

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Cadmium exposure and neuropsychological development in school children in southwestern Spain

Miguel Rodríguez-Barranco [a,b], Marina Lacasaña [a,b,c,*], Fernando Gil [d], Andres Lorca [e], Juan Alguacil [f,c], Diane S. Rohlman [g,h], Beatriz González-Alzaga [a,b], Isabel Molina-Villalba [d], Ramón Mendoza [i], Clemente Aguilar-Garduño [j]

[a] Andalusian School of Public Health (EASP), Campus Universitario de Cartuja, c/Cuesta del Observatorio 4, 18080 Granada, Spain
[b] Instituto de Investigación Biosanitaria de Granada (ibs.GRANADA), Granada, Spain
[c] CIBER of Epidemiology and Public Health (CIBERESP), Madrid, Spain
[d] Department of Legal Medicine and Toxicology, University of Granada, Granada, Spain
[e] Department of Clinical, Experimental and Social Psychology, University of Huelva, Huelva, Spain
[f] Research Center on Health and Environment (CYSMA), University of Huelva, Huelva, Spain
[g] Center for Research on Occupational and Environmental Toxicology, Oregon Health and Science University, USA
[h] Occupational and Environmental Health, University of Iowa, USA
[i] Department of Developmental and Educational Psychology, University of Huelva, Huelva, Spain
[j] Center for Public Health Research (CSISP-FISABIO), Valencia, Spain

ARTICLE INFO

Article history:
Received 13 March 2014
Received in revised form
27 June 2014
Accepted 28 June 2014

Keywords:
Neuropsychological development
Intelligence
Child
Cadmium
Gender
Biomonitoring

ABSTRACT

This study assessed the association between cadmium exposure and neuropsychological development in children from a region with high industrial and mining activities in southwestern Spain. We conducted a cross-sectional study with 261 children aged 6–9 years between January and March 2012. Cadmium exposure was measured in urine and hair of children, and neuropsychological development was assessed with the Wechsler Intelligence Scale for Children-Fourth Edition (WISC-IV) and with three computerized tests from the Behavioral Assessment and Research System (BARS): Reaction Time Test (RTT), Continuous Performance Test (CPT) and Selective Attention Test (SAT). Multivariate linear regression models, adjusted for potential confounders, were used to estimate the association between neuropsychological development and cadmium exposure measured in urine and hair samples. Geometric means of urine and hair cadmium levels were 0.75 μg/g creatinine and 0.01 μg/g, respectively. We observed that doubling of levels of cadmium in urine was associated with a reduction of two points (95% CI: −3.8 to −0.4) in the Full-Scale intelligence quotient (IQ) in boys. By domains, association was statistically significant for Verbal Comprehension ($\beta=-2.0$; $p=0.04$) and close to the significance level for Perceptual Reasoning ($\beta=-1.8$; $p=0.06$). Among girls, only Verbal Comprehension showed suggestive associations with cadmium exposure ($\beta=-1.7$; $p=0.06$). Cadmium exposure is associated with cognitive delays in boys in our region. Our results provide additional evidence of the neurotoxic effect of low-level postnatal cadmium exposure among children, and support the hypothesis of differences between sexes in the neurotoxic effect of metals on children.

1. Introduction

Over the past decades concern has risen about the potential effect of low-level exposure to toxic metallic compounds (including cadmium) among environmentally exposed populations, especially pregnant women and children (Grandjean and Landrigan, 2006). As reported by Landrigan et al. “children are not small adults”, and they present striking differences versus adults in both routes and magnitude of exposure (Landrigan et al., 2004). This makes children the preferred target group for the study of health effects of environmental xenobiotics.

Cadmium (Cd) is a natural element present in Earth's crust in concentrations ranging from 0.1 to 5 μg/g. Nonferrous metal mining and refining, manufacture and application of phosphate fertilizers, fossil fuel combustion, and waste incineration and disposal are the main anthropogenic sources of cadmium in the environment (ATSDR, 2012). Cadmium ranks seventh in the ATSDR list of elements posing the most significant potential threat to human health in the environment, and it ranks third in the heavy

* Corresponding author at: Andalusian School of Public Health (EASP), Campus Universitario de Cartuja, c/Cuesta del Observatorio 4, 18080 Granada, Spain.
E-mail address: marina.lacasana.easp@juntadeandalucia.es (M. Lacasaña).

http://dx.doi.org/10.1016/j.envres.2014.06.026
0013-9351/

metals subdivision of the same list, behind lead and mercury (ATSDR, 2011). Cadmium is a Group 1 IARC carcinogen and it is neurotoxic (Cao et al., 2009; ATSDR, 2012).

During pregnancy the placenta acts as a barrier for cadmium, and only a small amount ($<10\%$) is transferred to the fetus (Osman et al., 2000). A similar proportion is transferred to human milk (Hallen et al., 1995). Thus, the fetus and the newborn are protected against cadmium. However, cadmium exposure starts at a very young age through food, environmental tobacco smoke and household dust (Cao et al., 2009). Diet is the main source of environmental exposure to cadmium among non-occupationally exposed and non-smoker population and it has been reported that more than 80% of food-based cadmium comes from cereals and vegetables (Cho et al., 2013). While cadmium in blood reveals recent exposure status, cadmium in urine and hair reflects the body burden and is an indicator for cumulative long term exposure (Adams and Newcomb, 2014).

Although there is evidence of neurotoxic effects of cadmium from experimental studies in animals (Jin et al., 1998; Shagirtha et al., 2011; ATSDR, 2012), a few studies have evaluated the effect of cadmium exposure on child neuropsychological development, and their results are mostly inconclusive (Rodríguez-Barranco et al., 2013). Some studies have found association between prenatal cadmium exposure and neuropsychological development (Tian et al., 2009; Kippler et al., 2012), but there are not enough evidence related to postnatal exposure. Most studies that have evaluated postnatal exposure to cadmium do not observe a significant association with neuropsychological development in children (Torrente et al., 2005; Wright et al., 2006; Cao et al., 2009). In contrast, a recent study found a significant association between postnatal cadmium exposure and learning disability in U.S. children (Ciesielski et al., 2012).

Cadmium exposure can reach high levels in areas close to cadmium-emitting industries (Bao et al., 2009; Blasco et al., 2010), exceeding the 5 ng/m^3 safety threshold limit value recommended by the World Health Organization (WHO, 2004). The levels of cadmium in emission plumes in the proximity of the copper smelter factory in the Huelva area were 6.71 ng/m^3 in August 2005, though the average daily mean in the downtown area between 2001 and 2008 was below 1 ng/m^3 (Sánchez de la Campa et al., 2011).

The aim of this study was to assess the association between postnatal cadmium exposure and neuropsychological development in children living in a coastal industrialized region in southwestern Spain. This area has been dominated for decades by extensive chemical industrial and mining activities, and is crossed by an ecosystem named Ria de Huelva, a complex system of drainage channels that separate several areas of salt marshes. This ecosystem is controlled by the tidal regime and the inputs of the Odiel and Tinto rivers, as well as two channels which exchange water directly with the open sea. Ria of Huelva has high levels of metallic elements such as copper, zinc, iron, cadmium and manganese from three main sources of contamination: the industrial sewage from over 40 neighboring chemical industries, the urban sewage of the city of Huelva, and fluvial inputs from the Odiel and Tinto rivers, which have acidic waters and high levels of metallic trace elements (Blasco et al., 2010).

2. Methods

2.1. Design and study population

A cross-sectional study was conducted between January and March 2012 in a region in southwestern Spain (Huelva, Andalusia). Thirteen schools were randomly selected from a total of 38 public schools in seven municipalities within the Ria of Huelva area (Aljaraque, Huelva, Palos de la Frontera, Punta Umbría, San Juan del Puerto, Tharsis and Valdelamusa). A total of 2199 parents of the selected schools were invited to participate in the study, and 315 of them agreed to participate. A subset of 203 parents among those that declined to participate completed a brief non-participation questionnaire.

Finally, a sample of 261 children aged 6–9 years was randomly selected from those who met the inclusion criteria and whose parents gave signed informed consent. Inclusion criteria included uninterrupted residence in the study area for at least one year, and having one parent or guardian fluent in Spanish. Exclusion criteria included pre-and peri-natal problems, diabetes, neurological disorders, brain trauma, surgery under general anesthesia, and liver or kidney disease. No children were excluded from the study.

The sample size of 261 children was calculated a priori to provide a power of 80% at 0.05 of significance level in order to detect a decrease of -4 points in WISC Full-Scale intelligence quotient (IQ) due to a doubling of lead exposure, based on the results found by Tong et al. (1996).

2.2. Data collection

The day before the neuropsychological assessment, children were provided with a polypropylene container to collect a first morning spot urine sample. Before being used all polypropylene materials were cleaned by soaking in 10% (v/v) HNO_3 for 24 h. They were finally rinsed with several washes of Milli-Q® water and dried in a polypropylene container. Urine samples were collected by the field team in schools at the beginning of classes next day. Samples were refrigerated at -4 °C until transported to the laboratory, and stored at -20 °C until analysis. The same day a minimum of 100 mg of hair was collected from the scalp by a trained personal. Hair samples were cut from the back of the head as close as possible to the scalp and were transported and stored in plastic bags until analysis. Children were also weighed and measured in order to calculate body mass index (BMI).

Two questionnaires (one self-administrated and another by phone interview) were completed by the mother, father or guardian to obtain information about demographic and socioeconomic characteristics, environmental and home exposures, parent's occupational history, birth characteristics and lifetime residential history. Children's diet was assessed by means of a validated semi-quantitative food frequency questionnaire (FFQ) (Vioque et al., 2013). Number of servings per week of vegetables and cereals were calculated from the FFQ. We included pulses in the group of vegetables, and bread and pasta in the group of cereals.

In addition, a psychologist visited each family to assess the maternal intellectual quotient by the Kaufman Brief Intelligence Test (Kaufman and Kaufman, 1990).

2.3. Assessment of children's neuropsychological development

The Wechsler Intelligence Scale for Children-Fourth Edition (WISC-IV; Wechsler, 2003) was individually administered to assess intellectual function in children and administered in standard form. This scale provides measures of general intellectual ability by the Full-Scale IQ, and for four specific cognitive domains: Verbal Comprehension (with subtest of similarities, vocabulary, comprehension and information); Perceptual Reasoning (block design, matrix completion and picture completion); Working Memory (digit span, letter–number sequencing and arithmetic); and Processing Speed (digit symbol-coding and symbol search). In the event of any of the subtests being suspended, it was replaced with an equivalent subtest according to the author's guidelines, this occurred in five cases (Wechsler, 2003).

In addition, three computerized tests from the Behavioral Assessment and Research System (BARS; Rohlman et al., 2003) were used to assess additional attention functions: Reaction Time Test (RTT), Continuous Performance Test (CPT) and Selective Attention Test (SAT).

In the Reaction Time Test (RTT) a stimulus (square) is presented on the screen and the subject is instructed to press a button with their dominant hand as fast as possible to make it disappear. A total of 50 trials are presented. The Interstimulus Interval (ISI) was variable (between 1 and 3 s) and if the subject did not emit the response after 3 s the stimulus presentation screen disappeared. This task is designed to measure the speed of response to a visual stimulus (Lezak, 2004). Response latency in milliseconds (ms) and response latency excluding trials with latencies higher than 1000 ms were calculated from this test.

The Continuous Performance Test (CPT) measures attention, and consists of subjects pressing a button with their dominant hand as fast as they can when a target stimulus (closed circle) preceded by a cue stimulus (plus sign) is presented on the screen. A series of distractions are presented to subjects, specifically downward arrows, triangles, stars and hexagons. A total of 300 trials were presented, with a cue presentation fixed interval of 0.05 s and an ISI of 1 s. Percentage of false alarms, percentage of omission and response latency (ms) were calculated from this test.

The Selective Attention Test (SAT) presents two equidistant boxes on the screen, one on the right of the screen and one on the left. The subject is instructed to press a button whenever a small dot appeared inside the box (with his/her right hand if it appears on the right and with his/her left hand if it appears on the left) and to ignore dots appearing outside of the boxes. The task has a total duration of 600 s, where 80% of the presentations are within the box and 20% are outside the

box. The ISI begins initially at 2 s and is titrated based on the participants' response, decreasing by 0.1 s in the case of a hit, and increasing by 0.1 s in the case of a miss. This task assesses selective attention and sustained attention or concentration (Lezak, 2004). Percentage of false alarms, percentage of omission and response latency (ms) were calculated from this test.

The neuropsychological assessment team was composed of a coordinator (a neuropsychologist) and three previously trained psychologists. Apart from a few exceptions, all tests were done on the same day, with a 45 min break between the intelligence scale and the computerized tests. The intelligence tests were administered by two different psychologists and the computerized tests by a third psychologist. The intelligence scale for each child was administered by the same psychologist, following the standardized guidelines described in the WISC-IV administration manual, which ensures the uniqueness of criteria for the data collection (Wechsler, 2003).

2.4. Metallic elements measurement

Although the aim of this work was to assess cadmium exposure, the samples were analyzed to measure additional metallic elements: manganese (Mn), lead (Pb), mercury (Hg) and arsenic (As).

Urine and hair levels of Cd, Mn, Pb, Hg and As were measured by a Perkin-Elmer Analyst 800 Atomic Absorption Spectrometer (Perkin-Elmer, Norwalk, USA) equipped with Zeeman background correction and an AS-800 autosampler by a graphite furnace and graphite tubes with an integrated L'vov platform and hydride generation. Appropriate matrix modifiers were used for the selected metallic elements studied (for more details, see Gil et al. (2006) and Olmedo et al. (2010)). Reference samples for urine (Ref. 201205) were supplied by Seronorm (Billingstad, Norway) and Reference material NIES No. 5 for hair was obtained from the National Institute for Environmental Studies, Japan Environment Agency.

Prior dilution of each sample was critical and the best results were obtained with 1/2 and 1/5 dilutions for urine and hair samples, respectively. A calibration curve with different cadmium concentrations (0, 0.5, 1 and 1.5 μg/l) was prepared in 0.2% HNO_3. Aliquots of 10 μl of urine and digested hair previously diluted were introduced directly into the graphite furnace with an equal volume of matrix modifier (0.3 g/l of $Mg(NO_3)_2$ + 0.33 g/l of $Pd(NO_3)_2$ solution was prepared in 0.2% (v/v) nitric acid and 0.1% Triton X-100.

Hair samples were kept in polypropylene containers previously cleaned with acid and were washed by ultrasonic cleaning in a non-ionic detergent (Triton X-100, Merck, Madrid, Spain; Darmstadt, Germany) solution, and then the detergent was removed by copious rinsing with Milli-Q water and washed by ultrasonic cleaning in an ethanol solution (Merck, Darmstadt, Germany) and again with Milli-Q water. The clean hair were dried overnight at room temperature. Hair samples after addition of 1 ml of HNO_3 (Merck), 0.5 ml of HCl (Merck), 2 ml of H_2O_2 (Merck) and 2 ml of H_2O were digested for 30 min in a microwave oven Multiwave 3000 (Anton Parr, Graz, Austria) (Olmedo et al., 2010).

Following the recommendations of the IUPAC, the validation of analytical procedures developed for the determination of metal compounds in urine and hair samples, including the limit of detection (LOD) and quantification (LOQ), linear range, precision (minimal, intermediate and reproducibility), accuracy, recovery, characteristic mass and uncertainty, was previously reported by our research group (Gil et al., 2006; Olmedo et al., 2010). For cadmium, limit of detection (LOD) in urine was 0.03 μg/l and LOD in hair was 0.0033 μg/g.

2.5. Statistical analysis

Geometric means and 95% confidence interval (CI) were calculated to describe levels of cadmium in urine and hair. Values of cadmium level below the LOD were assigned the LOD divided by the square root of 2. Neuropsychological development scores were described by means of arithmetic mean and standard deviation. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared, and categorized into four groups according to the sex and age-based growth charts of the Spanish population (Sobradillo et al., 2004): underweight (percentile 3 or lower), normal (P3–P85), overweight (P85–P95) and obese (P95 or higher). The difference between boys and girls was tested with Mann–Whitney's test for exposure variables (with non-normal distribution according to Shapiro–Wilk's test) and with the Student's *t*-test for response variables (with normal distribution according to Shapiro–Wilk's test). Differences between participants and non-participants in demographic and socioeconomic characteristics were checked by means of the Chi-square test.

Multivariate linear regression models were used to evaluate the neurotoxic effect of cadmium exposure, taking as dependent variables the results of the neuropsychological tests used, and as independent variables the concentration of cadmium in urine and hair. Exposure variables were transformed using base 2 logarithm in order to smooth out their strongly asymmetric distributions. Thus, regression coefficients are interpreted as the change in the dependent variable associated with a doubling of the exposure (or a 100% increase). Potential confounding variables were assessed, including sex, child's age, body mass index (BMI), mother's age, IQ, education and occupation, father's education and occupation, monthly family income, residence area, family status, gestational age, weight, height and head circumference at birth, vegetables and cereals intake, and IQ assessor. Covariables not associated with the exposure and the outcome ($p > 0.20$ in Wald's test) were discarded as potential confounders. Levels of Mn, As, Pb and Hg were also retained in all models to control for exposure to other metals. Furthermore we included in the models interaction terms between sex and cadmium levels (log 2Cd × sex) in order to assess the possible role of sex as an effect modifier. Effect size for each sex was derived from these interaction models. The statistical analyses were developed with the Stata 11 statistical package software (Stata Corp LP. 2009, TX).

3. Results

A total of 261 children (126 girls and 135 boys) participated in the study. There were no significant differences between participants and non-participants in relation to children's sex and age, mother's age, and parent's education and occupation. Non-participant children only differed from participants in the place of residence: 46% of non-participants lived less than 1 km from industrial or mining areas versus 33% of participants (Table 1).

Average time of residence in the study area was seven years (interquartile range 6–8). Forty eight percent of children were girls, and average age was 7.4 years. Mean BMI was 17.2 (range: 8.6–27.9); 3% of the children were underweight, 9% were overweight, and 14% were obese. Mother's averaged age was 37 years, 55% of mothers were unemployed and 3.6% had no formal education. Among fathers, 25% were unemployed and 7% had no formal education. Most children lived in urban areas (90%), and 15% did not live with both of their biological parents (Table 1). Boys differed significantly from girls in weight, height, and head circumference at birth, but not in gestational age (Table 2). Vegetables intake was significantly higher in girls than in boys, and there were no significant differences in cereals intake (Table 2).

Geometric mean of urine cadmium levels was 0.75 μg/g creatinine, and 91.6% of samples were above the LOD. A total of 220 hair samples were available. Geometric mean of cadmium in hair was 0.01 μg/g, and 38.7% of samples were above the LOD. Correlation between hair and urine cadmium levels was negative and very low: Spearman correlation coefficient was equal to −0.048 in boys ($p = 0.641$) and −0.047 in girls ($p = 0.603$).

There was no significant difference in urine cadmium levels between boys and girls: geometric mean was 0.77 μg/g creatinine in boys and 0.73 μg/g creatinine in girls, with *p*-value 0.75 in Mann–Whitney's test. However, girls had higher levels in hair than boys (0.006 μg/g versus 0.003 μg/g; $p < 0.01$) (Table 3). Geometric means of urine As, Hg, Mn and Pb levels were 2.44, 1.06, 0.42 and 2.22 μg/g creatinine, respectively. Geometric means of hair As, Hg, Mn and Pb levels were 0.017, 0.407, 0.137 and 0.140 μg/g, respectively.

A total of 259 children completed the WISC-IV scale. Full-Scale IQ of children averaged 98.2 points (SD = 13.6), with a range between 56 and 126. In 9% of children, IQ score was lower than 79 points, 84% had an IQ between 80 and 119 points, and 7% showed a higher record of IQ (≥ 120 points). There was no significant difference between girls and boys in the Full-Scale IQ, but girls reached better scores in the Processing Speed subtest than boys (Table 4). The three computerized tests of the BARS were completed for 257 children. Girls showed lower percentage of false alarms in SAT and CPT and higher percentage of omissions in SAT than boys. Girls also showed higher response latency in two of the three tasks than boys (Table 4).

We observed a significant negative association between urine cadmium levels and the scores of WISC-IV, after adjustment for potential confounders (Table 5). In the overall sample, a doubling of levels of cadmium in urine was associated with 1.2 points less in the Full-Scale IQ (95% CI: −2.49 to 0.03), affecting Verbal Comprehension most ($\beta = -1.8$; 95% CI: −3.2 to −0.4). No significant associations between cadmium levels in urine and measures from the computerized tests were observed. Cadmium

Table 1
Demographic and socioeconomic characteristics of the study population.

	Participants		Non-participants		*p*-value
	n	(%)	*n*	(%)	
Total	**261**	**(100)**	**203**	**(100)**	
Sex					
Boys	135	(51.7)	90	(44.8)	
Girls	126	(48.3)	111	(55.2)	0.14[a]
BMI (Kg/m^2); mean (range)	17.2	(8.6–27.9)	–	–	–
Child's age; mean (range)	7.4	(6–9)	7.3	(6–9)	0.20[b]
Mother's age; mean (range)	37.2	(23–70)	37.5	(23–54)	0.65[b]
Mother's education					
No formal	9	(3.6)	12	(6.0)	
Primary	101	(40.2)	91	(45.7)	
Secondary	93	(37.1)	56	(28.1)	
Higher	48	(19.1)	40	(20.1)	0.18[a]
Mother's occupation					
Employed	112	(44.6)	92	(49.5)	
Unemployed	139	(55.4)	94	(50.5)	0.32[a]
Father's education					
No formal	16	(7.1)	21	(10.9)	
Primary	111	(49.6)	94	(49.0)	
Secondary	64	(28.6)	50	(26.0)	
Higher	33	(14.7)	27	(14.1)	0.58[a]
Father's occupation					
Employed	168	(75.0)	138	(74.2)	
Unemployed	56	(25.0)	48	(25.8)	0.85[a]
Monthly family income					
$\leq$ 1000 €	28	(11.2)	–	–	
1001–2000 €	159	(63.9)	–	–	
$>$ 2000 €	62	(24.9)	–	–	-
Residence area					
Urban	225	(89.6)	–	–	
Metropolitan	15	(6.0)	–	–	
Rural	11	(4.4)	–	–	–
Living near from mining or industrial area					
No	142	(67.0)	99	(53.8)	
Yes, less than 1 km	70	(33.0)	85	(46.2)	0.01[a]
Family status					
Living with biological mother and father	214	(85.3)	–	–	
Other situations[c]	37	(14.7)	–	–	–

[a] Chi-square test.
[b] Student's *t*-test.
[c] Living with single parent, biological mother/father and partner, adoptive parents, or grandparents.

Table 2
Children's characteristics at birth and current food intake of participants.

	n	Mean	SD	P25	P50	P75	P90	P95	Max
Boys									
Gestational age (weeks)	125	39.3	1.6	39.0	40.0	40.0	41.0	42.0	43.0
Weight (kg)	125	3.4[b]	0.5	3.1	3.4	3.7	4.0	4.1	4.8
Height (cm)	121	50.4[b]	2.3	49.0	51.0	52.0	53.0	54.0	58.0
Head circumference (cm)	75	34.5[b]	1.4	34.0	34.5	35.5	36.0	37.0	38.0
Vegetables consumption[a]	128	17.8[b]	6.6	14.5	17.8	20.8	25.0	29.5	45.0
Cereals consumption[a]	128	24.6	4.6	22.5	24.5	27.5	31.0	31.5	32.0
Girls									
Gestational age (weeks)	120	39.2	2.2	39.0	40.0	40.0	41.0	41.0	43.0
Weight (kg)	122	3.2[b]	0.6	2.9	3.2	3.6	3.9	4.0	4.5
Height (cm)	118	49.6[b]	2.9	48.0	50.0	51.0	53.0	54.0	57.0
Head circumference (cm)	65	33.8[b]	1.7	32.8	34.0	35.0	35.5	37.0	37.5
Vegetables consumption[a]	122	19.9[b]	6.5	16.0	19.0	23.0	29.0	33.0	40.5
Cereals consumption[a]	122	23.8	4.7	22.0	24.5	27.0	29.5	30.5	31.5

SD: standard deviation; P*x*: percentile *x*.
[a] Number of servings per week.
[b] Student's *t*-test *p*-value $<$ 0.05.

levels in hair were not associated with any of the outcomes, neither WISC-IV nor BARS scores (Table 6).

This inverse significant association between cadmium levels in urine and WISC-IV scores was observed in boys only, not in girls (Table 5 and Fig. 1). In boys, the Full-Scale IQ decreased two points (95% CI: −3.8 to −0.4) for a doubling of cadmium levels in urine, while in girls the effect size was not statistically significant (95% CI: −2.1 to 1.0). Furthermore, boys had poorer scores in all domains

Table 3
Levels of cadmium in urine and hair by sex.

	n	% < LOD	GM	95% CI	Maximum
Boys					
Cd in urine (μg/l)	135	5.9	**0.221**	(0.186–0.262)	0.986
Cd in urine (μg/g creatinine)	135	5.9	**0.769**	(0.633–0.934)	7.439
Cd in hair (μg/g)	96	83.3	**0.003**[a]	(0.003–0.004)	0.107
Girls					
Cd in urine (μg/l)	126	11.1	**0.208**	(0.170–0.255)	1.502
Cd in urine (μg/g creatinine)	126	11.1	**0.725**	(0.579–0.907)	10.074
Cd in hair (μg/g)	124	64.5	**0.006**[a]	(0.004–0.007)	0.320

LOD: limit of detection; GM: geometric mean; and CI: confidence interval.
[a] Mann–Whitney's test p-value < 0.05.

measured by WISC-IV for the increase of cadmium levels in urine, while in girls those effects were not observed (Fig. 1). Only the effect size on Verbal Comprehension was similar in both sexes, being statistically significant in boys and almost significant in girls. Domains most affected in boys for cadmium exposure were Verbal Comprehension ($\beta = -2.0$; 95% CI: −4.0 to −0.1) and Perceptual Reasoning ($\beta = -1.8$; 95% CI: −3.7 to 0.1), in the latter case, the association was close to statistical significance ($p = 0.06$).

4. Discussion

This is the first study that has found an association between cadmium exposure and neuropsychological development in children

Table 4
Outcomes of neuropsychological development tests by sex (mean ± SD).

	Boys ($n = 135$)	**Girls** ($n = 126$)	p-value[a]
WISC-IV scores			
Full-Scale IQ	98.3 ± 14.3	98.2 ± 12.8	0.94
Verbal Comprehension	100.8 ± 15.6	97.8 ± 13.2	0.10
Perceptual Reasoning	99.5 ± 14.7	99.4 ± 13.5	0.96
Working Memory	99.0 ± 16.4	98.4 ± 15.7	0.74
Processing Speed	99.1 ± 13.4	103.2 ± 11.4	0.01
Selective Attention Test (SAT)			
Percentage of false alarms	46.7 ± 11.9	41.0 ± 9.7	< 0.01
Percentage of omissions	36.6 ± 6.0	39.6 ± 4.4	< 0.01
Latency (ms)	279.5 ± 69.6	271.8 ± 46.5	0.30
Reaction Time Test (RTT)			
Latency (ms)	605.4 ± 149.7	654.3 ± 175.7	0.02
Latency (ms) excluding trials with latencies > 1000 ms	509.9 ± 74.2	538.6 ± 77.3	< 0.01
Continuous Performance Test (CPT)			
Percentage of false alarms	7.7 ± 7.6	5.7 ± 6.8	0.03
Percentage of omissions	25.5 ± 15.4	28.3 ± 15.9	0.14
Latency (ms)	435.4 ± 91.8	486.2 ± 81.3	< 0.01

[a] Student's t-test.

Table 5
Adjusted regression coefficients of association between urine cadmium levels and neuropsychological development measures.

Response variable	**Boys**			**Girls**			**Total**		
	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value
WISC-IV[a]									
Full-Scale IQ	−2.06	(−3.78;−0.35)	0.02	−0.59	(−2.14;0.96)	0.45	−1.23	(−2.49;0.03)	0.06
Verbal Comprehension	−2.02	(−3.96;−0.08)	0.04	−1.66	(−3.41;0.09)	0.06	−1.82	(−3.24;−0.40)	0.01
Perceptual Reasoning	−1.80	(−3.70;0.09)	0.06	−0.11	(−1.82;1.60)	0.90	−0.85	(−2.24;0.55)	0.23
Working Memory	−1.20	(−3.43;1.02)	0.29	−0.48	(−2.49;1.53)	0.64	−0.80	(−2.43;0.84)	0.34
Processing Speed	−1.32	(−3.13;0.49)	0.15	1.19	(−0.44;2.82)	0.15	0.09	(−1.25;1.44)	0.89
BARS									
Selective Attention Test (SAT)[b]									
Percentage of false alarms	0.87	(−0.49;2.22)	0.21	−0.22	(−1.45;1.00)	0.72	0.25	(−0.74;1.25)	0.62
Percentage of omissions	−0.35	(−1.01;0.30)	0.29	0.33	(−0.26;0.92)	0.28	0.03	(−0.45;0.51)	0.90
Latency (ms)	3.06	(−4.07;10.19)	0.40	2.06	(−4.37;8.49)	0.53	2.50	(−2.70;7.69)	0.35
Reaction Time Test (RTT)[a]									
Latency (ms)	0.16	(−21.16;21.47)	0.99	−10.13	(−30.47;10.2)	0.33	−5.29	(−21.4;10.82)	0.52
Latency (ms) excluding trials with latencies > 1000 ms	1.04	(−9.29;11.36)	0.84	1.37	(−8.48;11.22)	0.78	1.21	(−6.57;9.00)	0.76
Continuous Performance Test (CPT)[c]									
Percentage of false alarms	0.15	(−0.81;1.10)	0.76	−0.11	(−0.95;0.73)	0.80	0.00	(−0.69;0.69)	0.99
Percentage of omissions	1.04	(−0.89;2.97)	0.29	−0.73	(−2.43;0.97)	0.40	0.01	(−1.38;1.41)	0.98
Latency (ms)	3.32	(−7.71;14.35)	0.55	−0.22	(−9.92;9.48)	0.97	1.27	(−6.67;9.21)	0.75

[a] Adjusted by sex, child's age, body mass index (BMI), mother's age, intelligence quotient and education, monthly family income, family status, gestational age, vegetables and cereals intake, and levels of Mn, As, Pb, Hg in urine.
[b] Adjusted by sex, child's age, body mass index (BMI), mother's age and education, monthly family income, family status, gestational age, vegetables and cereals intake, and levels of Mn, As, Pb, Hg in urine.
[c] Adjusted by sex, child's age, body mass index (BMI), mother's age, education and occupation, monthly family income, residence area, family status, gestational age, weight and height at birth, vegetables and cereals intake, and levels of Mn, As, Pb, Hg in urine.

Table 6
Adjusted regression coefficients of association between hair cadmium levels and neuropsychological development measures.

Response variable	Boys			Girls			Total		
	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value
WISC-IV[a]									
Full-Scale IQ	0.30	(−1.75;2.35)	0.77	0.09	(−1.30;1.47)	0.90	0.15	(−0.97;1.28)	0.79
Verbal Comprehension	−0.05	(−2.32;2.23)	0.97	−0.57	(−2.11;0.96)	0.46	−0.41	(−1.65;0.84)	0.52
Perceptual Reasoning	1.01	(−1.29;3.32)	0.39	0.32	(−1.23;1.88)	0.68	0.54	(−0.72;1.81)	0.40
Working Memory	0.88	(−1.63;3.40)	0.49	−0.54	(−2.23;1.16)	0.53	−0.08	(−1.46;1.30)	0.91
Processing Speed	0.26	(−1.82;2.34)	0.81	0.93	(−0.47;2.33)	0.19	0.71	(−0.43;1.85)	0.22
BARS									
Selective Attention Test (SAT)[b]									
Percentage of false alarms	0.49	(−1.04;2.01)	0.53	0.46	(−0.59;1.51)	0.39	0.47	(−0.40;1.33)	0.29
Percentage of omissions	−0.41	(−1.18;0.35)	0.29	−0.10	(−0.63;0.43)	0.71	−0.20	(−0.63;0.23)	0.36
Latency (ms)	−5.80	(−14.62;3.02)	0.20	1.37	(−4.71;7.45)	0.66	−0.95	(−5.94;4.05)	0.71
Reaction Time Test (RTT)[c]									
Latency (ms)	−19.77	(−43.91;4.38)	0.11	2.03	(−14.30;18.36)	0.81	−4.81	(−18.38;8.77)	0.49
Latency (ms) excluding trials with latencies > 1000 ms	−6.06	(−17.12;4.99)	0.28	0.01	(−7.47;7.49)	0.99	−1.90	(−8.09;4.30)	0.55
Continuous Performance Test (CPT)[d]									
Percentage of false alarms	0.13	(−1.05;1.31)	0.83	0.14	(−0.70;0.98)	0.75	0.13	(−0.55;0.81)	0.70
Percentage of omissions	−0.46	(−2.77;1.84)	0.69	0.14	(−1.50;1.78)	0.87	−0.06	(−1.39;1.27)	0.93
Latency (ms)	−11.88	(−24.21;0.45)	0.16	2.34	(−6.42;11.09)	0.60	−2.43	(−9.62;4.76)	0.51

[a] Adjusted by sex, child's age, mother's age, intelligence quotient and education, monthly family income, family status, gestational age, vegetables and cereals intake, and levels of Mn, As, Pb, Hg in hair.
[b] Adjusted by sex, child's age, mother's age and education, monthly family income, family status, and levels of Mn, As, Pb, Hg in hair.
[c] Adjusted by sex, child's age, mother's age, family status, vegetables and cereals intake, and levels of Mn, As, Pb, Hg in hair.
[d] Adjusted by sex, child's age, body mass index (BMI), mother's age, education and occupation, monthly family income, family status, weight and height at birth, and levels of Mn, As, Pb, Hg in hair.

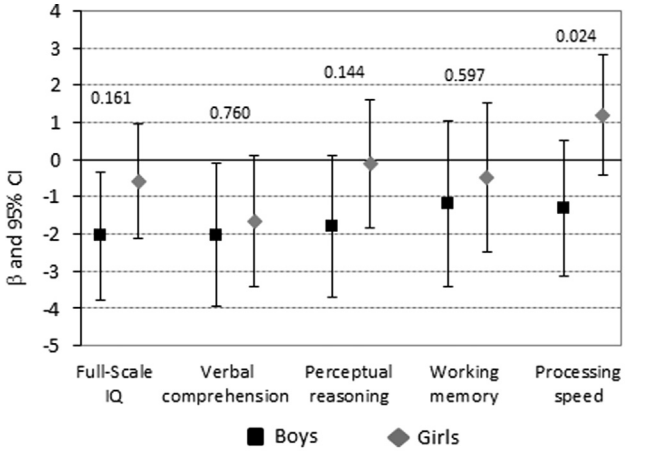


Fig. 1. Interaction between cadmium in urine and sex on WISC-IV scores.

living in a European country. Our results show an inverse association between postnatal cadmium exposure and neuropsychological development among boys, but not among girls. In boys, the Full-Scale IQ and the Verbal Comprehension and Perceptual Reasoning domains showed associations with cadmium exposure. Only the magnitude of the inverse association between urine cadmium and Verbal Comprehension had the same strength in both boys and girls. Association between cadmium exposure and attention function was not observed.

In a recent systematic review, four articles were identified published from January 2000 to March 2012 that evaluated the effects of cadmium exposure on children's neuropsychological development (Rodríguez-Barranco et al., 2013), and their results were inconclusive. Only one of these studies reported a significant negative effect of prenatal cadmium exposure on neuropsychological development (Tian et al., 2009). In this prospective cohort was found lower Full-Scale IQ and performance IQ at 4.5 years of age in children who had higher levels of cadmium in cord blood at birth. Two cross-sectional studies (Torrente et al., 2005; Wright et al., 2006) and one randomized clinical trial (Cao et al., 2009) did not observe a statistically significant association between postnatal cadmium exposure and neuropsychological development.

More recently Kippler et al. (2012) observed a decrease of −0.8 points (95% CI: −1.2 to −0.4) in Full-Scale IQ at 5 years of age for a doubling of cadmium in maternal urine in early pregnancy, with these associations being stronger in girls than boys. Ciesielski et al. (2012) observed a significant association between postnatal cadmium exposure and both learning disability and special education, this being stronger in boys.

Gender-related differences in the health effects of toxic elements have been reported elsewhere, and it is known that certain toxic elements can act differently in males and females due to difference in pattern of exposure, metabolism or individual susceptibility (Vahter et al., 2007; Gil and Hernández, 2009; Mergler, 2012). Neurotoxic effects of lead and mercury seem to be more pronounced in boys than in girls, but not all the epidemiological studies found this result (Llop et al., 2013). With respect to other metallic trace elements, such as manganese, cadmium or arsenic, there are not enough evidence in human studies to suggest gender-related neurotoxic effect.

Although we found a higher frequency of vegetables intake in girls than in boys, there was no significant difference in levels of cadmium in urine, so the difference in the association with neuropsychological development found in this study could not be due to differences in the long-term exposure levels. Thus, this difference observed between boys and girls could be related to biological issues rather than gender differences in diet patterns.

We observed no association between hair cadmium levels and neuropsychological development. Two recent studies in which cadmium hair levels were evaluated do not find a significant association with neuropsychological development in children (Torrente et al., 2005; Wright et al., 2006), so results from our study are consistent with previous literature. While blood concentrations clearly reflect recent exposure, hair reflects past

exposure providing an average of their growth period, but it strongly depends on the hair sample length. Hair grows approximately 1 cm per month so, on average, boys' levels reflect exposure over the past 1–3 months and in girls the exposure ranges from between 6 and 12 months in our population. In contrast, urine cadmium levels may reflect several years of exposure (Adams and Newcomb, 2014), so hair levels in boys cannot reflect long-term exposure as well as urine levels. In fact, correlation between urine and hair cadmium levels was very low in our study.

Moreover, hair has several limitations; one of them is the potential for external contamination and the failure to remove it completely by using different washing procedures. Another disadvantage is the lack of sufficient information to define a normal range of metal levels typically found in the general population, because metal hair content varies significantly according to age, sex, hair color, hair care, smoking habits and racial/ethnic factors (Olmedo et al., 2010; Gil et al., 2011).

The urinary cadmium levels observed in our study population are 10 times higher than those observed in children aged 6–11 years in the U.S. population (CDC, 2012) (median, 0.08 μg/g creatinine versus 0.85 μg/g creatinine in our study), or in German children at 6–8 years of age (Kolossa-Gehring et al., 2007) (median, 0.07 μg/l versus 0.29 μg/l). By contrast, cadmium levels in our population were similar to regions in Bangladesh (Gardner et al., 2013) (median, 0.21 μg/l versus 0.29 μg/l), or in Thailand (Chaiwonga et al., 2013) (mean, 1.01 μg/g creatinine versus 1.37 μg/g creatinine). A study in the same area of the Ria of Huelva (Aguilera et al., 2010) reported a geometric mean of 0.35 μg/l in children between 5 and 17 years of age, levels slightly higher than those observed in our sample (0.22 μg/l). Moreover, 66% of the children in the current study exceeded the reference value for children of 0.2 μg/l established by the Human Biomonitoring Commission of the German Federal Environment Agency; however no participants presented concentrations above the threshold limit value of 2 μg/l, above which it is considered that there is an increased risk of adverse health effects in children (Schulz et al., 2012). Thus, the effect on neuropsychological development observed in this study occurs at exposure levels below those considered to be a risk.

Twenty nine percent of children had levels of creatinine in urine below 20 mg/dl. We conducted a sensitivity analysis that excluded those children, and we observed changes less than 20% in the main effect sizes reported, so the current results included data from the overall sample.

There is biological evidence that cadmium can affect neuropsychological development in children (Provias et al., 1994; ATSDR, 2012). Animal experiments have shown that cadmium affects brain metabolism, inhibiting sulfhydryl-containing enzymes. Therefore, chronic exposure to cadmium has a depressant effect on the levels of several neurotransmitters such as norepinephrine, serotonin and acetylcholine (Stowe et al., 1972; Singhal et al., 1976). Animal studies have also shown that cadmium can cross the blood–brain barrier (Andersson et al., 1997), and this strengthens the hypothesis that the blood–brain barrier does not prevent cadmium from reaching the brain during early development stages in children (Provias et al., 1994). This evidence suggests that cadmium reaches the central nervous system directly, bringing about a neurotoxic effect on child's development (Petersson-Grawe et al., 2004; Cao et al., 2009). Other studies have shown the role of cadmium as an endocrine disruptor, acting on estrogens, thyroid and growth hormones, all of which have an important role in brain development (Schantz and Widholm, 2001; Takiguchi and Yoshihara, 2006). This interaction between cadmium and various hormones could also explain the differences between sexes observed in this study (Vahter et al., 2007).

Nevertheless, this study has some limitations. Although we did not find differences between participants and non-participants with regard to socioeconomic variables, we did observe a higher percentage of non-participants living closer to industrial or mining areas than participants. This fact could be related to the reluctance of some parents to participate in environmental impact studies of the population living near or currently working in industrial activity in the study area. However, we did not observe differences either in cadmium levels or in intelligence quotient between participants who live near industrial or mining organizations versus participants who live further away. On the other hand, one of the strengths of this study is that all of the children provided a urine sample, and 99% completed all the neuropsychological development tests, so we were able to maintain our power calculated a priori.

Some scientists point toward cadmium as the “new lead”, since it is a heavy metal which has been linked to learning difficulties in school children, and every child is exposed to it (Cone, 2012). New findings are a sign that cadmium can have dangerous properties similar to lead that alter the way children's brains develop. However, more research is necessary to confirm and refine the potential effects of cadmium on children.

5. Conclusions

Cadmium exposure was associated with cognitive delay in boys living in southwestern Spain. Our results provide additional evidence about the neurotoxic effect of low-level postnatal cadmium exposure on the child population and support the differences between sexes in the neurotoxicity of metals in children suggested in other studies.

Conflict of interest

Authors declare that they have no conflict of interest, except for Diane S. Rohlman. Oregon Health and Science University (OHSU) and Dr. Rohlman have a significant financial interest in Northwest Educational Training and Assessment, LLC, a company that may have a commercial interest in the results of this research and technology. This potential conflict of interest has been reviewed and managed by OHSU and the Integrity Program Oversight Council.

Acknowledgments

We would like to thank the Andalusian Health Department (Spain) for its financial support to this project (Project no.: PI0755/2010). This work is part of the Doctoral Thesis of Miguel Rodríguez Barranco from the University of Granada (Spain), in the context of the Doctoral Program in Clinical Medicine and Public Health. The authors would also like to thank the schools staff and the children and families, without whom this study would not have been possible.

References

Adams, S.V., Newcomb, P.A., 2014. Cadmium blood and urine concentrations as measures of exposure: NHANES 1999-2010. J. Expo. Sci. Environ. Epidemiol. 24, 163–170.

Andersson, H., Petersson-Grawe, K., Lindqvist, E., Luthman, J., Oskarsson, A., Olson, L., 1997. Low-level cadmium exposure of lactating rats causes alterations in brain serotonin levels in the offspring. Neurotoxicol. Teratol. 19, 105–115.

Aguilera, I., Daponte, A., Gil, F., Hernández, A.F., Godoy, P., Pla, A., Ramos, J.L., 2010. Urinary levels of arsenic and heavy metals in children and adolescents living in the industrialised area of Ria of Huelva (SW Spain). Environ. Int. 36, 563–569.

ATSDR, 2011. The Priority List of Hazardous Substances, 2011. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Health Services, Atlanta, GA (accessed 18.11.13.).
ATSDR, 2012. Toxicological Profile for Cadmium. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Health Services, Atlanta, GA.
Bao, Q.S., Lu, C.Y., Song, H., Wang, M., Ling, W., Chen, W.Q., Deng, X.Q., Hao, Y.T., Rao, S., 2009. Behavioural development of school-aged children who live around a multi-metal sulphide mine in Guangdong province, China: a cross-sectional study. BMC Public Health 9, 217.
Blasco, J., Gomes, T., García-Barrera, T., Rodríguez-Romero, A., Gonzalez-Rey, M., Morán-Roldán, F., Tromibini, C., Miotk, M., Gómez-Ariza, J.L., Bebianno, M.J., 2010. Trace metal concentrations in sediments from the southwest of the Iberian Peninsula. Sci. Mar. 74, 99–106.
Cao, Y., Chen, A., Radcliffe, J., Dietrich, K.N., Jones, R.L., Caldwell, K., Rogan, W.J., 2009. Postnatal cadmium exposure, neurodevelopment, and blood pressure in children at 2, 5, and 7 years of age. Environ. Health Perspect. 117, 1580–1586.
CDC (Centers for Disease Control and Prevention), 2012. Fourth National Report on Human Exposure to Environmental Chemicals: Updated Tables, September 2012. CDC, Atlanta, GA.
Chaiwonga, S., Sthiannopkao, S., Supanpaiboon, W., Chuenchoojit, S., Pupatwibul, K., Poodendaen, C., 2013. Urinary cadmium concentrations in a population downstream: from a zinc mining area in Mae Sot District, Tak Province, Thailand. Environ. Geochem. Health 35, 69–78.
Cho, Y.A., Kim, J., Woo, H.D., Kang, M., 2013. Dietary cadmium intake and the risk of cancer: a meta-analysis. PLoS One 8, e75087.
Ciesielski, T., Weuve, J., Bellinger, D.C., Schwartz, J., Lanphear, B., Wright, R.O., 2012. Cadmium exposure and neurodevelopmental outcomes in U.S. children. Environ. Health Perspect. 120, 758–763.
Cone M. 2012. Is cadmium the new lead? Link reported between the ubiquitous metal and kids with learning disabilities. Environmental Health News [Internet]. 2012 Feb 10. Available from ⟨http://www.environmentalhealthnews.org/ehs/news/2012/is-cadmium-the-new-lead⟩.
Gardner, R.M., Kippler, M., Tofail, F., Bottai, M., Hamadani, J., Grandér, M., Nermell, B., Palm, B., Rasmussen, K.M., Vahter, M., 2013. Environmental exposure to metals and children's growth to age 5 years: a prospective cohort study. Am. J. Epidemiol. 177, 1356–1367.
Gil, F., Capitan-Vallvey, L.F., De Santiago, E., Ballesta, J., Pla, A., Hernández, A.F., Gutiérrez-Bedmar, M., Fernández-Crehuet, J., Gómez, J., López-Guarnido, O., Rodrigo, L., Villanueva, E., 2006. Heavy metal concentrations in the general population of Andalusia, South of Spain. A comparison with the population within the area of influence of Aznalcollar mine spill (SW Spain). Sci. Total Environ. 372, 49–57.
Gil, F., Hernández, A.F., 2009. Significance of biochemical markers in applied toxicology. In: Ballantyne, B., Marrs, T.C., Syversen, T. (Eds.), General and Applied Toxicology, 2. John Wiley and Sons Ltd., Chichester, UK, pp. 847–858.
Gil, F., Hernández, A.F., Márquez, C., Femia, P., Olmedo, P., López-Guarnido, O., Pla, A., 2011. Biomonitorization of cadmium, chromium, manganese, nickel and lead in whole blood, urine, axillary hair and saliva in an occupationally exposed population. Sci. Total Environ. 409, 1172–1180.
Grandjean, P., Landrigan, P.J., 2006. Developmental neurotoxicity of industrial chemicals. Lancet 368, 2167–2178.
Hallen, I.P., Jorhem, L., Lagerkvist, B.J., Oskarsson, A., 1995. Lead and cadmium levels in human milk and blood. Sci. Total Environ. 166, 149–155.
Jin, T., Lu, J., Nordberg, M., 1998. Toxicokinetics and biochemistry of cadmium with special emphasis on the role of metallothionein. Neurotoxicology 19, 529–535.
Kaufman, A., Kaufman, N., 1990. Kaufman Brief Intelligence Test Manual. American Guidance Service, Circle Pines, MN.
Kippler, M., Tofail, F., Hamadani, J.D., Gardner, R.M., Grantham-McGregor, S.M., Bottai, M., Vahter, M., 2012. Early-life cadmium exposure and child development in 5-year-old girls and boys: a cohort study in rural Bangladesh. Environ. Health Perspect. 120, 1462–1468.
Kolossa-Gehring, M., Becker, K., Conrad, A., Lüdecke, A., Riedel, S., Seiwert, M., Schulz, C., Szewzyk, R., 2007. German Environmental Survey for Children (GerES IV)—first results. Int. J. Hyg. Environ. Health 210, 535–540.
Landrigan, P.J., Kimmel, C.A., Correa, A., Eskenazi, B., 2004. Children's health and the environment: public health issues and challenges for risk assessment. Environ. Health Perspect. 112, 257–265.
Lezak, M., Howieson, D., Bigler, E., Tranel, D., 2004. Neuropsychological Assessment. Oxford University Press, New York.
Llop, S., Lopez-Espinosa, M.J., Rebagliato, M., Ballester, F., 2013. Gender differences in the neurotoxicity of metals in children. Toxicology 311, 3–12.
Mergler, D., 2012. Neurotoxic exposures and effects: gender and sex matter! Hänninen Lecture 2011. Neurotoxicology 33, 644–651.
Olmedo, P., Pla, A., Hernández, A.F., López-Guarnido, O., Rodrigo, L., Gil, F., 2010. Validation of a method to quantify chromium, cadmium, manganese, nickel and lead in whole blood, urine, saliva and hair samples by electrothermal atomic absorption spectrometry. Anal. Chim. Acta 659, 60–67.
Osman, K., Akesson, A., Berglund, M., Bremme, K., Schütz, A., Ask, K., Vahter, M., 2000. Toxic and essential elements in placentas of Swedish women. Clin. Biochem. 33, 131–138.
Petersson-Grawe, K., Teiling-Gardlund, A., Jalkesten, E., Oskarsson, A., 2004. Increased spontaneous motor activity in offspring after maternal cadmium exposure during lactation. Environ. Toxicol. Pharmacol. 17, 35–43.
Provias, J.P., Ackerley, C.A., Smith, C., Becker, L.E., 1994. Cadmium encephalopathy: a report with elemental analysis and pathological findings. Acta Neuropathol. 88, 583–586.
Rodríguez-Barranco, M., Lacasaña, M., Aguilar-Garduño, C., Alguacil, J., Gil, F., González-Alzaga, B., Rojas-García, A., 2013. Association of arsenic, cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: a systematic review and meta-analysis. Sci. Total Environ. 454–455, 562–577.
Rohlman, D.S., Gimenes, L.S., Eckerman, D.A., Kang, S.K., Farahat, F.M., Anger, W.K., 2003. Development of the Behavioral Assessment and Research System (BARS) to detect and characterize neurotoxicity in humans. Neurotoxicology 24, 523–531.
Sánchez de la Campa, A.M., de la Rosa, J., González-Castanedo, Y., Fernández-Camacho, R., Alastuey, A., Querol, X., Stein, A.F., Ramos, J.L., Rodríguez, S., Orellana, I.G., Nava, S., 2011. Levels and chemical composition of PM in a city near a large Cu-smelter in Spain. J. Environ. Monit. 13, 1276–1287.
Schantz, S.L., Widholm, J.J., 2001. Cognitive effects of endocrine-disrupting chemicals in animals. Environ. Health Perspect. 109, 1197–1206.
Schulz, C., Wilhelm, M., Heudorf, U., Kolossa-Gehring, M., 2012. Reprint of "Update of the reference and HBM values derived by the German Human Biomonitoring Commission". Int. Hyg. Environ. Health 215, 150–158.
Shagirtha, K., Muthumani, M., Prabu, S.M., 2011. Melatonin abrogates cadmium induced oxidative stress related neurotoxicity in rats. Eur. Rev. Med. Pharmacol. Sci. 15, 1039–1050.
Singhal, R.L., Merali, Z., Hrdina, P.D., 1976. Aspects of the biochemical toxicology of cadmium. Fed. Proc. 35, 75–80.
Sobradillo, B., Aguirre, A., Aresti, U., Bilbao, A., Fernández-Ramos, C., Lizárraga, A., Lorenzo, H., Madariaga, L., Rica, I., Ruiz, I., Sánchez, E., Santamaría, C., Serrano, J. M., Zabala, A., Zurimendi, B., Hernández, M., 2004. Curvas y Tablas de Crecimiento. Estudios longitudinal y transversal. Fundación Faustino Orbegozo, Bilbao, ISBN: 84-607-9967-0.
Stowe, H.D., Wilson, M., Goyer, R.A., 1972. Clinical and morphologic effects of oral cadmium toxicity in rabbits. Arch. Pathol. 94, 389–405.
Takiguchi, M., Yoshihara, S., 2006. New aspects of cadmium as endocrine disruptor. Environ. Sci. 13, 107–116.
Tian, L.L., Zhao, Y.C., Wang, X.C., Gu, J.L., Sun, Z.J., Zhang, Y.L., Wang, J.X., 2009. Effects of gestational cadmium exposure on pregnancy outcome and development in the offspring at age 4.5 years. Biol. Trace Elem. Res. 132, 51–59.
Tong, S., Baghurst, P., McMichael, A., Sawyer, M., Mudge, J., 1996. Lifetime exposure to environmental lead and children's intelligence at 11–13 years: the Port Pirie cohort study. BMJ 312, 1569–1575.
Torrente, M., Colomina, M.T., Domingo, J.L., 2005. Metal concentrations in hair and cognitive assessment in an adolescent population. Biol. Trace Elem. Res. 104, 215–221.
Vahter, M., Akesson, A., Lidén, C., Ceccatelli, S., Berglund, M., 2007. Gender differences in the disposition and toxicity of metals. Environ. Res. 104, 85–95.
Vioque, J., Navarrete-Muñoz, E.M., Gimenez-Monzó, D., Hera, M.G., Granado, F., Young, I.S., Ramón, R., Ballester, F., Murcia, M., Rebagliato, M., Iñiguez, C., 2013. Reproducibility and validity of a food frequency questionnaire among pregnant women in a Mediterranean area. Nutr. J. 12, 26.
Wechsler, D., 2003. WISC-IV Administration and Scoring Manual. Harcourt Assessment, San Antonio, TX.
WHO, 2004. Guidelines for Drinking-Water Quality, 3rd ed. World Health Organization, Geneva, Switzerland (accessed 18.11.13.).
Wright, R.O., Amarasiriwardena, C., Woolf, A.D., Jim, R., Bellinger, D.C., 2006. Neuropsychological correlates of hair arsenic, manganese, and cadmium levels in school-age children residing near a hazardous waste site. Neurotoxicology 27, 210–216.

Postnatal arsenic exposure and attention impairment in Spanish school children

Rodríguez-Barranco M, Gil F, Hernández A, Alguacil J, Lorca A, Mendoza R, Molina-Villaba I, González-Alzaga B, Aguilar-Garduño C, Rohlman DS, Lacasaña M

CORTEX. 2014 [in press]

POSTNATAL ARSENIC EXPOSURE AND ATTENTION IMPAIRMENT IN SCHOOL CHILDREN

Miguel Rodríguez-Barranco [a,b], Fernando Gil [c], Antonio F. Hernández [c,*], Juan Alguacil [d,e], Andres Lorca [f], Ramón Mendoza [g], Inmaculada Gómez [h], Isabel Molina-Villalba [c], Beatriz González-Alzaga [a,b], Clemente Aguilar-Garduño [i], Diane S. Rohlman [j], Marina Lacasaña [a,b,e]

[a] Andalusian School of Public Health (EASP), Granada, Spain
[b] Instituto de Investigación Biosanitaria ibs.GRANADA. Hospitales Universitarios de Granada/Universidad de Granada, Granada, Spain
[c] Department of Legal Medicine and Toxicology, University of Granada, School of Medicine, Granada, Spain
[d] Research Center on Health and Environment (CYSMA), University of Huelva, Huelva, Spain
[e] CIBER of Epidemiology and Public Health (CIBERESP), Madrid, Spain
[f] Department of Clinical, Experimental and Social Psychology, University of Huelva, Huelva, Spain
[g] Department of Developmental and Educational Psychology, University of Huelva, Huelva, Spain
[h] Department of Psychology, University of Almería, Almería, Spain
[i] Center for Public Health Research (CSISP-FISABIO), Valencia, Spain
[j] Department of Occupational and Environmental Health, The University of Iowa, Iowa City, IA and the Oregon Institute for Occupational Health Sciences, Oregon Health & Science University, Portland OR, USA

* Corresponding author at: Dr. A.F. Hernández, Department of Legal Medicine and Toxicology, University of Granada School of Medicine, Avda. Madrid, 11. 18071-Granada, Spain. Email address: ajerez@ugr.es.

Abstract

Background: Over the last few decades there has been an increased concern about the health risks from exposure to metallic trace elements, including arsenic, because of their potential neurotoxic effects on the developing brain.

Objective: To assess whether urinary arsenic levels are associated with attention performance and Attention-Deficit/Hyperactivity Disorder (ADHD) in children living in an area with high industrial and mining activities in Southwestern Spain.

Methods: A cross-sectional study was conducted in 261 children aged 6-9 years between January and May 2012. Arsenic exposure was measured in urine samples. Attention was measured by using 4 independent tools: a) tests from the Behavioral Assessment and Research System (BARS) designed to measure attention function: Simple Reaction Time Test (RTT), Continuous Performance Test (CPT) and Selective Attention Test (SAT); b) the AULA Test, a virtual reality-based test that evaluates children's response to several stimuli in an environment simulating a classroom; c) the Child Behavior Checklist (CBCL) administered to parents; and d) the Teacher's Report Form (TRF) administered to teachers. These two last scales evaluate different problems or psychopathological syndromes with a clinical approach. Multivariate linear and logistic regression models, adjusted for potential confounders, were used to estimate the magnitude of the association between urinary arsenic levels and attention performance scores.

Results: Higher urinary arsenic levels (UAs) were associated with an increased latency of response in RTT (β = 12.3; 95% CI: 3.5 to 21.1) and SAT (β = 3.6; 95% CI: 0.4 to 6.8) as well as with worse performance on selective and focalized attention in the AULA test (β for impulsivity = 0.6; 95% CI: 0.1 to 1.1; β for inattention = 0.5; 95% CI: 0.03 to 1.0). A dose-response relationship was observed for two measures related with inattention and impulsivity. In contrast, results from the CBCL and TRF tests failed to show a significant association with UAs.

Conclusions: Urinary arsenic levels were associated with impaired attention/cognitive function, even at levels considered safe. These results provide additional evidence that postnatal arsenic exposure impairs neurological function in children.

Keywords: Attention; ADHD; neurobehavioral disorder; arsenic; neurotoxicity; child.

1. Introduction

Over the last few decades a concern has been raised about the potential neurotoxic effects of low-level exposure to metal trace elements, including arsenic, on vulnerable populations, especially pregnant women and children (Grandjean & Landrigan, 2014; Tyler & Allan, 2014). More than 1000 chemical substances have shown neurotoxic effects in experimental animals. Among them, metal trace elements such as arsenic, lead, methylmercury, fluoride and manganese have elicited developmental neurotoxicity and brain damage in humans (Grandjean & Landrigan, 2014). Continued exposure to these neurotoxic compounds could be creating a "silent pandemic" in the modern society, responsible for a subclinical and permanent decrease in IQ, ultimately leading to increased school failure, diminished economic productivity and increased risk of criminal and antisocial behavior (Grandjean & Landrigan, 2006, 2014). Millions of children around the world are exposed to high levels of arsenic in drinking water in countries such as Argentina, Chile, Mexico, China, Hungary, India, Bangladesh and Vietnam (Smedley & Kinniburgh, 2002), so the global nature of this pandemic could have a huge impact on public health.

There is growing evidence that arsenic exposure has detrimental effects on the intellectual function in children. Several studies have shown an inverse relationship between arsenic exposure and Full-Scale IQ (Calderon et al., 2001; Hamadani et al., 2010; Rocha-Amador, Navarro, Carrizales, Morales, & Calderon, 2007; Wang et al., 2007; Wasserman et al., 2007; Wasserman et al., 2004; Wasserman et al., 2011; Wright, Amarasiriwardena, Woolf, Jim, & Bellinger, 2006), Verbal IQ or verbal comprehension (Calderon et al., 2001; Hamadani et al., 2010; Rocha-Amador et al., 2007; von Ehrenstein et al., 2007; Wasserman et al., 2011; Wright et al., 2006), Performance IQ (Rocha-Amador et al., 2007; Wasserman et al., 2007; Wasserman et al., 2004), motor

function (Parvez et al., 2011), as well as memory function (Tsai, Chou, The, Chen, & Chen, 2003; Wasserman et al., 2011; Wright et al., 2006). A recent meta-analysis has estimated that a 50% increase in arsenic levels in drinking water is associated with a significant decrease of –0.6 points in the Full-Scale IQ and –0.3 points in Performance IQ in children aged 5-15 years (Rodriguez-Barranco et al., 2013).

In contrast, only a few studies have evaluated the relationship between arsenic exposure and child behavior, and their results are inconclusive (Rodriguez-Barranco et al., 2013). Roy and collaborators found a significant association between organic forms of arsenic in urine and oppositional behavior, cognitive problems and Attention-Deficit/Hyperactivity Disorder (ADHD) in children aged 6-7 years from Mexico (Roy et al., 2011). In contrast, other studies have no found significant association between total arsenic in blood or drinking water and behavior impairment (Khan et al., 2011; Yousef et al., 2011). However, there is evidence of a negative effect of occupational exposure to high doses of arsenic on adult's behavior (Bolla-Wilson & Bleecker, 1987; Hall, 2002). Animal studies have also shown behavioral disturbances after exposure to inorganic arsenic during development (Rodriguez, Carrizales, Mendoza, Fajardo, & Giordano, 2002; Xi, Sun, Wang, Jin, & Sun, 2009).

ADHD is the most commonly diagnosed neurobehavioral disorder in school children. The prevalence of ADHD in Spain is estimated to be between 4.9 and 8.8%, affecting around 361,580 children and adolescents in the country (Catala-Lopez et al., 2012). It is well established that attention disturbance is the main symptom of some of the clinical syndromes such as the ADHD (Barkley, 1997; Seidman, 2006). It has been suggested that exposure to environmental contaminants that impair the attention function would be a risk factor for such neurobehavioral disorder (Yousef et al., 2011).

The aim of this study was to assess the association of postnatal arsenic exposure with attention and ADHD risk in children living in an industrialized region in Southwestern Spain. This area has been dominated for decades by extensive chemical industrial and mining activities, with an important presence of high levels of metallic elements such as zinc, copper, lead, arsenic, nickel, mercury and cadmium (Blasco et al., 2010).

2. Methods

2.1. Design and study population

A cross-sectional study was conducted between January and March 2012 in a region from Southwestern Spain (Huelva, Andalusia). Details of the study area, population and sample size calculation have been reported elsewhere (Rodriguez-Barranco et al., 2014). In short, thirteen schools were randomly selected from a total of 38 public schools in seven municipalities within the Ria of Huelva area (Aljaraque, Huelva, Palos de la Frontera, Punta Umbría, San Juan del Puerto, Tharsis and Valdelamusa). A total of 2199 parents of the selected schools were invited to participate in the study, and 315 of them agreed to participate. Finally, a sample of 261 children aged 6-9 years was randomly selected from those who met the inclusion criteria and whose parents gave signed informed consent. Inclusion criteria included uninterrupted residence in the study area for at least one year, and having one parent or guardian fluent in Spanish. Exclusion criteria included pre and perinatal disturbances, diabetes, neurological disorders, brain trauma, surgery under general anesthesia, and liver or kidney disease.

2.2. Data collection

The day before the neuropsychological assessment, children were provided with a polypropylene container previously cleaned with nitric acid to collect a first morning

spot urine sample. Containers with urine samples were collected the next day in schools at the beginning of classes. Samples were transported refrigerated at 4°C and then stored at – 20°C until analysis. Children were also weighed and measured to calculate their body mass index (BMI).

Two questionnaires (one self-administrated and another administered by phone interview) were completed by the mother, father or guardian to obtain information about demographic and socioeconomic characteristics, environmental and home exposures, parent's occupational history, birth characteristics and daily hours doing leisure and sport activities.

Occupational exposure of parents to metal trace elements (arsenic, cadmium, chrome, iron, nickel and lead) during pregnancy and after children's birth was assessed by using the Job-Exposure Matrix for Research and Surveillance of Occupational Health and Safety in Spanish Workers (Garcia, Gonzalez-Galarzo, Kauppinen, Delclos, & Benavides, 2013). To achieve this, occupational history was codified according to the National Classification of Occupations from the National Statistics Institute, and linked with the job-exposure matrix.

Children's diet was assessed by means of a validated semi-quantitative food frequency questionnaire (FFQ). Numbers of servings per week of food groups were calculated from the FFQ.

In addition, a psychologist visited each family to assess the maternal intellectual quotient with the Kaufman Brief Intelligence Test (Kaufman & Kaufman, 1990), and the quality of the family context using an adapted version of the Etxadi-Gangoiti scale (Arranz Freijo, Olabarrieta Artetxe, Manzano Fernández, & Galende Pérez, 2014; Velasco et al., 2014). In this study the Etxadi-Gangoiti scale was adapted in order to be administered to children from 6 to 10 years of age; the resulting Cronbach's alpha

reliability coefficient was 0.72. The Etxadi-Gangoiti scale is a brief scale for assessing the quality of the family context that includes updated items of the Home Observation for Measurement of the Environment Inventory (HOME) (B. M. Caldwell & Bradley, 2003) and the Developmental History Scale (Pettit, Bates, & Dodge, 1997).

2.3. Assessment of children's attention impairment and ADHD

Three computerized tests from the Behavioral Assessment and Research System (BARS) (Rohlman et al., 2003) were used to assess attention/cognitive function: Simple Reaction Time Test (RTT), Continuous Performance Test (CPT) and Selective Attention Test (SAT).

In the Simple Reaction Time Test (RTT) a stimulus (square) is presented on the screen and the subject is instructed to press a button with their dominant hand as fast as possible to make it disappear. A total of 50 trials are presented. The Interstimulus Interval (ISI) was variable (between 1 and 3 s) and if the subject failed to respond after 3 s the stimulus presentation screen disappeared. This task is designed to measure the speed of response to a visual stimulus (Lezak, Howieson, Bigler, & Tranel, 2004). Response latency in milliseconds (ms) and response latency excluding trials with latencies higher than 1000 ms were calculated from this test.

In the Continuous Performance Test (CPT), which measures attention, subjects have to press a button with their dominant hand as fast as they can when a target stimulus (closed circle) preceded by a cue stimulus (plus sign) is presented on the screen. A series of distractions are presented to subjects, specifically downward arrows, triangles, stars and hexagons. A total of 300 trials were presented, with a cue presentation fixed interval of 0.05 s and an ISI of 1 s. Percentage of false alarms, percentage of omission and response latency (ms) were calculated from this test.

The Selective Attention Test (SAT) presents two equidistant boxes on the screen, one on the right side and the other one on the left. The subject is instructed to press a button whenever a small dot appeared inside the box (with his/her right hand if it appears on the right and with his/her left hand if it appears on the left) and to ignore dots appearing outside of the boxes. The task has a total duration of 600 s, where 80% of the presentations are within the box and 20% are outside the box. The ISI begins initially at 2 s and is titrated based on the participants' response, decreasing by 0.1 s in the case of a hit, and increasing by 0.1 s in the case of a miss. This task assesses selective attention and sustained attention or concentration (Lezak et al., 2004). Percentage of false alarms, percentage of omission and response latency (ms) were calculated from this test.

In addition, a virtual reality-based test for attention (AULA) was applied (Iriarte et al., 2012). AULA is based on the paradigm of continuous performance tests but performed in a virtual reality (VR) environment, and it is visualized by means of a special set of VR glasses with movement sensors. The scenario is similar to either a primary or a high school classroom, and the perspective (i.e., what the participant sees) is located in one of the classroom desks, looking towards the blackboard. AULA was developed with the goal of being used as an aid to the clinician in the diagnosis of ADHD, based on two attention models derived from the study of ADHD (Barkley, 1997; Sergeant, Oosterlaan, & Van der Meere, 1999). AULA presents a subset of both visual and auditory stimuli, in presence or absence of distractors, and is composed of two main exercises: No-X-paradigm-based exercise and X-paradigm based exercise. In the No-X-Task the subjects must press the button when they do not see or hear the stimuli. This task contains several stimuli and may induce an overstimulation that challenges individuals' skills to control their impulses. The fast presentation of stimuli may induce fast, inaccurate and inadequate responses, thus providing a measure of impulsivity. In turn, in X-Task

subjects must press the button when they see or hear the stimuli. This is a slower and monotonous task that leads to hypoactivation, thus making it difficult to sustain attention and/or concentration. In consequence, X-Task may induce slow, variable and inefficient answers, thus providing a measure of inattention (Iriarte et al., 2012).

For each task, the following measures are obtained: a) omission errors: this measure is related to selective and focalized attention, and occurs when the child does not press the button when he should do; b) commission errors: this measure is related to lack of motor control or lack of inhibition of response, and occurs when the child presses the button when he should not do it; c) reaction time: is the time required to answer a stimulus, and it tends to be longer in people with attention impairment or ADHD because they tend to process information slowly; d) motor activity: frequency and relevance of movements of user's head are registered (i.e., necessary vs. unnecessary movements) by the movement of sensors placed in the VR headset. Scores were calculated for both No-X-Task and X-Task and expressed as typified quantities that range 20 to 80 points. A higher score means worse results in the task, indicating more attention impairment. Scores between 65 and 70 point are considered to be in borderline clinical range whereas values above 70 points fall within the clinical range.

The neuropsychological assessment team consisted of a coordinator (a neuropsychologist) and one previously trained psychologist. Each child performed all tests on the same day with the same psychologist, with a 45 min break between tests.

Additionally, parents and teachers were asked to complete the Child Behavior Checklist (CBCL) (Achenbach & Rescorla, 2001) and the Teacher's Report Form (TRF) (Achenbach, 1991) respectively. The CBCL is a scale in which a parent rates a variety of behaviors and the combined responses create scores reflecting possible deficit areas, including attention problems. The TRF is a teacher-report measure parallel to the CBCL

that assesses behavioral changes and can identify 8 syndromes, including ADHD. Both, CBCL and TRF, have two distinct parts: the first consists of 20 questions about the child's social competence. The second, which is what was used in this study, consists of 113 items related to problem behaviors of children that are assessed using a Likert-type scale ranging from 0 (that behavior never appears) to 2 (often true). Scores from both the CBCL and TRF scales can be examined continuously or by the proportion above a standard cutoff score: > 93rd percentile (borderline clinical range) or > 98th percentile (clinical range).

2.4. Urine arsenic measurement

Urinary levels of total arsenic were measured by a direct flow-injection atomic absorption spectrometric technique through a hydride generation system (FI-HGAAS) as reported elsewhere (Gil et al., 2006). A Perkin-Elmer Analyst 800 Atomic Absorption Spectrometer (Perkin Elmer, Norwalk, USA) was used. Briefly, the arsenic contained in standard solutions (calibration curve 0, 0.5, 1.5 and 2.5 μg/l) or urine samples was reduced to As^{3+} prior to analysis with a mixture of potassium iodide and ascorbic acid. To 1 mL of sample or reference solution 1 mL of concentrated HCl and 1 ml of 5% (w/v) KI – ascorbic acid was added. After 45 min at room temperature the mixture was diluted to 10 ml with water. The reducing agent was an aqueous solution of 0.2% (w/v) $NaBH_4$ in a 0.05% (w/v) NaOH solution freshly prepared and filtered. Standard addition and an electrodeless discharge lamp were required. The validation of analytical procedures used for the determination of arsenic in urine has been described previously (Gil et al., 2006). The limit of detection (LOD) was 0.03 μg/l. Creatinine levels were determined by Jaffe method.

Before being used all polypropylene materials were cleaned by soaking in 10% (v/v) HNO_3 for 24 h. They were finally rinsed with several washes of Milli-Q® water and dried in a polypropylene container.

2.5. Statistical analysis

Geometric means and 95% confidence interval (CI) were calculated to describe levels of urinary arsenic (UAs). Values of UAs below the LOD were assigned LOD divided by the square root of 2. Body Mass Index (BMI) was calculated as weight (Kg) divided by height (m) square, and categorized into four groups according to the sex and age-based growth charts of the Spanish population (Sobradillo et al., 2004). Average hours per day doing several activities were calculated weighting the number of hours spent in the activity in weekdays and in weekend. Mann-Whitney or Kruskal-Wallis tests were used to assess differences between groups when categorical variable had two or more than two categories respectively.

Multivariate linear regression models were used to evaluate the neurotoxic effect of arsenic exposure, taking as dependent variables the results of the neuropsychological tests, and as independent variables the UAs levels (μg/l). Exposure variable was transformed using base 2 logarithm to smooth their strong asymmetric distribution. Thus, regression coefficients are interpreted as the change in the dependent variable associated with a two-fold increase in the exposure (or a 100% increase). Exposure to arsenic was also categorized into four categories: values bellow the LOD and tertiles of detectable values. For CBCL and TRF scales, multivariate logistic regression was used when cutoff of scores (P93 and P98) were considered to evaluate attention problems or ADHD. Potential confounding variables assessed included socioeconomic characteristics, environmental and home exposures, birth characteristics, food frequency

intake, child activity, parent occupational exposure to metallic trace elements, and the scale of assessment of quality family context.

Multivariate models were constructed in three steps: in the first step sex, age and urine creatinine level were introduced as fixed covariates; in a second step those variables associated with either exposure or the outcome ($p<0.20$ in bivariate analysis) were included into the models as potential confounders; in a third step a backward procedure with criteria of $p>0.10$ in the Wald's test were developed to remove non-relevant covariates from models.

Furthermore interaction terms between sex and UAs levels (log_2UAs x Sex) were included into the models to assess the possible role of sex as an effect modifier.

The statistical analyses were developed with the Stata 11 statistical package software (StataCorp LP. 2009, TX).

3. Results

A total of 261 children (126 girls and 135 boys) participated in the study. Average time of residence in the study area was seven years (interquartile range 6 – 8). Forty eight percent of children were girls, with an average age of 7.4 years. Geometric mean of UAs levels was 0.70 μg/l (95% CI: 0.58–0.85), and 85.8% of samples were above the LOD (Table 1). There was no significant difference in UAs in relation to sex and age (Table 2).

A gradient relationship between UAs levels and parent's education and monthly family income was observed; with higher education and incomes being associated with lower UAs levels (Table 2). Children living near industrial areas had higher average UAs levels than who lived farther away (geometric mean 0.93 vs 0.62 μg/l), with a p-value close to the significance level ($p=0.06$). Type of drinking water usually consumed was

also associated with UAs levels: while children who drank well of spring water showed highest UAs levels (1.73 μg/l), children who usually drank municipal filtered water had the lowest levels (0.49 μg/l). Surprisingly, children drinking bottled water had more than twice the UAs levels found in those who drank municipal water (Table 2). No association was found between UAs levels and parent's occupation or occupational exposure to metals during pregnancy or after birth (Table 2).

No significant differences were observed between the most and least exposed children (taking the 75th percentile as a breakpoint) in relation to birth characteristics (gestational age, weight, height, head circumference, breastfeeding, and alcohol and tobacco consumption during pregnancy), children's diet, and daily activity (active games, sport, computer or game console, study and sleep) of the study participants (Table 3).

A total of 257 children completed the BARS tests, and 249 the AULA test. The BARS tests were not completed in four cases because two children did not attend class on the evaluation period, and two other children refused to perform the test. Additionally, another eight children failed to complete the AULA test because of dizziness during the execution. The CBCL was completed by 256 parents and TRF was available for 185 participants (some teachers refused to complete the form). Table 4 shows the main scores obtained in the different tests. Prevalence of attention problems in our children population was 5.9% based in clinical range (>P98) and 11.3% based in borderline clinical range (>P93). Prevalence of ADHD was 1.6% and 7% according to clinical range and borderline clinical range, respectively.

A significant association between UAs levels and some scores from the BARS and AULA tests was observed. After adjustment for potential confounders, a two-fold increase in UAs levels was associated with a delay in latency of response of 3.6 ms (95% CI: 0.4 to 6.8) in SAT. Furthermore, the same change in exposure was associated

with an increase of 12.3 ms (95% CI: 3.5 to 21.1) in RTT latency, which dropped to 5.5 ms (95% CI: 1.3 to 9.8) when trials with latencies > 1000 ms were excluded (Table 5). Figure 1 shows the association between UAs categorized as tertiles and test scores. While a significant trend was observed for RTT latency (p-trend=0.002), no significant trend was found for SAT latency. In relation to the AULA test, a two-fold increase in UAs levels was associated with more omission errors in both No-X-Task, that reflects impulsivity, (β=0.6; 95% CI: 0.1 to 1.1) and X-Task, that reflects inattention, (β=0.5; 95% CI: 0.03 to 1.0) (Table 6). Analysis of tertiles for No-X-Task showed a significant trend with UAs (p-trend=0.021) whereas trend for X-Task did not reach the significance level (Figure 1).

No significant association was observed in relation to attention problems and ADHD from the CBCL and TRF respectively, neither with continuous scales nor with diagnosis criteria (Table 7).

The interaction terms between UAs and sex failed to be significantly associated with any of the outcomes assessed (data not shown).

4. Discussion

This study adds more evidence to the few studies available at this time supporting an association between arsenic exposure and cognitive and behavioral performance in children (Rodriguez-Barranco et al., 2013; Tyler & Allan, 2014). Our results showed a significant association of postnatal arsenic exposure with impaired selective and focused attention, and with a delayed reaction time. These two independent tests directly performed on children showed consistent results with each other. Moreover, a dose-response relationship was observed for two measures related with inattention and impulsivity.

In contrast, the two tests completed by parents and teachers used to assess attention problems and ADHD failed to show a significant association with UAs. These controversial results might be due to several reasons. On the one hand, attention problems may be occurring at subclinical level, which are detected for performance tests administered directly to children, but they have not yet resulted in a clinical discomfort that worry parents and teachers. On the other hand, prevalence of clinical attentional problems and ADHD is low, so an increased sample size may be required to find significant effects at the level of arsenic exposure found in this study. The lack of statistical power due to the loss in sample size on TRF data may have limited the possibility to study such association. Other studies also using scales for parents and teachers have found no significant association between arsenic exposure and adverse neurobehavioral effects (Khan et al., 2011; Yousef et al., 2011).

To the best of our knowledge, only one study published to date has found an association between arsenic exposure and attention impairment in children (Roy et al., 2011). In the later cross-sectional study higher urinary dimethylarsinic acid (DMA) levels (organic form of arsenic) was associated with higher ratings on ADHD index by teachers. Although the inorganic forms of arsenic are generally more toxic than the organic forms, some of the organic forms, such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), have been shown to have neurological effects in animal health (ATSDR, 2007) and in humans (Rosado et al., 2007; Roy et al., 2011). Unfortunately, it was not possible to measure organic and inorganic arsenic in our study to assess association with attention impairment separately. However, it is known that about 75% of the total urinary arsenic is composed of organic forms (K. L. Caldwell et al., 2009), and total urinary arsenic levels have been accepted as a good biomarker of dose (WHO, 2001).

Although the effect size from regression models may seem small, the change in terms of exposure is also small. The effect size for an increase from the 5th percentile (0.02 μg/l) to the 95th percentile (3.9 μg/l) is the beta coefficient multiplied by 7.6 ($\log_2(3.9/0.02)$). Thus, a change from P5 to P95 in exposure is associated with an increase of 27 ms in SAT latency (10% of mean latency), 94 ms in RTT latency (15% of mean), 4.3 points more in No-X-Task omission errors (7% of mean) and 3.8 points more in X-Task omission errors (6% of mean).

Our study was carried out in children from the municipalities in the Ria of Huelva, an area located in Southwestern Spain which has been dominated for decades by extensive chemical industry and mining activities. This Ria is controlled by the tidal regime and the inputs of the Odiel and Tinto rivers, as well as two channels which exchange water directly with the open sea. High levels of metallic elements (e.g. zinc, copper, lead, arsenic, nickel, mercury and cadmium) have been observed in Ria of Huelva (Blasco et al., 2010). Main sources of contamination in this area include: the industrial sewage from over forty neighboring chemical industries, urban sewage of the city of Huelva, and fluvial inputs from the Odiel and Tinto rivers, which drain acidic waters from mining activities carrying high levels of metallic trace elements (Blasco et al., 2010).

Environmental levels of arsenic in Ria of Huelva averaged 179 μg/g in sediments in 2010, ranging from 36 to 405 μg/g (Blasco et al., 2010). These levels are largely higher than average concentrations reported for soil and other surficial materials in the U.S., where mean concentration was 7.2 μg/g, ranging from <0.1 to 97 μg/g (ATSDR, 2007). Mean arsenic levels in the emission plumes in proximity of the copper smelter factory in the Ria of Huelva area exceeded the target value set by European Directive 2004/107/EC (6 ng/m^3) from 1st January 2013 onwards, and hourly peak concentrations

reached maximum hourly levels as high as 326 ng/m^3 (Sanchez de la Campa et al., 2011).

We found lower As levels in children living in the farthest municipality from the industrial and mining areas and vice versa. However, the total urinary arsenic levels found in our study population is almost ten-fold lower than the reported for children aged 6-11 years in the U.S. population (CDC, 2012) (geometric means 0.70 μg/l versus 6.63 μg/l respectively), and six-fold lower that those found in German children at 6-8 years of age (Kolossa-Gehring et al., 2007) (geometric means 0.70 μg/l versus 4.40 μg/l respectively). Levels found in our study are also far lower than those reported for areas with high environmental arsenic levels, such as Bangladesh (51 μg/l) (Hamadani et al., 2010), Mexico (55 μg/l) (Roy et al., 2011), India (78 μg/l) (von Ehrenstein et al., 2007) or China (73 μg/l) (Wang et al., 2007). A previous study carried out in the same area of the Ria of Huelva (Aguilera et al., 2010) reported a geometric mean of 1.36 μg/l in children 5 to 17 years, which is almost twice the figure found in our study. This downward trend might indicate that environmental pollution by arsenic has decreased over time in the same industrialized area. Moreover, no children in the current study exceeded the reference value established by the Human Biomonitoring Commission of the German Federal Environment Agency for children (15 μg/l). Levels above this limit are considered as a potential risk for adverse health effects in children (Schulz, Wilhelm, Heudorf, & Kolossa-Gehring, 2012). Thus, the adverse effects on attention observed in this study occur at arsenic levels below those considered to be safe.

Multivariate models used in this study were adjusted for urine creatinine levels rather than using creatinine-corrected arsenic concentrations (creatinine standardization). The latter has been questioned as the precursor of creatinine (creatine) is linked to one-carbon metabolism and is a strong predictor of arsenic methylation efficiency (Basu et

al., 2011; Gamble et al., 2005). On the other hand, the high creatinine seasonal variability in children produces differences in internal dose measurement independent of exposure (O'Rourke et al., 2000). So, following the recommendation made by Barr et al. (2005), creatinine levels were included as an independent covariate in the regression models. This approach allows: a) urinary arsenic concentrations to be appropriately adjusted for urinary creatinine; and b) independence of statistical significance of other variables entered into the models from the effects of creatinine concentration (Barr et al., 2005).

There is little evidence about the mechanistic pathways by which arsenic can impair neurobehavioral development in children. One of the potential mechanisms involved is related to increased oxidative stress, which may result in DNA damage. Although arsenic does not participate directly in redox reactions, it can induce oxidative stress via depletion of reduced glutathione (GSH) and inhibition of enzymes-containing thiol groups. Besides, arsenic induces up-regulation of several antioxidant genes, including superoxide dismutase (SOD) and thioredoxin (Trx-1), suggests compensatory mechanisms to overcome the oxidative byproduct effects of arsenic toxicity on thiol-containing enzymes related to arsenic metabolism (Rodriguez, Limon-Pacheco, Carrizales, Mendoza-Trejo, & Giordano, 2010). On the other hand, experimental animals treated with arsenic have shown alterations in hippocampal function, morphology and signaling, leading to altered cognitive behavior (Tyler & Allan, 2014). Both developmental and adult exposures to arsenic have shown deficits in adult neurogenesis in the dentate gyrus of the hippocampus through a reduced proliferation of neural progenitor cells and the number of mature neurons (Liu et al., 2012). Arsenic also affects NMDA receptors in the hippocampus, which play an essential role in synaptic plasticity. The resulting impairment of hippocampal neurons may have an impact on

learning and memory eventually leading to neurobehavioral disorders and cognitive dysfunctions (Kruger et al., 2009; Luo et al., 2009). Other brain targets for arsenic neurotoxicity include alteration of glutamatergic, cholinergic and monoaminergic signaling and alteration of glucocorticoid receptor signaling, responsible for hypothalamus-pituitary-adrenal axis dysregulation (Tyler & Allan, 2014).

Although some studies have pointed out gender-related differences in neurotoxicity of metallic trace elements (Llop, Lopez-Espinosa, Rebagliato, & Ballester, 2013; Mergler, 2012; Vahter, Akesson, Liden, Ceccatelli, & Berglund, 2007), the present study failed to show significant differences between sexes in the adverse effect of arsenic exposure on attention. It is known that certain toxic elements can act differently in males and females due to differences in pattern of exposure, metabolism or individual susceptibility. Although few studies have systematically evaluated sex differences in relation to arsenic exposure, a greater risk of neurological deficits is commonly found in females than in males (Tyler & Allan, 2014). However, these findings derive from studies assessing neurodevelopment or intelligence, but no study has identified so far such sex-related differences in behavior outcomes.

This study has several limitations. The cross-sectional design is the primary one because it does not allow causal inference. In addition, a single measurement of current arsenic levels in urine cannot assess past exposures, especially prenatal exposures. A better understanding of the exposure–outcome relationship can be obtained from prospective studies where children are follow-up from the fetal period and both arsenic exposure and behavioral outcomes are measured at regular intervals. Ninety two percent of our children were born in the study area and 89% of them had been living in the same residence since birth. If we assume that emission sources of arsenic to the environment have not changed over time in our study area, in particular for the last 9 years

(maximum age of our children), then the current arsenic exposure in our study population might be representative of past exposures. Another limitation of our study is a low rate response, as approximately 15% of invited parents accepted to participate in the study. Nonetheless, a potential selection bias related to differential exposure can be ruled out as all children (both those who participated in the study and those who refused to participate) lived in the same geographical area, and hence the environmental exposure to As is similar for both groups. On the other hand, as the prevalence of ADHD found in the study area is somewhat lower than that reported for Spain, a potential selection bias related to a higher participation of children having attention problems can be discarded. Accordingly, the effect size on attention found in this study might be underestimated.

One of the strengths of this study is that all of the selected children provided a urine sample, and between 95-99% completed the neuropsychological tests, so a potential selection bias influencing the results can be ruled out, except for TRF which was completed for 71% of the participants. However, comparison between children with and without TRF data showed no significant differences in scores from others tests (AULA, BARS and CBCL). Thus we can assume that results from TRF analysis are unbiased, though there is less statistical power to detect significant associations.

In sum, we found an association between increased urine arsenic levels and attention impairment, which may have important implications in later life. Attention deficit in first grade may affect interactions with peers and teachers, as well as modify the expected trajectory of school achievement. Moreover, attention impairment is related to an increased risk of developing neurobehavioral disorders such as ADHD. Because of the scarce evidence available to date, more research is needed to confirm and refine the potential effects of arsenic on children's behavior.

5. Conclusions

Increased urine arsenic levels were associated with attention impairment in school children living in an industrialized area from Southwestern Spain even at urinary levels of arsenic considered safe. Our results provide additional evidence about the neurotoxic effects of low-level postnatal arsenic exposure on children.

Acknowledgements

We would like to thank the Andalusian Health Department (Spain) for its financial support of this project (Project Number: PI0755/2010). This work is part of the Doctoral Thesis of Miguel Rodríguez Barranco from the University of Granada (Spain), in the context of the Doctoral Program in Clinical Medicine and Public Health. Authors would also like to thank the schools staff and the children and families, without whom this study would not have been possible.

Conflict of interest

Authors declare that they have no conflict of interest, except for Diane S. Rohlman. Oregon Health and Science University (OHSU) and Dr. Rohlman have a significant financial interest in Northwest Educational Training and Assessment, LLC, a company that may have a commercial interest in the results of this research and technology. This potential conflict of interest has been reviewed and managed by OHSU and the Integrity Program Oversight Council.

Figure legends

Figure 1. Regression coefficients from multivariate linear regression models that evaluate categorized urinary arsenic levels. Beta coefficients are adjusted for the same covariables than shown in tables 5 and 6. Values bellow the LOD are the reference category.

References

Achenbach, T. M. (1991). *Manual for the Teacher's Report Form and 1991 profile*. Burlington, VT: Department of Psychiatry, University of Vermont.

Achenbach, T. M., & Rescorla, L. A. (2001). *Manual for the ASEBA School-Age Forms & Profiles*. Burlington, VT: University of Vermont, Research Center for Children, Youth and Families.

Aguilera, I., Daponte, A., Gil, F., Hernandez, A. F., Godoy, P., Pla, A., . . . Sanchez-Parra, F. (2010). Urinary levels of arsenic and heavy metals in children and adolescents living in the industrialised area of Ria of Huelva (SW Spain). *Environ Int, 36*, 563-569.

Arranz Freijo, E. B., Olabarrieta Artetxe, F., Manzano Fernández, A., & Galende Pérez, N. (2014). Etxadi–Gangoiti scale: a proposal to evaluate the family contexts of two-year-old children†. *Early Child Development and Care, 184*, 933-948.

ATSDR. (2007). *Agency for Toxic Substances and Disease Registry, 2007. Toxicological Profile for Arsenic*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services.

Barkley, R. A. (1997). Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. *Psychol Bull, 121*, 65-94.

Barr, D. B., Wilder, L. C., Caudill, S. P., Gonzalez, A. J., Needham, L. L., & Pirkle, J. L. (2005). Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. *Environ Health Perspect, 113*, 192-200.

Basu, A., Mitra, S., Chung, J., Guha Mazumder, D. N., Ghosh, N., Kalman, D., . . . Smith, A. H. (2011). Creatinine, diet, micronutrients, and arsenic methylation in West Bengal, India. *Environ Health Perspect, 119*, 1308-1313.

Blasco, J., Gomes, T., García-Barrera, T., Rodríguez-Romero, A., Gonzalez-Rey, M., Morán-Roldán, F., . . . Bebianno, M. (2010). Trace metal concentrations in sediments from the southwest of the Iberian Peninsula. *Scientia Marina, 74*, 99-106.

Bolla-Wilson, K., & Bleecker, M. L. (1987). Neuropsychological impairment following inorganic arsenic exposure. *J Occup Med, 29*, 500-503.

Calderon, J., Navarro, M. E., Jimenez-Capdeville, M. E., Santos-Diaz, M. A., Golden, A., Rodriguez-Leyva, I., . . . Diaz-Barriga, F. (2001). Exposure to arsenic and lead and neuropsychological development in Mexican children. *Environ Res, 85*, 69-76.

Caldwell, B. M., & Bradley, R. H. (2003). *Home Observation for Measurement of Environment: Administration manual*. Little Rock, AR: University of Arkansas.

Caldwell, K. L., Jones, R. L., Verdon, C. P., Jarrett, J. M., Caudill, S. P., & Osterloh, J. D. (2009). Levels of urinary total and speciated arsenic in the US population: National Health and Nutrition Examination Survey 2003-2004. *J Expo Sci Environ Epidemiol, 19*, 59-68.

Catala-Lopez, F., Peiro, S., Ridao, M., Sanfelix-Gimeno, G., Genova-Maleras, R., & Catala, M. A. (2012). Prevalence of attention deficit hyperactivity disorder among children and adolescents in Spain: a systematic review and meta-analysis of epidemiological studies. *BMC Psychiatry, 12*, 168.

CDC. (2012). *Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, September 2012*. Atlanta, GA: Centers for Disease Control and Prevention.

Gamble, M. V., Liu, X., Ahsan, H., Pilsner, R., Ilievski, V., Slavkovich, V., . . . Graziano, J. H. (2005). Folate, homocysteine, and arsenic metabolism in arsenic-exposed individuals in Bangladesh. *Environ Health Perspect, 113*, 1683-1688.

Garcia, A. M., Gonzalez-Galarzo, M. C., Kauppinen, T., Delclos, G. L., & Benavides, F. G. (2013). A job-exposure matrix for research and surveillance of occupational health and safety in Spanish workers: MatEmESp. *Am J Ind Med, 56*, 1226-1238.

Gil, F., Capitan-Vallvey, L. F., De Santiago, E., Ballesta, J., Pla, A., Hernandez, A. F., . . . Villanueva, E. (2006). Heavy metal concentrations in the general population of Andalusia, South of Spain: a comparison with the population within the area of influence of Aznalcollar mine spill (SW Spain). *Sci Total Environ, 372*, 49-57.

Grandjean, P., & Landrigan, P. J. (2006). Developmental neurotoxicity of industrial chemicals. *Lancet, 368*, 2167-2178.

Grandjean, P., & Landrigan, P. J. (2014). Neurobehavioural effects of developmental toxicity. *Lancet Neurol, 13*, 330-338.

Hall, A. H. (2002). Chronic arsenic poisoning. *Toxicol Lett, 128*, 69-72.

Hamadani, J. D., Grantham-McGregor, S. M., Tofail, F., Nermell, B., Fangstrom, B., Huda, S. N., . . . Vahter, M. (2010). Pre- and postnatal arsenic exposure and child development at 18 months of age: a cohort study in rural Bangladesh. *Int J Epidemiol, 39*, 1206-1216.

Iriarte, Y., Diaz-Orueta, U., Cueto, E., Irazustabarrena, P., Banterla, F., & Climent, G. (2012). AULA--Advanced Virtual Reality Tool for the Assessment of Attention: Normative Study in Spain. *J Atten Disord*.

Kaufman, A., & Kaufman, N. (1990). *Kaufman Brief Intelligence Test Manual*: Circle Pines, MN: American Guidance Service.

Khan, K., Factor-Litvak, P., Wasserman, G. A., Liu, X., Ahmed, E., Parvez, F., . . . Graziano, J. H. (2011). Manganese exposure from drinking water and children's classroom behavior in Bangladesh. *Environ Health Perspect, 119*, 1501-1506.

Kolossa-Gehring, M., Becker, K., Conrad, A., Ludecke, A., Riedel, S., Seiwert, M., . . . Szewzyk, R. (2007). German Environmental Survey for Children (GerES IV)--first results. *Int J Hyg Environ Health, 210*, 535-540.

Kruger, K., Straub, H., Hirner, A. V., Hippler, J., Binding, N., & Musshoff, U. (2009). Effects of monomethylarsonic and monomethylarsonous acid on evoked synaptic potentials in hippocampal slices of adult and young rats. *Toxicol Appl Pharmacol, 236*, 115-123.

Lezak, M., Howieson, D., Bigler, E., & Tranel, D. (2004). *Neuropsychological Assessment*. New York: Oxford University Press.

Liu, S., Piao, F., Sun, X., Bai, L., Peng, Y., Zhong, Y., . . . Sun, W. (2012). Arsenic-induced inhibition of hippocampal neurogenesis and its reversibility. *Neurotoxicology, 33*, 1033-1039.

Luo, J. H., Qiu, Z. Q., Shu, W. Q., Zhang, Y. Y., Zhang, L., & Chen, J. A. (2009). Effects of arsenic exposure from drinking water on spatial memory, ultra-structures and NMDAR gene expression of hippocampus in rats. *Toxicol Lett, 184*, 121-125.

Llop, S., Lopez-Espinosa, M. J., Rebagliato, M., & Ballester, F. (2013). Gender differences in the neurotoxicity of metals in children. *Toxicology, 311*, 3-12.

Mergler, D. (2012). Neurotoxic exposures and effects: gender and sex matter! Hanninen Lecture 2011. *Neurotoxicology, 33*, 644-651.

O'Rourke, M. K., Lizardi, P. S., Rogan, S. P., Freeman, N. C., Aguirre, A., & Saint, C. G. (2000). Pesticide exposure and creatinine variation among young children. *J Expo Anal Environ Epidemiol, 10*, 672-681.

Parvez, F., Wasserman, G. A., Factor-Litvak, P., Liu, X., Slavkovich, V., Siddique, A. B., . . . Graziano, J. H. (2011). Arsenic exposure and motor function among children in Bangladesh. *Environ Health Perspect, 119*, 1665-1670.

Pettit, G. S., Bates, J. E., & Dodge, K. A. (1997). Supportive parenting, Ecological Context, and Children's Adjustment: A seven-Year Longitudianl Study. *Child development, 68*, 908-923.

Rocha-Amador, D., Navarro, M. E., Carrizales, L., Morales, R., & Calderon, J. (2007). Decreased intelligence in children and exposure to fluoride and arsenic in drinking water. *Cad Saude Publica, 23 Suppl 4*, S579-587.

Rodriguez-Barranco, M., Lacasana, M., Aguilar-Garduno, C., Alguacil, J., Gil, F., Gonzalez-Alzaga, B., & Rojas-Garcia, A. (2013). Association of arsenic, cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: a systematic review and meta-analysis. *Sci Total Environ, 454-455*, 562-577.

Rodriguez-Barranco, M., Lacasana, M., Gil, F., Lorca, A., Alguacil, J., Rohlman, D. S., . . . Aguilar-Garduno, C. (2014). Cadmium exposure and neuropsychological development in school children in southwestern Spain. *Environ Res, 134C*, 66-73.

Rodriguez, V. M., Carrizales, L., Mendoza, M. S., Fajardo, O. R., & Giordano, M. (2002). Effects of sodium arsenite exposure on development and behavior in the rat. *Neurotoxicol Teratol, 24*, 743-750.

Rodriguez, V. M., Limon-Pacheco, J. H., Carrizales, L., Mendoza-Trejo, M. S., & Giordano, M. (2010). Chronic exposure to low levels of inorganic arsenic causes alterations in locomotor activity and in the expression of dopaminergic and antioxidant systems in the albino rat. *Neurotoxicol Teratol, 32*, 640-647.

Rohlman, D. S., Gimenes, L. S., Eckerman, D. A., Kang, S. K., Farahat, F. M., & Anger, W. K. (2003). Development of the Behavioral Assessment and Research System (BARS) to detect and characterize neurotoxicity in humans. *Neurotoxicology, 24*, 523-531.

Rosado, J. L., Ronquillo, D., Kordas, K., Rojas, O., Alatorre, J., Lopez, P., . . . Stoltzfus, R. J. (2007). Arsenic exposure and cognitive performance in Mexican schoolchildren. *Environ Health Perspect, 115*, 1371-1375.

Roy, A., Kordas, K., Lopez, P., Rosado, J. L., Cebrian, M. E., Vargas, G. G., . . . Stoltzfus, R. J. (2011). Association between arsenic exposure and behavior among first-graders from Torreon, Mexico. *Environ Res, 111*, 670-676.

Sanchez de la Campa, A. M., de la Rosa, J., Gonzalez-Castanedo, Y., Fernandez-Camacho, R., Alastuey, A., Querol, X., . . . Nava, S. (2011). Levels and chemical composition of PM in a city near a large Cu-smelter in Spain. *J Environ Monit, 13*, 1276-1287.

Schulz, C., Wilhelm, M., Heudorf, U., & Kolossa-Gehring, M. (2012). Reprint of "Update of the reference and HBM values derived by the German Human Biomonitoring Commission". *Int J Hyg Environ Health, 215*, 150-158.

Seidman, L. J. (2006). Neuropsychological functioning in people with ADHD across the lifespan. *Clin Psychol Rev, 26*, 466-485.

Sergeant, J., Oosterlaan, J., & Van der Meere, J. (1999). *Information processing and energetic factors in attention-deficit/hyperactivity disorder. In C. Herbert & A.*

E. Hogan (Eds.), Handbook of disruptive behavior disorders. Dordrecht, Netherlands: Kluwer.

Smedley, P. L., & Kinniburgh, D. G. (2002). A review of the source, behaviour and distribution of arsenic in natural waters. *Applied Geochemistry, 17*, 517-568.

Sobradillo, B., Aguirre, A., Aresti, U., Bilbao, A., Fernández-Ramos, C., Lizárraga, A. L., H., . . . Hernández, M. (2004). *Curvas y tablas de crecimiento (Estudios Longitudinal y Transversal)*. Bilbao, Spain: Fundación Faustino Orbegozo.

Tsai, S. Y., Chou, H. Y., The, H. W., Chen, C. M., & Chen, C. J. (2003). The effects of chronic arsenic exposure from drinking water on the neurobehavioral development in adolescence. *Neurotoxicology, 24*, 747-753.

Tyler, C. R., & Allan, A. M. (2014). The Effects of Arsenic Exposure on Neurological and Cognitive Dysfunction in Human and Rodent Studies: A Review. *Curr Environ Health Rep, 1*, 132-147.

Vahter, M., Akesson, A., Liden, C., Ceccatelli, S., & Berglund, M. (2007). Gender differences in the disposition and toxicity of metals. *Environ Res, 104*, 85-95.

Velasco, D., Sanchez de Miguel, M., Egurza, M., Arranz, E., Aranbarri, A., Fano, E., & Ibarluzea, J. (2014). Family context assessment in a public health study. *Gac Sanit, 28*, 356-362.

von Ehrenstein, O. S., Poddar, S., Yuan, Y., Mazumder, D. G., Eskenazi, B., Basu, A., . . . Smith, A. H. (2007). Children's intellectual function in relation to arsenic exposure. *Epidemiology, 18*, 44-51.

Wang, S. X., Wang, Z. H., Cheng, X. T., Li, J., Sang, Z. P., Zhang, X. D., . . . Wang, Z. Q. (2007). Arsenic and fluoride exposure in drinking water: children's IQ and growth in Shanyin county, Shanxi province, China. *Environ Health Perspect, 115*, 643-647.

Wasserman, G. A., Liu, X., Parvez, F., Ahsan, H., Factor-Litvak, P., Kline, J., . . . Graziano, J. H. (2007). Water arsenic exposure and intellectual function in 6-year-old children in Araihazar, Bangladesh. *Environ Health Perspect, 115*, 285-289.

Wasserman, G. A., Liu, X., Parvez, F., Ahsan, H., Factor-Litvak, P., van Geen, A., . . . Graziano, J. H. (2004). Water arsenic exposure and children's intellectual function in Araihazar, Bangladesh. *Environ Health Perspect, 112*, 1329-1333.

Wasserman, G. A., Liu, X., Parvez, F., Factor-Litvak, P., Ahsan, H., Levy, D., . . . Graziano, J. H. (2011). Arsenic and manganese exposure and children's intellectual function. *Neurotoxicology, 32*, 450-457.

WHO. (2001). Arsenic and Arsenic Compounds. 2nd ed. Environmental Health Criteria 224. Geneva. Retrieved 11 September 2014, from http://www.inchem.org/documents/ehc/ehc/ehc224.htm

Wright, R. O., Amarasiriwardena, C., Woolf, A. D., Jim, R., & Bellinger, D. C. (2006). Neuropsychological correlates of hair arsenic, manganese, and cadmium levels in school-age children residing near a hazardous waste site. *Neurotoxicology, 27*, 210-216.

Xi, S., Sun, W., Wang, F., Jin, Y., & Sun, G. (2009). Transplacental and early life exposure to inorganic arsenic affected development and behavior in offspring rats. *Arch Toxicol, 83*, 549-556.

Yousef, S., Adem, A., Zoubeidi, T., Kosanovic, M., Mabrouk, A. A., & Eapen, V. (2011). Attention deficit hyperactivity disorder and environmental toxic metal exposure in the United Arab Emirates. *J Trop Pediatr, 57*, 457-460.

Table 1. Urinary arsenic levels (μg/l) in the study sample by sex and age.

	n	%<LOD	GM	95% CI	P25	P50	P75	P95
Total	261	14.2	0.70	(0.58 – 0.85)	0.50	1.17	1.93	3.90
Boys	135	13.3	0.72	(0.55 – 0.95)	0.48	1.22	2.06	3.76
Girls	126	15.1	0.68	(0.51 – 0.90)	0.51	1.07	1.80	4.36
6-8 years	145	15.2	0.64	(0.49 – 0.84)	0.50	1.06	1.93	4.04
9-10 years	116	12.9	0.78	(0.59 – 1.04)	0.52	1.34	1.96	3.90

Table 2. Demographic and socioeconomic characteristics of the study population and their relation with urinary arsenic levels (UAs).

	n	(%)	Geometric mean of UAs (μg/l) (95% CI)	p[a]
Overall	261	(100)	0.70 (0.58–0.85)	
Sex				0.473
Boys	135	(51.7)	0.72 (0.55–0.95)	
Girls	126	(48.3)	0.68 (0.51–0.90)	
Child's age				0.220
6-7 years	145	(55.6)	0.64 (0.49–0.84)	
8-9 years	116	(44.4)	0.78 (0.59–1.04)	
Body Mass Index (BMI)				0.157
Normal	201	(77.0)	0.76 (0.61–0.96)	
Overweight	24	(9.2)	0.54 (0.26–1.11)	
Obesity	36	(13.8)	0.54 (0.31–0.94)	
Mother's age				0.790
≤35 years	93	(37.1)	0.77 (0.57–1.05)	
>35 years	158	(62.9)	0.63 (0.48–0.82)	
Mother's education				0.007
No formal	9	(3.6)	1.45 (0.72–2.91)	
Primary	101	(40.2)	1.00 (0.77–1.31)	
Secondary	93	(37.1)	0.59 (0.42–0.82)	
Higher	48	(19.1)	0.34 (0.19–0.61)	
Mother's occupation				0.410
Employed	112	(44.6)	0.60 (0.44–0.82)	
Unemployed	139	(55.4)	0.75 (0.58–0.98)	
Father's education				0.069
No formal	16	(7.1)	1.71 (1.18–2.47)	
Primary	111	(49.6)	0.68 (0.50–0.91)	
Secondary	64	(28.6)	0.70 (0.48–1.02)	
Higher	33	(14.7)	0.34 (0.16–0.72)	
Father's occupation				0.381
Employed	168	(75.0)	0.62 (0.48–0.79)	
Unemployed	56	(25.0)	0.82 (0.55–1.21)	
Monthly family income				0.032
≤1000 €	28	(11.2)	0.88 (0.43–1.79)	
1001-2000 €	159	(63.9)	0.78 (0.62–0.98)	
>2000 €	62	(24.9)	0.42 (0.26–0.69)	
Residence area				0.529
Urban	225	(89.6)	0.68 (0.55–0.84)	
Metropolitan	15	(6.0)	0.72 (0.28–1.90)	
Rural	11	(4.4)	0.60 (0.23–1.62)	
Living near industrial areas				0.060
No	153	(72.9)	0.62 (0.47–0.81)	
Yes, less than 1 Km	57	(27.1)	0.93 (0.61–1.40)	

Living near mining areas				0.911
No	178	(90.8)	0.70 (0.55–0.89)	
Yes, less than 1 Km	18	(9.2)	0.83 (0.40–1.75)	
Living near agricultural areas				0.714
No	120	(58.0)	0.71 (0.53–0.95)	
Yes, less than 1 Km	87	(42.0)	0.73 (0.51–1.03)	
Maternal occupational exposure to metals during pregnancy				0.155
No	231	(92.0)	0.70 (0.56–0.86)	
Yes	20	(8.0)	0.51 (0.25–1.04)	
Current maternal occupational exposure to metals				0.526
No	228	(91.6)	0.67 (0.54–0.84)	
Yes	21	(8.4)	0.67 (0.36–1.23)	
Paternal occupational exposure to metals during pregnancy				0.116
No	147	(60.5)	0.76 (0.59–0.98)	
Yes	96	(39.5)	0.53 (0.37–0.76)	
Current paternal occupational exposure to metals				0.171
No	144	(59.3)	0.73 (0.57–0.95)	
Yes	99	(40.7)	0.56 (0.40–0.79)	
Pets at home				0.313
No	180	(71.7)	0.73 (0.58–0.92)	
Yes	71	(28.3)	0.57 (0.38–0.86)	
Cleaning frequency				0.079
> One per week	192	(76.5)	0.80 (0.65–0.98)	
≤ One per week	59	(23.5)	0.41 (0.24–0.68)	
Tobacco consumption at home				0.405
No	144	(56.5)	0.68 (0.52–0.87)	
Yes	111	(43.5)	0.72 (0.52–0.98)	
Usual drinking water consumption				<0.001
Municipal	133	(51.4)	0.49 (0.36–0.66)	
Municipal filtered	10	(3.9)	0.20 (0.05–0.84)	
Bottled	89	(34.4)	1.17 (0.91–1.49)	
Municipal + Bottled	22	(8.5)	1.13 (0.69–1.85)	
Well or spring	5	(1.9)	1.73 (0.59–5.06)	
Family status				0.472
Living with biological mother and father	214	(85.3)	0.67 (0.54–0.83)	
Other situations [b]	37	(14.7)	0.74 (0.43–1.28)	

[a] p-value from Mann-Whitney's test or Kruskal-Wallis' test.
[b] Living with single parent, biological mother/father and partner, adoptive parents, or grandparents.

Table 3. Description of lifestyle of the mother during pregnancy. anthropometric measures at birth. current food intake and activity of the children by urinary arsenic levels (µg/l).

		Overall	UAs < P75	UAs ≥ P75	
	n	Mean ± SE	Mean ± SE	Mean ± SE	p[c]
Birth characteristics					
Gestational age (wks)	245	39,24 ± 0,12	39,34 ± 0,11	38,95 ± 0,36	0,308
Weight (kg)	247	3296,49 ± 34,51	3308,67 ± 36,17	3256,83 ± 88,25	0,588
Height (cm)	239	49,98 ± 0,17	50,02 ± 0,18	49,83 ± 0,41	0,632
Head circumference (cm)	140	34,20 ± 0,13	34,22 ± 0,16	34,12 ± 0,25	0,744
Breastfeeding[a]	249	63.5%	64.0%	61.7%	0,741
Alcohol consumption during pregnancy[a]	247	0%	0%	0%	-
Tobacco consumption during pregnancy[a]	247	21.9%	23.4%	16.9%	0,295
Food intake[b]					
Dairy products	250	29,97 ± 0,62	29,78 ± 0,71	30,55 ± 1,29	0,595
Eggs and meat	250	14,63 ± 0,24	14,59 ± 0,28	14,74 ± 0,49	0,794
White fish	250	1,65 ± 0,08	1,61 ± 0,09	1,80 ± 0,16	0,276
Big oily fish	250	1,69 ± 0,09	1,68 ± 0,10	1,74 ± 0,19	0,774
Small oily fish	250	0,94 ± 0,06	0,88 ± 0,07	1,11 ± 0,13	0,117
Crustaceans and molluscs	250	0,53 ± 0,04	0,54 ± 0,05	0,48 ± 0,07	0,474
Cephalopods	250	0,61 ± 0,04	0,60 ± 0,05	0,65 ± 0,09	0,595
Shellfish	250	0,26 ± 0,03	0,28 ± 0,03	0,22 ± 0,04	0,386
Vegetables and pulses	250	18,84 ± 0,42	18,62 ± 0,46	19,52 ± 0,95	0,353
Fruits	250	16,75 ± 0,67	16,55 ± 0,69	17,38 ± 1,75	0,662

Bread, cereals, pasta and rice	250	29,36 ± 0,34	29,13 ± 0,40	30,07 ± 0,64	0,239
Oils, fat and confectionery	250	44,96 ± 0,99	43,99 ± 1,06	47,95 ± 2,37	0,131
Activity					
Hours per day spent sleeping	252	10,33 ± 0,03	10,36 ± 0,04	10,23 ± 0,07	0,105
Hours per day spent in active games	258	1,71 ± 0,09	1,71 ± 0,10	1,71 ± 0,19	0,999
Hours per day spent in sports	257	1,47 ± 0,09	1,45 ± 0,10	1,55 ± 0,20	0,625
Hours per day spent in computer or game console	257	0,61 ± 0,04	0,59 ± 0,05	0,69 ± 0,09	0,319
Hours per day spent studying	257	1,62 ± 0,06	1,66 ± 0,07	1,50 ± 0,08	0,148

[a] Percentage of "Yes".; [b] Number of servings per week.; [c] t-Student test; SE: standard error

Table 4. Scores obtained from BARS, AULA, CBCL and TRF tests.

	Mean	SD	Range
BARS (n=257)			
Selective Attention Test (SAT)			
Percentage of false alarms	44.0	11.3	(15.5 – 72.4)
Percentage of omissions	38.0	5.5	(21.4 – 56.3)
Latency (msec.)	275.8	59.6	(165.8 – 656.3)
Simple Reaction Time Test (RTT)			
Latency (msec.)	628.8	164.2	(313.4 – 1429.6)
Latency (msec.) excluding trials with latencies > 1000 msec.	523.6	76.9	(313.4 – 743.1)
Continuous Performance Test (CPT)			
Percentage of false alarms	6.7	7.3	(0 – 52.9)
Percentage of omissions	26.8	15.7	(0 – 78.3)
Latency (msec.)	459.7	90.4	(229.6 – 717.9)
AULA (n=249)			
No-X-Task (impulsivity)			
Omission errors	65.6	9.0	(36 – 80)
Commission errors	54.6	12.1	(23 – 80)
Reaction time in hits	60.7	12.3	(27 – 80)
Motor activity	60.2	10.8	(26 – 80)
X-Task (inattention)			
Omission errors	62.9	9.0	(39 – 80)
Commission errors	58.8	9.2	(35 – 80)
Reaction time in hits	61.4	12.9	(30 – 80)
Motor activity	56.7	11.2	(27 – 80)
CBCL (n=256)			
Attention problems	56.1	7.3	(50 – 88)
TRF (n=185)			
Attention deficit/hyperactive disorder	53.0	5.3	(50 – 74)

SD: Standard deviation

Table 5. Adjusted regression coefficients of the association between urinary arsenic levels (log2-transformed) and BARS test scores.

Response variable	β (95% CI)	p-value
Model 1[a]		
Selective Attention Test (SAT)		
Percentage of false alarms	0.18 (-0.42 to 0.78)	0.560
Percentage of omissions	-0.05 (-0.34 to 0.24)	0.727
Latency (msec.)	3.52 (0.34 to 6.70)	0.030
Simple Reaction Time Test (RTT)		
Latency (msec.)	9.98 (1.30 to 18.66)	0.024
Latency (msec.) excluding trials with latencies > 1000 msec.	4.03 (0.01 to 8.05)	0.049
Continuous Performance Test (CPT)		
Percentage of false alarms	0.14 (-0.26 to 0.54)	0.492
Percentage of omissions	0.14 (-0.71 to 0.99)	0.747
Latency (msec.)	-0.38 (-4.95 to 4.19)	0.869
Model 2		
Selective Attention Test (SAT)[b]		
Percentage of false alarms	0.30 (-0.34 to 0.94)	0.362
Percentage of omissions	-0.17 (-0.48 to 0.14)	0.279
Latency (msec.)	3.58 (0.37 to 6.79)	0.029
Simple Reaction Time Test (RTT)[c]		
Latency (msec.)	12.31 (3.51 to 21.11)	0.006
Latency (msec.) excluding trials with latencies > 1000 msec.	5.51 (1.27 to 9.75)	0.011
Continuous Performance Test (CPT[d]		
Percentage of false alarms	0.16 (-0.24 to 0.56)	0.433
Percentage of omissions	-0.39 (-1.25 to 0.47)	0.374
Latency (msec.)	0.08 (-4.72 to 4.55)	0.970

[a] Adjusted by sex, child's age and creatinine level.
[b] Adjusted by sex, child's age, creatinine level, mother's education, hours sleeping, usual drinking water, eggs and meat intake, and family status.
[c] Adjusted by sex, child's age, creatinine level, mother's education, residence area, paternal exposure to metals during pregnancy, gestational age, shellfish and white fish intake, and family status.
[d] Adjusted by sex, child's age, creatinine level, mother's occupation, pets at home, maternal exposure to metals during pregnancy, gestational age, weight at birth, breastfeeding, usual drinking water, white fish intake, and quality family context scale.

Table 6. Adjusted regression coefficients of the association between urinary arsenic levels (log2-transformed) and AULA test scores.

Response variable	β (95% CI)	p-value
Model 1[a]		
No-X-Task (impulsivity)		
Omission errors	0.48 (-0.02 to 0.97)	0.058
Commission errors	-0.14 (-0.82 to 0.54)	0.685
Reaction time in hits	0.21 (-0.47 to 0.89)	0.539
Motor activity	-0.07 (-0.66 to 0.53)	0.827
X-Task (inattention)		
Omission errors	0.51 (0.03 to 0.99)	0.039
Commission errors	0.38 (-0.12 to 0.89)	0.137
Reaction time in hits	0.16 (-0.53 to 0.86)	0.644
Motor activity	0.06 (-0.54 to 0.67)	0.840
Model 2		
No-X-Task (impulsivity)[b]		
Omission errors	0.57 (0.07 to 1.07)	0.026
Commission errors	-0.11 (-0.80 to 0.58)	0.754
Reaction time in hits	0.16 (-0.52 to 0.83)	0.644
Motor activity	-0.01 (-0.60 to 0.59)	0.981
X-Task (inattention)[c]		
Omission errors	0.50 (0.03 to 0.97)	0.039
Commission errors	0.36 (-0.15 to 0.87)	0.168
Reaction time in hits	0.22 (-0.45 to 0.89)	0.519
Motor activity	0.32 (-0.28 to 0.91)	0.299

[a] Adjusted by sex, child's age and creatinine level.

[b] Adjusted by sex, child's age, creatinine level, pets at home, maternal exposure to metals, weight at birth, hours of active games, usual drinking water, fruit, bread/cereals and oils/fat intake, family status, and quality family context scale.

[c] Adjusted by sex, child's age, creatinine level, mother's occupation, pets at home, maternal exposure to metals during pregnancy, paternal exposure to metals after birth, gestational age, weight at birth, breastfeeding, tobacco during pregnancy, hours of active games, usual drinking water, eggs/meat, fruit and vegetables intake, family status, and quality family context scale.

Table 7. Adjusted regression coefficients of the association between urinary arsenic levels (log2-transformed) and CBCL and TRF tests scores.

Response variable	n	Effect size (95% CI)	p-value
Model 1[a]			
CBCL Attention problems			
Continuous scale (β & 95% CI)	256	-0.11 (-0.51 to 0.29)	0.601
Score > P93 (OR & 95% CI)	29/256	0.98 (0.82 to 1.18)	0.858
Score > P98 (OR & 95% CI)	15/256	0.97 (0.77 to 1.23)	0.825
TRF Attention deficit/hyperactive disorder			
Continuous scale (β & 95% CI)	185	0.10 (-0.24 to 0.43)	0.572
Score > P93 (OR & 95% CI)	13/185	1.04 (0.80 to 1.35)	0.756
Score > P98 (OR & 95% CI)	3/185	0.99 (0.63 to 1.57)	0.969
Model 2			
CBCL Attention problems [b]			
Continuous scale (β & 95% CI)	224	-0.28 (-0.67 to 0.12)	0.166
Score > P93 (OR & 95% CI)	23/224	0.93 (0.76 to 1.15)	0.503
Score > P98 (OR & 95% CI)	14/224	0.97 (0.75 to 1.27)	0.835
TRF Attention deficit/hyperactive disorder [c]			
Continuous scale (β & 95% CI)	176	0.12 (-0.21 to 0.46)	0.472
Score > P93 (OR & 95% CI)	12/176	1.05 (0.78 to 1.42)	0.738
Score > P98 (OR & 95% CI)	3/176	1.12 (0.68 to 1.87)	0.652

[a] Adjusted by sex, child's age and creatinine level.
[b] Adjusted by sex, child's age, creatinine level, weight at birth, hours of study, and quality family context scale.
[c] Adjusted by sex, child's age, creatinine level, mother's age, residence area, family status, and hours of study.

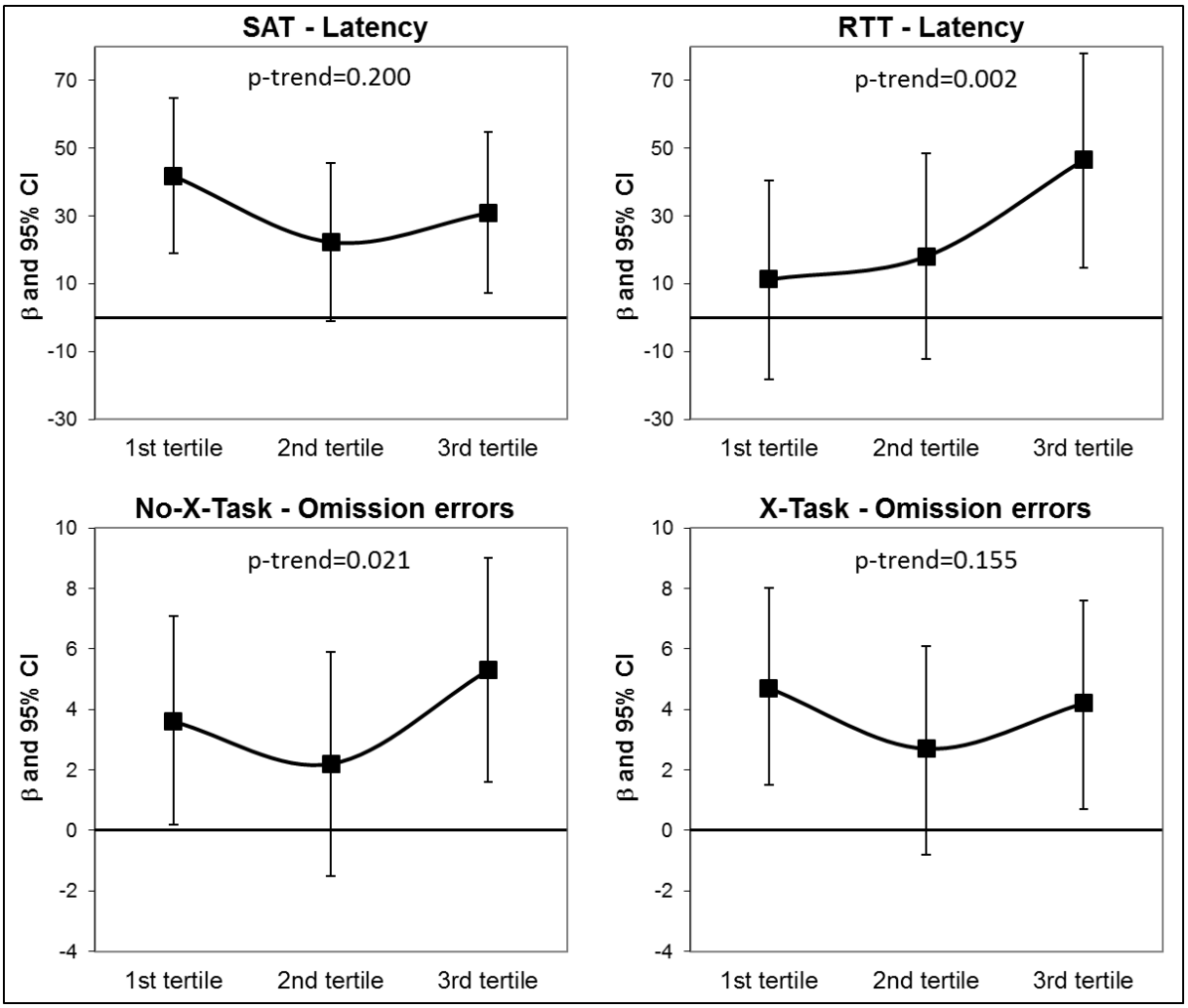


Figure 1. Regression coefficients from multivariate linear regression models that evaluate categorized urinary arsenic levels. Beta coefficients are adjusted for the same covariables than shown in tables 5 and 6. Values bellow the LOD are the reference category.