Efecto de la exposición a contaminación atmosférica durante el embarazo sobre el crecimiento fetal

Inmaculada Aguilera Jiménez

TESI DOCTORAL UPF / 2009

DIRECTOR DE LA TESI

Dr. Jordi Sunyer i Deu

(Departament de Ciències Experimentals i de la Salut)

UNIVERSITAT POMPEU FABRA

A *třešnička*, con la esperanza de que pueda vivir en
un mundo con mayor justicia ambiental y social

"Those who contemplate the beauty of the earth find reserves
of strength that will endure as long as life lasts".
Rachel Carson

"Si cerramos la puerta a todos los errores,
también la verdad se quedará fuera".
Rabindranath Tagore

"La utopía está en el horizonte. Me acerco dos pasos, ella se aleja dos pasos.
Camino diez pasos y el horizonte se desplaza diez pasos más allá. Por mucho
que camine, nunca la alcanzaré. Entonces, ¿para qué sirve la utopía?
Para eso: sirve para caminar".
Eduardo Galeano

Agradecimientos

Esta tesis es el resultado de varios años de trabajo, y son muchas las personas que me han ayudado, de una u otra manera, a que este trabajo por fin vea la luz en forma de tesis doctoral. Quisiera expresar mi especial agradecimiento a algunas de ellas.

En primer lloc, vull agrair al meu director per haver-me obert les portes de l'antiga URRA (ara CREAL), ara fa gairebé cinc anys, i haver-me permès treballar en un projecte tan fascinant com és l'estudi INMA. Gràcies, Jordi, per la teva confiança i suport, pel teu rigor científic i per tenir sempre oberta la porta del teu despatx. És admirable el teu entusiasme encomanador a la feina del dia a dia, el qual dóna lloc contínuament a noves idees i projectes.

A todas las familias que participan en el proyecto INMA, porque sin su colaboración desinteresada sería imposible llevar a cabo estudios tan ambiciosos como este. Y a Sílvia Fochs, Núria Pey, Anna Sànchez y Maribel López, enfermeras de la cohorte INMA-Sabadell, por su profesionalidad, esfuerzo y cercanía a las familias. Gran parte del éxito en el seguimiento de la cohorte es gracias a ellas.

A los compañeros de las otras cohortes INMA, y en especial al grupo de trabajo de contaminación atmosférica, por su ayuda en el diseño de las campañas y por el trabajo conjunto en el intercambio de información y desarrollo de modelos de exposición.

Al Servei de Salut de l'Ajuntament de Sabadell, y en particular a Maria Sala, Teresa Corbella, Mònica Jacas y Eli Puigdollers, sin cuya colaboración hubiese sido imposible realizar las campañas de medida de contaminación del aire. También quiero agradecer enormemente la ayuda de Josep Iglesias (Servei d'Informació Corporativa) en la obtención de las capas municipales de información geográfica y en la geocodificación.

A Xavier Querol, Andrés Alastuey y demás investigadores del Instituto Jaume Almera del CSIC, así como al Servei de Vigilància i Control de l'Aire del Departament de Medi Ambient, por aportar su experiencia y sus equipos de medición en la obtención de medidas de partículas y otros contaminantes en Sabadell.

I would like to thank David Briggs, Danielle Vienneau, Kees de Hoogh, Linda Beale, John Gulliver, Chloe Morris, and Daniela Fetch from the Imperial College London (Small Area Health Statistics Unit) for giving me the opportunity to stay in their unit to improve my knowledge on GIS. That stay was extremely helpful for me and I enjoyed one month in London with no rain at all! Danielle, many thanks for your help, your patience, and your hospitality.

I am grateful to Bert Brunekreef, Gerard Hoek and Kees Meliefste, from the Institute for Risk Assessment Sciences (Utrecht), for their implication in the field work design and their help on my first GIS analysis within the European GA^{2}LEN network. A special thank to the other partner of the GA^{2}LEN demonstration project, Christian

Madsen from the Norwegian Institute of Public Health, for his lessons on spatial autocorrelation and ArcGIS scripts.

Trabajar en el CREAL significa estar rodeada de excelentes investigadores y compañeros que hacen que el trabajo del día a día sea mucho más agradable. Del pequeño edificio que habitábamos en 2005 a la casi media planta del PRBB que ocupamos hoy han transcurrido muchos cambios, pero la esencia del grupo y el buen ambiente sigue siendo el mismo. Quiero agradecer especialmente a mis compañeros presentes y pasados de sala (Raquel, Estel, Gemma, Bene, Cristina, David, los JoFo's I y II, Carlos, Ana, Alejandro, María y todos los demás) por los buenos momentos pasados y las risas, por las salidas nocturnas y las cervezas en las terrazas del centro, ¡que siga siendo así por mucho tiempo! A Mark y Audrey por sus valiosos consejos sobre *exposure assessment*. A la pequeña gran familia del estudio INMA-Sabadell (Mònica, Mar, Aina, Michelle, Cristina y un largo etcétera), y en especial a quienes participaron en el duro trabajo de campo (escalera y alicates en mano) y a la "madrina" de INMA, Núria Ribas, por su entusiasmo, dedicación e implicación en el estudio.

Mis primeros pasos en la Epidemiología Ambiental los di en la Escuela Andaluza de Salud Pública, y, como tantas otras cosas en la vida, ocurrió más por casualidad que por vocación. Sin embargo, lo que inicialmente pensé que sería un trabajo breve en un proyecto, se acabó convirtiendo en una profesión la cual, ocho años después, me sigue entusiasmando. Por eso, me gustaría dar las gracias a Antonio Daponte por haberme introducido en este fascinante campo y haberme dado la oportunidad de trabajar en un proyecto tan interesante (y complejo) como el de la Ría de Huelva. A Piedad Martín y Ricardo Ocaña, por su profesionalidad y apoyo dentro de la Escuela y su amistad fuera de ella. A Carmen, Ceci, Pablo, Clara, Jaime, Silvia, Andrés, María, Julia y otros/as muchos/as compañeros/as que hicieron de mi paso por la Escuela una de las mejores etapas de mi vida, y a quienes, a pesar de la distancia, hoy en día me sigue uniendo una gran amistad.

A Rocío Quijano, de la Universidad de Jaén, por ser una excelente profesora y mejor compañera. Aunque he acabado dirigiendo mi vida profesional por otros caminos, gracias a ella guardo muy buen recuerdo de mis dos años en la Universidad, y aprendí lo importante que es creer en la capacidad de uno mismo para conseguir todo aquello que se proponga.

A la Fundació IMIM, por su ayuda concedida para la finalización de esta tesis doctoral.

Por último, quisiera dar las gracias a mis padres, porque gran parte de lo que soy es gracias a ellos y porque siempre han respetado y apoyado mis decisiones, por difíciles que fuesen. Y a Bořek, por ser parte de mi vida y haber cambiado mi manera de ver y entender muchas cosas, por lo que hemos vivido juntos y por lo que nos queda por vivir.

Resumen

Antecedentes y objetivo: Un creciente número de estudios epidemiológicos han asociado la exposición prenatal a contaminación atmosférica urbana con un menor crecimiento fetal, pero pocos están basados en cohortes prospectivas con modelos de exposición que capten la variabilidad espacial a pequeña escala de la contaminación dentro de una misma ciudad.

Métodos: A partir de modelos basados en medidas de contaminación con captadores pasivos y variables geográficas, se estimó la exposición prenatal a dióxido de nitrógeno (NO_2) y compuestos orgánicos volátiles (COVs) en una cohorte de 611 embarazadas de Sabadell. El crecimiento fetal se midió como peso al nacer y también longitudinalmente mediante ecografías obstétricas.

Resultados: Tras estratificar por determinados patrones de tiempo-actividad, se halló un efecto negativo de la exposición prenatal a COVs por incremento en el rango intercuartílico sobre el peso al nacer (-77 g, p<0.05). Al evaluar el crecimiento fetal mediante ecografías se halló una asociación entre exposición a NO_2 y COVs desde el inicio del embarazo y un menor crecimiento de varios parámetros fetales a partir de la semana 20 de gestación.

Conclusiones: La variabilidad en la exposición a contaminación atmosférica asociada al tráfico dentro de una misma ciudad tiene un efecto negativo sobre el crecimiento fetal. Este efecto comienza a manifestarse hacia la mitad del embarazo y parece persisitir hasta el nacimiento.

Abstract

Background and objective: A growing number of studies have found an association between prenatal exposure to urban air pollution and fetal growth, but few of them are based on prospective cohorts with exposure models developed to capture the small-scale spatial variability in air pollution levels within a city.

Methods: We developed models based on air pollution measurements with passive samplers and geographic variables. They were applied to estimate prenatal exposure to nitrogen dioxide (NO_2) and volatile organic compounds (VOCs) in a cohort of 611 pregnant women from Sabadell. Fetal growth was assessed as birth weight and also through obstetric ultrasounds.

Results: After stratifying by some specific time-activity patterns, a negative effect of prenatal exposure to VOCs was found on birth weight (-77 g, p<0.05 for an interquartilic range increase in VOCs levels). When fetal growth was longitudinally assessed through ultrasound examinations, an association was found between exposure to NO_2 and VOCs from early pregnancy and impaired growth in several fetal parameters from week 20 onwards.

Conclusiones: Within-city variations in exposure to traffic-related air pollution have an effect on fetal growth. This effect already manifests during mid-pregnancy and seems to persist until birth.

Prólogo

Esta tesis se presenta como compendio de publicaciones, según la normativa del Programa de Doctorat en Biomedicina del Departament de Ciències Experimentals i de la Salut. Consta de un resumen, un capítulo de introducción, justificación y objetivos, otro de material y métodos (el cual incluye dos artículos publicados, uno en prensa y uno enviado para publicación), una discusión general y las conclusiones.

Los cuatro artículos originales presentados en esta tesis pertenecen al estudio INMA (INfancia y Medio Ambiente), el cual se inició en el año 2003 con el objetivo de estudiar el papel de la exposición a contaminantes ambientales a través del aire, agua y dieta en el desarrollo y salud infantil desde la etapa prenatal. Concretamente, esta tesis presenta la metodología y resultados obtenidos sobre la exposición a contaminación atmosférica en la etapa prenatal en una de las cohortes del estudio INMA: la cohorte de Sabadell. Mi participación en este proyecto comenzó en el año 2005 como becaria predoctoral, y ha consistido principalmente en el diseño de todos los muestreos de contaminación atmosférica, coordinación del trabajo de campo y participación en el mismo, obtención y análisis de datos geográficos, revisión bibliográfica, análisis estadístico de parte de los datos y redacción de los manuscritos, como segunda autora en el segundo artículo y como primera autora en los tres restantes.

Abreviaturas

BTEX: benceno, tolueno, etilbenceno, *o*-xileno y *m,p*-xileno

CIUR: crecimiento intrauterino retardado

CO: monóxido de carbono

COVs: Compuestos Orgánicos Volátiles

DBP: diámetro biparietal

HAPs: Hidrocarburos Aromáticos Policíclicos

LF: longitud del fémur

LUR: *Land-use Regression*

NO: óxido nítrico

NO_2: dióxido de nitrógeno

NOx: óxidos de nitrógeno

PA: perímetro abdominal

PC: perímetro cefálico

PEG: pequeño para edad gestacional

PFE: peso fetal estimado

PM_{10}: partículas torácicas (de diámetro $\leq$ 10 μm)

$PM_{2,5}$: partículas finas (de diámetro $\leq$ 2,5 μm)

$PM_{0,1}$: partículas ultrafinas (de diámetro $\leq$ 0,1 μm)

SIG: Sistemas de Información Geográfica

SO_2: dióxido de azufre

Índice

1. INTRODUCCIÓN

1.1. Contaminación atmosférica urbana

La contaminación atmosférica asociada al tráfico representa uno de los problemas de salud pública más importantes en las ciudades. La evidencia de efectos adversos sobre la salud de la exposición a material particulado (especialmente a partículas finas) es cada vez más consistente, no habiéndose hallado un nivel umbral por debajo del cual no existan efectos en salud (WHO, 2005). Concretamente en Europa se ha estimado que la contaminación atmosférica procedente del tráfico es responsable cada año del 3% de la mortalidad total en adultos, de 25.000 nuevos casos de bronquitis en adultos y de casi 300.000 episodios de bronquitis en niños, entre otros efectos (Künzli et al., 2000). En consecuencia, la mejora de la calidad del aire en las ciudades es un objetivo prioritario (EEA, 2006).

En la mayoría de las ciudades europeas, el tráfico rodado es la fuente más importante de emisión de óxidos de nitrógeno (NOx), monóxido de carbono (CO), BTEX (benceno, tolueno, etilbenceno y xilenos), humos negros (excepto en ciudades con uso extendido de carbón para calefacción) y partículas ultrafinas ($PM_{0,1}$). Los contaminantes primarios emitidos por tubos de escape constituyen hasta un 30% del total de las partículas finas ($PM_{2,5}$), y otros contaminantes generados por la resuspensión de polvo y los frenos de los vehículos son la fuente más importante de partículas de fracción gruesa (entre 2,5 y 10 μm de diámetro) (Krzyzanowski et al., 2005).

Las concentraciones de los contaminantes asociados al tráfico varían dentro de las ciudades, siendo más elevadas en las proximidades de las fuentes de emisión. Este contraste entre zonas de proximidad al tráfico y zonas de fondo urbano es muy pronunciado para contaminantes primarios como el óxido nítrico (NO) o las partículas ultrafinas, los cuales muestran un descenso importante en su concentración a una distancia inferior a 100 m de calles con tráfico elevado (Henderson et al., 2007). Para contaminantes como el NO_2, que tienen origen primario y secundario, el gradiente es ligeramente más suave (Gilbert et al., 2007), mientras que $PM_{2,5}$ y PM_{10} muestran ya una distribución espacial considerablemente más homogénea (Henderson et al., 2007). En los cañones urbanos, donde la dispersión de contaminantes está muy limitada por la altura de los edificios circundantes, los contaminantes atmosféricos pueden presentar niveles mucho más elevados que las zonas de fondo urbano (Krzyzanowski et al., 2005).

1.2. Evaluación de la exposición a contaminación atmosférica urbana

En epidemiología ambiental, la exposición a una sustancia presente en el medio (ya sea en el agua, suelo, aire o alimentos) se define como el contacto de dicha sustancia con la superficie del cuerpo humano. Una vez ha entrado en el cuerpo por vía oral, inhalatoria o dérmica ya no se habla de exposición sino de dosis (Nieuwenhuijsen, 2004a).

La evaluación de la exposición a contaminación atmosférica urbana no es una tarea fácil, debido a la compleja mezcla de contaminantes presentes en el aire y a la gran variabilidad temporal y espacial que presentan la mayoría de ellos, incluso a pequeña escala. Un ejemplo de lo segundo es que la variabilidad espaciotemporal de contaminantes como los NOx o las partículas ultrafinas dentro de las ciudades puede superar a la variabilidad entre ciudades (Briggs, 2000). Este hecho, junto con la creciente contribución del tráfico rodado como fuente principal de contaminación del aire en ambientes urbanos, justifica que una correcta evaluación de la exposición humana a contaminantes atmosféricos dentro de las ciudades constituya actualmente un área de investigación prioritaria (Brunekreef y Holgate, 2002).

Uno de los métodos más comunes para evaluar la exposición de la población de una ciudad a contaminación atmosférica es el uso de los datos procedentes de las redes de vigilancia de la calidad del aire. Estos datos se han usado tradicionalmente en estudios ecológicos de series temporales, pero también en estudios individuales, donde a partir de la residencia de cada sujeto del estudio (en base a su dirección postal, sección censal o código postal), se le asignan los niveles de contaminación medidos en la estación o estaciones más cercanas durante el periodo de estudio. Su principal ventaja es, aparte de su bajo coste, que proporciona información sobre la variabilidad temporal de la contaminación atmosférica, pues según el contaminante se pueden obtener hasta niveles semihorarios. Sin embargo, la variabilidad espacial suele ser bastante pobre, lo cual plantea problemas importantes de mala clasificación de la exposición.

En los últimos años, los avances producidos en los Sistemas de Información Geográfica (SIG) y técnicas estadísticas asociadas se han expandido al campo del análisis de la exposición y de la epidemiología.

Esto ha permitido el desarrollo de varios tipos de modelos que, a partir de los datos de las estaciones fijas o de mediciones propias, consiguen caracterizar la variabilidad espacial de la contaminación atmosférica a diferentes niveles geográficos, incluido el intraurbano (Jerrett et al., 2005). Más adelante se describen las características de los principales modelos de exposición.

Por último, la monitorización personal es un método directo de evaluación de la exposición que, debido a su mayor coste, normalmente se utiliza sólo en estudios pequeños o en una submuestra de la población de estudio (Nieuwenhuijsen, 2004b). Para ello se emplean captadores personales que los individuos del estudio llevan durante un periodo que suele oscilar entre las 48 horas y los 7 días, al final del cual rellenan un cuestionario de tiempo-actividad. A menudo las medidas personales se complementan con medidas simultáneas en el exterior e interior de la vivienda (Brown et al., 2009; Choi et al., 2008a). Este método aporta información muy detallada, aunque durante un periodo de tiempo muy corto. Por tanto, es necesario realizar un correcto diseño para captar lo mejor posible la variabilidad estacional de la contaminación atmosférica urbana y de los patrones de tiempo-actividad que determinan la exposición individual.

a) Modelos de exposición

Existen varios tipos de modelos, cuya complejidad depende en parte de la metodología estadística, de la necesidad de medidas de contaminación atmosférica (niveles de inmisión y/o emisión) y de la demanda de variables geográficas y otras covariables. Los más utilizados son los siguientes:

Modelos de proximidad

Son los modelos más sencillos, ya que únicamente miden la proximidad de un individuo (en base a la localización de su vivienda, trabajo, colegio, etc.) a una fuente de contaminación, como por ejemplo una calle con tráfico elevado. Varios estudios han hallado que vivir cerca de calles con tráfico elevado es un factor de riesgo de padecer asma, sibilancias y menor función pulmonar en niños (Gauderman et al., 2005, 2007; McConnell et al., 2006). Sin embargo, la simplicidad de estos modelos aconseja su uso para análisis exploratorios previos a otros más sofisticados, ya que son poco válidos en ambientes heterogéneos como las ciudades, donde el efecto pantalla de las edificaciones, junto con factores meteorológicos y topográficos, determinan patrones de dispersión más complejos que la dispersión isotrópica (es decir, igual hacia todas las direcciones) asumida en esta metodología (Jerrett et al., 2005).

Métodos de interpolación espacial

Son técnicas geoestadísticas mediante las cuales se crean mapas de contaminación atmosférica a partir de una serie de puntos de medida. El método más conocido es el *kriging*, el cual predice la concentración de un contaminante en un punto determinado a partir del promedio ponderado de las concentraciones medidas en los puntos más cercanos. Su principal ventaja es que no necesita variables geográficas adicionales (a excepción de una de sus variantes conocida como *cokriging*, que sí usa covariables) y que permite cuantificar su grado de fiabilidad en forma de error estándar de los valores predichos. Sin embargo, la aplicación del *kriging* en ambientes urbanos está limitada, ya que tiende a suavizar la variabilidad de las concentraciones existente a distancias cortas dentro de una ciudad. Por ello, normalmente se aplica para estimar niveles de fondo a escalas geográficas mayores (Briggs, 2005; Leem et al., 2006).

Modelos de dispersión

Son modelos dinámicos de procesos de dispersión que se basan en la pluma de Gauss y que requieren de datos de emisión de contaminantes, meteorológicos y topográficos, mientras que usan los datos de inmisión de las estaciones fijas para su calibración. Su principal ventaja es que capturan no sólo la variabilidad espacial sino también la temporal, pero la alta demanda de datos de emisión de contaminantes y meteorológicos, tanto en cantidad como en calidad, hace que sean un método costoso y a menudo inviable, aunque se han utilizado a nivel intraurbano en estudios epidemiológicos sobre efectos en salud (Nyberg et al., 2000; Rosenlund et al., 2007).

Modelos Land-use Regression o mapas de regresión

Los modelos *Land-use Regression* (LUR) o mapas de regresión se han convertido en una herramienta muy prometedora para evaluar la variabilidad intraurbana de la contaminación atmosférica debido a su alta resolución espacial (Hoek et al., 2008; Marshall et al., 2008), habiéndose usado hasta la fecha en ciudades de Europa y Norteamérica para contaminantes con gran variabilidad espacial como los NOx (Brauer et al., 2003; Briggs et al., 1997; Henderson et al., 2007; Hochadel et al., 2006; Jerrett et al., 2007; Madsen et al., 2007; Morgenstern et al., 2007; Ross et al., 2006; Sahsuvaroglu et al., 2006), pero también para otros contaminantes menos heterogéneos como $PM_{2,5}$ (Brauer et al., 2003; Hochadel et al., 2006; Moore et al., 2007;

Morgenstern et al., 2007). Desarrollados por primera vez por Briggs et al. (1997), utilizan los niveles de contaminación medidos en varios puntos de muestreo, junto con una serie de variables predictoras alrededor de esos puntos obtenidas mediante SIG (como datos de tráfico, usos del suelo, densidad de población, altitud, etc.) para construir modelos de regresión. Estos modelos posteriormente se aplicarán para predecir los niveles en puntos no muestreados, los cuales deben disponer de las mismas variables predictoras. Típicamente los datos de contaminación proceden de varias campañas de medidas llevadas a cabo durante una o dos semanas a lo largo de varios meses, de manera que la media de las campañas representa con bastante fiabilidad la media anual para cada punto de muestreo (Lebret et al., 2000). Los modelos LUR suelen presentar coeficientes de determinación elevados (R^2 > 0.65-0.70) con solamente tres o cuatro variables predictoras, sobre todo en el caso de contaminantes con gran variabilidad a pequeña escala como los NOx. Además son sensibles a la distinción entre contaminantes primarios y secundarios, como muestran algunos estudios cuyos modelos para NO incluyen variables predictoras en distancias más cortas a las fuentes de emisión que los modelos para NO_2 (Henderson et al., 2007) (Figura 1). El principal inconveniente de este método es su especificidad ligada al área de estudio (es decir, su aplicabilidad a otros ambientes urbanos es limitada) y que requiere variables en formato SIG de calidad, así como un número de puntos de muestreo suficiente y bien repartido, especialmente entre las zonas de tráfico que son las que presentan mayor variabilidad espacial (Jerret et al., 2005). Una opción interesante cuando el área de estudio combina zonas urbanas, semiurbanas y rurales con diferente calidad en las variables geográficas disponibles es la combinación de modelos LUR + *kriging* (Freire et al., 2009; Iñiguez et al., 2009).

Dado que los mapas de regresión reflejan con precisión la variabilidad espacial pero no la temporal (pues representan medias anuales), en estudios epidemiológicos donde interese definir la exposición durante un periodo de tiempo concreto (como puede ser el embarazo) los modelos LUR se suelen ajustar temporalmente usando los niveles diarios de contaminación atmosférica medidos en una estación fija durante el periodo de interés (Slama et al., 2008).

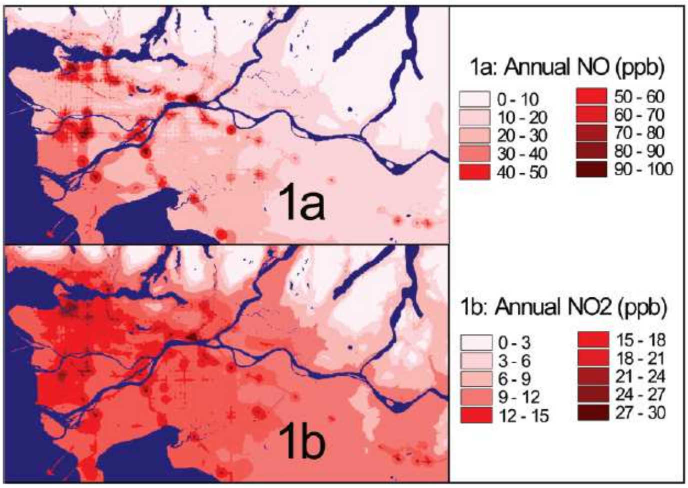


Figura 1. Mapas de regresión de la distribución espacial de NO (mapa 1a) y NO_2 (mapa 1b) en Vancouver, Canadá.

1.3. Vulnerabilidad a la contaminación atmosférica durante la etapa prenatal

Los primeros años de vida son una etapa de especial susceptibilidad a los efectos de la contaminación atmosférica, ya que el completo desarrollo de los pulmones y del sistema inmunitario no finaliza hasta pasados varios años. Además, los niños respiran más aire que los adultos en proporción a su masa corporal y tienen patrones de comportamiento que favorecen una mayor exposición a la contaminación atmosférica, como pasar más tiempo en el exterior realizando actividades que incrementan el ritmo respiratorio (Olsen, 2000; Schwartz, 2004). Existe suficiente evidencia científica para afirmar que la exposición a contaminación atmosférica en niños provoca efectos agudos, como descenso en la función pulmonar y exacerbación de neumonía y de episodios de asma (Brunekreef y Sunyer, 2003; Nicolai, 1999). También hay indicios crecientes de una asociación con efectos crónicos, como la broquitis crónica, asma, alergias, sibilancias o menor función pulmonar (Brauer et al., 2002; Gauderman et al., 2007; Jerrett et al., 2008).

Quizá menos previsibles han sido los resultados de estudios que, en la última década, vienen indicando que la etapa prenatal también parece ser un periodo de especial vulnerabilidad a la contaminación atmosférica. La mayoría de estos estudios han usado como indicadores de contaminación atmosférica aquellos contaminantes que se miden de manera rutinaria en las Redes de Vigilancia de la Calidad del Aire (principalmente partículas, SO_2, NOx, CO y ozono), así como varios indicadores de salud perinatal (crecimiento intrauterino retardado, prematuridad, mortalidad intrauterina, neonatal y postneonatal y malformaciones congénitas). Varias revisiones publicadas entre 2004 y 2005 concluyeron que había ciertos indicios de pequeños efectos de la exposición a contaminación atmosférica durante el embarazo sobre el crecimiento fetal (medido como pequeño para edad gestacional o como bajo peso al nacer) y sobre los nacimientos prematuros. Para otros efectos en salud, como las malformaciones congénitas, no existen indicios de un efecto o bien la disponibilidad de datos es muy limitada (Lacasaña et al., 2005; Glinianaia et al., 2004; Šrám et al., 2005; Maisonet et al., 2004). Desde entonces se ha publicado un considerable número de estudios que han tratado de cubrir las limitaciones identificadas en las revisiones anteriores, como por ejemplo mejorar la evaluación de la exposición mediante modelos basados en SIG (Brauer et al., 2008; Leem et al., 2006; Slama et al., 2007) o monitorización personal (Choi et al., 2008b; Jedrychowski et al., 2004; Perera et al., 2003). A pesar de ello, sigue habiendo una gran heterogeneidad entre los resultados publicados, la cual puede deberse en parte a diferencias en la evaluación de la exposición, en la definición de los efectos perinatales y en el ajuste por variables confusoras (Parker y Woodruff, 2008).

a) Mecanismos biológicos

Se han propuesto varios mecanismos biológicos a través de los cuales la contaminación atmosférica podría afectar al crecimiento fetal (Figura 2). Entre ellos se incluyen el estrés oxidativo e inflamación, cambios en la coagulación sanguínea, disrupción endocrina y alteraciones en el transporte de nutrientes y oxígeno a través de la placenta. Estos mecanismos podrían o no actuar independientemente (Kannan et al. 2006; Slama et al. 2008).

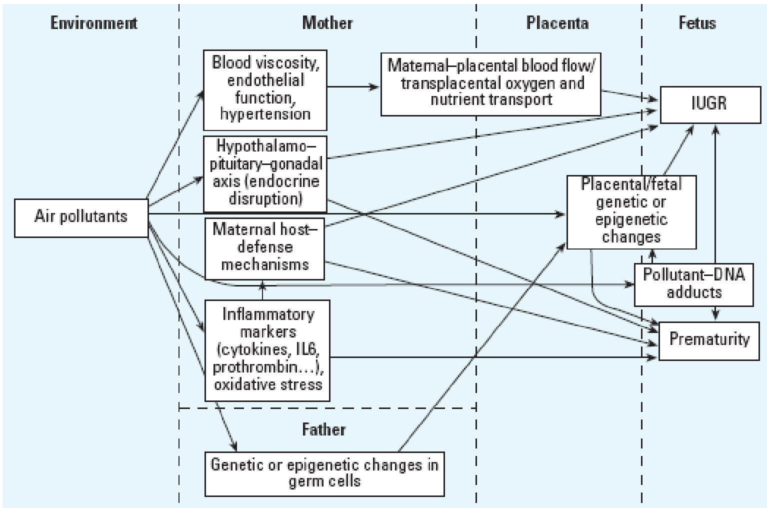


Figura 2. Posibles mecanismos biológicos a través de los cuales la contaminación atmosférica podría influir en el crecimiento intrauterino retardado o en el nacimiento prematuro (Slama et al., 2008).

Para las partículas ultrafinas, el estrés oxidativo e inflamación sea quizá el mecanismo de actuación más probable (Sioutas et al. 2005). Otros estudios experimentales y en humanos sugieren que los HAPs pueden atravesar la placenta y afectar al crecimiento fetal a través de la formación de aductos de ADN en la placenta y en los órganos del feto (Dejmek et al., 2000; Šrám et al. 1999). En cualquier caso, se requieren de más estudios experimentales que ayuden a identificar los mecanismos más relevantes.

b) Implicaciones en la edad adulta: teoría del origen fetal de las enfermedades

La teoría del origen fetal de las enfermedades surge a raíz de estudios epidemiológicos, clínicos y experimentales realizados en las últimas décadas, los cuales sugieren que los eventos implicados en el desarrollo fetal normal tienen efectos a largo plazo e influyen en la salud durante la vida adulta (Silveira et al., 2007). Esta teoría se originó a raíz de los estudios de Barker et al. (1989a, 1989b, 1993), los cuales hallaron una asociación entre bajo peso al nacer y mayor riesgo de enfermedades cardiovasculares en la edad adulta, como diabetes mellitus tipo 2, dislipidemias, hipertensión arterial o enfermedad isquémica del corazón. Estudios más recientes también sugieren que el patrón de crecimiento fetal influye en el patrón de crecimiento durante la etapa infantil, el cual a su vez es un factor determinante en el desarrollo posterior de enfermedades metabólicas y cardiovasculares (Bettiol et al., 2007; Eriksson et al., 2001).

Dentro de los factores que influyen en la programación fetal, los nutricionales han sido los más ampliamente estudiados, pudiendo inducir efectos permanentes en el crecimiento, metabolismo, neurodesarrollo y procesos patológicos (Sinclair et al., 2007). Sin embargo, y puesto que la placenta no protege al feto de la exposición a contaminantes ambientales presentes en la sangre materna, existe un interés creciente en evaluar la exposición a sustancias tóxicas como factores igualmente influyentes en el desarrollo fetal (Olsen, 2000).

1.4. Aspectos metodológicos clave en la investigación sobre contaminación atmosférica y efectos reproductivos

La combinación de la epidemiología perinatal y la epidemiología de la contaminación atmosférica plantea una serie de retos para ambas disciplinas. Por ello, desde el año 2007 se han llevado a cabo varias reuniones de trabajo de grupos de expertos, en las que se han identificado una serie de aspectos metodológicos clave para avanzar en esta relativamente joven línea de investigación (Ritz y Wilhelm, 2008; Slama et al., 2008; Woodruff et al., 2009). Algunos de estos aspectos metodológicos son:

a) Identificación de los contaminantes de interés

La mayoría de estudios se han centrado en evaluar la exposición a los contaminantes medidos de manera rutinaria por las redes de vigilancia de control de la calidad del aire: partículas (PM_{10} y $PM_{2,5}$), SO_2, NOx, CO y ozono, pero sus resultados no coinciden en la identificación de asociaciones más consistentes para alguno de ellos en concreto. Algunos de estos estudios han usado modelos multicontaminante con el objetivo de separar el efecto individual de cada uno, pero su uso está limitado cuando los contaminantes evaluados proceden de una fuente común (por ejemplo, CO y NOx procedentes del tráfico o PM y SO_2 de emisiones industriales) (Kim et al., 2007). La ausencia de hipótesis específicas sobre los mecanismos biológicos implicados para la mayoría de los contaminantes (con la excepción quizá de los HAPs y las $PM_{0,1}$) representa una complicación añadida. En cualquier caso, los resultados hallados para CO, NOx, PM y HAPs, así como para indicadores indirectos de contaminación asociada al tráfico (como la distancia de la residencia a calles con tráfico elevado) apuntan con bastante claridad a una causalidad entre las sustancias tóxicas emitidas por los vehículos y los efectos perinatales observados (Bell et al., 2007; Brauer et al., 2008; Choi et al., 2006, 2008b; Darrow et al., 2009; Dejmek et al., 1999; Gouveia et al., 2003; Ha et al., 2001; Jedrychowski et al., 2004; Leem et al., 2006; Liu et al., 2003, 2007; Maisonet et al., 2001; Mannes et al., 2005; Parker et al., 2005; Rich et al., 2009; Ritz et al., 1999, 2007; Salam et al., 2005; Slama et al., 2007; Wilhelm et al., 2003, 2005). En esta línea, se propone la realización de más estudios que evalúen contaminantes con hipótesis específicas de mecanismos de actuación (como HAPs y $PM_{0,1}$) o que puedan ser por sí mismos tóxicos de interés emitidos por los vehículos (como metales, HAPs o COVs). También sería de interés determinar la composición química del material particulado, ya que presenta variaciones temporales y geográficas que podrían modificar su toxicidad (Hopke et al., 2006; Viana et al., 2008).

b) Definición de los efectos perinatales más relevantes

La mayoría de estudios realizados se han centrado en el crecimiento fetal y en los nacimientos prematuros (definidos como los ocurridos antes de completar la semana 37 de gestación). El término crecimiento intrauterino retardado (CIUR) indica un desarrollo del feto por debajo de su potencial genético de crecimiento, debido a un proceso patológico ocurrido durante la etapa prenatal. Ante la falta de una definición clínica del CIUR, se han utilizado diferentes indicadores del mismo: peso al nacer como variable continua, bajo peso al nacer (inferior a 2.500 g), muy bajo peso al nacer (inferior a 1.500 g) o pequeño para edad gestacional (PEG), definido como peso inferior al percentil 10 para una edad gestacional determinada. El uso de PEG ha sido criticado por ser un concepto puramente descriptivo de una población en particular, de manera que un recién nacido puede ser pequeño con respecto a su población de referencia a pesar de haber alcanzado su crecimiento potencial genéticamente determinado. Por este motivo se ha sugerido que el interés debería centrarse en la desviación del potencial de crecimiento genéticamente determinado y no en el peso al nacer en términos absolutos (Olsen y Basso, 2005). Otro problema común que surge en los estudios sobre contaminación atmosférica y efectos perinatales es cómo diferenciar los efectos derivados del CIUR de los efectos derivados de la prematuridad.

Como los estudios basados en certificados de nacimiento a menudo carecen de muchas covariables que son necesarias para dar respuesta a los problemas mencionados, se plantea la necesidad de disponer de estudios de cohortes que puedan examinar definiciones alternativas de efectos perinatales, como por ejemplo la evaluación del crecimiento fetal a través de ecografías durante el embarazo.

c) Identificación de los periodos de mayor susceptibilidad durante el embarazo

El embarazo constituye un intervalo de tiempo claramente definido y relativamente corto, lo cual permite el estudio de posibles ventanas temporales (como meses o trimestres) en las que el feto podría ser más susceptible a los efectos de un factor ambiental con importante variación estacional como es la contaminación atmosférica. Sin embargo, la identificación de la/s ventana/s de exposición más críticas (si es que existen) es complicada por varias razones: (a) diferentes contaminantes podrían actuar durante diferentes etapas del embarazo; (b) los contaminantes comúnmente evaluados (aquellos medidos en las redes de vigilancia) podrían actuar solamente como indicadores de los verdaderos contaminantes que causan los efectos perinatales; y (c) la mezcla de contaminantes atmosféricos presenta variación geográfica y temporal. Otra dificultad añadida es la falta de suficiente información toxicológica que pudiese orientar en la selección de periodos de mayor vulnerabilidad durante el embarazo para la mayoría de efectos perinatales adversos (con la excepción tal vez de determinadas malformaciones congénitas, cuyo periodo crítico correspondería con el de la formación del órgano afectado).

Los primeros meses de embarazo podrían ser una época de mayor susceptibilidad, coincidiendo con la adherencia de la placenta y su desarrollo. Alternativamente, la susceptibilidad podría aumentar hacia el final del embarazo, cuando la velocidad de crecimiento del feto es mayor (Figura 3). La mayoría de estudios publicados han utilizado el trimestre como ventana temporal de estudio, pero de nuevo las diferencias metodológicas dificultan la comparación de unos resultados que, por otra parte, no han podido identificar claramente cuáles son los periodos de mayor asociación. Además, al igual que ocurre con los modelos multicontaminante, los modelos multitrimestre están limitados por la alta correlación que suele haber entre las exposiciones trimestrales, aunque se han propuesto algunas técnicas estadísticas alternativas para corregir este problema (Bell et al., 2007).

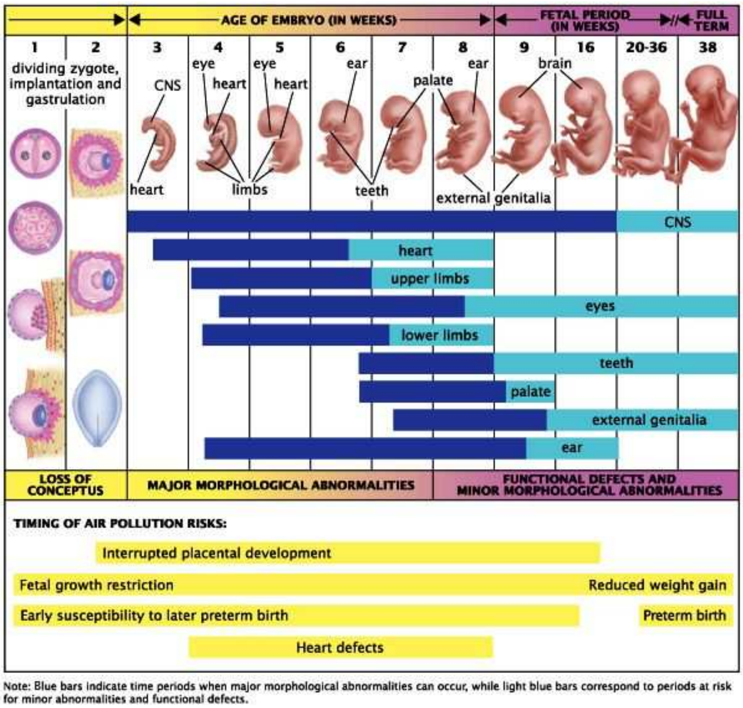


Figura 3. Desarrollo fetal y ventanas de riesgo para los efectos de la exposición a contaminación atmosférica (adaptado de Moore, 1973).

d) Mejora en la evaluación de la exposición

Debido a la alta heterogeneidad espacial de la mayoría de contaminantes emitidos por el tráfico, el uso de técnicas de modelización basadas en SIG, y más concretamente los modelos LUR, son una opción muy prometedora para estimar exposiciones individuales en estudios sobre efectos perinatales realizados en ambientes urbanos (Brauer et al., 2008; Slama et al., 2007).

Puesto que la asignación individual de los niveles de exposición a contaminación atmosférica normalmente se basa en la localización de la vivienda, un aspecto fundamental para mejorar la clasificación de la exposición es conocer los patrones de tiempo-actividad durante el embarazo. Por ejemplo, los cambios de residencia son relativamente frecuentes, habiéndose reportado porcentajes de cambio de domicilio de entre un 12% (Fell et al., 2004) y un 35% (Brauer et al., 2008) de la población estudiada. Si estos cambios de residencia no se tienen en cuenta es muy probable incurrir en una mala clasificación de la exposición. Por tanto, variables de tiempo-actividad durante el embarazo como cambios de residencia, trabajar o no fuera de casa o el tiempo pasado en el interior de la casa, en el trabajo y en desplazamientos, entre otras, ofrecen la oportunidad de realizar análisis de sensibilidad con el objetivo de evaluar el impacto de la mala clasificación de la exposición sobre las asociaciones halladas (Fell et al., 2004; Nethery et al., 2009).

e) Variables confusoras y modificadoras de efecto

Los estudios que han analizado los efectos de la contaminación atmosférica en el crecimiento fetal con datos procedentes de certificados de nacimiento (la mayoría de ellos en EE.UU.) presentan la ventaja de tener un tamaño muestral grande pero su principal inconveniente es la disponibilidad limitada de covariables que podrían confundir o modificar los efectos hallados. Mientras que variables como estacionalidad (medida en el momento del nacimiento), edad, nivel educativo, raza y paridad sí suelen estar disponibles en las bases de datos de certificados de nacimiento, a menudo faltan otras variables como tabaquismo activo y pasivo, consumo de alcohol y medicamentos, estatus nutricional antes y durante el embarazo, medidas antropométricas de la madre y el padre, ocupación u otras variables socioeconómicas que podrían estar confundiendo las asociaciones (Strickland et al., 2009). Por este motivo, se identifica la necesidad de llevar a cabo estudios individuales que compensen un menor tamaño muestral con la recogida detallada de datos de exposición, covariables y muestras biológicas.

1.5. El estudio INMA (Infancia y Medio Ambiente)

El estudio INMA es un proyecto que se inicia en 2003 en forma de red temática de investigación cooperativa, con la financiación del Instituto de Salud Carlos III (Ministerio de Sanidad). Su objetivo principal es estudiar el papel de la exposición a contaminantes ambientales a través del aire, del agua y de la dieta en el desarrollo y salud infantil desde la etapa prenatal (Ramón et al., 2005; Ribas-Fitó et al., 2006). La creación de esta red responde a la llamada de la Organización Mundial de la Salud (OMS), quien considerando la vulnerabilidad de los niños a los factores ambientales declaró prioritaria esta línea de investigación, así como a la estrategia europea SCALE (*Science, Children, Awareness, Legal instrument & Evaluation*), formulada para reducir las enfermedades relacionadas con exposiciones ambientales, con especial atención a la infancia (http://ec.europa.eu/environment/health/strategy.htm).

Coordinado desde el Centre de Recerca en Epidemiologia Ambiental de Barcelona, el estudio INMA está formado por siete cohortes, tres de ellas preexistentes (cohortes de Ribera d'Ebre, Menorca y Granada) y cuatro de novo (cohortes de Valencia, Sabadell, Asturias y País Vasco). Las siete cohortes en total realizan un seguimiento a casi 4.000 mujeres embarazadas y a sus hijos.

Los criterios de inclusión en las cohortes de novo fueron: (a) pertenecer al área de estudio específica de cada cohorte; (b) tener al menos 16 años de edad; (c) tener un embarazo único; (d) acudir a la primera visita prenatal (entre las semanas 10 y 13 de embarazo) al centro de salud u hospital público del área de estudio; (e) no haber seguido ningún programa de reproducción asistida; (f) tener intención de dar a luz en el hospital de referencia y (g) no tener problemas de comunicación (Ribas-Fitó et al., 2006).

El estudio INMA ha estrenado recientemente una nueva página web (www.proyectoinma.org) que pretende ser una herramienta de divulgación científica, comunicación de resultados e interacción con las familias que participan en el proyecto.

a) La cohorte de Sabadell

La ciudad de Sabadell está situada en la comarca del Vallès Occidental, en la provincia de Barcelona. El municipio tiene un área de 38 km^2 dividida en siete distritos administrativos (Figura 4), una altura media de 190 m y una población de aproximadamente 200.000 habitantes. Durante el siglo XIX y comienzos del XX experimentó un fuerte impulso industrial, llegando a convertirse junto con la vecina Terrassa en la ciudad de la industria textil por excelencia. Sin embargo, la mayor parte de la industria acabó desmantelándose a partir de la década de los 70 y hoy en día Sabadell es principalmente una ciudad de servicios con una actividad industrial residual, sin exposiciones específicas, representando un ambiente promedio de los ciudadanos residentes en el sur de Europa.

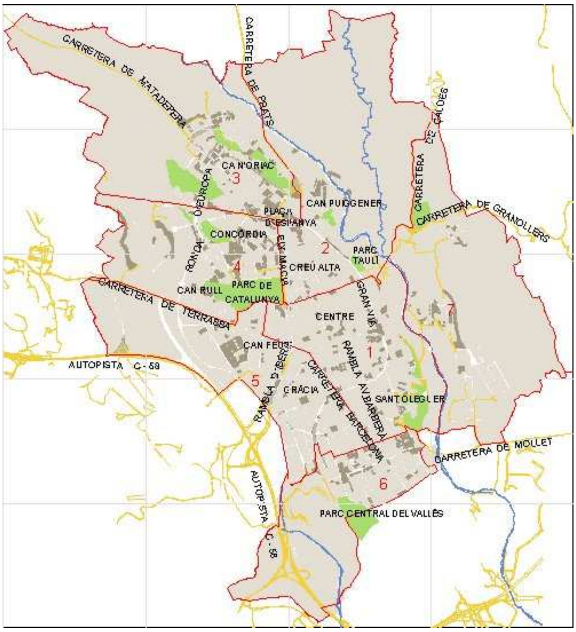


Figura 4. Plano de Sabadell

La cohorte de Sabadell se formó entre junio de 2004 y julio de 2006, periodo en el cual se reclutaron 657 mujeres embarazadas que acudieron al Centro de Atención Primaria (CAP) Sant Fèlix con intención de dar a luz en el Hospital de Sabadell y que cumplían el resto de criterios de inclusión. Dado que el CAP Sant Fèlix es el único en Sabadell especializado en control prenatal y que el Hospital de Sabadell es el hospital de referencia para el parto, los dos centros cubren aproximadamente el seguimiento del 85% de las mujeres embarazadas de la ciudad. Por razones logísticas, 49 de las mujeres incluidas en la cohorte no residían en Sabadell, sino en dos poblaciones colindantes (Barberà del Vallès y Sant Quirze del Vallès) que comparten el mismo CAP y hospital de referencia.

A diferencia del resto de cohortes del estudio INMA, la cohorte de Sabadell es exclusivamente urbana y por tanto representa una oportunidad única para estudiar los posibles efectos en salud de las diferencias en los niveles de la contaminación atmosférica a pequeña escala.

2. JUSTIFICACIÓN

El crecimiento fetal es un importante indicador no sólo de la salud postnatal sino que también puede influir en el estado de salud en la edad adulta. Por otro lado, la contaminación atmosférica, cuya exposición es de carácter ubicuo, constituye un problema de salud pública en los entornos urbanos. Ambos factores han contribuido al creciente interés en evaluar los efectos de la contaminación atmosférica urbana sobre el crecimiento fetal. Esta línea de investigación se ha desarrollado mayoritariamente a partir de estudios basados en datos procedentes de certificados de nacimiento y de estaciones fijas de medición de la calidad del aire. Sin embargo, existen aún pocos estudios de cohortes en este campo, los cuales permitirían avanzar en algunas cuestiones relevantes que aún quedan por resolver, como por ejemplo mejorar la evaluación de la exposición, identificar posibles ventanas de mayor susceptibilidad al efecto de los contaminantes durante el embarazo o explorar el papel de posibles variables modificadoras del efecto.

Esta tesis, basada en una cohorte urbana con niveles de contaminación equiparables a otras ciudades del sur de Europa, pretende dar respuesta a algunas de estas cuestiones. Para ello se han utilizado modelos de exposición que reflejan la variabilidad a pequeña escala de los contaminantes atmosféricos, así como medidas personales y domiciliarias. En cuanto al crecimiento fetal, no sólo se ha medido al nacimiento sino también mediante ecografías durante el embarazo.

3. OBJETIVOS

Objetivo general

- Estudiar la relación entre exposición a contaminación atmosférica urbana durante el embarazo y el crecimiento fetal.

Objetivos específicos

1. Desarrollar un modelo de exposición a NO_2 y COVs durante el embarazo, basado en la variabilidad geográfica de estos contaminantes en la ciudad de Sabadell.
2. Describir la exposición personal y los niveles NO_2 en el interior y exterior del domicilio en mujeres embarazadas de la cohorte INMA-Sabadell y evaluar sus determinantes.
3. Analizar la asociación entre exposición prenatal a NO_2 y COVs, usados como marcadores de contaminación asociada al tráfico, y el crecimiento fetal medido como peso al nacer.
4. Analizar la asociación entre exposición prenatal a NO_2 y COVs, usados como marcadores de contaminación asociada al tráfico, y el crecimiento fetal medido durante el embarazo a través de ecografías.

4. MÉTODOS Y RESULTADOS

ARTÍCULO 1: Estimation of outdoor NOx, NO_2 and BTEX exposure in a cohort of pregnant women using Land Use Regression modelling

Environmental Science & Technology 2008; 42(3): 815-821

Inmaculada Aguilera[1,2], Jordi Sunyer[1,2], Rosalía Fernández-Patier[3], Gerard Hoek[4], Amelia Aguirre-Alfaro[3], Kees Meliefste[4], M. Teresa Bomboi-Mingarro[3], Mark J Nieuwenhuijsen[1], Dolores Herce-Garraleta[3] and Bert Brunekreef[4,5]

[1] Centre for Research in Environmental Epidemiology, Institut Municipal d'Investigació Mèdica, Barcelona, Spain.

[2] Pompeu Fabra University, Barcelona, Spain.

[3] National Centre for Environmental Health, Carlos III Health Institute, Majadahonda (Madrid), Spain.

[4] Institute for Risk Assessment Sciences, Utrecht University, The Netherlands.

[5] Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands.

Corresponding author:

Inmaculada Aguilera

E-mail: iaguilera@imim.es

Phone: +34 933160643 / Fax: +34 933160635

Aguilera I, Sunyer J, Fernández-Patier R, Hoek G, Aguirre-Alfaro A, Meliefste K, *et al*. *Estimation of outdoor NO(x), NO(2), and BTEX exposure in a cohort of pregnant women using land use regression modeling.* Environ Sci Technol. 2008 Feb 1;42(3):815-21.

ABSTRACT

Land use regression (LUR) has been successfully used to assess the intraurban variability of air pollution. In the INMA (Environment and Childhood) Study, ambient nitrogen oxides (NOx and NO_2) and aromatic hydrocarbons (BTEX) were measured at 57 sampling sites in Sabadell (North-east Spain). Multiple regression models were developed to predict residential outdoor concentrations in a cohort of pregnant women (n=657), using geographic data as predictor variables. The models accounted for 68% and 69% of the variance in NOx and NO_2 levels, respectively, with four predictor variables (altitude, land coverage and two road length indicators). These percentages of explained variability could be further improved by replacing the two road length indicators with an ordinal indicator (road type). To our knowledge, this is the first study using LUR to assess the intraurban variability of BTEX in Europe, with a model including altitude and source-proximity variables that explained 74% of the variance in BTEX levels. These models will be used to study the association between prenatal exposure to air pollution and adverse pregnancy outcomes and early childhood effects in the cohort.

INTRODUCTION

Assessing exposure to traffic-related air pollution within cities in health studies has been identified as a research priority (*1*). GIS-based methods are increasingly used for mapping air pollution and assessing individual exposure to specific pollutants.

Several techniques have been applied to air pollution mapping at different geographic scales. Interpolation methods such as kriging have been used mainly at the regional and national scale (*2, 3*). However, kriging presents some difficulties when it is used at the intraurban scale because of the marked variation in air pollutant concentrations at very short distances (*4*). Dispersion models can potentially incorporate both spatial and temporal variation without the need for additional air pollution monitoring, but their high and costly data demand is an important disadvantage (*5, 6*).

Land use regression (LUR) has appeared as a promising alternative technique for air pollution mapping within cities to obtain estimates for epidemiological studies. This method uses geographic, traffic and monitoring data from several locations to build regression models which can be used to predict air pollution concentrations at unmeasured areas where the same geographic and traffic variables are available (*6*). It has been successfully used in Europe and North America to model the intraurban variability of nitrogen dioxide (NO_2) (*5, 7-9*) and to assess the differences in the spatial patterns between primary and secondary pollutants (*10*). The variability of fine particulate matter ($PM_{2.5}$) has been assessed in larger areas (*7, 11, 12*) since it is a more regionally dispersed pollutant.

There is emerging evidence that prenatal exposure to air pollution is associated with elevated risk of adverse pregnancy outcomes and childhood health effects (*13, 14*). However, the strength of the evidence differs between air pollutants and outcomes, and priorities for future research are to improve prenatal air pollution exposure assessment (*13, 15*) and to identify the most vulnerable period of exposure in pregnancy (*15*).

The INMA (Environment and Childhood) study is a research network that includes several birth cohorts in Spain. The general aim of the project is to study the impact of the most important environmental pollutants in air, water and diet on children's development and health (*16*). Here we aim to assess the prenatal exposure to urban air pollution, specifically nitrogen oxides (NOx and NO_2) and aromatic hydrocarbons (BTEX: benzene, toluene, ethylbenzene, *m/p*-xylene and *o*-xylene), at the residential addresses in the cohort of Sabadell (North-east Spain). Nitrogen oxides (NO + NO_2) are emitted mostly in the form of NO, whereas NO_2 is formed mainly from the oxidation of NO. This process makes NOx a more specific indicator of vehicle emissions than NO_2 alone. BTEX are some of the most common air toxics found in urban settings but there are limited studies assessing BTEX exposure during susceptible periods such as pregnancy.

METHODS

Study area

Sabadell is situated at 41º32'53"N, 2º6'33"E, in the metropolitan area of Barcelona. The city has an area of 38 km^2, an altitude of 190 meters and a population of nearly 200,000 in 2003. The municipal area is composed of a main continuous urban area and two small districts separated from the main area by a river and agricultural lands. In the past decades the city was one of the most important areas of the textile industry in Spain, but now Sabadell is mainly a city of services with a residual industrial activity located along the river basin.

Study population

The study population consists of pregnant women who visit the public health centre of Sabadell in the 12th week of pregnancy and fulfill the inclusion criteria of the study (*16*). All eligible women were interviewed and received written and oral information about the study. A total of 657 women were recruited between June 2004 and July 2006.

Monitoring site locations

Criteria used for the selection of the monitoring sites were geographic distribution, population density and traffic density. First, a 500-meter grid was overlaid onto a city map. The city centre was an area of particular interest because of its higher road density and economic activity, so the grid size was reduced to 250 meters in this district. Then, one sampling site was selected in each grid cell taking into account the road distribution in the cell. Eleven sampling sites were located at points that were difficult to be clearly defined as traffic sites, so these sites were moved closer to major and medium roads.

A total of 57 sites (29 urban background and 28 traffic sites) were selected to represent the gradient of exposure in the study population and the small-area variation in air pollution levels (see Figure S1, Supporting Information). Background sites were situated more than 50 meters from major and secondary roads, according to the local classification of the streets. Traffic sites had no other air pollution sources than nearby traffic (*11*).

Air pollution measurements

Air pollution measurements were carried out from April 2005 to March 2006, in a total of four sampling periods of one week. For budgetary reasons we could not measure the three pollutants in all periods. As a result, BTEX was measured during three periods (June 2005, October 2005 and March 2006), NOx was measured during two periods (April and June 2005) and NO_2 during four periods (April 2005, June 2005, October 2005 and March 2006). Measurements at the 57 sites were performed simultaneously in all the sampling periods to avoid the influence of temporal variation.

Because this study was funded by two projects with different air pollution protocols, two different passive samplers were used to measure NO_2. Ogawa™ samplers were used in the 1st and 2nd periods to measure NOx and NO_2, while Radiello® monitors were used in the 1st, 3rd and 4th periods to measure NO_2 and BTEX (see Table S1, Supporting Information). To compare the methods and be able to make adjustments, NO_2 was measured with both samplers in the 1st period.

The passive samplers were deployed at a height of 2.5 meters, on lampposts and traffic signs. After sample collection, Ogawa™ and Radiello® samplers for NOx and NO_2 analysis were stored at 4ºC. NOx and NO_2 in Ogawa™ samplers were determined following the Ogawa Protocol (Ogawa & Co V3.98, USA, Inc) by adding a reagent to the reactive filter and measuring the developed color by spectrophotometry at 540 nm. NO_2 in Radiello® samplers was adsorbed in a cartridge coated with triethanolamine and then determined by spectrophotometry at 540 nm. NOx and NO_2 concentrations of both samplers were corrected for temperature and relative humidity during the exposure period. Radiello® samplers for BTEX determination were stored at room temperature. BTEX were adsorbed in a graphitised charcoal cylinder and then recovered by thermal desorption. Analysis was performed by capillary gas chromatography and flame ionization detection. The separation of BTEX was performed on a 60 m × 0.32 mm × 1 μm J&W capillary column (J&W DB-1) with helium as carrier gas. The GC oven was maintained at 45 ºC for 2 minutes, then was increased to 150 ºC at a rate of 4 ºC min^{-1} during 10 min.

Forty-eight additional samplers (40 for NOx/NO_2 and 8 for BTEX) were used to collect duplicates (only for NOx/NO_2), laboratory blanks, transport blanks and field blanks. The average field blank value was subtracted from all results and the coefficient of variation (CV) was estimated to document the reproducibility of the NO and NO_2 measurements.

Since the most polluted areas in an urban setting tend to be the same throughout the year (*17*), the NO_2, NOx and BTEX concentrations used in this analysis were the average of the four, two and three measurement campaigns, respectively.

Geographic data

Geographic variables were stored and derived using ArcGIS 9.1 (ESRI, Redlands, California) and were classified into five categories: (1) land use (focusing on industrial and urban land cover); (2)

topography (elevation of the sampling site); (3) population density; (4) roads (road type, length of different road types, distance to nearest major and secondary road); and (5) distance to local sources of BTEX (parking lots, petrol stations and vehicle repair shops).

Land use data were obtained from CORINE Land Cover 2000 (European Environment Agency, Copenhagen), a database that provides information on vegetation and land use on a 100-m grid at EU level. Since our study area is mainly urban, only 9 of the 44 existing land cover classes were included in the area. For the purpose of this study we simplified these 9 classes in 3 different areas: urban, industrial and agricultural/semi-natural.

The remaining four groups of geographic variables were obtained from the City Council of Sabadell. Traffic intensity is not counted in the city on a regular basis but only during short periods for specific purposes. For this reason, the streets were classified in three categories: (a) major roads, including main city access roads and main intra city roads; (b) secondary intra city roads and (c) minor or residential roads. This classification was chosen according to the dominant use of the roads (provided by the City Council) and the limited availability of traffic intensity counts.

The coordinates of the sampling locations were determined by global positioning system (GPS). The cohort addresses were geocoded by using the local GIS of the municipality (see Figure S1, Supporting Information). 46 women did not live in Sabadell but in nearby cities covered by the health service of the hospital of Sabadell. Because air pollution measurements were carried out only in the municipal area of Sabadell, these women were removed from this analysis. Finally, 611 women were included in the analysis.

Buffering

For some of the geographic variables, different spatial scales were calculated around the monitoring sites. Length of major, secondary and all roads was calculated within buffers of 50, 75, 100, 250 and 500 meters; population density within buffers of 100, 250 and 500 meters; and area of urban and industrial land use (in hectares) within buffers of 300, 400 and 500 meters. In order to avoid overlapping buffers in the regression analysis, concentric bands (i.e. 50-250 and 250-500 meters) were also used. The buffer sizes were selected according to previous studies and the characteristics of the study area. Given the dense structure of roads and buildings within the city, we did not expect a significant contribution of sources more than 500 meters away from the sampling sites.

A similar approach to the SAVIAH study (*5*) was used for land use variables. The combination of urban and industrial land cover in different distances was tested in regression models as predictors of air pollution mean levels and weights were identified by examination of the slope coefficients. This resulted in a weight of 1.6, 1.5 and 1.4 for urban cover within 300, 400 and 500 meters buffers, respectively, while industrial cover was weighted as 1.

Exposure modeling

The association between the geographic variables and the mean levels of measured air pollutants was analyzed using multiple linear regression. Because of a slight right skew of the data, natural log transformation of the NO_2, NOx and BTEX values were used.

Firstly, each independent variable was tested against the dependent variable by using a univariate regression model. Because several geographic variables were calculated at different spatial scales around the sampling sites, for each variable we selected the buffer with the highest adjusted R^2. Next, we identified the most relevant univariate relationships and then we conducted a stepwise multiple regression to find the best-fit combination of variables (defined by the adjusted R^2 coefficient). Variables retained in the model had to be significant at the 95% level and have low collinearity with the other variables (defined by a Variance Inflation Factor <10.0).

After developing the models, regression diagnostics were conducted to identify outliers and leverage values and residuals were tested for heteroscedasticity and spatial autocorrelation. For the spatial autocorrelation analysis, Thiessen poligons were used to assign a first-order neighborhood structure and Moran's *I* values were calculated.

We used two cross-validation procedures to evaluate the precision of the exposure models. The first one involved removing one of the locations, re-deriving the model, and predicting the concentration at the omitted site. This procedure was repeated for all the sampling sites and the prediction error was expressed as root mean squared error

(RMSE), calculated as the square root of the sum of the squared differences of the observed concentration at site i and the predicted concentration at site i from the model developed without site i (*11*). A second cross-validation approach was developed by testing the models in three random selections of 85% of the sample to predict the air pollution levels at the remaining 15%.

Statistical analyses were performed using STATA 8.2 (Stata Corp., USA) and spatial autocorrelation analysis was carried out using ArcGIS 9.1.

RESULTS

Descriptive analyses

Nine BTEX and seven NO_2 samplers were damaged or vandalized in one of the periods, whereas two sites had BTEX samplers that were damaged during two periods. Since annual BTEX average levels were calculated as the mean of three sampling periods, these two sites were removed from the analysis. As a result, 57 sites were included in the NO_2 and NOx analysis and 55 sites in the BTEX analysis.

For quality control purposes 38 blanks and 10 duplicate measurements were obtained. The mean (in $\mu g/m^3$) of the field blanks was 0.64 (SD = 1.48) for NO, 0.80 (SD = 0.48) for NO_2 measured with Ogawa™ samplers, 2.56 (SD = 1.82) for NO_2 measured with Radiello® samplers, and below the limit of quantification (LOQ) for the five BTEX compounds. The mean NO_2 concentration of the Radiello® laboratory blanks (2.11 $\mu g/m^3$) was slightly lower than the mean of the field blanks. The coefficients of variation were 4.2% for NO and 3.3%

for NO_2. All NOx and NO_2 values were above the LOQ, while 7 benzene, 2 toluene, 3 ethylbenzene, 4 *m/p*-xylene and 21 *o*-xylene values were below the LOQ (0.01 μg/m^3 for benzene, toluene, and *o*-xylene; 0.02 μg/m^3 for ethylbenzene and 0.03 μg/m^3 for *m/p*-xylene). In the statistical analysis, we used LOQ/2 for values below the LOQ.

The comparison of the Ogawa™ and Radiello® samplers in the 1st campaign showed that the NO_2 mean (38.13 μg/m^3) and range (19.75 - 72.85 μg/m^3) measured with Ogawa™ was lower than the mean (43.42 μg/m^3) and range (18.44 - 80.44 μg/m^3) measured with Radiello®. The linear R^2 value between the two groups of measurements was 0.85. Since the values differed systematically, the NO_2 levels measured with Ogawa™ were adjusted using this comparison. Thus, we used the results from Radiello® samplers for the 1st, 3rd and 4th campaigns and the adjusted results from Ogawa™ samplers for the 2nd campaign.

Summary statistics of the 57 sampling sites by the three pollutants are presented in Table 1. Among BTEX, toluene was the most abundant compound at all the sampling sites, followed by *m/p*-xylene, as observed in other urban areas (*18*). Correlation coefficients among the five compounds (based on site average concentrations) were high ($r > 0.75$), particularly among toluene, ethylbenzene and xylenes ($r > 0.85$) (see Table S2, Supporting Information). Correlation coefficients were also high between BTEX and NOx/NO_2 (r = 0.74 and 0.80, respectively), suggesting that traffic is the main source of BTEX in the study area. Mean concentrations measured at traffic sites were significantly higher than those measured at the corresponding background sites (see Figure S2, Supporting Information). The NO_2 average of the four campaigns at traffic sites (49.37 μg/m^3, SD = 12.28 μg/m^3) was very similar to the NO_2 mean level measured at the fixed monitoring station of Sabadell (an urban traffic station of the regional Air Pollution Monitoring Network) for the study period (50.98 μg/m^3, SD = 16.47 μg/m^3).

Table 1. Distribution of average air pollution concentrations (μg/m^3) at the measurement sites

				Percentiles			
	Mean	**SD**	**Min**	**25th**	**50th**	**75th**	**Max**
NOx (n=57)	60.05	30.49	26.50	37.62	51.93	80.26	160.67
NO_2 (n=57)	40.27	13.84	18.75	29.78	38.42	50.52	74.86
BTEX (n=55)	17.21	8.27	3.53	9.82	17.47	22.81	34.12
benzene	0.98	0.52	0.36	0.56	0.91	1.30	3.08
toluene	8.55	4.30	1.92	5.24	8.25	11.97	16.65
ethylbenzene	1.68	0.83	0.24	0.97	1.64	2.15	3.58
m/*p*-xylene	4.79	2.25	0.74	2.82	4.68	6.23	9.89
o-xylene	1.21	0.66	0.11	0.64	1.19	1.67	3.11

Correlation coefficients between consecutive sampling periods varied depending on the pollutant and the number of periods. For NOx, the correlation between the two periods was very high (r > 0.90). For NO_2 and BTEX, the correlation coefficients varied from 0.94 to 0.53 and from 0.68 to 0.50, respectively.

Regression analyses

Univariate (see Table S3, Supporting Information) and multiple linear regression models were developed for NOx, NO_2 and BTEX. Because of the high correlation among BTEX components in the three sampling periods and the evidence of traffic as the main source of all of them in the study area, we used the sum of the five BTEX components as the dependent variable in the regression analysis.

We identified two outliers in the BTEX model with studentized residuals larger than +3 and -3, respectively. They corresponded to two background sites with BTEX levels higher than expected, indicating the possible influence of local sources not available in the GIS (i.e. painting works, solvent usages…). These outliers were removed and the regression analysis was rerun to check whether any changes in the predictor variables were produced. The final model included the same variables as the model with the outliers but the coefficients of the traffic-related variables changed substantially and the R^2 value increased from 0.63 to 0.74 after excluding the outliers.

Final regression models are presented in Table 2 ("GIS-surface" models). NOx and NO_2 models included the same variables (altitude, road length indicators and land cover) and produced an adjusted R^2 of 0.68 and 0.69, respectively. The BTEX regression model included altitude and source-proximity variables and accounted for 74% of the intraurban variation. The R^2 of both NOx and NO_2 models were further improved by replacing the road length indicators with road type as an ordinal indicator ("best" models in Table 2). These models have the limitation of not being able to visualise GIS-based pollution surfaces.

Table 2. Results of regression models for NOx, NO_2 and BTEX

	"GIS-surface" model[a]			"Best" model[a]		
Variable	Slope	SE[b]	R^2 full model (individual variables)	Slope	SE[b]	R^2 full model (individual variables)
Model for Ln(NOx)			**0.68**			**0.77**
Constant	3.682			4.025		
Altitude	-0.002	0.001	0.02	-0.003	0.001	0.04
Land cover factor (500m)	0.010	0.003	0.42	0.007	0.002	0.26
Length of major roads (50m)	2.171	0.459	0.20	-	-	-
Length of secondary roads (500m)	0.073	0.038	0.04	-	-	-
Road type (reference: minor road)						
Major road	-	-	-	0.754	0.083	0.43
Secondary road	-	-	-	0.307	0.092	0.04
Model for Ln(NO_2)			**0.69**			**0.75**
Constant	3.468			3.627		
Altitude	-0.003	0.001	0.03	-0.003	0.001	0.04
Land cover factor (500m)	0.009	0.002	0.54	0.009	0.002	0.54
Length of major roads (50m)	1.468	0.307	0.10	-	-	-
Length of secondary roads (500m)	0.057	0.026	0.02	-	-	-
Road type (reference: minor road)						
Major road	-	-	-	0.445	0.066	0.15
Secondary road	-	-	-	0.179	0.073	0.02
Model for Ln(BTEX)[b]			**0.74**			
Constant	5.022					
Altitude	-0.010	0.002	0.53			
Distance to nearest major road[c]	-0.055	0.020	0.18			
Distance to nearest secondary road[c]	-0.026	0.012	0.02			
Distance to nearest parking lot[c]	-0.043	0.020	0.01			

[a] "GIS-surface" model refers to the best-fit combination of variables that provide a GIS-based air pollution map. The "best" model includes variables that produce the highest R^2 but are not able to provide air pollution maps. Both models are the same for BTEX.
[b] SE = standard error of the slope; BTEX = sum of benzene, toluene, ethylbenzene, *m/p*-xylene and *o*-xylene.
[c] distances are divided by 100 meters.

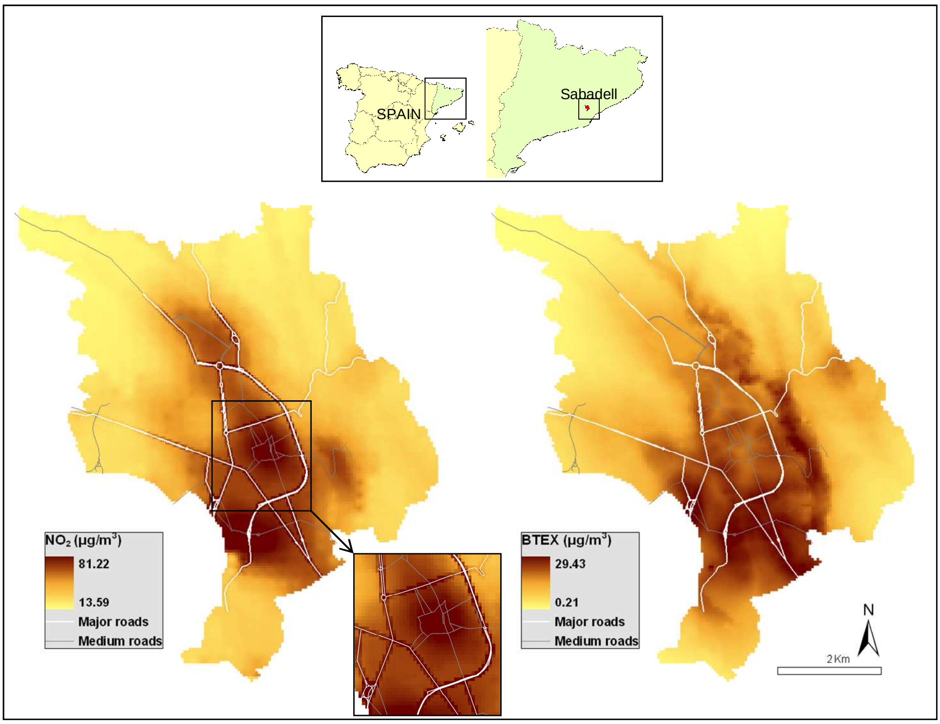
Since the number of sampling periods was different for the three pollutants and NO_2 was measured with two different passive samplers, as a sensitivity analysis we developed two new NO_2 models, one with the Ogawa™ measurements (which included the two same campaigns as NOx) and other with the Radiello® measurements (which included the three same campaigns as BTEX). These two models included the same predictor variables as the general NO_2 model and produced an adjusted R^2 of 0.65 and 0.66, respectively. The coefficients of the predictor variables did not change substantially. To evaluate the pollutant-specificity of LUR technique, we also tested a second BTEX model by introducing the same variables as the NO_2 and NOx models. This model produced an adjusted R^2 of 0.71, although the variable length of secondary roads within 500 m was not statistically significant (data not shown).

Two cross-validation analyses were conducted to evaluate the validity of the regression models. The "leave-one out procedure" estimated a root mean squared error (RMSE) of 2.38 $\mu g/m^3$ for NOx, 1.59 $\mu g/m^3$ for NO_2 and 2.15 $\mu g/m^3$ for BTEX. The analysis in which we developed models for three random selections of 85% of the sites estimated RMSE of 1.92 $\mu g/m^3$ for NOx, 1.30 $\mu g/m^3$ for NO_2 and 1.72 $\mu g/m^3$ for BTEX, and produced adjusted R^2 in the range of 0.67 - 0.75 for NOx, 0.68 - 0.74 for NO_2 and 0.71 - 0.75 for BTEX. Examination of the residuals indicated homogeneity of variance in all the models. There were no significant spatial autocorrelation in NO_2 and NOx models based on a first-order connectivity matrix. A slightly dispersed pattern of the residuals was found in the BTEX model, although Moran's *I* value was consistently low (-0.07).

The three models were then applied to predict outdoor air pollution levels at the cohort addresses (see Table S4, Supporting Information). Mean levels of NOx (52.73 µg/m^3), NO_2 (37.38 µg/m^3) and BTEX (15.67 µg/m^3) in the cohort addresses were lower than in sampling sites (Table 1) and ranges were more narrow in the cohort addresses (24.30 - 131.39 µg/m^3 for NOx, 18.73 - 76.65 µg/m^3 for NO_2 and 3.72 - 26.13 µg/m^3 for BTEX).

Surfaces of predicted NO_2 and BTEX concentration were created by applying the equation of the regression models on a 50m-cell by cell basis to all locations in the city area (Figure 1).

Figure 1. Regression maps of modelled annual mean NO_2 and BTEX concentrations in Sabadell



DISCUSSION

Most studies on prenatal exposure to air pollution and pregnancy outcomes have relied on routine monitoring of air pollution and area-based exposures (*19-21*), with few studies assessing individual exposure using spatial analysis techniques (*22*) or personal monitoring (*23*). The extrapolation from routine measurements to individual exposures is a limitation that may be related to the lack of strong evidence of the effects of air pollution on several adverse pregnancy outcomes (*15*). In this context, this study provides a more accurate exposure assessment during pregnancy for future research of the effects of prenatal exposure to air pollution in children's health.

We derived multiple linear regression models that explained 68% and 69% of the variance in NOx and NO_2 levels, respectively, with four predictor variables. The percentage of explained variability was higher when replacing the two road length indicators with an ordinal indicator (road type). This variable showed the highest association for NOx concentrations, which are more specific for traffic emissions. However, the application of models including this ordinal indicator is limited because they can only predict concentrations at locations next to roads.

According to our annual NO_2 model, 36% of the pregnant women live in areas with annual mean NO_2 levels higher than 40 $\mu g/m^3$, the EU limit value which comes into force in 2010 (*24*). This percentage varies from 41% to 29% when we use the two NO_2 models developed with the Ogawa™ and Radiello® measurements, respectively. Overall, there is a significant fraction of the cohort living in high polluted areas, which is an issue of management intervention and regulatory concern. These results demonstrate the capability of LUR to produce stable models using few short-term sampling periods and to identify local populations at potential risk of chronic exposure to high air pollution levels.

Exposure assessment of BTEX becomes necessary due to their prevalence in urban areas and the magnitude of their potential adverse health effects from chronic environmental exposure to low levels, including the carcinogenic effect of benzene (*25*). To our knowledge, this is the first study in Europe using LUR to assess the intraurban variability of BTEX, with a model accounting for almost 75% of the variation. Less predictive intraurban models were obtained in the RIOPA study by using source-proximity and meteorological variables, with R^2 between 0.16 and 0.45 for the different BTEX compounds (*18*). In our model, two background sites were identified as outliers and the possible influence of unknown local BTEX sources was suggested. Additional data on other local sources would probably lead to a better exposure estimate of the subjects living near such unknown sources.

The BTEX annual mean concentration in Sabadell (17.1 $\mu g/m^3$) was similar to mean levels measured in other European urban areas (*26, 27*). Benzene levels, however, were similar to the values reported in Spanish rural areas (*28*), with a maximum concentration below the EU annual mean limit value of 5 $\mu g/m^3$ (*29*). Whereas toluene, ethylbenzene and xylenes are mainly emitted by vehicle exhausts, benzene can have additional sources of emission at local levels that may not exist in Sabadell, such as wood combustion or landfills.

Since most studies have relied on NO_2 measurements because of the low cost and logistic advantages of passive samplers, one question that arises is whether NO_2 is a reasonably proxy for other relevant air pollutants such as particulate matter (PM)(*6*). In Sabadell, the fixed monitoring station measures daily levels of NO_2, PM_{10}, CO, SO_2 and ozone, and the correlation between NO_2 and PM_{10} for the study period was relatively low ($r = 0.25$), probably due to the relevant contribution of natural sources to PM_{10} in the area (*30*).

We found high correlations between BTEX and NOx/NO_2 concentrations at the sampling sites. Furthermore, we 'forced' a second BTEX model including the same predictor variables as the NO_2 and NOx models. The resultant model explained a similar percentage of variation as the original BTEX model, although the length indicator for secondary roads was not statistically significant. This could be due to one limitation of this study, which is the possible misclassification of some roads due to the lack of more reliable traffic data. The use of more specific variables such as total and heavy vehicle traffic intensity could have increased the predictive capability of the LUR models. Still, our models showed a high predictive capability with fewer predictor variables than other studies including traffic intensity data (*8, 9, 11*).

The range in predicted air pollution levels at the cohort addresses was smaller than the range in measured levels at the sampling sites. This confirms that the sampling sites were properly selected to provide enough estimation of the gradient of the exposure in the cohort. However, the use of annual mean air pollution levels at the home address as an indicator of individual exposure do not take into account that individual exposure to air pollution during a specific period involves both spatial and temporal variations.

The combination of GIS models and information on temporal-spatial patterns in epidemiologic studies of pregnant women and children has been identified as necessary in exposure assessment (*31*). In the INMA study, term specific individual exposure to urban air pollution during pregnancy will be assessed considering both spatial and temporal components. The spatial component will be estimated as a combination of residential and workplace outdoor-modeled levels, using the regression models presented in this article. The temporal component will be calculated as the exposure during each trimester of pregnancy. Since our regression models represent the annual average of the pollutants, modeled air pollution levels will be adjusted for temporal variation using the continuous air pollution data from the fixed monitoring station for the pregnancy period of each woman.

Although a model involving spatial and temporal variations could provide better exposure estimation than other using residential outdoor levels alone, a remaining question is to what extent outdoor-modeled levels represent personal exposure to traffic-related pollution. People spend most of their daily time indoors and therefore exposure to traffic-related pollutants will be determined by the degree to which they affect indoor air quality (*26*). In this context, several studies have investigated the influence of outdoor air pollution in residential and workplace indoor levels and in personal exposure. The AMICS Study found a significant correlation between outdoor and indoor NO_2 concentrations in Barcelona and a significant increase of indoor NO_2 levels with ventilation (*32*). For volatile organic compounds (VOCs), the differences in outdoor levels between homes located in high and low traffic streets in Amsterdam was smaller than the differences found in indoor levels of the same homes, due to the influence of multiple indoor VOC sources (*26*). Regarding the influence of outdoor air pollution on personal exposure, Rijnders et al. (*33*) found that personal NO_2 levels in children were significantly influenced by the degree of urbanization of the city district where they lived and by the traffic intensity in the nearby highway. The EXPOLIS-Helsinki Study found that personal exposure to BTEX was higher than time weighted residential and workplace indoor levels, suggesting the influence of traffic emissions (*34*). Overall, outdoor traffic-related air pollution affects indoor levels and therefore personal exposure in both outdoor and indoor environments, although indoor sources of some pollutants such as VOCs seem to have a higher impact on personal exposure than outdoor concentrations.

Despite these limitations, the development of LUR models is a relatively low-cost approach that clearly offers an advantage over traditional approaches that use ambient monitoring data alone, particularly in cohort studies where estimation of individual exposure is essential for assessing health effects from air pollution.

Acknowledgements

We thank Laura Bouso, Laura Fernández, Bénédicte Jacquemin, Laura Muñoz, Gemma Perelló, Núria Pey, Estel Plana and Cristina Rebordosa for their help on the field work; Danielle Vienneau and Kees de Hoogh (Imperial College London) for valuable advise on GIS analysis; and Josep Iglesias (Ajuntament de Sabadell) for his help on GIS data collection.

INMA Study has the financial support of the 'Instituto de Salud Carlos III' (G03/176) and 'RCESP' (C03/09). Air pollution measurements have been partially funded by the EU FP6 Network of Excellence GA^{2}LEN.

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SUPPORTING INFORMATION

Figure S1. Location of sampling sites and home addresses of the cohort in Sabadell

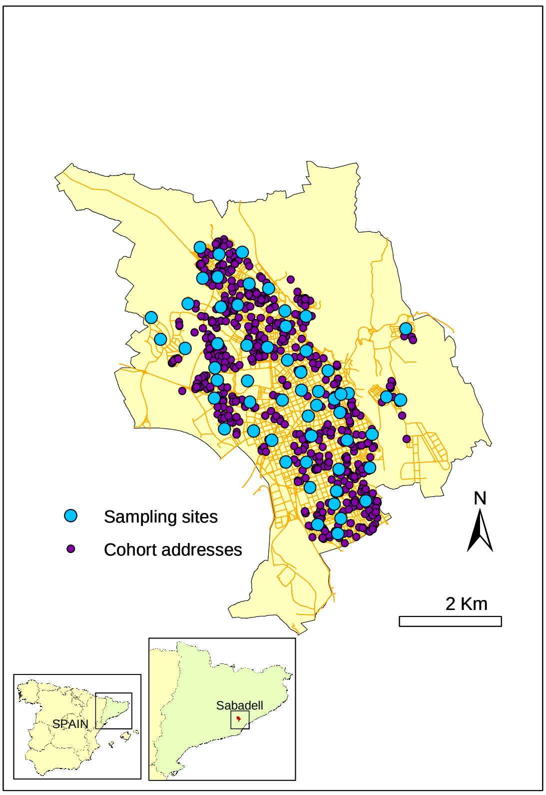


Figure S2. Mean concentrations of NOx, NO_2 and BTEX by type of site. Line in the box is the median, box limits are the 25th and 75th percentiles and whiskers are the 10th and 90th percentiles

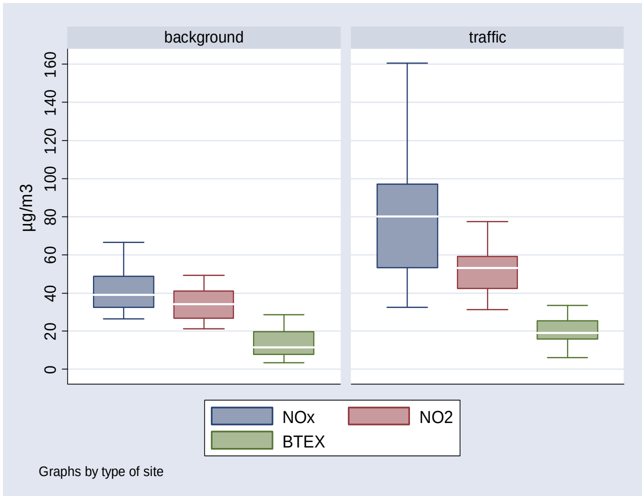


Table S1. Summary of the sampling periods and type of passive samplers used in the study

Sampling periods			
1st (April 2005)	2nd (June 2005)	3rd (October 2005)	4th (March 2006)
NOx (Ogawa™)	NOx (Ogawa™)	-	-
NO_2 (Ogawa™)	NO_2 (Ogawa™)	-	-
NO_2 (Radiello®)	-	NO_2 (Radiello®)	NO_2 (Radiello®)
BTEX (Radiello®)	-	BTEX (Radiello®)	BTEX (Radiello®)

Table S2. Pearson correlation coefficients between measured air pollutants[a]

Pollutants	NOx	NO_2	benzene	toluene	ethyl-benzene	*m*/*p*-xylene	*o*-xylene
NOx	1	0.95	0.77	0.66	0.72	0.72	0.76
NO_2		1	0.79	0.67	0.73	0.74	0.77
benzene			1	0.77	0.83	0.87	0.93
toluene				1	0.96	0.95	0.89
ethylbenzene					1	0.99	0.94
m/*p*-xylene						1	0.97
o-xylene							1

[a] All correlations are significant at the 0.01 level

Table S3. Associations between NOx, NO_2 and BTEX concentrations at sampling sites (n=57 for NOx and NO_2; n=55 for BTEX) and geographic variables by univariate regression models[a]

	Ln(NOx)		**Ln(NO_2)**		**Ln(BTEX)**	
Variable, unit	Slope	Adj R^2	Slope	Adj R^2	Slope	Adj R^2
Altitude, m	-0.006	0.12	-0.005	0.19	-0.014	0.43
Distance to nearest major road, m	-0.001	0.37	-0.001	0.34	-0.001	0.33
Distance to nearest secondary road, m	-0.001	0.11	-0.0004	0.16	-0.0005	0.09
Distance to nearest parking lot, m	-0.001	0.22	-0.0007	0.33	-0.001	0.39
Population density (100m), dwellings	0.001	0.09	0.001	0.11	0.001	0.11
Population density (250m), dwellings	0.0003	0.06	0.0002	0.12	0.0003	0.12
Population density (500m), dwellings	0.0001	0.15	0.0001	0.21	0.0001	0.21
Sum length major roads (50m), Km	2.607	0.34	1.868	0.28	1.981	0.09
Sum length major roads (250m), Km	0.388	0.21	0.244	0.13	0.322	0.09
Sum length major roads (500m), Km	0.210	0.32	0.146	0.26	0.224	0.26
Sum length secondary roads (50m), Km	1.773	0.02	1.652	0.04	2.051	0.02
Sum length secondary roads (250m), Km	0.284	0.06	0.248	0.08	0.261	0.03
Sum length secondary roads (500m), Km	0.149	0.14	0.128	0.18	0.150	0.09
Land cover factor[b] (300m), Has	0.040	0.36	0.033	0.41	0.051	0.39
Land cover factor[c] (400m), Has	0.025	0.41	0.021	0.50	0.030	0.44
Land cover factor[d] (500m), Has	0.016	0.42	0.014	0.54	0.020	0.46

[a] All univariate relationships between the pollutants and the geographic variables had *p*-values < 0.05 except "Sum length secondary roads (50m)" (*p*-value=0.20 for NOx, 0.12 for NO_2 and 0.23 for BTEX).
[b] Computed as (1.60 × urban hectares) + industrial hectares
[c] Computed as (1.50 × urban hectares) + industrial hectares
[d] Computed as (1.40 × urban hectares) + industrial hectares

Table S4. Mean air pollutant concentrations (μg/m^3) measured at the sampling sites and estimated at the home addresses of the cohort

	Sampling sites (n = 57)[a]			Cohort (n= 611)		
	Mean	Range	SD	Mean	Range	SD
NOx	60.05	26.50- 160.67	30.49	52.73	24.30 - 131.39	15.23
NO_2	40.12	19.33 - 76.26	13.87	37.39	18.73 - 76.65	8.62
BTEX	17.21	3.53 - 34.12	8.27	15.67	3.72 - 26.13	5.78

[a] n=53 for BTEX

ARTÍCULO 2: Concentrations and determinants of outdoor, indoor and personal nitrogen dioxide in pregnant women from two Spanish birth cohorts

Environment International doi:10.1016/j.envint.2009.08.002 (en prensa)

Natalia Valero[1], Inmaculada Aguilera[2,3,4,5], Sabrina Llop[4,6], Ana Esplugues[4,6], Audrey de Nazelle[2,3,4], Ferran Ballester[4,6] and Jordi Sunyer[2,3,4,5]

[1] Agència de Salut Pública de Barcelona, Spain

[2] Centre for Research in Environmental Epidemiology, Barcelona, Spain

[3] Municipal Institute of Medical Research (IMIM-Hospital del Mar), Barcelona, Spain

[4] CIBER en Epidemiología y Salud Pública, Barcelona, Spain

[5] Pompeu Fabra University, Barcelona, Spain

[6] Centre for Research on Public Health, Conselleria de Sanitat, Generalitat Valenciana, Valencia, Spain

Corresponding author:

Inmaculada Aguilera

Centre for Research in Environmental Epidemiology

Barcelona Biomedical Research Park

Doctor Aiguader 88. 08003 Barcelona, Spain

Tel. +34 932147300 / Fax + 34 932147301

E-mail: iaguilera@creal.cat

Valero N, Aguilera I, Llop S, Esplugues A, de Nazelle A, Ballester F, Sunyer J.
Concentrations and determinants of outdoor, indoor and personal nitrogen dioxide in pregnant women from two Spanish birth cohorts.
Environ Int. 2009 Nov;35(8):1196-201.

ABSTRACT

Determinants of outdoor, indoor and personal concentrations of nitrogen dioxide (NO_2) were assessed in a subset of pregnant women of the Spanish INMA (Environment and Childhood) Study. Home indoor and outdoor NO_2 concentrations were measured during 48 hours with passive samplers for 50 and 58 women from the INMA cohorts of Valencia and Sabadell, respectively. Women from Sabadell also carried personal NO_2 samplers during the same period. Data on time-activity patterns, socio-economic characteristics, and environmental exposures were obtained through questionnaires. Multiple linear regression models were developed to predict NO_2 levels. In Valencia, median outdoor NO_2 levels (42 μg/m^3) were higher than median indoor levels (36 μg/m^3). In Sabadell, personal NO_2 showed the highest median levels (40 μg/m^3), followed by indoor (32 μg/m^3) and outdoor (29 μg/m^3) levels. Personal exposure to NO_2 correlated best with the indoor NO_2 levels. Temporal and traffic-related variables were significant predictors for outdoor NO_2 levels. Thirty-two percent of the indoor NO_2 variability in the two cohorts was explained by outdoor NO_2 levels and the use of the gas appliances. The model for personal exposure accounted for 59% of the variance in NO_2 levels in Sabadell with four predictor variables (outdoor and indoor NO_2 levels, time spent in outdoor environments and time exposed to a gas cooker). No significant association was found between personal or indoor NO_2 levels and exposure to environmental tobacco smoke (ETS) at home. Personal NO_2 levels were found to be strongly influenced by indoor NO_2 concentrations. The study supports the use of time-activity patterns along with indoor

measurements to predict personal exposure to traffic-related air pollution.

INTRODUCTION

Fetuses and children are specially vulnerable and sensitive to environmental toxics, in comparison to adults (Schwartz, 2004). Fetuses are considered to be highly susceptible to a variety of toxicants because of their physiological immaturity and exposure patterns (Šrám et al., 2005). Several studies have investigated the association between maternal exposure to environmental pollution during pregnancy and adverse effects at birth such as intrauterine and postneonatal mortality, low birth weight, preterm birth, and intrauterine growth restriction (reviewed by Glinianaia et al., 2004; Lacasaña et al., 2005; Maisonet et al., 2004; Šrám et al., 2005; Wang and Pinkerton, 2007).

Nitrogen dioxide (NO_2) is an irritant gas that can have both acute and chronic respiratory effects (Latza et al., 2009). It causes inflammation of the airways at relatively high concentrations (Blomberg et al, 1997) and several studies suggest that long term exposure to NO_2 may affect lung function and increase the risk of respiratory tract infections in children (Ciencewicki and Jaspers, 2007; Fuentes-Leonarte et al., 2009; Oftedal et al., 2008). In addition, a negative association has been found between early-life exposure to indoor NO_2 and neuropsychological development through the first 4 years of life (Morales et al., 2009). In outdoor environments where concentrations are typically low, its epidemiological interest lies mainly on being a good marker of traffic-related pollution (Brunekreef, 2001). Besides outdoor sources of NO_2, indoor NO_2 is also generated by environmental tobacco smoke (ETS) and by gas appliances (Baxter et al., 2006; García-Algar et al., 2004; Lai et al., 2006; Monn et al., 1998).

Several studies, in particular the multicentric EXPOLIS (Air Pollution Exposure Distributions Within Adult Urban Population in Europe) study (Hänninen et al., 2004) and the Swiss SAPALDIA (Swiss Cohort Study on Air Pollution and Lung Diseases in Adults) study (Ackermann-Liebrich et al., 2005), have assessed the relationship between personal, indoor, and outdoor NO_2 concentrations (Kousa et al., 2001; Levy et al., 1998; Monn et al., 1998; Ramírez-Aguilar et al., 2002; Rojas-Bracho et al., 2002; Rotko et al., 2001; Sørensen et al., 2005; Spengler et al., 1994; Zipprich et al., 2002). All of them found that personal exposure correlated better with indoor than NO_2 outdoor concentrations, while some of them concluded that personal exposure can might be estimated using home indoor and outdoor levels and time-activity patterns, when personal exposure measurements are not possible, or too difficult, to obtain (Ramírez-Aguilar et al., 2002; Rotko et al., 2001; Zipprich et al., 2002). To date, only one study of personal monitoring of NO_2 has been conducted on a sample of pregnant women, a specific population for which there is very little exposure data (Nethery et al., 2008).

Some studies have observed elevated outdoor NO_2 levels in highly urbanized areas (Rijnders et al., 2001) and nearby high-traffic roads (Ramírez-Aguilar et al., 2002; Rijnders et al., 2001; Rotko et al., 2001). Although partly emitted directly by vehicles, NO_2 is mainly a secondary pollutant resulting from the oxidation of traffic-related nitrogen monoxide (NO) (Rodríguez et al., 2001). During summer this reaction is produced at a faster rate due to photochemical reactions that contribute to the oxidation of NO. Seasonal variations are therefore expected in the results of outdoor NO_2 sampling campaigns (Monn, 2001).

The association between socio-demographic factors and personal exposure to NO_2 has been less studied. Rotko et al. (2001) observed higher personal exposures to NO_2 in subjects with lower compared to higher educational level in Helsinki.

To our knowledge, only one study has analyzed the determinants of indoor NO_2 levels in Spain (García-Algar et al., 2004), but it has not studied personal exposure or time-activity patterns. The INMA (Environment and Childhood) study is the first to assess exposure to environmental pollution during pregnancy in Spain. INMA is a population-based prospective cohort aimed at assessing the impact of the most important environmental pollutants in air, water, and diet on children's development and health. The research is conducted in aproximately 4,000 couples of pregnant women and children from seven Spanish regions (Ribas-Fitó et al., 2006).

The objective of the present study was to determine home outdoor and indoor NO_2 levels as well as personal NO_2 exposure in two subsets of pregnant women of the INMA cohorts from Valencia and Sabadell (east and northeast Spain, respectively) and to assess their determinants.

MATERIALS AND METHODS

Study population

The study population consists of 855 and 657 pregnant women from the INMA cohorts of Valencia and Sabadell, respectively. The study area of the Valencia cohort includes the city of Valencia and 34 more municipalities. The area is divided in four categories (urban, metropolitan, semi-urban and rural) representing a gradient from higher to lower degree of urbanization which is typical of many Mediterranean cities (Muñoz, 2003). The cohort of Sabadell is exclusively urban.

Two subsets of 50 women in Valencia and 58 women in Sabadell were selected during their third trimester of pregnancy to participate in the study. Both subsets were selected to represent the geographical distribution of the dwellings of their cohorts (Esplugues et al., 2007), given the geographical differences in air pollution levels and socioeconomic factors.

Sampling campaigns

Indoor, outdoor and personal NO_2 concentrations were measured during a period of 48 hours with passive Radiello samplers (Fondazione Salvatore Maugeri, Padova, Italy). Although longer sampling periods would better reflect the influence of seasonality in air pollution levels, NO_2 personal monitoring is often performed for no more than 48 hours for practical reasons such as minimizing sample losses (Nethery et al., 2008; Rotko et al., 2001; Sørensen et al., 2005). Indoor samplers were placed in the living room, at 2 – 2.5 m above ground and far from any window or air conditioner. Outdoor measurements were taken at the home balcony or outside a window, facing the main street of the building when possible. Women carried the personal samplers attached to clothes as close to the face as possible. For budgetary reasons we could not measure personal exposure to NO_2 in the Valencia cohort, so personal concentrations only refer to Sabadell.

Four sampling campaigns were carried out in Valencia (November 2003, April 2004, November 2004, and February 2005) and three in Sabadell (April 2005, October 2005, and March 2006). The number of sampling campaigns and the dates are different between the two cohorts due to differences in the recruitment periods and funding.

The methodology, technical and analytical specifications of the air pollution sampling campaigns, as well as the inclusion criteria and recruitment strategy for the INMA cohorts have been described elsewhere (Aguilera et al., 2008; Esplugues et al., 2007; Ribas-Fitó et al., 2006).

Covariates

After the sampling campaigns, women answered a questionnaire about their activity patterns during the sampling period. Other available sources of information were the questionnaires administered in the 12^{th} and 32^{nd} week of pregnancy as part of the INMA study that collected data on sociodemographic characteristics and environmental exposures (Ribas-Fitó et al., 2006). Additional traffic-related variables (type of road at home address and distance to nearest major road) were obtained in Sabadell using Geographic Information System (GIS) (Aguilera et al., 2008).

The predictor variables were classified into the following groups: (1) time-activity patterns during the 48-h sampling period: time spent at home, time spent in outdoor environments (in a park, walking, etc), and time spent commuting; (2) time exposed to indoor sources of NO_2 during the 48-h sampling period: ETS, gas appliances (heater, cooker and water heater), and open windows to ventilate; (3) traffic variables collected by questionnaire: frequency of cars and heavy vehicles on the closest road (none, not many, moderate or many); (4) traffic-related variables collected with GIS (only in Sabadell): type of road (major, secondary or minor road) and distance to nearest major road ($\leq$ 50 meters and > 50 meters); and (5) socioeconomic characteristics: educational level and working status in the 32nd week of pregnancy. Other independent variables were the degree of urbanization of the place of residence (only for the Valencia cohort), the indoor NO_2 concentration (when the dependent variable was personal concentration), the outdoor NO_2 concentration (when the dependent variable was either indoor or personal concentration), the height of the balcony or window where the outdoor sampler was placed (classified as ground or 1st floor and $\geq$ 2nd floor), and the month of sampling, defined by two categories: October to November and February to April.

Statistical analysis

A descriptive analysis was performed for all the variables in the two subsets. Bivariate associations between categorised variables and outdoor, indoor and personal NO_2 levels were tested using ANOVA, both in the two subsets combined and separately. Finally, multiple linear regression models were developed to analyze the determinants of outdoor, indoor and personal NO_2 levels. The variables included in the multivariate models were selected through a backward elimination process forcing the entrance of all the variables and removing consecutively those with non significant association. The level of statistical significance was established at $p < 0.10$.

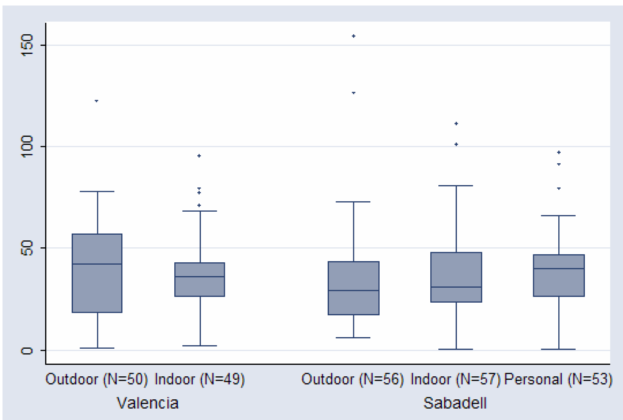
Since outdoor NO_2 concentrations in Sabadell and indoor concentrations in Valencia did not follow a normal distribution, regression diagnostics were conducted after developing the models to verify the residuals' distribution and to identify extreme values and influential observations. As a result, two observations were excluded from the analysis: one outdoor measurement in Sabadell with a NO_2 concentration over the 98^{th} percentile that was not correlated with the corresponding indoor value, and one indoor measurement in a dwelling from Valencia where the sampler was exposed to a kerosene heater during 14 hours, which is not a source of interest in this study. Three personal NO_2 measurements were also excluded from the analysis due to damaged samplers, as well as all NO_2 measurements corresponding to a woman from Sabadell because the samples could not be collected after the 48-h period.

All statistical analyses were performed using SPSS 12.0 (Chicago, IL, USA) for Windows.

RESULTS

Figure 1 shows the median concentrations of outdoor, indoor, and personal NO_2 measurements. Median indoor levels of NO_2 were similar in the two subsets (32 µg/m^3 in Sabadell and 36 µg/m^3 in Valencia) whereas median outdoor level of NO_2 was higher in Valencia (42 µg/m^3) than in Sabadell (29 µg/m^3). As the study area in Valencia is more heterogeneous with regard to the urbanization degree, the interquartile ranges are wider in Valencia than in Sabadell. Personal NO_2 exposure, available only for Sabadell, had a median value of 40 µg/m^3.

Figure 1. Median concentrations of outdoor, indoor and personal NO_2 (in µg/m^3) with 25th and 75th percentiles in the Valencia and Sabadell subsets of the INMA Study



The Pearson correlation coefficient in Sabadell between personal and indoor concentrations was 0.69 while the correlation between personal and outdoor concentrations was lower ($r = 0.39$). The Pearson correlation coefficient between indoor and outdoor levels was higher in Sabadell ($r = 0.43$) than in Valencia ($r = 0.25$).

Both subsets showed similar socio-economic characteristics. In Sabadell, 29.8% of the women had finished primary education, 45.6% had finished secondary education and 24.6% had a university degree. In Valencia, the percentages were 39.5%, 44.2% and 16.3% for primary, secondary and university education, respectively. Working status was also similar between the two subsets, with a percentage of women working in the 32nd week of pregnancy of 42.1% in Sabadell and 39% in Valencia.

The use of cooking/heating appliances during the 48-h sampling period was different between the two subsets. The percentage of women that used gas cookers was 50.0% in Valencia and 66.7% in Sabadell, whereas the use of gas water heaters was higher in Valencia (58.0%) than in Sabadell (29.8%). Gas heaters were infrequently used in either of the two subsets (8.0% in Valencia and 3.5% in Sabadell). Most of the participants had not been exposed to ETS in their homes during the sampling period (75.4% in Sabadell and 60.0% in Valencia). Regarding the height of the balcony or window where the outdoor NO_2 sampler was deployed, 54.8% of the women in Valencia and 33.3% in Sabadell lived on a ground or 1st floor.

Table 1 shows the descriptive statistics of NO_2 concentrations for the predictor variables.

Table 1. Determinants [a] of indoor, outdoor and personal NO_2 levels in Valencia and Sabadell (median and interquartilic range in µg/m^3). Time-activity variables refer to the 48-hour sampling period

	Valencia			Sabadell			
Variables	**N (%)**	**Outdoor NO_2**	**Indoor NO_2**	**N (%)**	**Outdoor NO_2**	**Indoor NO_2**	**Personal NO_2**
Month of sampling							
October to November [b]	27 (54.0)	54.0 (42.0-65.0)	n.a.[d]	19 (33.3)	40.0 (26.0-43.5)	n.a.	n.a.
February to April [c]	23 (46.0)	19.5 (12.0-42.0)	n.a.	38 (66.7)	24.0 (14.0-43.0)	n.a.	n.a.
Urbanization degree							
Urban area	8 (16.0)	52.0 (42.0-66.5)	61.0 (36.0-67.0)	56 (100.0)	29.0 (17.0-43.75)	31.0 (23.048.0)	40.0 (26.0-47.0)
Metropolitan area	20 (40.0)	46.0 (37.0-55.5)	36.0 (27.5-40.5)	n.a.	n.a.	n.a.	n.a.
Semi-urban area	14 (28.0)	17.0 (32.0-65.0)	33.0 (18.0-36.0)	n.a.	n.a.	n.a.	n.a.
Rural area	8 (16.0)	12.0 (10.0-33.0)	35.0 (18.0-48.0)	n.a.	n.a.	n.a.	n.a.
Road type at home address							
Major or secondary	n.a.	n.a.	n.a.	9 (15.8)	44.0 (40.5-49.5)	n.a.	n.a.
Minor	n.a.	n.a.	n.a.	48 (84.2)	25.0 (15.5-41.0)	n.a.	n.a.
Cars at home address							
Many	17 (40.5)	42.0 (24.0-61.0)	n.a.	18 (31.6)	37.0 (22.0-43.0)	n.a.	n.a.
Moderate	11 (26.2)	28.0 (17.5-44.5)	n.a.	12 (21.1)	23.5 (17.0-35.5)	n.a.	n.a.
None / not many	14 (33.3)	45.0 (18.0-56.0)	n.a.	27 (47.4)	8.0 (14.5-44.5)	n.a.	n.a.
Heavy vehicles at home address							
Many	5 (11.9)	61.0 (22.0-61.0)	n.a.	10 (17.5)	38.0 (36.0-44.0)	n.a.	n.a.
Moderate	7 (16.7)	42.0 (25.5-73.0)	n.a.	5 (8.8)	40.0 (27.0-44.0)	n.a.	n.a.
None / not many	30 (71.4)	39.0 (17.0-50.0)	n.a.	42 (73.7)	25.0 (15.0-42.0)	n.a.	n.a.

Table 1 continued

Variables	Valencia N (%)	Valencia Outdoor NO_2	Valencia Indoor NO_2	Sabadell N (%)	Sabadell Outdoor NO_2	Sabadell Indoor NO_2	Sabadell Personal NO_2
Time using a gas cooker							
0 minutes	25 (50.0)	n.a.	32.0 (18.0-41.0)	19 (33.0)	n.a.	23.0 (17.5-28.5)	22.0 (18.0-46.0)
10-60 minutes	9 (18.0)	n.a.	49.0 (28.5-69.5)	16 (28.1)	n.a.	40.5 (27.0-58.0)	41.0 (30.0-56.5)
> 60 minutes	16 (32.0)	n.a.	38.0 (32.0-46.5)	22 (38.6)	n.a.	40.0 (24.0-44.0)	41.5 (34.5-50.0)
Time using a gas heater							
0 minutes	46 (92.0)	n.a.	34.0 (24.0-41.0)	55 (96.5)	n.a.	29.0 (22.5-46.0)	40.0 (26.0-47.5)
≥1 minute	4 (8.0)	n.a.	63.0 (40.5-86.0)	2 (3.5)	n.a.	43.0 (33.0-53.0)	31.0 (21.0-41.0)
Time using a gas water heater							
0 minute	21 (42.0)	n.a.	34.0 (24.0-40.0)	40 (70.2)	n.a.	33.0 (24.0-43.5)	41.0 (26.0-48.0)
≥1 minute	29 (58.0)	n.a.	38.0 (28.5-47.0)	17 (29.8)	n.a.	24.0 (21.0-51.0)	35.0 (23.0-47.0)
Time spent at home							
≤ 36 hours	19 (44.2)	n.a.	n.a.	33 (57.9)	n.a.	n.a.	35.0 (26.0-47.0)
> 36 hours	24 (55.8)	n.a.	n.a.	24 (42.1)	n.a.	n.a.	42.0 (25.5-47.0)
Time spent commuting							
≤ 2 hours	31 (62.0)	n.a.	n.a.	33 (57.9)	n.a.	n.a.	40.0 (27.0-53.0)
> 2 hours	19 (38.0)	n.a.	n.a.	24 (42.1)	n.a.	n.a.	32.0 (21.0-45.0)
Time spent in outdoor environments							
≤ 2 hours	44 (88.0)	n.a.	n.a.	48 (84.2)	n.a.	n.a.	39.0 (23.5-46.0)
> 2 hours	6 (12.0)	n.a.	n.a.	9 (15.8)	n.a.	n.a.	47.0 (37.0-63.0)
Exposed to ETS at home							
Yes	20 (40.0)	n.a.	36.5 (31.0-41.5)	14 (24.6)	n.a.	n.a.	41.0 (30.0-57.0)
No	30 (60.0)	n.a.	34.0 (22.0-47.0)	43 (75.4)	n.a.	n.a.	40.0 (23.5-47.0)

Table 1 continued

Variables	Valencia			Sabadell			
	N (%)	Outdoor NO_2	Indoor NO_2	N (%)	Outdoor NO_2	Indoor NO_2	Personal NO_2
Time with open windows to ventilate							
≤ 10 hours	39 (78.0)	n.a.	35.0 (26.0-47.0)	47 (82.5)	n.a.	29.0 (21.5-43.5)	37.0 (22.0-49.5)
> 10 hours	11 (22.0)	n.a.	38.0 (28.0-42.0)	10 (17.5)	n.a.	39.0 (25.0-56.0)	44.0 (40.0-47.0)
Height of the balcony or window where the outdoor sampler was placed							
Ground or 1st flooor	23 (54.8)	42.0 (17.5-54.0)	n.a.	n.a.	19 (33.3)	36.0 (22.0-42.0)	n.a.
≥ 2nd floor	19 (45.2)	42.0 (18.0-60.5)	n.a.	n.a.	38 (66.7)	27.0 (14.0-44.0)	n.a.
Educational level							
Primary	17 (39.5)	n.a.	37.5 (26.5-44.0)	17 (29.8)	n.a.	24.0 (21.0-39.0)	28.0 (20.5-43.0)
Secondary	19 (44.2)	n.a.	32.0 (20.5-38.0)	26 (45.6)	n.a.	39.0 (25.0-48.0)	42.0 (31.0-57.0)
University	7 (16.3)	n.a.	30.0 (26.0-52.5)	14 (24.6)	n.a.	27.5 (21.0-51.0)	37.0 (25.0-47.0)
Working status at 32th week of pregnancy							
Employed	16 (39.0)	n.a.	32.0 (21.0-45.5)	24 (42.1)	n.a.	27.5 (19.5-46.0)	32.0 (24.0-45.5)
Unemployed/work leave	25 (61.0)	n.a.	32.5 (25.0-40.5)	33 (57.9)	n.a.	33.0 (23.0-48.0)	41.0 (26.0-53.0)

[a] The following variables did not show significant association in the multivariate analysis ($p > 0.10$): (1) traffic-related variables collected by questionnaire: frequency of cars and heavy vehicles at home address; (2) traffic-related variables collected with GIS: distance to nearest major road; (3) time-activity variables: time spent at home, time spent commuting; (4) socio-economic determinants: educational level, working status; (5) time exposed to indoor sources of NO_2: ETS, gas water heater, open windows to ventilate; and (6) height of the balcony/window where the outdoor sampler was placed.

[b] This category includes the following sampling campaigns: November 2003 and November 2004 (from Valencia), and October 2005 (from Sabadell)

[c] This category includes the following sampling campaigns: April 2004 and February 2005 (from Valencia), and April 2005 and March 2006 (from Sabadell)

[d] Not available or not applicable

Multiple linear regression models for outdoor NO_2 concentrations in each subset are presented in Table 2. Month of sampling showed a strong influence on outdoor NO_2 concentrations, particularly in Valencia. In Sabadell, 15.2% of the outdoor NO_2 variability was explained by the month of sampling and type of road, calculated with GIS. In Valencia, month and urbanization degree explained 58.5% of the variability of outdoor NO_2 concentrations. No significant associations were observed between outdoor NO_2 and traffic variables collected through questionnaires in either of the two subsets.

Table 2. Multiple linear regression models for outdoor NO_2 levels (in μg/m^3) in Valencia (n=50) and Sabadell (n = 56)

	Valencia			Sabadell		
Variable	Slope	SE[a]	*p*-value	Slope	SE[a]	*p*-value
Constant	9.078	5.927	0.133	26.900	4.084	<0.001
Month of sampling (ref: February to April[b]) October to November[c]	28.793	4.605	<0.001	12.915	7.102	0.075
Road type at home address (ref: minor road) Major or secondary road	n.a.			18.903	9.609	0.054
Urbanization degree (ref: rural area)						
Semi-urban area	12.326	7.164	0.092	n.a.[d]		
Metropolitan area	19.386	6.757	0.006	n.a.		
Urban area	30.927	8.100	<0.001	n.a.		
R^2 full model	**0.585**			**0.152**		

[a] Standard error
[b] This category includes the following sampling campaigns: April 2004 and February 2005 (from Valencia), and April 2005 and March 2006 (from Sabadell)
[c] This category includes the following sampling campaigns: November 2003 and November 2004 (from Valencia), and October 2005 (from Sabadell)
[d] Not available or not applicable

Table 3 shows that the determinants of indoor levels of NO_2 in the two subsets are outdoor NO_2 concentrations, use of gas cooker, and time using gas heater. Outdoor NO_2 concentration and use of gas cooker are significantly associated with indoor levels in the two subsets, taken together and separately. However, the association between time using a gas heater and indoor NO_2 concentrations is statistically significant in Valencia (p = 0.002) but not in Sabadell (p = 0.747). No significant association was found between indoor NO_2 levels and time exposed to ETS at home. Likewise, educational level, working status, time using a gas water heater, and time with open windows at home were not found to be significant predictors of indoor NO_2.

Table 3. Multiple linear regression models for indoor NO_2 levels (in μg/m^3) in Valencia (n = 49), Sabadell (n = 57) and both (n = 106)

	Valencia			**Sabadell**			**Both**[a]		
Variable	Slope	SE[b]	*p*-value	Slope	SE[b]	*p*-value	Slope	SE[b]	*p*-value
Constant	20.895	5.023	<0.001	10.833	6.370	0.095	27.232	7.421	<0.001
Outdoor NO_2 (μg/m^3)	0.201	0.104	0.060	0.446	0.104	<0.001	0.322	0.071	<0.001
Use of gas cooker during 48-h[c]	13.410	4.753	0.007	16.156	5.736	0.007	13.177	3.544	<0.001
Use of gas heater during 48-h[c]	0.050	0.015	0.002	0.036	0.111	0.747	0.058	0.017	<0.001
R^2 full model	**0.342**			**0.297**			**0.322**		

[a] Adjusted by cohort and month of sampling
[b] Standard error
[c] Reference category: no

The predictive model for personal exposure in Sabadell accounted for almost 60% of the variation in NO_2 personal levels with four variables: outdoor and indoor NO_2 concentrations, time spent outdoors and time using a gas cooker (Table 4). VIF (Variance Inflation Factor) values were lower than 2, indicating low levels of collinearity in the model. The influence of indoor NO_2 levels on personal exposure was more than 2.5 times greater than the influence of outdoor levels. We did not find any significant association between personal NO_2 levels and educational level, working status, time spent at home, time spent commuting, time exposed to ETS at home, time using a gas water heater or gas heater, and time with open windows at home.

Table 4. Multiple lineal regression model (R^2 = 0.59) for personal NO_2 exposure (in μg/m³) in Sabadell (n = 53)

Variable	Slope	SE[a]	*p*-value	VIF[b]
Constant	9.235	4.538	0.048	
Outdoor NO_2 (μg/m³)	0.156	0.082	0.065	1.49
Indoor NO_2 (μg/m³)	0.403	0.101	<0.001	1.56
Time spent outdoors during 48-h (reference: < 2 hours)				
≥ 2 hours	14.441	4.882	0.005	1.06
Time exposed to a gas cooker during 48-h (reference: < 10 minutes)				
10-60 minutes	10.514	4.996	0.041	1.73
> 60 minutes	11.101	4.888	0.028	1.66

[a] Standard error
[b] VIF: Variance Inflation Factor

The results of the regression diagnostics conducted in the three multiple linear regression models (outdoor, indoor and personal NO_2) verified the normality of residuals.

DISCUSSION

Characterizing the relationship between home outdoor, home indoor, and personal levels of air pollution in pregnant women is important to better understand the influence of different sources of air pollution on personal exposure. This may lead to improve exposure assessment for use in epidemiological studies aimed at assessing the effect of prenatal exposure to air pollution on reproductive and children's health (Gilliland et al., 2005). To our knowledge, there is only one published study on personal monitoring of NO_2 among pregnant women, which was conducted in Vancouver, Canada, but home outdoor levels were modeled with GIS techniques and home indoor levels were not measured (Nethery et al., 2008).

This study shows that temporal and traffic-related variables are the main determinants of outdoor NO_2 levels in the INMA cohorts of Sabadell and Valencia. It also indicates that personal exposure to NO_2 of the Sabadell participants is influenced by both home outdoor and indoor NO_2 levels. Outdoor NO_2 levels and use of a gas cooker are determinants of indoor NO_2 concentration in both subsets, unlike the use of gas heater which shows a different influence in Valencia than in Sabadell. In addition, the results indicate that educational level, working status and exposure to ETS at home are not associated with indoor or personal NO_2 levels.

Despite including rural and semi-urban areas, outdoor NO_2 levels were higher in Valencia than in Sabadell, which is exclusively an urban area. The median NO_2 concentration of Sabadell (29 $\mu g/m^3$) was lower than the annual median predicted for the whole cohort using Land Use Regression modeling (38.42 $\mu g/m^3$) (Aguilera et al., 2008). The high percentage of women from the subset living in minor streets (84.2%) could explain these low outdoor NO_2 levels observed in Sabadell. Furthermore, because the sampling campaigns were not performed simultaneously in the two cohorts, these differences can be also attributed to temporal or weather-related variations in air pollution levels.

The results agree with other studies (Janssen et al., 2001; Rijnders et al., 2001; Rotko et al., 2001) in which home outdoor NO_2 levels are shown to be associated with traffic-related variables. Outdoor NO_2 levels in Valencia, where no GIS data on traffic-related variables could be obtained, showed a gradual increase from the rural to the urban area and were significantly associated with the degree of urbanization. Similar results were reported in the Netherlands, where home outdoor NO_2 levels in children were significantly influenced by the degree of urbanization of the city district where they lived (Rijnders et al., 2001). These results suggest that the degree of urbanization can be used to estimate exposure to traffic-related air pollution when no outdoor NO_2 data are available.

As it has been shown in other studies, we found that personal NO_2 levels correlated better with home indoor than outdoor NO_2 levels and the multivariate analysis showed a strong influence of indoor NO_2 levels in personal exposure (Kousa et al., 2001; Levy et al., 1998; Monn et al., 1998; Ramírez-Aguilar et al., 1996). People spend most of their daily time indoors and particularly pregnant women tend to spend more time at home during the last trimester of pregnancy (Nethery et al., 2009). In our study, 42.1% of the women from Sabadell spent more than 75% of their time at home during the 48-h sampling period. In addition, the results support the use of questionnaires on time-activity patterns to improve the prediction of personal exposure levels. Despite spending most of their time at home, the introduction of time spent in outdoor environments improved the personal NO_2 prediction model significantly. This variable could reflect exposure to traffic-related NO_2 in other outdoor locations away from the home address, although other studies did not find higher NO_2 exposures for individuals spending more time outdoors (Brown et al., 2009).

The influence of the use of gas cookers on indoor and personal NO_2 levels has been reported in previous studies (Baxter et al., 2006; García-Algar et al., 2004; Lai et al., 2006; Monn et al., 1998). The association was less consistent for the use of gas heaters, since we found a significant association with indoor NO_2 levels only in Valencia and no association with personal NO_2 levels in either of the two subsets. This can be explained by the small number of women using gas heaters in Sabadell (n = 2; 3.5%), probably because the sampling campaigns took place in rather mild months (April, October and March). Outdoor NO_2 levels were also determinants of indoor concentrations, although a weaker influence was observed in Valencia than in Sabadell. This modest association between outdoor and indoor NO_2 levels has also been observed in other studies (Cyrys et al., 2000; Ponzio et al., 2006; Rojas-Bracho et al., 2002). One possible explanation for the differences between the two cohorts is that the higher use of gas water heaters in Valencia could result in a greater contribution of indoor sources and consequently a weaker association between indoor and outdoor levels than in Sabadell.

Surprisingly, we did not find any influence of time exposed to ETS at home on either indoor or personal NO_2 concentrations. Although there is another example of such no association in the literature (Zipprich et al., 2002), most studies have found higher indoor or personal concentrations of NO_2 in people exposed to ETS (García-Algar et al., 2004; Lai et al., 2006; Levy et al., 1998; Monn et al., 1998). Because our study subjects are pregnant women, time exposed to ETS at home could has been affected by their pollution-avoiding behavior.

The study is not very conclusive about the role of the season on NO_2 levels. Outdoor NO_2 levels in Valencia were higher when the sampling campaigns were performed between October to November than between February to April, and they were generally higher than indoor levels. This could be due to specific weather conditions that could have provoked higher outdoor NO_2 levels in certain campaigns. Moreover, the sampling campaigns were not undertaken simultaneously in the two subsets, the sampling period (48 hours) was too short to observe clear seasonal differences and the two categories (from October to November and from February to April) may not represent enough variation in weather conditions over the year.

The multivariate analysis was not able to explain all the variability in NO_2 levels. Additional determinants such as weather-related variables (wind direction and speed, humidity, temperature, etc.) could explain outdoor NO_2 variability but were not able to be assessed in this study due to the lack of accurate area-specific meteorological data. In terms of indoor NO_2, the unexplained variation could be in part due to the unknown use of kitchen extractor fans, the living room size or the distance between the indoor samplers (which were located in the living room) and the indoor sources of NO_2 (particularly gas appliances).

Finally, additional data on NO_2 levels at work and other microenvironments would probably lead to a better personal exposure estimate.

Another limitation of the study is the small sample size and the fact that both subsets are non-random samples, although these two factors are present in most other studies of personal exposure to air pollution (Brown et al., 2008; Nethery et al., 2008; Sørensen et al., 2005). Therefore, our findings are not extrapolable to the general population of pregnant women. Despite these limitations, the results of this study can be used to develop NO_2 personal exposure models in women of the INMA Study for whom there is available information on the determinants here studied. These personal exposure models could be further applied to assess the relationship between prenatal exposure to air pollution and early childhood effects.

Considering NO_2 as a marker of exposure to other more harmful traffic-related air pollutants such as fine particles, this study has shown the influence of traffic emissions on indoor and personal levels. In addition, our results should contribute to a better understanding of the relationship between outdoor, indoor and personal NO_2 concentrations in southern Europe, where there is limited information on this issue. In the case of epidemiological studies that are not able to sample personal exposure, the study supports the use of time-activity patterns along with indoor measurements to predict personal exposure to traffic-related air pollution.

Acknowledgements

We thank the women from the INMA cohorts of Valencia and Sabadell who participated in this study and our colleagues from CREAL and CSISP for their help on the field work. We also thank Rosalía Fernández-Patier and Amelia Aguirre-Alfaro (Centro Nacional de Sanidad Ambiental, Instituto de Salud Carlos III) for the laboratory analysis of passive samplers and Anna Sillero for her help in editing the manuscript. The INMA Study has the financial support of the 'Instituto de Salud Carlos III' (G03/176) and 'RCESP' (C03/09). This study has also been supported in part by the 'Fondo de Investigaciones Sanitarias' for the Valencia cohort (FIS 03/1615 and FIS 04/1509).

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ARTÍCULO 3: Association between GIS-based exposure to urban air pollution during pregnancy and birth weight in the INMA-Sabadell cohort

Environmental Health Perspectives 2009; 117(8): 1322-1327

Inmaculada Aguilera[1,2,3,4], Mònica Guxens[1,2,3], Raquel Garcia-Esteban[1,2,3], Teresa Corbella[5], Mark J Nieuwenhuijsen[1,2,3], Carles M Foradada[6], and Jordi Sunyer[1,2,3,4]

[1] Centre for Research in Environmental Epidemiology, Barcelona, Spain

[2] Municipal Institute of Medical Research (IMIM-Hospital del Mar), Barcelona, Spain

[3] CIBER en Epidemiología y Salud Pública, Barcelona, Spain

[4] Pompeu Fabra University, Barcelona, Spain

[5] Servei de Salut, Ajuntament de Sabadell, Spain

[6] Servei de Ginecologia i Obstetrícia, Hospital de Sabadell, Spain

Corresponding author:
Inmaculada Aguilera
Centre for Research in Environmental Epidemiology (CREAL)
Barcelona Biomedical Research Park
Doctor Aiguader 88. 08003 Barcelona, Spain
Tel. +34 932147300 / Fax + 34 932147301
E-mail: iaguilera@creal.cat

Short running head: Traffic-Related Air Pollution and Birth Weight

Aguilera I, Guxens M, Garcia-Esteban R, Corbella T, Nieuwenhuijsen MJ, Foradada CM, Sunyer J.
Association between GIS-based exposure to urban air pollution during pregnancy and birth weight in the INMA Sabadell Cohort.
Environ Health Perspect. 2009 Aug;117(8):1322-7.

ABSTRACT

Background: There is growing evidence that traffic-related air pollution reduces birth weight. Improving exposure assessment is a key issue to advance in this research area.

Objective: We investigated the effect of prenatal exposure to traffic-related air pollution via geographic information system (GIS) models on birth weight in 570 newborns from the INMA-Sabadell cohort.

Methods: We estimated pregnancy and trimester-specific exposures to nitrogen dioxide and aromatic hydrocarbons [benzene, toluene, ethylbenzene, *m/p*-xylene, and *o*-xylene (BTEX)] by using temporally-adjusted land-use regression (LUR) models. We built models for NO_2 and BTEX using four and three 1-week measurement campaigns, respectively, at 57 locations. We assessed the relationship between prenatal air pollution exposure and birth weight with linear regression models. We performed sensitivity analyses considering time spent at home and time spent in nonresidential outdoor environments during pregnancy.

Results: In the overall cohort, neither NO_2 nor BTEX exposure was significantly associated with birth weight in any of the exposure periods. When considering only women who spent < 2 hr/day in nonresidential outdoor environments, the estimated reductions in birth weight associated with an interquartile range increase in BTEX exposure levels were 77 g [95% confidence interval (CI), 7-146] and 102 g (95% CI, 28-176) for exposures during the whole pregnancy and the second trimester, respectively. The effects of NO_2 exposure were less clear in this subset.

Conclusions: The association of BTEX with reduced birth weight underscores the negative role of vehicle exhaust pollutants in

reproductive health. Time-activity patterns during pregnancy complement GIS-based models in exposure assessment.

INTRODUCTION

Fetal growth is an important indicator of the health of newborns and infants that may influence the health status in the adulthood (Sinclair et al. 2007). In the recent years, a growing body of research has associated prenatal exposure to air pollution with adverse pregnancy outcomes including intrauterine growth restriction (IUGR), low birth weight (LBW), preterm birth (PTB) and intrauterine mortality. Detailed reviews of these studies have concluded that the strength of the evidence differs among air pollutants, birth outcomes and exposure periods, although differences in study design, exposure assessment, and definition of outcomes make comparability of results difficult (Glinianaia et al. 2004; Lacasaña et al. 2005; Šrám et al. 2005; Wang and Pinkerton 2007).

To advance in this emerging and fast-growing field, some key methodological issues have been highlighted (Gilliland et al. 2005; Ritz and Wilhelm 2008; Slama et al. 2008a). Because most studies have linked birth outcomes and covariates from birth certificate records with routinely measured air pollutants, one priority is to develop prospective cohort studies that are able to obtain high-quality individual data on outcomes, covariates and exposure estimates. Because pregnancy is a well-defined and relatively narrow period of exposure, identification of windows of greater susceptibility to air pollution is also a key issue, but is difficult because of the lack of biological knowledge and the correlations among trimester or month-specific exposures. Furthermore, exposure assessment can be improved by using approaches based on geographic information systems (GIS) that take into account small area variations in vehicle exhaust pollutants, such as land-use regression (LUR). Because LUR models have mainly been used for estimating annual average exposures, being able to accurately incorporate temporal variability in the LUR models is key for studies of birth outcomes, where shorter-term exposures are of interest. Improving exposure assessment also requires consideration of women's residential mobility (Fell et al. 2004) and time-activity patterns during pregnancy (Nethery et al. 2009).

In this study we assessed the relationship between GIS-based exposure to traffic-related air pollution during pregnancy and birth weight in an urban cohort from the Spanish INMA (Environment and Childhood) Study. We also examined the influence of time-activity patterns during pregnancy in the association between air pollution and birth weight.

METHODS

Cohort

The study area is Sabadell, a city of nearly 200,000 inhabitants situated in the metropolitan area of Barcelona, Spain. Women who visited the public health center of Sabadell in the 12th week of pregnancy and fulfilled the inclusion criteria were eligible to participate in the study (Ribas-Fitó et al. 2006). Main exclusion criteria were being < 16 years of age, nonsingleton pregnancy, not planning to deliver at the Hospital of Sabadell, and having followed an assisted reproduction program.

Women were interviewed in the 12th and 32nd weeks of pregnancy and answered several questionnaires on sociodemographic characteristics, health status, use of drugs, occupational data, environmental exposures, time-activity patterns, and a food-frequency questionnaire. The protocol of the INMA study including a detailed description of data collection and assessment of determinants and outcomes has been published elsewhere (Ribas-Fitó et al. 2006). The study was approved by the Ethical Committees of the Municipal Institute of Medical Research and the Hospital of Sabadell, and all subjects gave written informed consent before participating.

A total of 657 women were enrolled in the study between June 2004 and July 2006. This sample was representative of the target population in terms of women's attendance at prenatal care in the public health system (used by 85% of the pregnant women in Sabadell), but the educational level of our sample was higher than the target population average. From the initial sample we followed 619 (94%) women until the child's birth. We excluded 44 children from the analysis because their mothers did not live in Sabadell during pregnancy but in nearby cities covered by the health service of the municipal hospital. We also excluded three children with no recorded birth weight and two with gestational duration of 28 and 32 weeks, respectively, because of missing data in the covariates obtained in the 32nd week interview. Finally, 570 (87 %) children were included in the analysis.

Air pollution exposure model

We used Land-use regression (LUR) modeling in the study area to estimate individual exposure to nitrogen dioxide and BTEX (benzene, toluene, ethylbenzene, *m/p*-xylene, and *o*-xylene) as markers of motor vehicle exhaust pollution. A complete description of the methodology on exposure modeling has been reported previously (Aguilera et al. 2008). Briefly, we measured NO_2 and BTEX with passive samplers in four and three sampling campaigns of 1 week, respectively, between April 2005 and March 2006. Measurements were conducted simultaneously at 57 sampling sites (29 urban background and 28 traffic sites) representing the gradient of exposure in the study population. For each pollutant, we calculated average concentrations of all the sampling campaigns, assuming that they are representative of annual mean levels of NO_2 and BTEX (Lebret et al. 2000), and fitted linear regression models using five groups of geographic data (land coverage, topography, population density, roads, and distance to local sources of pollution) as predictor variables. Geographic variables were stored and derived in ArcGIS, version 9.1 (ESRI, Redlands, CA, USA). The final model for NO_2 ($R^2 = 0.75$) included altitude, road type (major, secondary or minor road), and a land cover factor within a 500-m buffer as predictor variables. Geographic variables included in the final BTEX model ($R^2 = 0.74$) were altitude and three source-proximity variables (distance to nearest major road, secondary road, and parking lot). We used two cross-validation procedures to evaluate the precision of the regression models.

We then applied models to predict outdoor air pollution levels at the cohort addresses. For women who changed their home address during pregnancy (n = 25, 4%), exposure was calculated using the estimated concentrations at both the old and new home address, weighted by the percentage of the pregnancy period spent in each of them.

We adjusted models for temporal variations to calculate term-specific individual exposures, as it has been done in other studies relying on LUR exposure models (Brauer et al. 2008; Slama et al. 2007). To obtain an average exposure for the whole pregnancy period and for each trimester, we used temporal variations of air pollution measured in the only fixed monitoring station operating in Sabadell. The station is located on a traffic island in the middle of a main road, in a relatively open area with unobstructed air flow. Daily measurements of air pollution conducted simultaneously in this traffic site and in an urban background location during 1 month showed similar temporal variations in NO_2 levels between the two sites, with a correlation coefficient of 0.96 (Rivas-Lara 2008). We averaged daily means of NO_2 measured at the fixed station over the pregnancy period for each woman. The resulting value was divided by the average NO_2 concentration corresponding to the whole sampling period (from April 2005 to March 2006) and multiplied by the predicted value obtained in the LUR model. We applied the same procedure to estimate trimester-specific exposures for each woman. We defined the first trimester of pregnancy as weeks 1-13, the second trimester as weeks 14-26, and the third trimester as the period from week 27 until birth.

Regarding BTEX, the fixed monitoring station measures daily mean levels of benzene and toluene but the high percentage of missing data (65% of the sampling period) did not allow us to "seasonalize" the BTEX model with them. Because NO_2 showed higher correlation with benzene and toluene in the fixed monitoring station than with other traffic-related pollutants such as carbon monoxide or particulate matter (PM), and given the high correlation between NO_2 and BTEX levels measured with the passive samplers (r=0.80 for the whole sampling period), we used NO_2 daily levels to make the temporal adjustment, assuming that the temporal variations in both pollutants were similar.

Birth weight and gestational age

Birth weight was recorded by specially trained midwives at delivery. We calculated gestational age from the date of the last menstrual period (LMP) reported at recruitment and confirmed using estimates based on ultrasound examination in the 12th week of gestation. When the difference between the LMP reported at recruitment and estimated from the ultrasound was ≥ 7 days (n = 91; 16%), we estimated LMP using a quadratic regression formula defined by Westerway et al. (2000).

Statistical analysis

We examined the association between birth weight and prenatal exposure to NO_2 and BTEX by simple and multiple linear regression models. Other reproductive outcomes such as LBW or small for gestational age were not considered for analysis because of the relatively low sample size. Given the high correlation among the five BTEX compounds (r > 0.75) and because the relative fetal toxicity of each of them is not well known (Agency for Toxic Substances and Disease Registry 2004), we used the LUR estimate of the sum of the five compounds to assess the relationship between BTEX exposure and birth weight.

We chose covariates included in the analysis based on previous knowledge on their influence on birth weight. We collected some through questionnaire in the two interviews carried out during pregnancy for each woman: maternal age, maternal education, maternal ethnicity, parity, maternal height and prepregnancy weight, and paternal height and weight were obtained in the 12th week interview; tobacco use and passive smoking information were collected in the 32nd week interview. Birth date and child sex were collected from the child neonatal anthropometry record filled in by the midwives. We calculated season of conception using date of LMP. Because season of birth is influenced by the duration of pregnancy, we used season of conception in the analysis rather than season of birth.

With linear regression models, we estimated the change in birth weight for an interquartile range (IQR) increase in NO_2 and BTEX exposure (in $\mu g/m^3$), for each trimester and for the entire pregnancy. We retained as adjustment factors only covariates that modified the association between air pollution and birth weight by > 10%. Because fetal weight gain per week is not constant throughout pregnancy, we examined the association between birth weight and gestational age by using fractional polynomial models to identify the best-fit transformation of gestational age and allow polynomial terms for gestational age in the linear regression models (Blair et al. 2005).

We performed a sensitivity analysis considering time-activity patterns during pregnancy. In the 32nd week interview, women answered the following question for a typical weekday and for a typical weekend:

‘Since you have gotten pregnant, how much time have you typically spent daily in these environments? The answer options were (a) home indoors, (b) work indoors, (c) in other people’s houses, (d) in other indoor environments, (e) home outdoors, (f) work outdoors, (g) in other outdoor environments, and (h) in means of transportation’. The question was designed to obtain a 24-hr sum. We weighted the data to account for weekdays (5 of 7) and weekends (2 of 7) and then calculated time spent at home (answers a+e) and time spent in nonresidential outdoor environments (answers f+g). We used the median (rounded to the nearest whole number) as a cut-off value to restrict our analysis to two subsets: (1) women who spent more time at home; and (2) women who spent less time in nonresidential outdoor environments. Because we based LUR estimates on the women’s residential addresses, we assumed that these two subsets suffered less from exposure misclassification and that misclassification was non-differential.

We performed statistical analyses using Stata 8.2 (Stata Corp., College Station, TX, USA).

RESULTS

Mean birth weight of included births was 3,247 g (10th, 50th and 90th percentiles: 2,721, 3,288 and 3,760 g) and mean maternal age was 31.4 years (minimum and maximum: 18.2 and 43 years, respectively). Table 1 shows other characteristics of the study population and mean birth weight for each categorized variable. Birth weight was associated ($p < 0.10$) with child’s sex, season of conception, parity, tobacco smoke, passive smoking, maternal ethnicity, gestational age, maternal height and prepregnancy weight, and paternal height and weight.

Table 1. Characteristics of the study population (n = 570)

Categorized variables	N (%)	Birth weight (g)	*p*-value[a]
Child's sex			<0.001
Male	288 (50.5)	3316	
Female	282 (49.5)	3177	
Season of conception			0.04
Spring	158 (27.7)	3217	
Summer	167 (29.3)	3248	
Fall	132 (23.2)	3332	
Winter	113 (19.8)	3188	
Tobacco smoking during pregnancy			<0.001
0	467 (83.7)	3277	
1-5 cigarettes/day	61 (10.9)	3200	
> 5 cigarettes/day	30 (5.4)	3023	
Passive smoking during pregnancy			0.03
Yes	241 (43.2)	3207	
No	317 (56.8)	3285	
Maternal parity			0.09
0	323 (56.9)	3221	
≥1	245 (43.1)	3283	
Maternal education			0.11
Primary education	166 (29.3)	3197	
Secondary education	237 (41.8)	3258	
University degree	164 (28.9)	3274	
Maternal race/ethnicity			0.01
White/Caucasian	551 (96.8)	3238	
Latin American	14 (2.5)	3595	
Black	4 (0.7)	3136	
Continuous variables	**Median**	**IQR**	***p*-value[b]**
Maternal age (years)	31.1	5.7	0.43
Gestational age (weeks)	39.9	1.9	<0.001
Maternal height (cm)	162	8.3	0.01
Maternal pre-pregnancy weight (kg)	60	14	<0.001
Paternal height (cm)	175	8	0.02
Paternal weight (kg)	79	15	0.01

[a] *p*-values for comparing means by *t*-test or ANOVA
[b] *p*-values of Pearson correlation coefficients between each continuous variable and birth weight

We examined whether air pollution exposure was associated with maternal education as a surrogate of socioeconomic status (SES). We found a small but statistically significant association between LUR estimates of BTEX levels and maternal education (p=0.02). Predicted annual mean levels of BTEX were 17.6, 16.0 and 16.1 μg/m^3 for women with a university degree, secondary education and primary education, respectively. The corresponding NO_2 values for the three categories were 37.4, 35.7 and 35.7 μg/m^3 (p=0.14).

Tables 2 and 3 provide the distribution of 9-month and trimester-specific exposures to NO_2 and BTEX and the correlation coefficients among them, respectively. We found only slight differences between mean exposure levels by trimester and 9-month exposures, although the range of exposure was wider for the three trimester exposures than for the whole pregnancy period. According to these estimates, 14 % of the women had an average NO_2 exposure > 40 μg/m^3 for the entire pregnancy period, which is the European Union limit value to come into force in 2010 (European Commission 1999). Correlation coefficients among the three trimesters ranged from 0.45 to 0.50 for NO_2 and from 0.72 to 0.74 for BTEX, reflecting small seasonal variation in exposure.

Table 2. Distribution of nine-month and trimester exposures to NO_2 and BTEX (μg/m^3)

Pollutant (period)	Mean (SD)	Min	P25	Median	P75	Max
NO_2 (9-month)	32.17 (8.89)	17.37	26.40	30.77	35.91	68.45
NO_2 (1st trimester)	32.66 (10.56)	9.59	25.83	31.81	38.10	74.30
NO_2 (2nd trimester)	31.86 (10.57)	10.33	24.98	30.97	36.98	77.47
NO_2 (3rd trimester)	32.67 (10.60)	10.18	25.19	31.81	37.66	74.37
BTEX (9-month)	14.65 (5.52)	3.95	10.25	14.65	18.67	27.63
BTEX (1st trimester)	14.91 (6.21)	2.44	9.78	14.68	19.57	29.51
BTEX (2nd trimester)	14.49 (6.05)	2.69	9.42	13.82	19.46	31.30
BTEX (3rd trimester)	14.88 (6.24)	2.62	9.86	14.03	19.58	31.69

Table 3. Spearman correlation coefficients between estimated air pollutant's concentrations by nine-month and trimester exposures

Exposure	NO_2				BTEX			
	pregnancy	first trimester	second trimester	third trimester	pregnancy	first trimester	second trimester	third trimester
NO_2								
pregnancy	1							
1st trimester	0.79	1						
2nd trimester	0.79	0.50	1					
3rd trimester	0.80	0.45	0.46	1				
BTEX								
pregnancy	0.77	0.62	0.60	0.63	1			
1st trimester	0.69	0.80	0.45	0.43	0.90	1		
2nd trimester	0.71	0.48	0.80	0.44	0.90	0.74	1	
3rd trimester	0.72	0.46	0.43	0.81	0.91	0.74	0.72	1

All correlation coefficients are significantly different from 0 (p-value<0.01)

Table 4 shows time-activity patterns reported in the 32nd week interview and referring to the entire pregnancy. Differences between weekdays and weekends were statistically significant for all the activities. During weekdays, women who did not work during pregnancy or worked only during part of it (n=350) spent more time at home and in nonresidential outdoor environments, and less time in means of transportation, compared with women who worked during the entire pregnancy (n=210) (Mann-Whitney test, P<0.05). we found no differences in total time spent in indoor environments between the two groups.

Table 4. Hours/day in specific activities/locations during pregnancy (reported in the 32nd week of pregnancy)

	Weekdays				Weekends			
Activity[a]	**Mean (SD)**	**P10**	**Median**	**P90**	**Mean (SD)**	**P10**	**Median**	**P90**
Indoor								
a. Home	15.1 (3.3)	11.2	14.5	19.6	16.0 (3.1)	12.0	16.0	20.0
b. Work	4.4 (3.8)	0.0	5.0	9.0	0.2 (1.2)	0.0	0.0	0.0
c. Other people's houses	1.0 (1.4)	0.0	0.5	3.0	2.3 (1.9)	0.0	2.0	4.0
d. Other indoor environments	1.0 (0.8)	0.0	1.0	2.0	1.6 (1.3)	0.0	2.0	3.0
Outdoor								
e. Home	0.1 (0.4)	0.0	0.0	0.0	0.2 (1.0)	0.0	0.0	0.3
f. Work	0.2 (0.8)	0.0	0.0	0.0	0.0 (0.5)	0.0	0.0	0.0
g. Other outdoor environments	1.4 (1.1)	0.3	1.0	3.0	2.7 (1.8)	1.0	2.0	5.0
Walking[b]	0.9 (0.9)	0.3	0.8	0.2	1.3 (1.1)	0.5	1.0	2.0
Means of transportation[c]	0.8 (0.8)	0.0	0.5	2.0	1.0 (0.7)	0.0	1.0	2.0
Car	0.6 (0.8)	0.0	0.5	1.5	0.9 (0.7)	0.0	1.0	2.0
Bus	0.2 (0.9)	0.0	0.0	0.5	0.0 (0.1)	0.0	0.0	0.0
Metro/train	0.1 (0.3)	0.0	0.0	0.0	0.0 (0.1)	0.0	0.0	0.0
Total in non-residential outdoor environments[d] (f+g)	1.6 (1.3)	0.3	1.0	3.0	2.7 (1.8)	1.0	2.0	5.0
Total at home (a+e)	15.1 (3.4)	11.5	14.5	20.0	16.2 (3.0)	12.5	16.4	20.0
Total in indoor environments (a+b+c+d)	21.5 (1.6)	20.0	22.0	23.0	20.1 (2.2)	17.0	20.5	22.5

See "Materials and Methods" for time-activity questions (a-h).
[a] Differences between weekdays and weekends are statistically significant for all the activities (Wilcoxon signed ranks test, $p < 0.05$).
[b] Women reported specifically the amount of time spent walking as part of the time spent in other outdoor environments.
[c] Mean, 10th percentile, median, and 90th percentile values for bicycle and motorcycle categories were 0.
[d] This activity refers to time spent in outdoor environments other than at the home address.

Table 5 presents the effect of air pollution exposure during pregnancy and during each trimester on birth weight. Neither NO_2 nor BTEX exposure was significantly associated with the outcome in any of the exposure periods. Associations for BTEX were more pronounced in the subset of women who spent ≥ 15 hr/day at home (n = 276), but they were also not statistically significant. However, when considering only women who spent < 2 hr/day in nonresidential outdoor environments (n = 259), BTEX exposure both during the whole pregnancy period and the second trimester showed a statistically significant negative effect on birth weight. Estimated reductions in birth weight for an IQR increase of BTEX exposure were 76.6 g and 101.9 g during pregnancy and the second trimester, respectively. The negative effect of NO_2 exposure variables in this subset of women was less clear, but showed some stronger effects during the second trimester of pregnancy (p=0.09).

Because the three trimester exposures of both pollutants (particularly BTEX) were correlated, we also adjusted models for trimester-specific exposures (Table 5). Associations found for BTEX and NO_2 exposures in the second trimester were more pronounced in the whole cohort and in the two subsets, but only statistically significant among women who spent < 2 hr/day in nonresidential outdoor environments. Variance Inflation Factor values ranged from 1.76 to 1.96 for NO_2 and from 2.53 to 2.89 for BTEX, indicating acceptable levels of collinearity in the multitrimester models.

Table 5. Change (coefficient) in birth weight (g) for an IQR increase (μg/m^3) in exposure to NO_2 and BTEX at the entire pregnancy period and each trimester in 570 newborns from INMA-Sabadell.

	Birth weight [g (95% CI)]		
	All women (n=570)	Women who spent ≥ 15 hours/day at home (n=276)	Women who spent < 2 hours/day in nonresidential outdoor environments (n=259)
Crude			
NO_2			
9 months	-6.1 (-43.3 to 31.2)	-21.4 (-75.9 to 33.2)	-14.4 (-68.5 to 39.6)
1st trimester	-4.5 (-45.2 to 36.2)	-6.1 (-67.4 to 55.2)	-1.2 (-61.4 to 59.1)
2nd trimester	-18.1 (-57.5 to 21.4)	-45.2 (-103.4 to 12.9)	-46.2 (-103.7 to 11.2)
3rd trimester	4.8 (-36.2 to 45.7)	-7.4 (-67.4 to 52.6)	6.7 (-52.2 to 65.6)
BTEX			
9 months	-9.6 (-62.8 to 43.6)	-37.2 (-114.5 to 40.1)	-61.9 (-140.0 to 17.6)
1st trimester	-10.9 (-66.1 to 44.3)	-27.3 (-110.3 to 55.7)	-50.0 (-132.0 to 32.1)
2nd trimester	-26.4 (-84.5 to 31.7)	-68.0 (-152.8 to 16.8)	-98.5 (-184.0 to -13.0)*
3rd trimester	6.5 (-47.7 to 60.8)	-15.8 (-94.7 to 63.0)	-29.7 (-110.7 to 51.2)
Adjusted (each trimester separately)[a]			
NO_2			
9 months	8.8 (-23.8 to 41.5)	8.6 (-37.9 to 55.2)	-18.6 (-66.3 to 29.1)
1st trimester	3.3 (-33.2 to 39.7)	9.8 (-43.2 to 62.9)	4.2 (-49.3 to 57.6)
2nd trimester	3.7 (-31.1 to 38.4)	-1.5 (-50.9 to 47.9)	-42.3 (-92.1 to 7.4)
3rd trimester	16.8 (-18.8 to 52.4)	15.8 (-35.1 to 66.6)	-9.0 (-60.5 to 42.6)
BTEX			
9 months	-7.6 (-54.9 to 39.8)	-13.0 (-79.3 to 53.3)	-76.6 (-146.3 to -7.0)*
1st trimester	-12.0 (-62.0 to 38.0)	-16.3 (-87.8 to 55.2)	-52.5 (-125.8 to 20.8)
2nd trimester	-13.3 (-65.1 to 38.4)	-22.5 (-95.1 to 50.0)	-101.9 (-176.2 to -27.6)*
3rd trimester	2.5 (-45.3 to 50.4)	-1.4 (-68.8 to 66.0)	-59.7 (-130.9 to 11.5)
Adjusted (all trimesters together)[b]			
NO_2			
1st trimester	-7.6 (-56.8 to 41.5)	12.7 (-63.0 to 88.4)	54.8 (-18.5 to 128.0)
2nd trimester	-5.8 (-52.7 to 41.0)	-22.8 (-90.7 to 45.0)	-74.7 (-140.4 to -9.0)*
3rd trimester	24.6 (-22.1 to 71.3)	22.1 (-46.1 to 90.2)	0.9 (-64.4 to 66.3)
BTEX			
1st trimester	-19.2 (-101.0 to 62.6)	-13.4 (-139.9 to 113.0)	60.8 (-61.8 to 183.5)
2nd trimester	-21.7 (-104.6 to 61.3)	-42.3 (-161.4 to 77.0)	-137.3 (-252.6 to -22.0)*
3rd trimester	30.0 (-44.8 to 104.9)	35.8 (-74.8 to 146.4)	-16.7 (-123.3 to 89.8)

[a] Adjusted for child's sex, gestational age, season of conception, parity, maternal educational level, maternal smoking during pregnancy, maternal height and pre-pregnancy weight and paternal height.
[b] Adjusted for above variables and exposures to the same pollutant during the other two trimesters
* *p*-value <0.05

DISCUSSION

We found an effect of exposure to BTEX, and to a lesser extent NO_2, during the second trimester of pregnancy on birth weight among a subset of women who spent < 2 hr/day in outdoor environments during pregnancy, after controlling for exposure to the same pollutant during the other two trimesters. Exposure to BTEX during the whole pregnancy period was also significantly associated with birth weight for the same subset. The magnitude of the association was higher for BTEX in all the exposure periods. Overall, exposure during the second trimester appeared to be the most harmful, and the association became larger after adjusting for trimester-specific exposures.

Identifying critical exposure windows is a research need but a difficult task because of differences in mixture of pollutants across space and time, as well as possible different effects of specific pollutants during specific exposure periods (Slama et al. 2008a). In addition, there is currently a lack of toxicologic information to help guide selection of relevant exposure periods for most fetal growth end points (Ritz and Wilhelm 2008). To our knowledge, this is the first study assessing the relationship between prenatal exposure to ambient BTEX and birth weight, so we cannot compare our results with those of other studies. Regarding NO_2, the evidence of a susceptible window of exposure is unclear. Some studies found an adverse effect of NO_2 on birth weight in the second trimester of pregnancy (Lee et al. 2003; Mannes et al. 2005), whereas others identified first trimester of exposure to NO_2 as the only period influencing fetal growth, measured as continuous birth weight, LBW or IUGR (Bell et al. 2007; Ha et al. 2001; Salam et al., 2005). Two studies found an association between NO_2 and birth weight for the whole pregnancy but did not identify any specific harmful exposure period (Brauer et al. 2008; Liu et al. 2007). Finally, other studies did not observe any significant association between NO_2 and fetal growth (Gouveia et al. 2004; Hansen et al. 2007; Liu et al. 2003; Slama et al. 2007). However, between-study comparisons are limited by differences in study design, exposure assessment and different outcome definitions (IUGR or birth weight treated as continuous or dichotomous variable).

We found reductions in birth weight with increases in BTEX concentrations only among women who spent < 2 hr/day in nonresidential outdoor locations. This could potentially be due to less exposure misclassification (assumed nondifferential) in residence-based LUR estimates for this subset. Although representing a small portion of total daily activity, time spent outdoors can signify direct exposure to traffic-related pollutants. Thus, women who spent a considerable amount of time (≥ 2 hr/day) in nonresidential outdoor environments could have been exposed to a high variability of traffic-related NO_2 and BTEX levels, very different than those reflected by the LUR estimates based on the residential address. This hypothesis is supported by results obtained for a subset of 53 women of this cohort in their third trimester of pregnancy, selected to represent the geographic distribution of the cohort addresses, and for which personal levels of NO_2 were measured with passive samplers during 48 hr. In this subset, women who spent ≥ 2 hr/day in nonresidential outdoor environments (reported for the 48-hour measurement period) showed higher personal levels of NO_2 (β=14.4 $\mu g/m^3$; 95% confidence interval, 4.6-24.3 $\mu g/m^3$), compared with the reference group (< 2 hr/day) (Valero N et al., submitted). A study conducted in Athens also found that time spent outdoors in the city center was a major contributor to personal exposure to toluene and xylenes (Alexopoulos et al. 2006). Nethery et al. (2008) found a better correlation ($r = 0.72$) between 48-hour personal exposure to nitric oxide and LUR estimates based on home address in a subset of pregnant women who spent > 65% of sampling time at home, compared with those who spent ≤ 65% ($r = 0.31$). Although not statistically significant, effect estimates for BTEX in our cohort were also more pronounced among women who spent more time at home, compared with the whole cohort. Overall, results reinforce the need of considering time-activity patterns during pregnancy to better characterize the exposure (Ritz and Wilhelm 2008).

The inclusion of LUR estimates based on work addresses also could improve exposure assessment among employed women (Nethery et al. 2008). In our study, we were unable to account for work-based LUR estimates for the whole subset of employed women because approximately 25% of them worked outside the area covered by our LUR models and 16% reported imprecise work addresses that we were unable to geocode. We did not conduct a sensitivity analysis by working status because 37% (n=160) of the 437 women who were employed at the beginning of the study changed their working status during the 12th and 32nd-week interviews, making the trimester-specific classification of working status in this subset prone to error, particularly for the second trimester of pregnancy. Instead, we investigated differences in time-activity patterns by working status during the whole pregnancy and found that women who worked during the entire pregnancy spent less time in nonresidential outdoor environments. This suggests that this time-activity variable, although reported mainly as a walking activity, is not an indicator of commuting but of a wider variety of transit activities.

Several studies have reported seasonal patterns both in air pollution levels and in birth weight (Hazenkamp-von Arx et al. 2004; Murray et al. 2000). In Sabadell, daily mean levels of NO_2, benzene and toluene (measured at the fixed monitoring station) were higher in winter and lower in summer during the study period, probably due to seasonal differences in meteorologic conditions and traffic intensity. We also have found a seasonal pattern in birth weight, with lowest birth weights seen in infants conceived in winter. This effect is larger than that observed in other studies (Jedrychowski et al. 2004; Slama et al. 2007; Wilhelm and Ritz 2005) and independent from the air pollution effects (p for interaction > 0.10), suggesting that seasonal effects on birth weight could be related to seasonal factors other than air pollution, such as ambient temperature and sunlight (Murray et al. 2000; Tustin et al. 2004).

LUR estimates of NO_2 and BTEX levels were higher among women with higher educational level, compared with women with secondary or primary education. This could be explained by the higher percentage of women with university degree living in the city center, which is one of the districts with higher air pollution levels because of its higher road density and economic activity (Aguilera et al. 2008). O'Neill et al. (2003) found that people with lower SES tend to live in areas with higher levels of air pollution in North America. This evidence is more limited in Europe, with some studies suggesting that the inverse association may occur (Forastiere et al. 2007; Hoek et al. 2002). Differences between European and North American cities in their structure and social class distribution could explain these discrepancies. Within Europe, southern European cities have a more dense structure of roads and buildings, and thus higher traffic emissions, particularly in central districts (Muñoz 2003). Although preliminary, our results support the hypothesis that higher SES can be associated with living in more polluted areas in south European cities.

One of the strengths of our study is estimation of individual exposure to traffic-related air pollutants based on temporally-adjusted LUR models applied to geocoded home addresses, whereas most studies assess exposure by using routinely measured air pollution levels and community-level residence (census data or postal codes). To date, only two studies have also applied temporally-adjusted LUR models in Munich, Germany, and Vancouver, Canada (Brauer et al. 2008; Slama et al. 2007), although they did not take into account time-activity patterns as potential factors affecting exposure misclassification. In addition, we accounted for residential mobility during pregnancy when assigning exposures and we were able to control for a considerable number of potential confounders not always available in studies relying on data from birth certificates, such as quantitative measures of maternal smoking, passive smoking, or maternal and paternal weight and height.

Because we used ultrasound measurements to correct reported LMP dates that differed in $\geq$ 7 days from the ultrasound-based estimates, corrected gestational age could be biased if air pollution exposure shows an early effect on fetal growth (Slama et al. 2008b). However, analysis of gestational age (both corrected and noncorrected) by exposure categories during the first trimester indicated that the correction of gestational age was not biased by air pollution effects.

A limitation of this study was the relatively small sample size, which limited our ability to investigate other birth outcomes (i.e. PTB or IUGR) and evaluate interactions between air pollution exposure and potential effect modifiers such as maternal nutrition (Kannan et al. 2006). In addition, because we used daily mean levels of NO_2 to temporally adjust the BTEX exposure model, identification of the second trimester as the most susceptible to BTEX exposure needs careful interpretation. Because NO_2 and BTEX were highly correlated in space and time and both originate mainly from vehicle emissions in the study area, it remains unclear whether the more pronounced effect found for BTEX was independent of other traffic-related pollutants. Considering that NO_2 is mainly a secondary pollutant (formed from the oxidation of NO primary emissions) and that LUR estimates of BTEX capture the influence of additional traffic emission sources such as parking lots, our results suggest that BTEX could be a more specific marker for exhaust toxins of concern for pregnancy in studies conducted within urban areas.

CONCLUSIONS

We found an effect of exposure to traffic-related air pollutants (BTEX, and to a lesser extent NO_2) on birth weight among pregnant women who live in an urban area and spent < 2 hr/day in nonresidential outdoor locations. Although the magnitude of the association was higher for BTEX, the independent effect of different air pollutants with common emission sources remains to be determined. When possible, time-activity patterns during pregnancy should be considered to examine whether they may affect exposure misclassification. Overall, our findings add to a growing body of research linking intraurban variations of vehicle exhaust pollutants and reduced birth weight. Even being small, adverse reproductive effects of air pollution may have a considerable public health impact at the population level given the ubiquity of air pollution exposure (Slama et al. 2008a). This study reinforces the importance of developing strategies for air pollution prevention in a context of urban planning and management.

Acknowledgements/grant support

We thank Kees Meliefste (IRAS, Utrecht University, The Netherlands) and Rosalía Fernández-Patier, Amelia Aguirre, Teresa Bomboi and Dolores Herce (National Centre for Environmental Health, Madrid, Spain) for the laboratory analysis of passive samplers. The INMA Study has the financial support of the 'Carlos III Health Institute, Spanish Ministry of Health' (G03/176), 'RCESP' (C03/09) and CIBERESP.

The authors declare they have no competing financial interests.

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ARTÍCULO 4: Prenatal exposure to traffic-related air pollution and ultrasound measures of fetal growth in the INMA-Sabadell cohort

Environmental Health Perspectives (en revisión)

Inmaculada Aguilera[1,2,3,4], Raquel Garcia-Esteban[1,2,3], Carmen Iñiguez[3,5], Mark J Nieuwenhuijsen[1,2,3], Àgueda Rodríguez[6], Ferran Ballester[3,5] and Jordi Sunyer[1,2,3,4]

[1] Centre for Research in Environmental Epidemiology, Barcelona, Spain

[2] Municipal Institute of Medical Research (IMIM-Hospital del Mar), Barcelona, Spain

[3] CIBER de Epidemiología y Salud Pública, Barcelona, Spain

[4] Pompeu Fabra University, Barcelona, Spain

[5] Centre for Public Health Research, Conselleria de Sanitat, Generalitat Valenciana, Valencia, Spain

[6] Servei de Ginecologia i Obstetrícia, Hospital de Sabadell, Spain

Corresponding author:

Inmaculada Aguilera

Centre for Research in Environmental Epidemiology

Barcelona Biomedical Research Park

Doctor Aiguader 88. 08003 Barcelona, Spain

Tel. +34 932147300 / Fax + 34 932147301

E-mail: iaguilera@creal.cat

Aguilera I, Garcia-Esteban R, Iñiguez C, Nieuwenhuijsen MJ, Rodríguez A, Paez M, Ballester F, Sunyer J.
Prenatal Exposure to Traffic-Related Air Pollution and Ultrasound Measures of Fetal Growth in the INMA Sabadell Cohort.
Environ Health Perspect. 2010 May;118(5):705-11.

ABSTRACT

Background: Few studies have used longitudinal ultrasound measurements to assess the effect of traffic-related air pollution on fetal growth.

Objective: We examined the relationship between exposure to nitrogen dioxide and aromatic hydrocarbons (BTEX) on fetal growth assessed by 1,692 ultrasound measurements among 562 pregnant women from the INMA (Environment and Childhood) Sabadell cohort.

Methods: We used temporally-adjusted land-use regression (LUR) models to estimate exposures to NO_2 and BTEX. We fitted linear mixed models to estimate longitudinal growth curves for femur length (FL), head circumference (HC), abdominal circumference, biparietal diameter (BPD), and estimated fetal weight (EFW). Unconditional and conditional standard deviation (SD) scores were calculated at 12, 20 and 32 weeks of gestation. Sensitivity analyses were performed considering time-activity patterns during pregnancy.

Results: Exposure to BTEX from early pregnancy was negatively associated with

growth in BPD from weeks 20 to 32. None of the other fetal growth parameters were associated with exposure to air pollution during pregnancy. When considering only women who spent < 2 hr/day in nonresidential outdoor locations, effect estimates were stronger and statistically significant for the association between NO_2 and growth in HC from weeks 12 to 20 and growth in AC, BPD and EFW from weeks 20 to 32.

Conclusions: Our results lend some support to an effect of exposure to traffic-related air pollutants from early pregnancy on fetal growth during mid-pregnancy.

INTRODUCTION

Children are likely to be one of the most vulnerable populations to air pollution, particularly traffic-related (Schwartz, 2004). Perhaps less expectedly, numerous studies published in the last decade have reported that adverse effects of traffic-related air pollution already manifest during the prenatal period by increasing the risk of intrauterine growth restriction (IUGR), low birth weight (LBW) and preterm birth (PTB), even at low air pollution levels (reviewed by Glinianaia et al., 2004; Lacasaña et al., 2005; Maisonet et al., 2004; Šrám et al., 2005). Results from these studies show considerable evidence for some birth outcomes (particularly IUGR and LBW) but are still inconclusive in identifying the most harmful pollutants and the most susceptible periods within gestation. Heterogeneity in these findings may be explained in part by differences in study design, air exposure assessment, adjustment for confounding, and definition of birth outcomes (Woodruff et al., 2009).

Given the ubiquity of air pollution exposure and the importance of fetal growth as an indicator of children's health which may be associated with the development of chronic diseases in adulthood (Sinclair et al., 2007), more research is needed to disentangle the effects of individual pollutants, understand the underlying biological mechanisms, and identify specific periods of pregnancy where fetal growth may be more susceptible to air pollution effects (Slama et al, 2008). Regarding exposure assessment, the use of spatio-temporal modelling approaches based on Geographic Information Systems (GIS) and supported by subject-derived questionnaire data are encouraged (Gilliland et al., 2005).

To date, most of the studies investigating the relationship between prenatal exposure to air pollution and fetal growth have relied on neonatal anthropometric measurements as proxies of fetal growth, particularly birth weight (Brauer et al., 2008; Choi et al., 2008; Gouveia et al., 2004; Ha et al., 2001 ;Liu et al., 2007; Mannes et al., 2005; Parker et al., 2005 ;Rich et al., 2009; Slama et al., 2007; Wilhelm and Ritz, 2005) and, to less extent, birth height and head circumference at birth in addition to birth weight (Choi et al., 2006; Hansen et al., 2007; Jedrychowski et al., 2004). One limitation of these studies is that they are unable to assess fetal growth patterns. In addition, it has been suggested that birth weight poorly reflects IUGR during the first two trimesters of pregnancy (Hemachandra and Klebanoff, 2006). Since different patterns of fetal growth and exposures may result in the same neonatal anthropometric measurement, studies using longitudinal ultrasound measurements may be helpful for identifying specific critical periods for the effect of air pollution on fetal growth (Hansen et al., 2008; Slama et al., 2009).

We previously reported an association between prenatal exposure to traffic-related air pollution (particularly during the second trimester of pregnancy) and birth weight in an urban cohort of pregnant women within the Spanish INMA (Environment and Childhood) study (Aguilera et al., 2009). Here we aim to investigate the relationship between prenatal exposure to air pollution and longitudinally measured fetal growth characteristics in the same cohort.

METHODS

Study population

The INMA Study is a multicenter population-based mother and child cohort study established in seven areas of Spain. The cohort of Sabadell (Catalonia, Spain) is formed by 657 pregnant women who were recruited at their first routine prenatal care visit in the primary care center between June 2004 and July 2006. From the total sample of pregnant women, 93% (n = 611) lived in Sabadell at the beginning of the study, whereas 7% (n = 46) lived in two adjacent towns (Sant Quirze del Vallès and Barberà del Vallès) covered by the health service of the hospital of Sabadell. Main exclusion criteria were being < 16 years of age, non singleton pregnancy, not planning to deliver at the hospital of Sabadell, and to have followed an assisted reproduction program (Ribas-Fitó et al., 2006). Information on education, socioeconomic background, maternal health and obstetric history, parity, medication use, dietary intake, active and passive smoking, and time-activity patterns during pregnancy were obtained through questionnaires administered during the first and third trimester of pregnancy. All participating women signed written informed consent and the study was approved by the Ethical Committees of the Municipal Institute of Medical Research and the Hospital of Sabadell.

For this study, we restricted the analysis to women who were followed until child's birth, had at least two ultrasound measurements during pregnancy, and lived in Sabadell during the whole pregnancy (n=562).

Ultrasound measurements and fetal growth models

Routine fetal ultrasound examinations were conducted in early, mid-, and late pregnancy both in the primary care center (first and third trimester ultrasounds) and in the hospital of Sabadell (second trimester ultrasound). Fetal parameters recorded (in mm) were femur length (FL), head circumference (HC), abdominal circumference (AC), and biparietal diameter (BPD). Estimated fetal weight (EFW) was calculated using the Hadlock algorithm (Hadlock et al., 1985). Because FL, HC and AC are not routinely measured in the first trimester of pregnancy, ecographists from both centers were trained to follow the same protocol before the study start.

Gestational age was estimated using the date of the last menstrual period (LMP) reported at recruitment and confirmed by the first ultrasound examination. For women with a difference between reported LMP and ultrasound-based LMP $\geq$ 7 days (n = 89; 16%), crown-rump length was used for establishing gestational age (Westerway et al., 2000).

We fitted linear mixed models (LME) to estimate longitudinal growth curves for the five fetal parameters using R 2.6.0 software. We estimated the transformation of each of the fetal parameter by modeling the mean transformed outcome as a cubic polynomial in gestational age (in days), using a box-cox function from the MASS library (Royston, 1995). We used generalized least squares (gls function from the nlme library) to account for dependence among observations. Variable selection using Akaike information criterion was carried out through a backward selection procedure (likelihood ratio test p-value < 0.10) on covariates considered to be determinants either of size or growth: child's sex, maternal age, parity, ethnicity, height and pre-pregnancy weight, and paternal height and weight. Then variance-covariance structure of the errors with no random effects was modelled. Child's sex, gestational age, and factors identifying ultrasounds close in time (18, 20, and 30 days) were considered to estimate variance function of the errors (heteroscedasticity). Finally, random effects at intercept or/and slope (lme function) were allowed and tested using likelihood ratio test and residual diagnosis (ACF). Growth models for each fetal parameter are shown in Supplemental Material.

Growth models were applied to calculate unconditional standard deviation (SD) scores at 12, 20 and 32 weeks of gestation, representing estimates of fetal size, and conditional SD scores over the week intervals 12-20 and 20-32, representing estimates of fetal growth (Gurrin et al., 2001; Owen et al., 2000). For model validation, we compared estimated fetal parameters at birth with neonatal anthropometric measurements (weight, length, AC, and HC), which were recorded by specially trained midwives at delivery. Comparisons resulted in the following correlation coefficients: 0.46 between estimated and measured HC, 0.43 between estimated BPD and measured HC, 0.46 between estimated FL and measured height, 0.25 between estimated and measured AC, 0.48 between estimated AC and birth weight, and 0.45 between estimated and measured weight. All correlations were statistically significant (p-value<0.01).

Air pollution exposure assessment

Land-use regression (LUR) modelling was used to estimate individual exposure to traffic-related air pollution in the cohort (Aguilera et al., 2008). We chose this GIS-based technique because of its ability to capture small-scale variations in air pollution levels within urban areas (Briggs et al., 2000). Briefly, passive samplers were used to measure NO_2 and BTEX (benzene, toluene, ethylbenzene, *m/p*-xylene and *o*-xylene) as markers of motor vehicle exhausts. One-week measurements were carried out at 57 sampling sites in four and three sampling campaigns for NO_2 and BTEX, respectively. Concentrations of all the sampling campaigns were averaged to represent annual mean levels of each pollutant (Lebret et al., 2000), and linear regression models were fitted for NO_2 (R^2 = 0.75) and BTEX (R^2 = 0.74) using five groups of geographic characteristics (land coverage, topography, population density, roads, and distance to local sources of pollution) as predictor variables. Models were then applied to predict outdoor air pollution levels at the cohort addresses, accounting for different home addresses for those women who moved during pregnancy within Sabadell (n = 25, 4%). For budgetary reasons we were not able to perform air pollution measurements in the two adjacent towns of Sant Quirze del Vallès and Barberà del Vallès, therefore LUR estimates were not available for the 46 women who lived in these two cities.

To calculate individual exposures to air pollutants during specific periods of pregnancy, both LUR models were adjusted for temporal variations of daily NO_2 levels measured in the fixed monitoring station of Sabadell, assuming similar temporal variations in NO_2 and BTEX levels (Aguilera et al., 2009). Using this procedure, for each woman we calculated average cumulative exposures to NO_2 and BTEX from the LMP until 12, 20, and 32 weeks of pregnancy, as well as average exposures between weeks 12-20 and weeks 20-32. These five windows of exposure were chosen to be comparable to the periods where fetal size and fetal growth were estimated using LME models.

Statistical analysis

Statistical analyses were conducted using STATA 10.1 (Stata Corp., College Station, TX, USA). Associations between unconditional and conditional SD scores and levels of exposure to NO_2 and BTEX were examined by simple and multiple linear regression models. Because we consider LUR estimates of NO_2 and BTEX as potential markers of vehicle exhaust toxins rather than potential causative agents by themselves, and given the high correlation among the five BTEX compounds, we used the LUR estimate of the sum of the five BTEX compounds in the statistical analysis.

To be able to compare the results from this analysis with our previous study on air pollution and birth weight conducted in the same cohort (Aguilera et al., 2009), we included the same covariates after examining for potential collinearity: season of conception, child's sex, maternal age, maternal education, maternal ethnicity, parity, smoking during pregnancy, maternal height and pre-pregnancy weight, and paternal height and weight. However, covariates already included in each LME model were not considered again for adjustment in the multivariate analysis.

Finally, since in our previous study we found more pronounced associations between prenatal exposure to air pollution and birth weight among two subsets of women potentially less prone to exposure misclassification due to some specific time-activity patterns during pregnancy (Aguilera et al., 2009), as a sensitivity analysis here we also examined the associations for the same two subsets: women who spent ≥ 15 hr/day at home (n = 274), and women who spent < 2 hr/day in non-residential outdoor environments (n = 255), being 15 and 2 hr/day the median of the distribution. Both variables were reported at the third trimester of pregnancy and referred for a typical week during pregnancy. Because exposure estimates were residential-based, we assumed that these two subsets suffered less from exposure misclassification (assumed non-differential).

RESULTS

The characteristics of the study population are shown in Table 1. Most women were nulliparous (56.6%), non smokers during the entire pregnancy (68.2%), exposed to passive smoking either at home or at work (53.7%), and with intermediate educational level (41.7%).

A total of 1,692 ultrasound examinations were performed for the 562 pregnancies (Table 2). Most women had one routine ultrasound examination in each trimester of pregnancy (n=556), however 17 women (3%) had four to six examinations.

Table 1. Characteristics of the study population (n = 562)

Categorized variables	N	%
Child's sex		
Male	286	50.9
Female	276	49.1
Season of conception		
Spring	156	27.8
Summer	165	29.4
Fall	129	23.0
Winter	112	19.9
Smoking during pregnancy		
Never	231	42.0
Former, quit before pregnancy	144	26.2
Former, quit during pregnancy	84	15.3
Current	91	16.6
Passive smoking		
At home (ref: no)	197	35.8
At work (ref: no)	149	27.0
Either at home or at work (ref: no)	296	53.7
Maternal education		
Primary education	164	29.3
Secondary education	233	41.7
University degree	162	29.0
Maternal race/ethnicity		
White/Caucasian	543	96.8
Latin American	14	2.5
Black	4	0.7
Child birth order		
First	317	56.6
Second	206	36.8
Third or more	37	6.6
Continuous variables	**Mean (SD)**	**Range**
Maternal age (years)	31.0 (4.3)	17 - 43
Gestational age (weeks)	39.7 (1.4)	34 - 42
Maternal height (cm)	162.4 (6.1)	146 -180
Maternal pre-pregnancy weight (kg)	62.6 (12.8)	39 – 143
Paternal height (cm)	175.9 (7.1)	150 – 197
Paternal weight (kg)	80.2 (12.5)	50 – 130
Birth weight (g)	3247.4 (423.4)	1750 – 4480
Birth height (cm)	49.4 (1.9)	44 – 55
Birth head circumference (cm)	34.2 (1.2)	30 – 38
Birth abdominal circumference (cm)	31.3 (2.0)	25 – 37

Table 2. Descriptive statistics of ultrasound measurements (n = 1,692)

	Gestational age (weeks)	Fetal characteristics (mm)			
		FL	HC	AC	BPD
First ultrasound					
No. of scans		512	512	498	553
Mean (SD)	12.2 (1.0)	7.8 (2.5)	75.3 (12.8)	62.1 (11.5)	20.7 (3.7)
Second ultrasound					
No. of scans		560	556	560	561
Mean (SD)	21.1 (1.0)	35.0 (3.3)	189.1 (13.9)	164.0 (14.2)	49.5 (3.8)
Third ultrasound					
No. of scans		555	554	554	553
Mean (SD)	34.0 (1.3)	66.1 (3.2)	309.2 (13.3)	300.0 (16.2)	86.1 (3.8)
Fourth ultrasound					
No. of scans		17	17	17	17
Mean (SD)	35.1 (3.0)	66.9 (6.5)	318.3 (19.5)	306.9 (28.8)	86.7 (7.1)
Fifth ultrasound					
No. of scans		2	2	2	2
Mean (SD)	33.7 (3.2)	67.5 (10.6)	331.5 (17.7)	305.5 (20.5)	85.5 (7.8)
Sixth ultrasound					
No. of scans		1	1	0	1
Mean (SD)	34.43 (0)	64.6 (0)	342 (0)	-	85.8 (0)

FL, femur length; HC, head circumference; AC, abdominal circumference; BPD, biparietal diameter.

Table 3 provides the distribution of exposures to NO_2 and BTEX during specific periods of pregnancy and the Spearman correlation coefficients among them. There were only slight differences between mean levels of both pollutants by exposure interval. Correlation coefficients were higher among BTEX exposures (r=0.71-0.73) than among NO_2 exposures (r=0.46-0.52). Mean cumulative exposures from weeks 1 to 20 (32.1 $\mu g/m^3$ for NO_2 and 14.7 $\mu g/m^3$ for BTEX) and from weeks 1 to 32 (32.0 $\mu g/m^3$ for NO_2 and 14.7 $\mu g/m^3$ for BTEX) were very similar to mean exposures from weeks 1 to 12, and therefore highly correlated for both NO_2 (r=0.81-0.89) and BTEX (r=0.91-0.96).

Table 3. Descriptive statistics of NO_2 and BTEX exposure periods and Spearman correlation coefficients between different exposure periods

Pollutant	Exposure period	Mean (SD)	Range	IQR	Spearman correlation coefficients* weeks 1-12	weeks 12-20	weeks 20-32
NO_2	weeks 1-12	32.45 (10.51)	8.91-76.16	12.19	1		
	weeks 12-20	31.68 (10.81)	6.65-75.74	11.47	0.52	1	
	weeks 20-32	32.13 (10.84)	9.83-73.01	13.23	0.46	0.48	1
BTEX	weeks 1-12	14.89 (6.24)	2.27-30.31	9.66	1		
	weeks 12-20	14.47 (6.15)	1.99-31.83	9.99	0.73	1	
	weeks 20-32	14.72 (6.30)	2.79-28.90	10.19	0.72	0.71	1

* p-value<0.01 for all correlation coefficients

The non-adjusted mean change in SD scores of fetal size (at weeks 12, 20, and 32) and fetal growth (from week 12 to 20, and from week 20 to 32) for an IQR increase in exposure to NO_2 and BTEX during three different periods of pregnancy (weeks 1 to 12, weeks 12 to 20, and weeks 20 to 32) showed that exposure to both NO_2 and BTEX from weeks 1 to 12 was negatively associated with growth in BPD between weeks 20 and 32 of pregnancy ($\beta = -0.075$, $p = 0.03$ for NO_2 and $\beta = -0.124$, $p = 0.01$ for BTEX). Moreover, cumulative exposure from weeks 1 to 20 was also associated with the same outcome (data not shown), but not was the exposure between weeks 12 and 20. We found an association between exposure to BTEX from weeks 1 to 12 and size in BPD at week 32 of pregnancy ($\beta = -0.095$, $p = 0.05$). None of the other fetal parameters were statistically significant associated with any of the exposure periods to air pollution. After adjustment for the potential confounders, associations between NO_2 and BTEX exposure from weeks 1 to 12 and growth in BPD between weeks 20 and 32 were very similar to the non-adjusted ones but only statistically significant at the $p<0.05$ level for BTEX (Table 4). We repeated the analysis removing the exceeding scans of the 17 women who had more than the three routine ultrasound measurements, but effect estimates did not change substantially from those reported in Table 4.

When we restricted the analysis to women who spent ≥ 15 hr/day at home during pregnancy, we found stronger associations between BTEX exposure from weeks 1 to 12 and SD scores for most of the fetal parameters, although not statistically significant (Table 5). However, when considering only women who spent < 2 hr/day in non-residential outdoor environments, we found consistently higher associations between NO_2 exposure from weeks 1 to 12 and all the SD scores, being statistically significant ($p<0.05$) for growth in HC between weeks 12 and 20, growth in AC, BPD and EFW between weeks 20 and 32, size in HC, AC and EFW at week 32, and size in HC at week 20. The largest effect on fetal growth was seen for BPD. Associations for BTEX exposure during the same period were also stronger but only statistically significant for the same outcomes as in the whole cohort. Given their high correlation with exposure from weeks 1 to 12, cumulative exposures from weeks 1 to 20 and from weeks 1 to 32 showed similar associations among these two subsets (data not shown), but neither the exposure from weeks 12 to 20 nor from weeks 20 to 32 was associated with any SD score.

Table 4. Adjusted[a] associations between an IQR increase (in μg/m^3) in exposure to NO_2 and BTEX during different periods of pregnancy and standard deviation scores of fetal size (at 12, 20 and 32 weeks of gestation) and fetal growth (between weeks 12-20 and 20-32).

		Periods of exposure during pregnancy					
		weeks 1 – 12 β (SE)		weeks 12 – 20 β (SE)		weeks 20 – 32 β (SE)	
	z-score	**NO_2**	**BTEX**	**NO_2**	**BTEX**	**NO_2**	**BTEX**
FL	week 12	-0.033 (0.041)	-0.019 (0.055)	n.a.	n.a.	n.a.	n.a.
	week 20	-0.017 (0.046)	-0.054 (0.062)	-0.026 (0.042)	-0.079 (0.064)	n.a.	n.a.
	week 32	-0.007 (0.037)	0.005 (0.050)	0.039 (0.034)	0.049 (0.052)	0.006 (0.040)	0.005 (0.052)
	weeks 12-20	-0.006 (0.046)	-0.051 (0.061)	-0.032 (0.041)	-0.087 (0.063)	n.a.	n.a.
	weeks 20-32	-0.003 (0.037)	0.017 (0.050)	0.045 (0.034)	0.068 (0.051)	0.021 (0.039)	0.031 (0.052)
HC	week 12	0.004 (0.047)	-0.002 (0.063)	n.a.	n.a.	n.a.	n.a.
	week 20	-0.036 (0.047)	-0.009 (0.063)	0.026 (0.042)	0.035 (0.064)	n.a.	n.a.
	week 32	-0.023 (0.039)	0.010 (0.052)	0.019 (0.035)	0.048 (0.053)	0.004 (0.041)	0.041 (0.055)
	weeks 12-20	-0.042 (0.046)	-0.009 (0.062)	0.028 (0.042)	0.048 (0.063)	n.a.	n.a.
	weeks 20-32	-0.013 (0.039)	0.013 (0.053)	0.012 (0.036)	0.039 (0.054)	-0.001 (0.042)	0.034 (0.055)
AC	week 12	-0.046 (0.045)	-0.048 (0.060)	n.a.	n.a.	n.a.	n.a.
	week 20	-0.012 (0.048)	-0.029 (0.064)	-0.002 (0.043)	-0.040 (0.066)	n.a.	n.a.
	week 32	-0.033 (0.040)	-0.026 (0.054)	-0.002 (0.036)	-0.004 (0.055)	0.013 (0.042)	0.014 (0.056)
	weeks 12-20	0.011 (0.048)	-0.008 (0.064)	0.021 (0.043)	-0.004 (0.066)	n.a.	n.a.
	weeks 20-32	-0.031 (0.040)	-0.018 (0.053)	-0.001 (0.036)	0.008 (0.055)	0.021 (0.042)	0.031 (0.056)
BPD	week 12	0.063 (0.049)	0.055 (0.066)	n.a.	n.a.	n.a.	n.a.
	week 20	0.074 (0.045)	0.060 (0.060)	0.075 (0.041)	0.046 (0.062)	n.a.	n.a.
	week 32	-0.041 (0.041)	-0.094 (0.056)	0.000 (0.038)	-0.046 (0.057)	0.026 (0.044)	-0.020 (0.058)
	weeks 12-20	0.050 (0.046)	0.036 (0.062)	0.070 (0.042)	0.048 (0.064)	n.a.	n.a.
	weeks 20-32	-0.069 (0.042)	-0.121** (0.056)	-0.026 (0.038)	-0.065 (0.057)	0.015 (0.044)	-0.026 (0.058)

Table 4 continued

		Periods of exposure during pregnancy					
		weeks 1 – 12 β (SE)		weeks 12 – 20 β (SE)		weeks 20 – 32 β (SE)	
	z-score	**NO_2**	**BTEX**	**NO_2**	**BTEX**	**NO_2**	**BTEX**
EFW	week 12	-0.036 (0.043)	-0.028 (0.058)	n.a.	n.a.	n.a.	n.a.
	week 20	0.005 (0.054)	-0.022 (0.072)	0.007 (0.049)	-0.046 (0.074)	n.a.	n.a.
	week 32	-0.034 (0.039)	-0.035 (0.052)	0.015 (0.035)	0.010 (0.054)	0.016 (0.041)	0.011 (0.055)
	weeks 12-20	0.029 (0.051)	-0.009 (0.069)	0.015 (0.047)	-0.035 (0.071)	n.a.	n.a.
	weeks 20-32	-0.040 (0.039)	-0.028 (0.052)	0.013 (0.035)	0.030 (0.054)	0.032 (0.041)	0.043 (0.055)

FL, femur length; HC, head circumference; AC, abdominal circumference; BPD, biparietal diameter; n.a.: non applicable.
[a] All associations are adjusted for season of conception, parity, maternal educational level, and maternal smoking. Other variables also included in some adjusted models: maternal pre-pregnancy weight (in models for BPD and FL) and child's sex (in model for FL).
** p-value < 0.05

Table 5. Adjusted[a] associations between an IQR increase (in µg/m^3) in exposure to NO_2 and BTEX between weeks 1 and 12 of pregnancy and standard deviation scores of fetal size (at 12, 20 and 32 weeks of gestation) and fetal growth (between weeks 12-20 and 20-32) in two subsets of the cohort according to their specific time-activity patterns during pregnancy.

		women who spent ≥ 15 hr/day at home (n=274)		women who spent < 2 hr/day in nonresidential outdoor environments (n=255)	
		β (SE)		β (SE)	
	z-score	**NO_2**	**BTEX**	**NO_2**	**BTEX**
FL	week 12	-0.035 (0.058)	-0.019 (0.077)	-0.058 (0.071)	-0.050 (0.090)
	week 20	-0.084 (0.067)	-0.106 (0.089)	-0.080 (0.075)	-0.082 (0.095)
	week 32	0.044 (0.056)	0.030 (0.074)	-0.047 (0.061)	0.044 (0.078)
	weeks 12-20	-0.077 (0.066)	-0.106 (0.087)	-0.064 (0.073)	-0.069 (0.093)
	weeks 20-32	0.064 (0.055)	0.054 (0.073)	-0.030 (0.061)	0.063 (0.078)
HC	week 12	0.014 (0.066)	-0.048 (0.087)	-0.081 (0.079)	-0.080 (0.100)
	week 20	-0.085 (0.068)	-0.067 (0.090)	-0.177 (0.076)**	-0.084 (0.098)
	week 32	-0.009 (0.057)	-0.048 (0.076)	-0.136 (0.063)**	-0.047 (0.081)
	weeks 12-20	-0.101 (0.067)	-0.051 (0.090)	-0.157 (0.075)**	-0.055 (0.096)
	weeks 20-32	0.017 (0.058)	-0.030 (0.077)	-0.088 (0.066)	-0.023 (0.084)
AC	week 12	-0.078 (0.066)	-0.067 (0.087)	-0.107 (0.077)	-0.066 (0.099)
	week 20	0.019 (0.071)	-0.030 (0.095)	-0.052 (0.080)	-0.084 (0.101)
	week 32	-0.006 (0.059)	-0.008 (0.079)	-0.132 (0.064)**	-0.088 (0.082)
	weeks 12-20	0.062 (0.072)	0.001 (0.096)	-0.004 (0.076)	-0.060 (0.097)
	weeks 20-32	-0.013 (0.061)	0.001 (0.081)	-0.122 (0.062)**	-0.065 (0.080)
BPD	week 12	0.065 (0.069)	0.050 (0.092)	0.048 (0.081)	0.077 (0.103)
	week 20	0.082 (0.061)	0.076 (0.082)	0.123 (0.070)	0.118 (0.089)
	week 32	-0.053 (0.064)	-0.116 (0.085)	-0.086 (0.067)	-0.137 (0.085)
	weeks 12-20	0.058 (0.064)	0.060 (0.084)	0.114 (0.074)	0.093 (0.095)
	weeks 20-32	-0.085 (0.065)	-0.150 (0.086)	-0.135 (0.069)**	-0.186** (0.087)
EFW	week 12	-0.067 (0.061)	-0.053 (0.080)	-0.075 (0.075)	-0.046 (0.095)
	week 20	-0.003 (0.079)	-0.046 (0.104)	-0.043 (0.090)	-0.065 (0.115)
	week 32	-0.007 (0.059)	-0.030 (0.079)	-0.127 (0.062)**	-0.080 (0.079)
	weeks 12-20	0.039 (0.077)	-0.021 (0.103)	-0.003 (0.084)	-0.048 (0.107)
	weeks 20-32	-0.006 (0.061)	-0.013 (0.081)	-0.120 (0.061)**	-0.060 (0.078)

FL, femur length; HC, head circumference; AC, abdominal circumference; BPD, biparietal diameter; n.a.: non applicable

[a] All associations are adjusted for season of conception, parity, maternal educational level, and maternal smoking. Other variables also included in some adjusted models: maternal pre-pregnancy weight (in models for BPD and FL) and child's sex (in model for FL).

** p-value < 0.05

DISCUSSION

In this cohort of pregnant women from Sabadell, Spain, we found an association between exposure to traffic-related air pollution from the beginning of the pregnancy and impaired growth in BPD from mid- to late pregnancy. None of the other fetal growth characteristics were associated with exposure to air pollution in any of the periods studied for the whole cohort. The magnitude of most of the associations for BTEX exposure was more pronounced, although not statistically significant, among women who spent ≥ 15 hr/day at home, compared to the whole cohort. It is among women who spent < 2 hr/day in non-residential outdoor environments that associations were statistically significant. Adverse effects of exposure to NO_2 from the beginning of pregnancy were found on growth in HC from weeks 12 to 20 and growth in AC and EFW (in addition to BPD) from weeks 20 to 32. Size in HC at weeks of gestation 20 and 32, and size in AC and EFW at week 32 was also associated with NO_2 exposure in this subset.

This is the first study to use exposure assessment based on LUR models to investigate the effect of prenatal exposure to traffic-related air pollution on ultrasound-based fetal growth. So far, only two studies have assessed fetal growth by ultrasound measurements, using different exposure assessment approaches. Hansen et al. (2008) assigned air pollution data from the closest monitoring station to each woman's residential postcode. They found an association between exposure to low levels of air pollution during early pregnancy and decreased fetal growth characteristics in mid-pregnancy, although they only included scans between weeks 13 and 26 of pregnancy and therefore were not able to study the effect of air pollution on fetal growth either in the first or in the third trimester of pregnancy. Slama et al. (2009) assessed benzene exposure by using personal monitoring during one week and found an association with BPD at each of the trimester ultrasound examinations with HC at both the second and third trimester ultrasound examinations. Although we used a different approach from these two studies in terms of exposure assessment and statistical analysis, our results lend some support to an effect of air pollution exposure on fetal growth starting during mid-pregnancy. These findings are also in accordance with some studies assessing the relationship of maternal smoking on fetal growth during pregnancy, which found adverse effects on fetal growth from mid-pregnancy onwards (Jaddoe et al., 2007; Vik et al., 1996).

Exposure to a specific environmental factor in early, mid, and late pregnancy is likely to affect the fetus differently. In addition, according to the fetal programming hypothesis, the timing when an adverse effect occurs as a result of the exposure is crucial in determining the risk for diseases during adulthood (Nathanielsz, 2000). The second trimester is the period of maximal growth velocity of the placenta, therefore an abnormal pattern of placental growth earlier in gestation may result in abnormal fetal growth during mid- or late pregnancy and lead to an IUGR newborn (Lestou and Kalousek, 1998). One of the proposed biological mechanisms by which air pollution may affect fetal growth is by binding receptors for placental growth factors and consequently decreasing placental-fetal exchange of oxygen and nutrients (Kannan et al., 2006). If so, this could explain the influence of exposure from early pregnancy on fetal growth during mid-pregnancy.

An accurate assessment of air pollution exposure is particularly important in studies on reproductive outcomes, where the exposure period is clearly defined and there is concern about the existence of potential windows of susceptibility. If exposure assessment is based on residential location during pregnancy, to what extent air pollution levels around the residence represent personal exposure will depend on several factors, including activity and mobility patterns during the exposure period (Nethery et al., 2009). Therefore, sensitivity analysis taking into account differences in time-activity patterns (such as residential and occupational mobility, work status, time spent at or near home, etc.) are needed to verify the impact of exposure misclassification on effect estimates (Ritz and Wilhelm, 2008). In a previous study (Aguilera et al., 2009) we found an association between exposure to NO_2 and BTEX during the second trimester of pregnancy on birth weight, but only statistically significant among women who spent < 2 hr/day in non-residential outdoor environments. Given that LUR estimates were based in women's residential addresses, we argued that women spending ≥ 2 hr/day in non-residential outdoor were potentially more exposed to different levels of traffic-related air pollutants not reflected by the residence-based LUR estimates and therefore were more prone to exposure misclassification. The stronger effects found in the same subset for most of the fetal parameters support our previous findings.

Because levels of NO_2 and BTEX were not directly measured at the residential addresses but estimated with LUR models (which predictor variables were mainly traffic-related), we considered these pollutants as markers of vehicle exhaust toxins rather than potential causative agents by themselves. Whereas in our previous study on air pollution and birth weight we found consistently stronger associations for BTEX than for NO_2, here we did not clearly identify one of them as a potential better marker of traffic-related air pollution. Overall, the high correlation in space and time between pollutants sharing similar sources, together with the lack of enough knowledge on underlying casual pathways, makes it difficult to separate the etiological agents and to disentangle the role of independent pollutants in causing adverse health effects (Kim et al., 2007).

We used unconditional and conditional SD scores to estimate fetal size and growth, respectively. Because conditional SD scores for a given fetal parameter take into account the fetal size earlier in gestation, which is a determinant of subsequent fetal growth, they are more appropriate for assessing fetal growth and identifying IUGR than using cross-sectional data (Owen et al, 2000; Royston, 1995). This difference between conditional and unconditional SD scores may explain why an association between air pollution exposure and SD scores of fetal size at two different weeks do not imply an association for SD scores of fetal growth between these two weeks (as shown in Table 6 for HC).

One of the strengths of our study is that it is a population-based cohort followed from early pregnancy onwards, with information on many potential confounders at individual level and a well-controlled quality of the data. In addition, prenatal exposure to air pollution was estimated using temporally-adjusted LUR models applied to geocoded residential addresses and accounting for residential mobility during pregnancy.

One concern about using ultrasound measurements to assess the effects of any exposure of interest on fetal growth is measurement error. Potential measurement errors in clinical practice include the use of different ultrasound units and inter-observer variability (Perni et al., 2004). In our cohort, the ultrasound examinations were carried out in two centres for all the women, which limited the number of ultrasound units and ecographists performing the measurements. In addition, all the ecographists participating in the study were specifically trained to follow the same protocol.

One limitation of our study is that we did not account for indoor exposures to air pollution. Some traffic-related air pollutants (such as NO_2, particulate matter, polycyclic aromatic hydrocarbons or volatile organic compounds) have also relevant indoor sources that could contribute to important inter-individual variations in exposure, although the limited data about the identification of the most harmful pollutants and their biological pathways do not allow us to evaluate the real importance of indoor exposure on the relationship between air pollution and fetal growth. A second limitation is the multiple comparisons performed between exposures and outcomes, which may have led to spurious findings. Finally, because of the small number of ultrasound measurements performed from week 35 onwards, we were not able to assess the influence of air pollution exposure on fetal growth during late pregnancy (i.e. from weeks 32 to 38). However, the combination of findings from the present and the previous study among the subset potentially less prone to exposure misclassification indicate that the effect of air pollution persist until birth (at least for fetal weight).

CONCLUSIONS

We found an effect of prenatal exposure to urban air pollution on growth in BPD between from weeks 20 to 32 of gestation. Among women who spent < 2 hr/day in non-residential outdoor locations, associations were stronger and statistically significant for growth in HC from weeks 12 to 20 and growth in AC, BPD and EFW from weeks 20 to 32. Overall, air pollution exposure from early pregnancy seems to affect fetal growth during mid pregnancy. Sensitivity analysis using time-activity patterns during pregnancy should be performed to examine potential variations in effect estimates. Considering both NO_2 and BTEX as markers of a complex mixture of vehicle exhaust toxins, no consistently higher associations were found for one of them.

Acknowledgements/grant support

We are grateful to Silvia Fochs, Anna Sànchez, Maribel López and Nuria Pey for their assistance in contacting the families and administering the questionnaires, and to the ecographists who conducted the ultrasound measurements. We also thank Kees Meliefste, Rosalía Fernández-Patier, Amelia Aguirre, Teresa Bomboi and Dolores Herce for the laboratory analysis of passive samplers.

The INMA (Environment and Childhood) study has the financial support of the 'Carlos III Health Institute, Spanish Ministry of Health' (G03/176), Red de Centros de Investigación en Epidemiología y Salud Pública (C03/09) and Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública.
The authors declare they have no competing financial interests.

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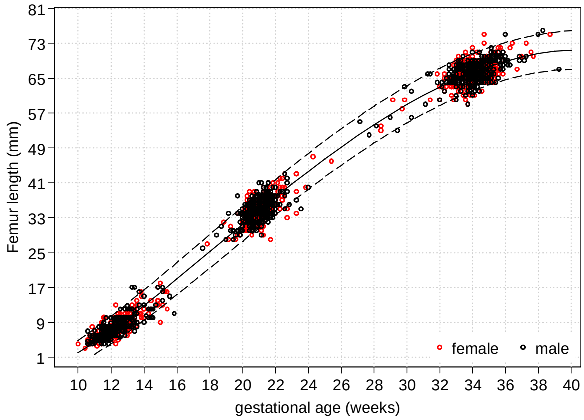
SUPPLEMENTAL MATERIAL

This online supplement contains the linear mixed models (LME) and growth curves for the five fetal parameters: femur length, head circumference, abdominal circumference, biparietal diameter and estimated fetal weight.

Abbreviations: GA = gestational age in days

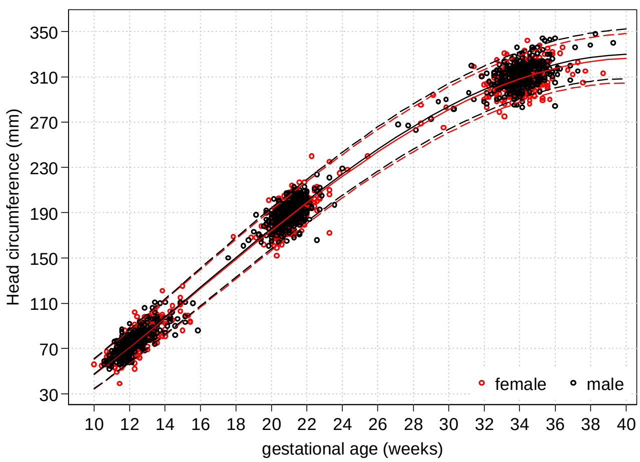
1. Femur length (FL)

$FL^{0.81} = -14.674 + 0.208\ GA + 1.210E^{-4}\ GA^2 - 1.204E^{-6}\ GA^3 + 0.018$ maternal age $+ 5.793E^{-5}\ GA \cdot$ maternal height $+ 4.139E^{-5}\ GA \cdot$ paternal height



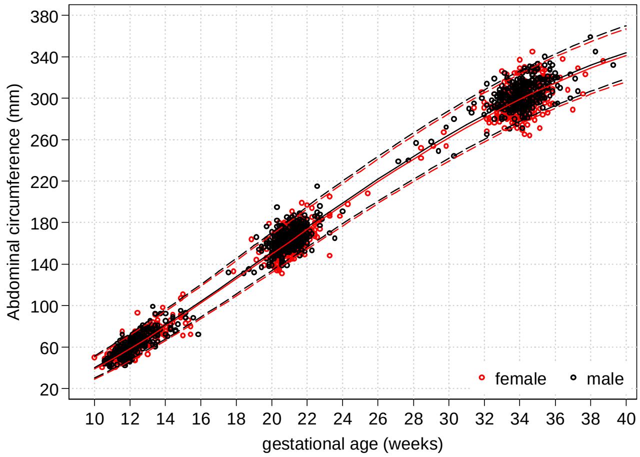
2. Head circumference (HC)

$HC^{0.76} = -24.847 + 0.555\ GA - 2.877E^{-4}\ GA^2 - 1.701E^{-6}\ GA^3 + 0.027$ maternal height $+0.027$ maternal age $+ 5.895E^{-5}\ GA \cdot$ prepregnancy weight $+ 8.807E^{-5}\ GA \cdot$ paternal height $+2.611E^{-3}\ GA \cdot$ female



3. Abdominal circumference (AC)

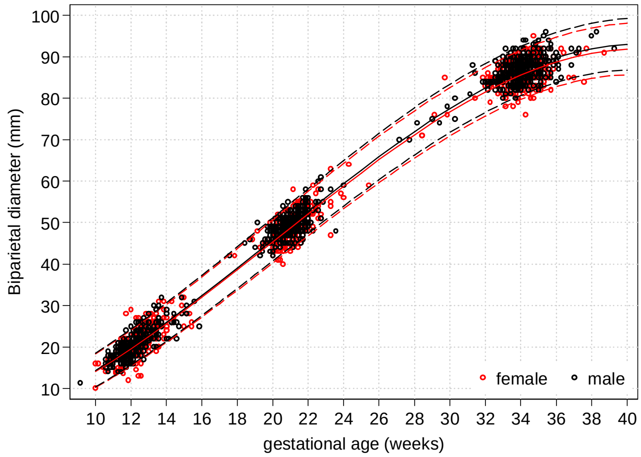
$AC^{0.59} = -6.274 + 0.213\ GA - 3.075E^{-4}\ GA^2 + 0.157\ \text{female} + 0.015\ \text{maternal age} + 2.558E^{-5} GA \cdot \text{pre-pregnancy weight} + 5.366E^{-3}\ GA \cdot \text{paternal height}$



4. Biparietal diameter (BPD)

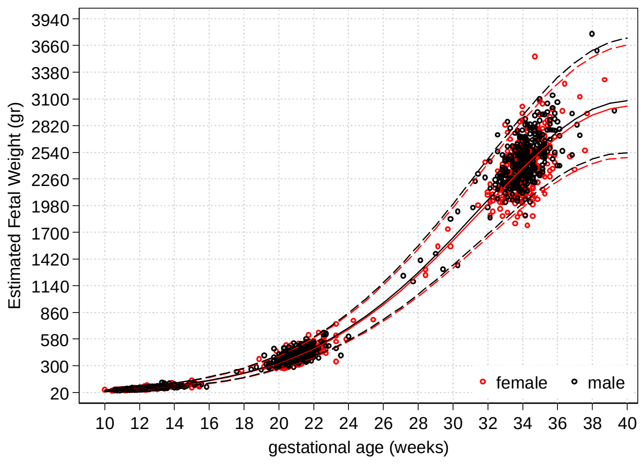
$BPD^{0.79} = -2.061 + 0.096\ GA + 6.170E^{-4}\ GA^2 - 1.909E^{-6}\ GA^3 + 0.071$ 1st tertile of maternal age + 0.156 3rd tertile of maternal age + $9.180E^{-5}\ GA \cdot \text{maternal height} + 1.281E^{-3}\ GA \cdot \text{female}$

Tertiles of maternal age:
1st tertile: 17.28 – 29.08 years
2nd tertile: 29.09 – 32.65 years (reference)
3rd tertile: 32.66 – 42.48 years



5. Estimated fetal weight (EFW)

$EFW^{0.06} = 1.062 + 1.800E^{-3}\ GA + 6.750E^{-6}\ GA^2 - 0.024E^{-6}\ GA^3 + 2.280E^{-4}$ maternal age $+1.920E^{-3}$ female $+$ 0.00008 maternal age $+0.284E^{-6}\ GA \cdot$ prepregnancy weight $+0.499E^{-6}\ GA \cdot$ paternal height



5. DISCUSIÓN

5.1. Sobre la evaluación de la exposición

La mayoría de estudios epidemiológicos sobre contaminación atmosférica y efectos perinatales han usado datos procedentes de las redes de vigilancia de la calidad del aire para asignar niveles de exposición durante el embarazo. La técnica más común consiste en tomar directamente los datos de la estación fija más cercana a la residencia, o bien calcular los niveles medios de las estaciones ubicadas en un radio determinado alrededor de la vivienda. Estos estudios a menudo no han podido disponer de la dirección exacta de cada mujer, usando en su lugar el código postal o la sección censal (Bell et al., 2007; Bobak et al., 2000; Gouveia et al., 2003; Ha et al., 2001; Hansen et al., 2007; Liu et al., 2003, 2007; Maisonet et al., 2001; Mannes et al., 2005; Parker et al., 2005; Rich et al., 2009; Ritz et al., 2007; Salam et al., 2005; Wilhelm et al., 2005). Esta metodología asume que todos los individuos residentes en un área determinada están expuestos a los mismos niveles de contaminación atmosférica, lo cual no tiene en cuenta la heterogeneidad espacial a pequeña escala de algunos contaminantes. Se ha indicado que los problemas de mala clasificación derivados del uso de esta metodología podrían ser una de las causas de la falta de consistencia en los efectos reproductivos hallados en estos estudios (Šrám et al., 2005).

En esta tesis se han desarrollado modelos de exposición que explican la variabilidad espacial intraurbana de los niveles de NOx, NO_2 y BTEX en un 77%, 75% y 74%, respectivamente. Estos modelos se han elaborado usando las concentraciones medias de varias campañas de medidas, ya que de esta manera se consigue un modelo más estable que los que se obtendrían para cada campaña de manera independiente (Briggs et al., 1997; Lebret et al., 2000). No obstante, para los análisis posteriores de asociación con crecimiento fetal solamente se usaron los modelos para NO_2 y BTEX basados en cuatro y tres campañas, respectivamente, excluyendo de estos análisis el modelo para NOx por estar basado en únicamente dos campañas muy próximas en el tiempo (abril y junio de 2005). Tras aplicar los modelos a las direcciones postales de las mujeres de cohorte, el rango obtenido de exposición a NO_2 y BTEX fue considerablemente amplio, lo cual es fundamental en un estudio epidemiológico (Navidi et al., 1999).

El aumento de la resolución espacial conseguida con los modelos LUR no debe producirse a costa de una pobre resolución temporal. Puesto que estos modelos representan los niveles medios anuales, es necesario realizar un ajuste temporal que permita obtener estimaciones de exposición durante el embarazo, utilizando para ello los niveles diarios medidos en una estación fija (Brauer et al., 2008; Slama et al., 2007). Tras realizar el ajuste temporal de los modelos de exposición a NO_2 y BTEX para adaptarlos al periodo de embarazo, las estimaciones obtenidas fueron ligeramente inferiores. En la muestra de 570 mujeres para las que se estudió la relación entre exposición a contaminación atmosférica y peso al nacer, el modelo LUR de NO_2 asignó una exposición media anual de 36,2 $\mu g/m^3$, mientras que tras el ajuste temporal la exposición media durante el embarazo resultó ser de 32,2 $\mu g/m^3$. Mayor fue la reducción en el porcentaje de mujeres expuestas a niveles de NO_2 >40 $\mu g/m^3$, pasando de un 25% según el modelo LUR sin ajustar a un 14% tras ajustar por el periodo de embarazo.

Con el uso de técnicas basadas en SIG para elaborar modelos de exposición a contaminantes atmosféricos, surge la pregunta de hasta qué punto los niveles estimados en el exterior de la vivienda representan la exposición personal a contaminantes procedentes del tráfico. En los países desarrollados, las personas pasan la mayoría del tiempo en ambientes interiores, de manera que la exposición a contaminantes asociados al tráfico en el interior del domicilio dependerá del grado en que los niveles exteriores afecten a los interiores (Fischer et al., 2000). Además, los desplazamientos por la ciudad a pie o en diferentes medios de transporte pueden representar periodos de exposición importantes a niveles no reflejados por la estimación en el exterior del domicilio (Briggs et al., 2008). El segundo artículo de esta tesis doctoral pretende en parte responder a esta cuestión, evaluando la relación entre los niveles personales, interiores y exteriores de NO_2, así como sus determinantes, en una submuestra de 58 mujeres. De él se concluye que parte de la variabilidad en los niveles de NO_2 en el interior del domicilio vienen explicados por los niveles en el exterior, de manera que existe una contribución del tráfico existente en las inmediaciones del domicilio en los niveles interiores. Por otro lado, la exposición personal a NO_2 viene determinada por los niveles en el exterior e interior del domicilio y por patrones de comportamiento que implican exposición a fuentes exteriores (tráfico) e interiores (cocina de gas). Aunque están basados en una muestra pequeña cuya exposición se ha evaluado durante un periodo de tiempo corto (48 horas), los resultados de este artículo apoyan la importancia de los patrones de tiempo-actividad en la exposición personal. Otros estudios hallaron resultados similares. Choi et al. (2008) analizaron durante 48 horas los niveles personales, interiores y exteriores de HAPs en 78 mujeres no fumadoras de Cracovia (Polonia), hallando que los niveles interiores de HAPs estaban fuertemente influenciados por los exteriores y que ciertos patrones de comportamiento como la exposición pasiva a tabaco, el tiempo pasado en ambientes exteriores y el uso de determinados medios de transporte eran determinantes de los niveles personales. Nethery et al. (2008a, 2008b) hallaron una clara influencia de los niveles de NO y NO_2 estimados para el exterior de la vivienda con modelos LUR en la exposición personal a estos contaminantes durante el embarazo. Además, el efecto de los niveles exteriores en la exposición personal no se vio afectado por la presencia de fuentes interiores, lo cual apoya el uso de estimaciones de niveles en el exterior del domicilio como indicadores de exposición personal a contaminantes asociados al tráfico.

En los artículos tercero y cuarto de esta tesis, se realizaron análisis de sensibilidad utilizando los patrones de tiempo-actividad durante el embarazo con el objetivo de evaluar el impacto de una posible mala clasificación de la exposición sobre las asociaciones entre contaminación atmosférica y crecimiento fetal (Ritz y Wilhelm, 2008). En ambos artículos se hallaron mayores asociaciones dentro del grupo de mujeres que pasaron $<$ 2 horas/día en ambientes exteriores, excluyendo el exterior del domicilio. Dado que el tiempo pasado en ambientes exteriores resultó ser un determinante de la exposición personal a NO_2, y que, según el cuestionario de tiempo-actividad, la mayor parte de este tiempo se dedicó a desplazamientos a pie, es posible que las mujeres que pasaron $\geq$ 2 horas/día en ambientes exteriores estuvieran expuestas a una variabilidad considerable de niveles de contaminantes asociados al tráfico no representada por las estimaciones de los modelos LUR (y por tanto sujetas a una peor clasificación de la exposición). No obstante, para confirmar esta hipótesis sería necesario disponer de información adicional sobre los desplazamientos realizados (como rutas habituales, cercanía a la vivienda, etc.) que permitiese caracterizar esta supuesta variabilidad en la exposición. En cualquier caso, no existen diferencias significativas en covariables como situación laboral, nivel educativo, paridad o estación de la concepción entre las mujeres que pasaron < 2 horas/día en ambientes exteriores y las que pasaron ≥ 2 horas/día. La otra variable de tiempo-actividad utilizada en los análisis de sensibilidad fue el tiempo pasado en el domicilio, ya que pasar más tiempo en casa está relacionado con una mejor correlación entre exposición personal y niveles en el exterior de la vivienda estimados con modelos LUR (Nethery et al., 2008b). Para el subgrupo de mujeres que pasaron ≥ 15 horas/día en casa, las asociaciones halladas con peso al nacer fueron en general más altas (aunque no estadísticamente significativas) en comparación con el total de la cohorte. En cambio, las asociaciones halladas para este mismo subgrupo con los diferentes parámetros de crecimiento fetal evaluados mediante ecografías fueron ligeramente superiores respecto al total de la cohorte para BTEX, pero no para NO_2.

Aunque las preguntas de tiempo-actividad hacen referencia a todo el embarazo, el hecho de que se respondieran en la visita de las 32 semanas podría implicar una sobrerrepresentación de los patrones de tiempo-actividad durante el tercer trimestre frente a los dos anteriores.

Teniendo en cuenta que en los últimos meses de embarazo existe una tendencia a pasar más tiempo en casa, relacionada en parte con cambios en la situación laboral (Nethery et al., 2009), una sobrerrepresentación del tercer trimestre no reflejaría correctamente los patrones medios de tiempo-actividad durante el embarazo. Sin embargo, la comparación de las variables referentes al tiempo pasado en el trabajo con la situación laboral referida en los cuestionarios de las semanas 12 y 32 no apunta a una sobrerrepresentación del tercer trimestre (por ejemplo, las mujeres que trabajaban en la semana 12 y estaban de baja laboral en la semana 32 reportaron horas pasadas en el trabajo en el cuestionario de tiempo-actividad). Por tanto, los patrones de tiempo-actividad referidos efectivamente deben ser considerados como patrones medios de todo el embarazo. Esto plantea la ventaja de no asignar más peso a un trimestre sobre los demás, pero también el inconveniente de no reflejar los cambios producidos durante el embarazo. En consecuencia, la identificación de trimestres de exposición más relevantes para efectos perinatales tras estratificar la muestra por patrones de tiempo-actividad debe ser interpretada con cautela.

Por último, es necesario aclarar el papel de los contaminantes evaluados en este estudio. Puesto que esta tesis centra su interés en los efectos de la contaminación atmosférica urbana (asociada mayoritariamente al tráfico) y que los niveles individuales de exposición han sido estimados a partir de modelos de regresión cuyas variables predictoras (excepto la altitud) están relacionadas con el tráfico, ni NO_2 ni BTEX han sido utilizados como potenciales sustancias tóxicas por sí mismas, sino como marcadores de contaminantes emitidos por los vehículos y que presentan gran variabilidad a pequeña escala.

El NO_2 ha sido ampliamente utilizado como marcador de contaminación asociada al tráfico para elaborar modelos LUR aplicados a estudios epidemiológicos, ya que se puede medir fácilmente con captadores pasivos a un bajo coste (Jerrett et al., 2005). Sin embargo, entre los numerosos contaminantes emitidos por los vehículos, existe bastante consenso en que el NO_2 no parece ser el principal causante de los efectos observados en salud (Brunekreef, 2001). Diferente es el caso de los BTEX, para los que sí hay indicios de efectos en salud derivados de la exposición crónica a niveles bajos en aire exterior, incluido el efecto carcinógeno del benceno (Suh et al., 2000), así como efectos teratogénicos para exposiciones elevadas a tolueno y xilenos (Bowen y Hannigan, 2006). Pero una hipotética interpretación de BTEX como compuestos potencialmente tóxicos por sí mismos pasaría primero por elaborar un modelo LUR para cada uno de sus cinco componentes (ya que la toxicidad relativa de cada uno es diferente), lo cual no ha sido posible en Sabadell porque la alta correlación existente entre ellos impide obtener modelos LUR suficientemente fiables por separado. Además, sería necesario tener en cuenta las numerosas fuentes interiores de BTEX, cuyo impacto sobre la exposición personal puede ser mayor que el de las fuentes exteriores (Saarela et al., 2003). Por estos motivos, y dadas las características de este estudio, parece más razonable atribuir las asociaciones halladas al efecto en general de la contaminación atmosférica asociada al tráfico y no específicamente a la exposición a NO_2 o BTEX.

5.2. Sobre los efectos en el crecimiento fetal

En esta tesis se ha evaluado el crecimiento fetal de dos maneras: usando las medidas antropométricas al nacimiento (en este caso el peso) y de manera longitudinal a lo largo del embarazo a partir de ecografías. La primera es una opción sencilla (pues utiliza una única medida obtenida al final de un largo periodo de crecimiento) cuya principal ventaja es que permite comparar nuestros resultados con los ya publicados en otros estudios, además de contribuir a la necesidad constatada de datos procedentes de estudios longitudinales que sean capaces de incluir medidas de exposición más precisas y mayor número de covariables. Teniendo en cuenta que diferentes trayectorias de crecimiento fetal pueden acabar en un mismo peso al nacer, la segunda opción es metodológicamente más interesante, ya que permite evaluar el crecimiento fetal de forma directa y durante diferentes periodos del embarazo, pero el escaso número de estudios publicados hasta la fecha (Hansen et al., 2008; Slama et al., 2009) limita la comparabilidad e interpretación de resultados.

En el tercer artículo de la tesis se halló un efecto negativo de la exposición prenatal a BTEX (durante todo el embarazo y específicamente durante los trimestres primero y segundo) sobre el peso al nacer, pero este efecto sólo fue estadísticamente significativo en el grupo de mujeres que pasaron menos tiempo en ambientes exteriores distintos del residencial. Los modelos multitrimestre mostraron efectos negativos para las exposiciones a NO_2 y BTEX durante el primer y segundo trimestre, aunque los análisis de sensibilidad apuntaron al segundo trimestre como periodo de mayor susceptibilidad.

La figura 5 muestra un resumen de los estudios que hasta la fecha han investigado la relación entre exposiciones trimestrales (o mensuales) y peso al nacer o PEG, e indica el periodo o periodos para los que se halló el mayor efecto. En ella se constata la disparidad de resultados incluso para un mismo contaminante, aunque en general se observa que los trimestres primero y tercero son los que aparecen con más frecuencia como los más relevantes. Por el contrario, otros estudios no hallaron ningún periodo específico de mayor susceptibilidad para ninguno de los contaminantes evaluados (Brauer et al., 2008) o bien no hallaron asociaciones estadísticamente significativas para ningún trimestre del embarazo con peso al nacer o PEG (Bobak, 2000; Hansen et al., 2007; Maroziene et al., 2002).

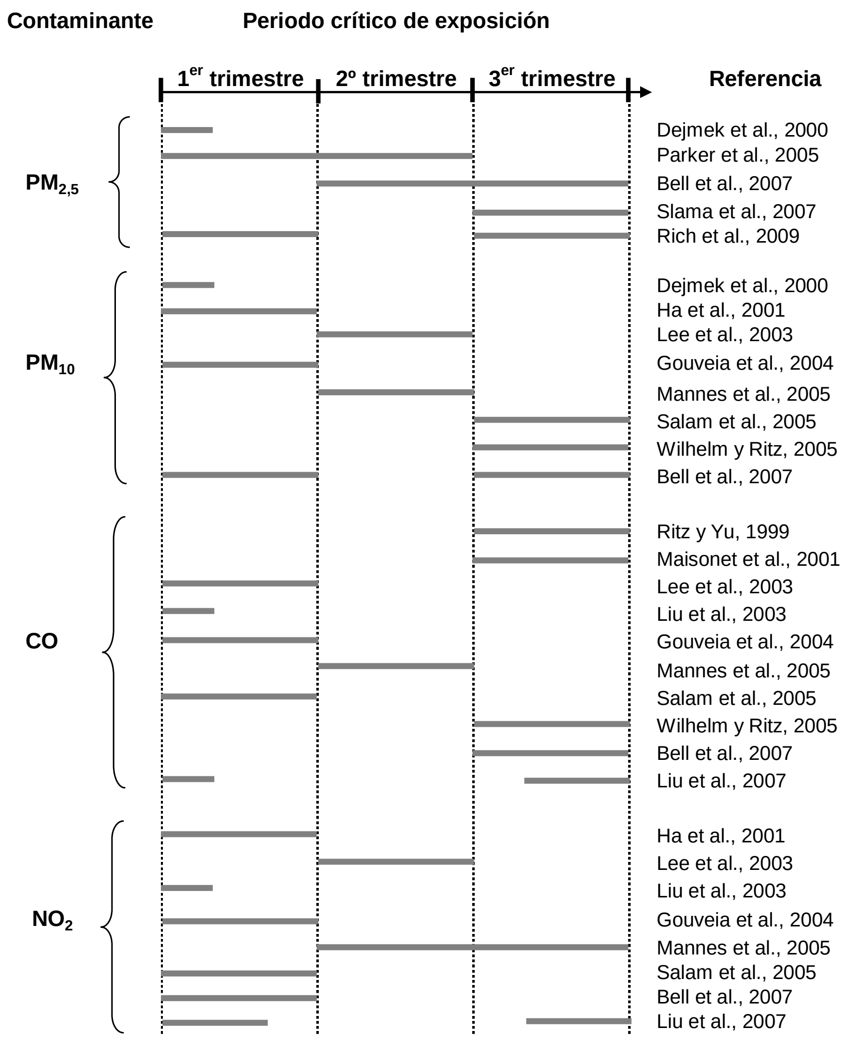


Figura 5. Períodos críticos de exposición publicados en la literatura correspondientes al mayor efecto estimado de la contaminación atmosférica sobre el crecimiento fetal, medico como peso al nacer o PEG (adaptado de Slama et al., 2008).

En el cuarto artículo de la tesis, el objeto de análisis fue la evaluación directa del crecimiento fetal a través de medidas ecográficas de cinco parámetros: longitud del fémur (LF), perímetro abdominal (PA), perímetro cefálico (PC), diámetro biparietal (DBP) y peso fetal estimado (PFE). Para el total de la cohorte, se halló un efecto negativo de la exposición a BTEX desde el inicio del embarazo sobre el crecimiento en el DBP entre las semanas de gestación 20 y 32. El DBP está correlacionado con el volumen cerebral del feto, y se ha asociado un menor crecimiento prenatal del DBP con una reducción del volumen ventricular cerebral en la edad infantil, aunque se desconoce si las variaciones en dicho volumen pueden o no tener consecuencias sobre el neurodesarrollo (Roza et al., 2008).

Al restringir el análisis a la muestra de mujeres que pasaron menos tiempo en ambientes exteriores distintos del residencial, las asociaciones fueron más fuertes, especialmente para la exposición a NO2 desde el comienzo del embarazo, mostrando una asociación estadísticamente significativa con el crecimiento en el DBP, PA, y PFE entre las semanas 20 y 32. Estos resultados coinciden con dos estudios publicados anteriormente (Hansen et al., 2008; Slama et al., 2009) en que el efecto de la contaminación atmosférica sobre el crecimiento fetal comienza a manifestarse alrededor de la mitad del embarazo. Además, analizando en conjunto los resultados de los artículos tercero y cuarto de esta tesis para la submuestra de mujeres potencialmente menos susceptible a una mala clasificación de la exposición, se pone de manifiesto que el efecto de la contaminación atmosférica sobre el crecimiento fetal durante el embarazo (evaluado como un menor crecimiento de los parámetros fetales medidos en las ecografías) persiste hasta el momento del nacimiento (evaluado como un descenso en el peso al nacer), coincidiendo de nuevo con los resultados de Slama et al. (2009).

Las consecuencias pediátricas del CIUR son muy importantes, ya que muchos procesos patológicos que se observan en la edad pediátrica están asociados a las condiciones del crecimiento intrauterino. Los niños nacidos con CIUR presentan una mayor incidencia de trastornos neurológicos como parálisis cerebral, mayor mortalidad perinatal, neonatal y postneonatal, trastornos del neurodesarrollo y del comportamiento y problemas de crecimiento durante la infancia (Rosenberg, 2008; Yanney y Marlow, 2004). Específicamente, los niños con bajo crecimiento craneal tanto en la etapa prenatal como en la postnatal son los que presentan un mayor riesgo de padecer trastornos del neurodesarrollo y del crecimiento (Yanney y Marlow, 2004). Además, como se ha indicado en la introducción, las secuelas del CIUR pueden permanecer hasta la edad adulta, influyendo en el desarrollo de enfermedades cardiovasculares y del síndrome metabólico (obesidad, diabetes mellitus tipo 2 e hipertensión arterial). Concretamente se ha estimado que entre un 25% y un 63% de los casos de diabetes, hipertensión arterial y enfermedad coronaria pueden ser atribuibles al efecto del bajo peso al nacer en combinación con una posterior ganancia acelerada de peso en las etapas infantil y adolescente (Ross y Beall, 2008).

La inclusión de un considerable número de potenciales variables confusoras es otra de las fortalezas a destacar en este estudio. Esto es un factor importante en cualquier estudio epidemiológico, pero adquiere aún más relevancia en esta línea de investigación, ya que los efectos causales de la contaminación atmosférica, en el caso de existir, son probablemente pequeños (Strickland et al., 2009). En nuestro estudio, las covariables que modificaron la asociación entre contaminación atmosférica y peso al nacer en más de un 10% fueron, de mayor a menor magnitud: sexo del recién nacido, altura de la madre, altura del padre, consumo de tabaco, estación de la concepción, nivel educativo materno, edad gestacional, paridad y peso previo al embarazo. En otro estudio prospectivo realizado en Munich, las covariables que más modificaron la asociación entre exposición prenatal a contaminación atmosférica y nacer con un peso < 3.000 g fueron la altura de la madre, su nivel educativo y la edad gestacional (Slama et al., 2007). Estos resultados ponen de manifiesto la importancia de determinadas covariables que normalmente no están disponibles en los certificados de nacimiento, como por ejemplo las medidas antropométricas de los padres.

5.3. Limitaciones del estudio

Este estudio presenta varias limitaciones. La primera de ellas está relacionada con el tamaño muestral, el cual no ha permitido analizar la asociación entre exposición prenatal a contaminación atmosférica y otros efectos perinatales como la prematuridad, debido al reducido número de nacimientos prematuros en la muestra (n=17). La prematuridad es un claro factor de riesgo de mortalidad y morbilidad infantil con un importante impacto en salud pública, cuya relación con la exposición prenatal a contaminación atmosférica merece sin duda ser objeto de más investigaciones (Darrow et al., 2009).

Otra limitación es que la evaluación de la exposición a contaminantes asociados al tráfico está basada únicamente en la localización del domicilio. Para las mujeres que trabajan fuera de casa, la correlación entre la exposición personal a NO_2 y la estimada con modelos LUR aumenta cuando se añade la localización del centro de trabajo a la del domicilio, especialmente en mujeres que pasan menos tiempo en casa (Nethery et al., 2008a). En esta tesis no ha sido posible calcular los niveles de contaminación atmosférica en el exterior del centro de trabajo, debido sobre todo al alto número de mujeres que trabajaban fuera del área de estudio. No obstante, en la cohorte de Valencia del estudio INMA sí se pudo comparar la estimación de exposición personal a NO_2 (calculada con una combinación de *kriging* y LUR) basada únicamente en el domicilio con la estimación ponderada en base al domicilio y al centro de trabajo, hallando una correlación del 99% entre ambas estimaciones y niveles de exposición muy similares (Iñiguez et al., 2009).

Puesto que esta tesis está centrada en el efecto de los contaminantes asociados al tráfico, en la evaluación de la exposición no se han tenido en cuenta las fuentes interiores de contaminantes atmosféricos. Sin embargo, como se ha indicado anteriormente, no se puede descartar que los BTEX no sean por sí mismos agentes causales de una disminución en el crecimiento fetal. Si este fuera el caso, y dada la importancia de las fuentes interiores de BTEX, el error en la medida de la exposición (el cual asumimos como no diferencial) podría ser importante. Esto también es aplicable a otros contaminantes emitidos por los vehículos y que podrían ser potenciales agentes causales, como los HAPs o las partículas, cuyas fuentes interiores son tan relevantes que pueden incrementar considerablemente los niveles de exposición personal estimados únicamente a partir de fuentes exteriores (Gilliland et al., 2005). Ante esta situación, los resultados presentados en esta tesis estarían infraestimando el efecto de los BTEX, al tener en cuenta solamente la exposición a fuentes exteriores.

Por último, el hecho de que la zona de estudio sea una ciudad con una extensión relativamente pequeña significa que los niveles de fondo de la contaminación atmosférica son bastante homogéneos, a diferencia de otras cohortes del estudio INMA, en donde la combinación de zonas urbanas, semiurbanas y rurales implica una mayor variabilidad en los niveles de fondo. Por tanto, la variabilidad en la exposición individual de las mujeres de la cohorte de Sabadell no procede de diferencias tanto en los niveles de fondo como a pequeña escala, sino casi exclusivamente de lo segundo, aumentando así la complejidad de la evaluación de la exposición. Aunque en este estudio ha sido posible obtener información sobre los patrones de tiempo-actividad mediante cuestionario, no se pudo obtener la localización geográfica de los lugares frecuentados ni calcular rutas de desplazamiento, variables que hubiesen ayudado a caracterizar mejor la exposición en el exterior como consecuencia de las variaciones de la contaminación a pequeña escala.

5.4. Futuras líneas de investigación

Este trabajo abre diversas líneas de investigación futuras, tanto en la cohorte de Sabadell como en todo el estudio INMA.

El estudio INMA pretende seguir de manera prospectiva el desarrollo de los niños nacidos en cada cohorte hasta la adolescencia. Por lo tanto, el estudio de los efectos de la contaminación atmosférica sobre la salud no se puede limitar solamente a la etapa prenatal, sino que ha de extenderse a la edad infantil y adolescente. Hasta la fecha, en la cohorte de Sabadell se han llevado a cabo visitas de seguimiento a la edad de 6 meses, 14 meses, 2 años y medio y 4 años. Durante este tiempo se han realizado nuevas medidas de contaminación atmosférica en la ciudad y se han llevado a cabo nuevas entrevistas con cuestionarios específicos sobre exposición a contaminación atmosférica, patrones de tiempo-actividad y localización geográfica de guarderías y colegios. Además, durante la visita de los 4 años se han realizado medidas de niveles de NO_2 en el exterior e interior de aproximadamente 100 domicilios de la cohorte. Toda esta información se utilizará para evaluar la exposición a contaminación atmosférica durante los primeros años de vida y estudiar su relación con la salud infantil y el neurodesarrollo.

Por otro lado, y dado que el tamaño de la cohorte de Sabadell es una limitación para el estudio de determinados efectos en salud o de interacciones entre exposición a contaminación atmosférica con otras covariables, se plantea la realización en el futuro de varios análisis conjuntamente con el resto de cohortes de novo del estudio INMA (Valencia, Asturias y País Vasco):

- Estudio de otros efectos perinatales, como los nacimientos prematuros o la mortalidad intrauterina.
- Estudio de la interacción entre factores nutricionales de la madre y la exposición a contaminantes atmosféricos. Puesto que el estrés oxidativo materno juega un papel importante en el crecimiento fetal, es posible que la ingesta de nutrientes

antioxidantes, como las vitaminas C y E, pueda actuar como atenuante del efecto negativo de los contaminantes atmosféricos sobre el desarrollo fetal.

- Uso de marcadores de susceptibilidad genética a los efectos de la contaminación atmosférica. En este sentido, sería interesante estudiar el papel de determinados polimorfismos de genes que regulan procesos de detoxificación, como el *GSTP1*, ya que es posible que los sujetos que presentan un determinado genotipo sean más susceptibles al efecto dañino de la contaminación atmosférica.

5.5. Implicaciones en Salud Pública y en las políticas ambientales

La exposición a contaminantes atmosféricos en ambientes urbanos afecta a toda la población y, salvo en situaciones muy puntuales, es difícil reducir la exposición mediante el comportamiento individual. Por este motivo, aunque el efecto negativo de la contaminación atmosférica sobre el crecimiento fetal sea relativamente pequeño, el impacto en salud pública puede ser considerable en términos de casos atribuibles a nivel poblacional. Se ha estimado que, solamente en EE.UU., entre 1990 y 2010 se prevendrán unos 10.000 casos de bajo peso al nacer como consecuencia de la aplicación de la ley de aire limpio (*U.S. Clean Air Act*) (Wong et al., 2004).

Desde el punto de vista científico, los resultados de este estudio han ayudado a conocer un poco mejor el impacto de la variabilidad intraurbana de la contaminación del aire sobre el crecimiento fetal, haciendo hincapié en la importancia de tener en cuenta los patrones de tiempo-actividad de las mujeres embarazadas para caracterizar mejor su exposición a la contaminación atmosférica. De cara a futuros estudios en este campo, sería conveniente no sólo conocer los patrones de tiempo-actividad sino también las variaciones de estos patrones a lo largo del embarazo y sus implicaciones en la exposición individual a contaminantes a través de diferentes fuentes (exteriores e interiores) en diferentes momentos del embarazo.

A nivel global, conseguir niveles de contaminación del aire suficientemente bajos como para producir efectos negligibles sobre la salud reproductiva e infantil es algo que probablemente requiera de cambios drásticos en el funcionamiento de los vehículos a motor, sistemas de transporte y procesos industriales. Sin embargo, el impacto de la contaminación atmosférica en la población infantil y en las mujeres embarazadas no se está teniendo en cuenta actualmente a la hora de establecer estándares ambientales en las administraciones competentes en esta materia. Ante esta situación, surge la pregunta de cuánta evidencia científica es necesario acumular para que deba considerarse dicho impacto, tal y como se ha hecho con anterioridad en relación a los efectos sobre la mortalidad y las enfermedades respiratorias y cardiovasculares. Dada la especial vulnerabilidad de los niños y las mujeres embarazadas al efecto de los contaminantes ambientales, y que algunos de los efectos perinatales observados (como el bajo peso al nacer y la prematuridad) son eventos frecuentes en la población general, sería muy importante que los resultados de los estudios publicados hasta la fecha, incluido el presente trabajo, fuesen tenidos en cuenta en los procesos de toma de decisiones ambientales, sin esperar a rellenar los huecos de incerteza científica con futuros estudios. Son en casos como este cuando se pone de manifiesto más que nunca que el principio de precaución debería ser el eje vertebrador de las políticas sobre medio ambiente y salud.

A nivel local, el hecho de que, dentro de una misma ciudad, las diferencias en la exposición a contaminación atmosférica estén asociadas con efectos perinatales (además de otros efectos adversos publicados en un creciente número de estudios) debería tener implicaciones sobre el planeamiento urbanístico. El diseño de una ciudad influye en numerosos factores relacionados con la exposición a contaminantes atmosféricos, desde la cantidad de emisiones hasta la actividad física (incluyendo los desplazamientos a pie) de sus habitantes (Marshall et al., 2009). Cada vez más ciudades cuentan con planes municipales de mejora de la calidad del aire, los cuales suelen contemplar medidas como limitar la circulación de vehículos en zonas céntricas, aumentar el número de aparcamientos subterráneos en detrimento de los superficiales o posibilitar el uso de medios de transporte alternativos al automóvil como la bicicleta. Aunque estas medidas contribuyen a reducir las emisiones de contaminantes a nivel general, no siempre son suficientes para reducir el impacto de la contaminación sobre determinadas zonas habitadas que se hallan bajo la influencia directa del tráfico. En este sentido, los resultados de este trabajo recomiendan la necesidad de tener en cuenta la cercanía a las fuentes de emisión de contaminantes atmosféricos como un factor más a la hora de planificar la construcción de nuevas viviendas y la ubicación de determinados equipamientos como guarderías, centros educativos, hospitales o instalaciones deportivas.

6. CONCLUSIONES

1. Existe una considerable variabilidad espacial en las concentraciones de NO_2 y COVs en la ciudad de Sabadell, con niveles más elevados en las inmediaciones de calles con tráfico medio y alto, indicando que el tráfico es la principal fuente de emisión de ambos contaminantes en la ciudad.

2. La exposición personal a NO_2 en mujeres embarazadas de la cohorte INMA-Sabadell está relacionada con los niveles de NO_2 en el interior y exterior de la vivienda (siendo mayor la correlación con los niveles interiores que con los exteriores), así como con el uso de cocina de gas en la vivienda y con el tiempo pasado en ambientes exteriores. Parte del NO_2 medido en el interior de la vivienda procede del exterior, es decir, de las emisiones del tráfico rodado.

3. La exposición prenatal a BTEX y, en menor medida, a NO_2, particularmente durante el segundo trimestre de embarazo, tiene un efecto negativo sobre el crecimiento fetal medido como peso al nacer, aunque solamente es estadísticamente significativo en mujeres que pasaron < 2 horas/día en ambientes exteriores, probablemente debido a una mejor clasificación de la exposición en este subgrupo.

4. Tras evaluar longitudinalmente el crecimiento fetal a través de las ecografías realizadas durante el embarazo, se halló un efecto negativo de la exposición a BTEX desde el inicio del

embarazo en el crecimiento del DBP a partir de la semana 20. En la submuestra de mujeres que pasaron < 2 horas/día en ambientes exteriores, el efecto de la exposición a NO_2 desde el inicio del embarazo sobre el crecimiento fetal fue mayor, hallándose asociaciones estadísticamente significativas con un menor crecimiento en el DBP, PA y PFE a partir de la semana 20 y con un menor crecimiento en el PC entre las semanas 12 y 20. Estos resultados sugieren que la exposición a contaminación atmosférica desde el inicio del embarazo podría afectar al crecimiento fetal hacia la mitad de la gestación.

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ANEXO 1: Protocolo general del estudio INMA

Ribas-Fitó N, Ramón R, Ballester F, Grimalt J, Marco A, Olea N et al. **Child health and the environment: the INMA Spanish Study.** *Paediatr Perinat Epidemiol* 2006; 20(5):403-410

ANEXO 2: Protocolo específico de exposición a contaminación atmosférica

Esplugues A, Fernández-Patier R, Aguilera I, Iñiguez C, García Dos Santos S, Aguirre A, et al. **Exposición a contaminantes atmosféricos durante el embarazo y desarrollo pre y neonatal: Protocolo de investigación en el proyecto INMA (Infancia y Medio Ambiente)** *Gac Sanit* 2007; 21(2): 162-171

ANEXO 3: Cuestionario correspondiente al muestreo personal de 48 horas

IDNUM

Fecha entrevista |__|__|__|__|__|__|

CUESTIONARIO DE EXPOSICIÓN DE 48 HORAS

COMPLETAR AL TERMINAR LAS 48 HORAS DE PARTICIPAR EN EL ESTUDIO

SITUACIÓN ACTUAL:

1- Trabaja
2- Trabaja pero en este momento está de baja

3- En paro / estudiante / ama de casa (exclusivamente)

Dirección completa de su lugar de trabajo (si trabaja fuera de Sabadell, anotar sólo la población):

I: EN CASA Y EN EL TRABAJO

En las 48 horas que ha llevado el captador, y mientras estuvo en casa o en su lugar de trabajo:

1) ¿Durante cuántas horas y minutos estuvo utilizando los siguientes aparatos?
(*rellene el tiempo en las casillas correspondientes y por favor conteste con un 0 si no tiene ese aparato o si no lo ha utilizado*):

(Aproximar por 5 minutos)

	CASA Horas	CASA Minutos	TRABAJO Horas	TRABAJO Minutos
- Una estufa de gas				
- Una estufa de madera				
- Una chimenea				
- Otro tipo de estufa *(especificar)*				
....				
....				
- El aire acondicionado				
- Un humidificador (incluso humidificadores del sistema de calefacción o aire acondicionado)				
- Un purificador de aire, ionizador o filtro de aire				
- Calentador de gas				
- Un horno eléctrico/microondas				
- Una cocina de gas				
- Una cocina con combustible sólido (madera, coque, carbón etc.)				
2) ¿Cuánto tiempo ha tenido las ventanas abiertas?				
3) ¿Durante cuánto tiempo ha utilizado velas o estuvo presente en un lugar en el que hubiera alguna encendida?				
4) ¿Durante cuánto tiempo ha utilizado incienso o estuvo presente en un lugar en el que hubiera encendido?				
5) ¿Durante cuántas horas y minutos ha estado usted u otra persona utilizando una fotocopiadora o una impresora en la misma habitación?				

6) ¿Preparó usted o estuvo presente en la preparación de alguna comida tipo fritura? *(Por favor haga un círculo alrededor de la respuesta correcta)*

1.-Sí
2.-No

7) ¿Preparó usted o estuvo presente en la preparación de alguna comida a la parrilla (asada)? *(Por favor haga un círculo alrededor de la respuesta correcta)*

1.-Sí
2.-No

8) ¿Ha comido estos dos días? *(anotar número de veces)*

	CARNE	PESCADO
Plancha		
Parrilla		
Asada		
Frita		
Guisada		

9) Ha aspirado, barrido o limpiado su casa (esto incluye cualquier actividad generadora de polvo, no incluye fregar, lavar los platos, etc.....)? *(Por favor haga un círculo alrededor de la respuesta correcta)*

1.- Sí
2.-No

4.1.- Si sí, ¿lo hizo usted?
1. Sí
2. No

4.2.- Si no, ¿estaba usted en casa cuando se llevó a cabo?
1. Sí
2. No

10) ¿Ha utilizado en su casa productos químicos de limpieza durante estas 48h?

1.- Sí *(por favor, diga el nombre comercial)* →
2.-No

II: VARIAS ACTIVIDADES

Durante las 48 horas que ha llevado el dispositivo:

1) ¿Cuántas horas y minutos ha estado ocupada en las siguientes actividades, en casa, en el trabajo o en cualquier otro sitio ?

	Horas	Minutos
- Revelando y positivando fotografía		
- Pintando/dibujando		
- Utilizando algún tipo de pegamento		
- Bricolaje o pequeñas reparaciones		
- En una gasolinera		
- Haciendo una barbacoa		
- Estando en el interior de un garaje		
- Otros: gimnasio, sauna, pista de hielo etc		
-		
....		

2) ¿Ha utilizado desodorante, perfume o laca?*(Por favor haga un círculo alrededor de la respuesta correcta)*

1-Sí
2-No
3-No recuerda

III: TABACO

(Por favor haga un círculo alrededor de la respuesta correcta)

1) En su casa, ¿han fumado en la misma habitación donde usted estaba?
1-Sí
2-No
1.1.- Si sí, ¿por cuánto tiempo? ____horas____minutos

2) En su trabajo, ¿han fumado en la misma habitación donde usted estaba
1-Sí
2-No
2.1.- Si sí, ¿por cuánto tiempo? ____horas____minutos

3) En un bar o restaurante, ¿han fumado en la misma habitación donde usted estaba?
1-Sí
2-No
3.1.- Si sí, ¿por cuánto tiempo? ____horas____minutos

4) En algún otro sitio,¿han fumado en la misma habitación donde usted estaba
1-Sí
2-No
4.1.- Si sí, ¿por cuánto tiempo? ____horas____minutos
4.2.- ¿Dónde?

5) ¿Durante el periodo de las 48 horas ha fumado?

1-Sí
2-No
5.1.- Si sí, ¿cuántos cigarrillos? ________

IV: CAPTADORES PASIVOS

1) ¿Durante las 48 horas hubo algún momento en que NO tuviese colgados en la ropa los captadores pasivos, inclusive mientras dormía o estaba en el baño ?
1-Sí
2- No, los lleve puestos todo el tiempo

1.1.- Si sí, rellenar la tabla:

1. ¿Que tipo de actividad realizó sin tener el dispositivo colgado en la ropa?
2. ¿Cuánto tiempo duró esa actividad (aproximar a los 15 minutos)?
3. ¿Dónde se encontraba el dispositivo? (Especificar si estaba en la misma habitación y la distancia al dispositivo en metros. Si es un espacio abierto, se considera la misma habitación)

1. Actividad	**2. ¿Duración?** Horas	Min.	**3. ¿En la misma habitación?**	**4. Distancia al dispositivo**
			1- Sí 2- No	
			1- Sí 2- No	
			1- Sí 2- No	
			1- Sí 2- No	
			1- Sí 2- No	
			1- Sí 2- No	

2) ¿Con qué frecuencia pasan coches al lado de su casa?

1- Continuamente
2- Con bastante frecuencia
3- Poco
4- Nunca

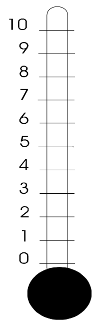
3) ¿Con qué frecuencia pasan vehículos pesados (por ej. camiones/autobuses) al lado de su casa?

1- Continuamente
2- Con bastante frecuencia
3- Poco
4- Nunca

4) ¿Hasta que punto le ha molestado la **contaminación atmosférica del exterior** durante las 48 horas que ha llevado el dispositivo (nos referimos a gases, humos, polvo etc procedente del tráfico, la industria etc.)?
(Esta escala es como un termómetro, marque el nivel de molestia que siente usted, utilizando esta escala del 0 al 10; el 0 no le molesta en absoluto, hasta el 10 es una molestia insoportable)

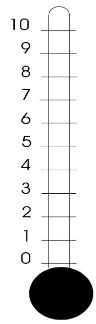
En casa

Molestia insoportable



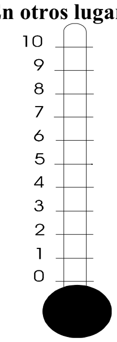
Ninguna molestia

En el trabajo



No procede

En otros lugares



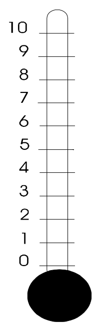
Indicar dónde:

No procede

5)¿Hasta que punto le ha molestado el **ruido del exterior** (procedente del tráfico la industria, etc.) durante las 48 horas que ha llevado el dispositivo?

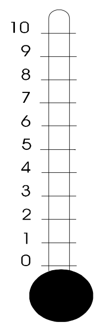
En casa

Molestia insoportable



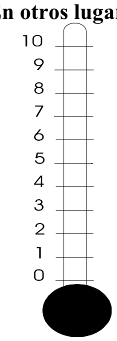
Ninguna molestia

En el trabajo



No procede

En otros lugares



Indicar dónde:

No procede

V: TIEMPO - ACTIVIDAD

1) Durante las 48 horas que llevó el dispositivo, ¿cuántas horas al día pasa en los siguientes lugares?
(Día 1: Las primeras 24h en las que llevó el captador; Día 2: Las 24h siguientes)

	Día 1 (horas:min)	**Día 2 (horas:min)**
En el trabajo:		
-Interior		
-Exterior		
En otros edificios (centros comerciales, lugares públicos.		
Desplazándose (al trabajo, compra, con los niños, otros familiares…)		
Exterior (en la calle, paseando, en un parque...)		
En su vivienda:		
-Interior de la casa		
-Exterior de la casa		
En interior de otras casas		
Total		

2) ¿Cuánto tiempo al día emplea en desplazarse (al trabajo, compra, con los niños, otros familiares...)?

	Día 1 (min/día)	**Día 2 (min/día)**
Caminando		
Bicicleta		
Motocicleta o ciclomotor		
Coche o taxi		
Autobús o tranvía		
En tren o metro		
Total		

OBSERVACIONES:

ENTREVISTADOR:

1. ¿El domicilio tiene balcón y/o ventanas que den a la calle? SI / NO

2. ¿El captador se ha puesto en un balcón o ventana que de a calle? SI / NO

Si la respuesta es NO, indicar si da a patio de manzana, descampado, patio de luces, etc.

…………………………………………………………………