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Internal exposure to pollutants and body size in Flemish adolescents and adults: Associations and dose–response relationships ☆

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ABSTRACT

Flanders is densely populated with much industry and intensive farming. Body size of 14- to 15-year old adolescents and of adults aged 50–65 was studied in relation to internal exposure to pollutants. 1679 adolescents (887 boys and 792 girls), 775 men and 808 women were selected as a random sample of the population. Concentrations of pollutants in blood or urine were measured in accordance with quality control/quality assurance procedures. Self-assessment questionnaires provided information on personal and life-style factors. Height and weight of subjects were measured. Confounding factors and significant covariates were taken into account. For boys and girls, height and body mass index (BMI) showed a negative association with urinary concentration of cadmium and BMI also with serum concentration of hexachlorobenzene (HCB) and with the sum of serum concentrations of polychlorinated biphenyls (PCBs) 138, 153, and 180 (marker PCBs), whereas BMI showed a positive association with serum concentration of PCB 118. For boys, height showed a negative association with urinary concentration of 1-hydroxypyrene (1-OHP) and positive associations with serum concentrations of HCB and PCB 118. For adults no significant associations between internal exposure and height were observed. For men, BMI showed negative associations with urinary cadmium concentration and with serum levels of marker PCBs and positive associations with serum levels of HCB, p,p′-dichlorodiphenyldichloroethylene (p,p′-DDE), PCB 118 and the dioxin fraction of dioxin-like activity. For women, BMI showed a negative association with urinary cadmium concentration, with blood lead concentration and with the concentration of marker PCBs in serum, and a positive association with serum concentrations of HCB, p,p′-DDE and PCB 118. Associations between biological effects and internal exposures were, in terms of the regression coefficient, often stronger at exposures below the median. Environmental exposures to pollutants resulting in "normal" levels of internal exposure were associated with quite substantial differences in body mass index.

1. Introduction

Flanders is one of the most populated areas in Europe, with a dense network of traffic roads, industrial activities and intensive farming close to habitation. The five-year (2001–2006) biomonitoring program on neonates, adolescents and adults (50–65 years) by the Flemish Centre for Environment and Health aimed at measuring internal exposure to pollutants in areas differing in pollution pressure and assessing whether place of residence or observed differences in internal concentrations of pollutants were associated with biological and health effects. All public information on the project can be found on the website www.milieu-en-gezondheid.be.

In this study, we report on body size of 50- to 65-year old adults and 14- to 15-year old adolescents in relation to internal exposure to environmental pollutants suspected to affect hormonal equilibrium. PCBs are known to have estrogenic, anti-estrogenic and anti-androgenic activities (Bonefeld-Jorgensen et al., 2001); p,p′-DDE was reported to

☆ The study was approved by the medical–ethical committee of the University of Antwerp on the 4th of July 2002.

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have anti-androgenic properties (Kelce et al., 1995); HCB was reported to affect oestradiol levels in animals (Alvarez et al., 2000; Foster et al., 1995) and to interact with hormone receptors (Li et al., 2008); cadmium was observed to be able to interact with both estrogen and androgen receptors (Stoica et al., 2000; Martin et al., 2002); lead was reported to have xeno-estrogenic activity (Martin et al., 2003) and to affect pubertal development in girls (Selevan et al., 2003); polycyclic aromatic hydrocarbons were reported to affect development (Choi et al., 2006) and display AhR as well as estrogen receptor-mediated activity (Hilscherova et al., 2000). We wanted to test the hypothesis that low differences in levels of internal exposure (such as these occurring in the general population in Flanders) to endocrine disrupting substances result in differences in body size parameters. In addition, will this effect be larger at the lower end of the range of measured internal exposures than at the higher end of this range? This is what could happen if the above mentioned pollutants interact with receptors, as their dose–response curves might be expected to follow Michaelis–Menten kinetics (Sheehan et al., 1999; Castano and Flores-Saaib, 2008).

2. Materials and methods

2.1. Selection and recruitment of participants

2.1.1. Adolescents

A Stratified Clustered Multi-Stage Design was used to select 1600 participants as a random sample of the adolescents residing in the study areas, comprising 22% of the Flemish territory, 20% of the Flemish population and 20% of the Flemish municipalities as described in detail in Schroijen et al. (2008). The study areas were chosen to represent different types of environmental pressure occurring in Flanders. Sampling took place in three steps: first by study area, second by entities for access to participants (i.e. the schools), and third by selection of the participants in accordance with the inclusion criteria. The adolescents were enrolled via 42 schools located in the nine selected regions, and sampled between October 2003 and July 2004. For the areas around waste incinerators it was not possible to enroll adolescents through schools, because each separate area around a particular incinerator was small and comprised only a few streets. Therefore, adolescents living near an incinerator received a home addressed letter for participation. Inclusion criteria were: being born in 1988 or 1989, studying in the third year of secondary education, living for at least five years in the same study area, and giving informed consent (both adolescent and parents). Of all pupils who received an invitation, 28.4% did not respond, because they did not fulfill the inclusion criteria or because they were not interested, and of those who did respond, 14.7% refused to participate. So 61.07% of the pupils contacted wanted to participate. Among the pupils who wanted to participate, 2.3% were excluded by the researchers because they did not reside in the area since 5 years, and 1.9% because of incomplete questionnaires or insufficient blood or urine. So, finally 58.5% of the pupils contacted participated in the study. The recruitment resulted in a total of 1679 adolescents.

2.1.2. Adults

A Stratified Clustered Multi-Stage Design was used to select 775 men and 808 women ($n = 1583$) aged 50 to 65 as a random sample of the population of the areas under study as described by De Coster et al. (2008). Sampling took place in three steps: first by study area, secondly by sub-municipality entities for access to participants, and thirdly by selection of the participants in accordance with the inclusion criteria.

All participants signed an informed consent form and had the right to withdraw from the study at any time. The study design was approved by the medical–ethical committee of the University of Antwerp on July 4th, 2002.

2.2. Blood and urine collection

Length and body weight of the participants were measured by a study nurse. Each participant donated a urine sample of about 200 mL and a blood sample of 40 mL for subsequent analysis. Serum samples were prepared by immediate centrifugation of the coagulated blood. Urine, whole blood and serum samples were fractionated immediately and stored at −20 °C until analysis.

2.3. Measurement of biomarkers of exposure

Lead and cadmium concentrations in whole blood were determined after an acid digestion pre-treatment destroying the organic matrix and a ten times dilution, followed by high resolution-inductively coupled plasma-mass spectrometry detection (ICP-MS) as described by Schroijen et al. (2008). Detection limits for cadmium and lead in the whole blood were 0.09 and 2.0 µg/L respectively for digested blood samples diluted 10 times. Isotope Cd114 was used to quantify the amount of cadmium in urine using ICP-MS. Urinary cadmium levels were expressed in µg/g creatinine. Urine samples were diluted in nitric acid (0.7%). Rhodium was used as an internal standard. The detection limit for urinary cadmium was 0.002 µg/L. The creatinine content in urine was determined by spectrophotometry.

Polychlorobiphenyl (PCB) 118, PCB 138, PCB 153, PCB 180, hexachlorobenzene (HCB) and p,p′-dichlorodiphenyldichloroethylene (p,p′-DDE) were measured in serum using gas-chromatography-electron capture detection (GC-ECD) as described by Schroijen et al. (2008). The detection limit of all chlorinated compounds in serum was 0.02 µg/L. Blood fat was calculated on the basis of serum cholesterol and serum triglycerides (Covaci et al., 2006). Levels of chlorinated compounds were expressed in ng/g lipid.

CALUX analyses of the dioxin fraction of dioxin-like activity in blood plasma were performed (only in the study on adults) as described by Van Wouwe et al. (2004) and Schroijen et al. (2006). Briefly, 5 mL of blood plasma was extracted with acetone and *n*-hexane and dried on a Celite/Na_2SO_4 column. The extract was then transferred on an acid silica column in series with an activated carbon column (XCARB column). After elution of the sample with *n*-hexane, the acid silica column was discarded and the XCARB column was then differentially eluted to yield 3 fractions:

1. a mixture of *n*-hexane/acetone allows the elution of some toxic or interfering compounds
2. the PCB fraction is eluted with a mixture of *n*-hexane/toluene/ethyl acetate
3. the fraction with polychlorodibenzodioxins (PCDDs) and polychlorodibenzofurans (PCDFs) is collected with 20 mL of toluene.

After this clean-up, fractions 1 and 2 were discarded and only the dioxin fraction was used for the bio-analysis. The solution containing the dioxins was then evaporated and exposed to the mouse hepatoma H1L6.1 cell line developed by Xenobiotic Detection System, Inc. After an exposure time of 20 h, cells were lysed and measurements were made with a luminometer. TEQ-values were calculated after comparison of the obtained signals to a 2,3,7,8-tetrachlorodibenzodioxin calibration curve.

The determination in urine of 1-hydroxypyrene (1-OHP), a metabolite of pyrene, was performed with high performance liquid chromatography (HPLC) as described by Schroijen et al. (2008). The detection limit was 0.030 µg/L.

t,t′-Muconic acid (t,t′-MA), a metabolite of benzene, was determined in urine by means of ion chromatography using SPE-SAX columns as described by Schroijen et al. (2008). The detection limit was 0.0086 mg/L. Levels of 1-OHP and t,t′-MA were expressed in µg/g creatinine and mg/g creatinine respectively.

All laboratories involved in the analyses of biomarkers applied standard agreed quality control/quality assurance procedures.