Host and Environmental Determinants of Polychlorinated Aromatic Hydrocarbons in Serum of Adolescents

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This study investigated host factors and environmental factors as potential determinants of polychlorinated aromatic hydrocarbons (PCAHs) in serum of adolescents. We recruited 200 participants (80 boys and 120 girls), with a mean age of 17.4 years (SD, 0.8), in Belgium from a rural control area (Peer) and from two polluted suburbs of Antwerp where a nonferrous smelter (Hoboken) and waste incinerators (Wilrijk) are located. We quantified polychlorinated biphenyls (PCBs; congeners 138, 153, and 180) in serum by gas chromatography and obtained the toxic equivalents (TEQs) of PCAHs in serum with the chemically activated luciferase gene expression bioassay (CALUX). Serum PCB concentration was higher in boys than in girls (1.67 vs. 1.02 nmol/L or 377 vs. 210 pmol/g serum lipids; $p < 0.001$). In the whole adolescent group, multiple regression showed that serum PCB concentration decreased 0.06 nmol/L per 1% increase in body fat content ($p < 0.001$) and increased 0.39 nmol/L and 0.14 nmol/L per 1 mmol/L increase in serum concentrations of triglycerides ($p < 0.001$) and cholesterol ($p = 0.002$), respectively. Host factors explained 44% of the serum PCB variance. In the same model, serum PCB concentration increased 0.14 nmol/L with 10 weeks of breast-feeding ($p < 0.001$) and 0.06 nmol/L with intake of 10 g animal fat per day ($p < 0.001$), and was associated with residence in the waste incinerator area (9% higher; $p = 0.04$); 11% of the variance could be explained by these environmental factors. The geometric mean of the serum TEQ value was similar in boys and girls (0.15 TEQ ng/L or 33.0 pg/g serum lipids). In multiple regression, TEQ in serum decreased 0.03 ng/L per centimeter increase in triceps skinfold ($p = 0.006$) and was 29% higher in subjects living close to the nonferrous smelter ($p < 0.001$). This study showed that in 16- to 18-year-old teenagers host factors are important determinants of serum concentrations of PCAHs, whereas environmentally related determinants may to some extent contribute independently to human exposure to these persistent chemicals in the environment. *Key words:* **adolescents, biomonitoring of exposure, body fat, breast-feeding, CALUX TEQ bioassay, dietary exposure, dioxinlike compounds, environmental pollution, PCBs, serum.** *Environ Health Perspect* **110:583–589 (2002). [Online 25 April 2002]** *http://ehpnet1.niehs.nih.gov/docs/2002/110p583-589nawrot/abstract.html*

Exposure of humans to xenobiotic chemicals can be estimated via environmental and biologic monitoring. Environmental monitoring usually assesses the external exposure to pollutants in air, water, regular foodstuffs, and so forth. This approach, however, does not take into account personal differences (e.g., age, sex, height, weight, physiologic and nutritional status, duration of exposure, and the like), the possibility of exceptional dietary intake and dermal exposure, or interindividual differences in absorption, distribution, biotransformation, and excretion. Assessment of the absorbed dose of a xenobiotic compound (internal dose) through biomonitoring takes into account most of these parameters. Hence, biomarkers of exposure are likely to be more directly associated with possible adverse health effects (*1*).

Polychlorinated aromatic hydrocarbons (PCAHs) such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) are contaminants of the food chain (*2*), are present in tobacco smoke (*3,4*) and air (*5,6*), and accumulate in fat tissue (*1,2*). Lower chlorinated PCB congeners (e.g., PCB 28, 52, and 101) are less cumulative (shorter half-life in the body) than are higher chlorinated congeners (e.g., PCB 138, 153, and 180). Consequently, the blood serum concentration of lower chlorinated PCBs probably reflects recent exposure to these compounds rather than body burden (*1*).

Determinants of serum PCAHs are well documented in newborns and young children (*7,8*). In the present study, however, we focused on 16- to 18-year-old teenagers, for whom no data are available in the literature, and aimed to assess the contribution of host factors (e.g., sex, body fat, blood fat) and environmental factors (e.g., lifestyle, pre- and postnatal exposure, dietary pattern, and geographical location) to the concentrations of PCAHs in serum.

Materials and Methods

Study group. The protocol of the study has been published in detail elsewhere (*9*). Briefly, we recruited the study population from adolescents in the last two years of high school and who resided in Belgium either in a suburb of Antwerp (Hoboken or Wilrijk) or, as controls, in the small town of Peer and its countryside surroundings. This rural control area is 15–25 km away from the nearest nonferrous and chemical plants and is not near motorways. Environmental monitoring showed that Peer was less polluted than the suburbs of Hoboken and Wilrijk, which are located 11–13 km southeast of the chemical and petrochemical industry established in the seaport of Antwerp. These locations are also the seat of a large nonferrous metal smelter (Hoboken) (*10*), two municipal waste incinerators (Wilrijk), a crematory (Wilrijk), and several small or medium-size enterprises manufacturing electronic equipment, plastics, and nonferrous products (*9*). The two waste incinerators in Wilrijk started their activities in 1970 and 1980, respectively. They had annual turnovers of 23,000 and 110,000 metric tons. They were shut down in November 1997 because in 1996

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We gratefully acknowledge the collaboration of school directorates, the school children, and the school physicians G. Avonts, G. Mertens, A. Nelissen, N. Nuyt, and C. Vandermeulen. Fieldwork was coordinated by S. Van Hulle and done with the assistance of S. Benoy, R. Bollen, I. Calders, L. De Pauw, L. Gijsbers, A. Gijselaar, A. Hermans, M. Jehoul, M. Lommaert, K. Rombouts, Y. Toremans, G. Thijs, M. Vandermaesen, and R. Wolfs. We also thank A. Fastabend and J. Bergerow (Heinrich-Heine-Universität, Düsseldorf, Germany) for the PCB analyses, J. Kleinjans (Universiteit Maastricht, Maastricht, The Netherlands), and W. Slob (RIVM, Bilthoven, The Netherlands) for their useful comments, and A. Brouwer (Vrije Universiteit Amsterdam, The Netherlands) for his gift of the H4IIE rat hepatoma cells used for the measurements of Ah receptor activity of dioxinlike compounds in serum.

The Environment and Health (Milieu en Gezondheid) Study was commissioned and financed by the Ministry of the Flemish Community (Brussels, Belgium).

Received 7 May 2001; accepted 13 November 2001.

and 1997 their dioxin emissions had exceeded the standard [> 0.1 ng toxic equivalents (TEQ)/m^3] (*11*). The dioxin levels in topsoil samples obtained at 15 sites in a radius of 0.5–3.0 km around the incinerators ranged from 3.9 to 27.2 ng TEQ/kg dry weight (*11*). In Hoboken, the deposition of dioxins from fallout dust greatly (> 27 pg TEQ/m^2/day) exceeded the standard (≤ 6.8 pg TEQ/m^2/day) at the time of the study (*12*).

In total, 524 adolescents born in 1980–1983 were eligible. Of these, 169 were not invited because they did not fulfill the requirement of lifelong residence in the study area (n = 7) or because the male/female quota by area had already been satisfied (n = 162). Of the 355 invited youngsters, 207 (58.3%) volunteered to participate. We did not examine seven adolescents because they had recently moved out of the study area (n = 3) or were not immediately available for study because of illness (n = 2) or travel (n = 2). Information on pre- and postnatal variables was missing for three participants. The ethics committee of the University of Leuven approved the study. We obtained written informed consent from the adolescents and their parents.

Questionnaire and clinical measurements. Registered nurses administered questionnaires to gather data on the use of tobacco and alcohol, intake of medicines, social class of the parents, dietary habits, and the consumption of locally produced foods. The mother provided information on birth order of the child, the weeks of gestation, and the duration of breast-feeding. We used a food frequency questionnaire to record information on the intake of meat, fish, eggs, milk, and cheese during the year preceding the present study by asking how many times per day, week, or month they consumed the above-mentioned food categories. From these results, we calculated the food frequencies per month. We computed the amount of fat intake per day from the recorded food frequencies using mean consumption portions and a Dutch food composition table (*13*).

We also recorded anthropometric characteristics (triceps skinfold thickness, waist, and hip circumference). We calculated body mass index (BMI) by dividing body weight (kg) by body height (m^2) and body fat content according to the formula of Deurenberg et al. (*14*): percent body fat = 1.20 × BMI + 0.23 × age – 10.8 × sex – 5.4, in which sex is 0 for females and 1 for males. Deurenberg et al. validated their formula for ages above 16 years against direct densitometric determination of body fat and BMI, taking age and sex into account (correlation coefficient = 0.89).

Laboratory analyses. We collected approximately 50 mL of venous blood in the morning using polyethylene syringes and transferred an aliquot of whole blood into hexane/acetone-cleaned glassware. After clotting the blood, we separated the serum by centrifugation and kept it frozen at –20°C. The analyses of the lower chlorinated PCB congeners (PCBs 28, 52, and 101) and higher chlorinated congeners (PCBs 138, 153, and 180) in serum were performed at the Analytical Chemistry Department of the Medical Institute of Environmental Hygiene (Düsseldorf, Germany). This laboratory is a reference laboratory of the German Society for Occupational and Environmental Medicine and routinely and successfully participates in external quality control studies on PCBs in blood. The method was worked out and described by Fastabend (*15*). Briefly, we mixed aliquots of 2.5 mL of serum sample with formic acid and homogenized them, followed by solvent-extraction (n-heptane) of PCBs and cleanup of the extracts on silica gel columns. We used a high-resolution gas chromatograph equipped with electron capture detection (HR-GC Mega 2; Fisons, Mainz, Germany) and two injectors and two capillary columns of different polarity (DB-5 and DB-1701, both 30 m × 0.32 mm × 0.25 µm; J & W Scientific, Köln, Germany) for the quantification of PCBs. We injected a 1 µL aliquot of each extract at an injector port temperature of 130°C, which we then increased to 280°C. The initial temperature of the columns was 130°C, which we held for 4 min. We then increased the temperature to 210°C at a rate of 8°C/min and finally to 280°C at a rate of 4°C/min and held the column at 280°C for 2 min. The carrier gas was helium at a flow rate of 2 mL/min. At a flow rate of 36 mL/min, argon containing 5% methane (vol/vol) served as makeup gas for the detector, and we calibrated the equipment by analyzing standard solutions. We identified the PCBs by means of the retention times and performed quantification using Mirex (Promochem, Wesel, Germany) as internal standard and included a blind and a control sample in each series of measurements for quality control. The detection limits for the six PCB congeners were in the range of 0.03–0.07 µg/L or 0.08–0.21 nmol/L.

We assessed exposure to dioxinlike compounds via the *in vitro* activation of the aryl hydrocarbon receptor (AhR) of cultured H4IIE rat hepatoma cells by the dioxinlike compounds present in 2 mL of serum [chemically activated luciferase gene expression bioassay (CALUX assay); BioDetection Systems BV, Amsterdam, The Netherlands]. We performed the extraction and cleanup procedures as described in detail elsewhere (*16–18*). Briefly, the method involved n-hexane extraction of the sample and removal of acid-labile matrix components, fat, and nonstable PCAHs by passage through a silica column containing concentrated H_2SO_4 (33%, w/v). We then quantitatively transferred the extract to a conical vial for evaporation till almost dry, followed by adding 7.5 µL dimethyl sulfoxide (Acros Organics, Geel, Belgium) and dilution to a total volume of 750 µL with minimal essential medium (Gibco, Merelbeke, Belgium). The CALUX assay uses rat hepatoma H4IIE cell line that is transfected with an AhR-controlled luciferase reporter gene construct. We grew cells in 96-well plates in minimal essential medium with 10% fetal calf serum (Gibco) at 37°C and 5% CO_2. When the cell layer reached 70–80% confluency, we dosed the cells with 100 µL of the sample extract in triplicate together with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) standards and then incubated them at 37°C for 24 hr. We then removed the medium and washed the cells with phosphate-buffered saline without Ca^{2+}/Mg^{2+} (Gibco) and added 30 µL of a cell-lysis reagent (Promega, Leiden, The Netherlands). We shook the well plates for 45 min and stored them at –80°C for at least 1 hr. We determined the luciferase activity after we thawed the cells in the well plates on ice and added 100 µL luciferin assay mix (Promega) at room temperature. We measured the light production by a Victor 2 Luminometer (EG&G Wallac, Oosterhout, The Netherlands). On each 96-well plate, we added standards of TCDD from 0.3 to 100 pmol/L to construct a TCDD-based calibration curve, which we then used to quantify the TCDD TEQ content of the individual serum samples measured. We express results in nanograms of TCDD TEQs per liter of serum. We ran all 200 serum sample extracts in one experiment to avoid interexperiment variation in cell culture. We calculated the limit of detection as the light signal measured from the dimethyl sulfoxide control plus three times its standard deviation on each well plate (0.10 ± 0.04 TEQ ng/L serum). We also included on each well plate a known fetal calf serum sample for internal quality control.

We determined total serum cholesterol and triglycerides by enzymatic methods using commercially available assay kits (Olympus System Reagents, San Jose, CA, USA). We calculated total serum lipids from total serum cholesterol and triglycerides by using the formula:total serum lipids = [(2.27 × total cholesterol) + triglycerides + 0.623] (*19*).

Statistical methods. We performed database management and statistical analysis with SAS software, version 6.12 (SAS Institute, Cary, NC, USA). We log-transformed log-normal distributed variables and describe them by their geometric means and 95% confidence intervals (CIs), or by the

median and the interquartile range (IQR; 25th to 75th percentiles). We used the Student's *t*-test to compare unpaired data, chi-square test to compare proportions, and Wilcoxon's rank test to compare non-normally distributed data. We identified determinants of the serum concentration of PCAHs by single regression and subsequently by stepwise regression procedures in which we set the *p*-value at 0.10 for the independent variables to enter and to stay in the model. In single and multiple stepwise regression analyses, we tested all variables listed in Table 1 as explanatory variables.

Results

General characteristics. Compared with the 200 participants, the 155 nonparticipants (148 nonrespondents and 7 adolescents excluded from participation) had similar mean age (17.3 vs. 17.4 years; $p = 0.67$), sex distribution (67.9 vs. 60.0% girls; $p = 0.13$), and parental social class (low, medium, and high, 28.8, 63.0, and 8.2 vs. 23.5, 64.5, and 12.0%; $p = 0.30$). In the suburbs, nonparticipants and participants resided at similar distances from the smelter (1,896 vs. 1,993 m; $p = 0.61$) and the largest waste incinerator (1,297 vs. 1,376 m; $p = 0.71$). This indicates that our results are not likely to be confounded by a selection bias.

The 200 adolescents included 120 girls (60.0%). Mean age was 17.4 years (SD, 0.8 years) for the total group and was not different between girls and boys (17.3 vs. 17.4 years). Table 1 lists all variables studied. Triceps skinfold thickness and the waist-to-hip ratio were higher in girls than in boys, whereas BMI did not differ. Because of differences in body composition between males and females, the relationship between BMI and body fat content is sex dependent: Mean calculated body fat content was 24.1% (SD, 3.5%) in girls and 13.1% (SD, 3.5%) in boys. Serum total cholesterol was higher in girls. Serum cholesterol and serum triglycerides did not correlate with the time interval between the last meal and blood sampling. Serum total cholesterol was positively correlated with serum triglycerides ($r = 0.44$; $p < 0.0001$) and with the calculated body fat content ($r = 0.35$; $p < 0.0001$), but not with fat intake. Furthermore, the correlations between triglycerides, body fat content, and fat intake were weak and not significant. In view of these weak correlations, the statistical parameters estimated by multiple regression will not be influenced by collinearity between serum total cholesterol, serum triglycerides, calculated body fat content, and dietary fat intake.

Girls and boys did not differ in pre- and postnatal variables, parental social class, dietary habits, and distribution over geographical location. Although the prevalence of tobacco use by girls and boys was the same, the number of cigarettes smoked per day was higher in boys: Median daily use was 11 cigarettes (IQR, 6–16) in 19 male smokers and only 6 cigarettes (IQR, 4–9) in 31 female smokers ($p = 0.04$). Median number of food servings per month were, for meat, 30 (IQR, 20–30); for fish, 3 (IQR, 1–8); and for milk, 30 (IQR, 8–30). Compared with adolescents in the two suburbs, more adolescents in the rural area consumed locally produced meat (33.0% vs. 5.0%; $p = 0.001$), dairy products (47.0% vs. 20%; $p = 0.001$) and vegetables (39.8% vs. 24.8%; $p = 0.02$). However, in Wilrijk compared with Hoboken, more participants

Table 1. Characteristics of the study population.

	Girls ($n = 120$)	Boys ($n = 80$)	*p*-Value	Total ($n = 200$)
Host factors				
Anthropometric measurements				
Mean (SD) weight (kg)	58.0 (9.3)	67.7 (11.9)	< 0.001[a]	61.9 (11.4)
Mean (SD) BMI (kg/m^2)	21.2 (2.9)	21.1 (2.9)	0.65[a]	21.2 (2.9)
Mean (SD) triceps skinfold (cm)	1.7 (0.6)	1.0 (0.5)	< 0.001[a]	1.4 (0.7)
Mean (SD) waist-to-hip ratio	0.8 (0.1)	0.7 (0.0)	< 0.001[a]	0.7 (0.1)
Lipids				
Mean (SD) total serum cholesterol (mmol/L)	4.5 (0.7)	4.1 (0.8)	< 0.001[a]	4.3 (0.8)
Mean (SD) serum triglycerides (mmol/L)	1.1 (0.5)	1.1 (0.5)	0.85[a]	1.1 (0.4)
Environmental factors				
Pre- and postnatal variables[b]				
Mean (SD) maternal age at birth (years)	27.0 (3.8)	27.9 (4.0)	0.12[a]	27.3 (3.9)
Mean (SD) duration of gestation (weeks)	39.3 (1.6)	39.5 (1.2)	0.37[a]	39.4 (1.4)
Order of birth				
Number (%) first child	50 (41.7)	34 (42.5)	—	84 (42.0)
Number (%) second child	48 (40.0)	32 (40.0)	—	80 (40.0)
Number (%) subsequent children	22 (18.3)	14 (17.5)	0.98[c]	36 (18.0)
Mean (SD) birth weight (kg)	3.3 (0.5)	3.4 (0.5)	0.06[a]	3.3 (0.5)
Number (%) breast-fed subjects	66 (55)	46 (58)	0.48[c]	112 (56)
Median (IQR) weeks of breast-feeding	8 (5–13)	12 (6–15)	0.99[d]	9 (6–13)
Lifestyle				
Social class of parents				
Number (%) workers	34 (28)	13 (16)	—	47 (24)
Number (%) middle class	75 (63)	54 (68)	—	129 (64)
Number (%) learned professionals	11 (9)	13 (16)	0.08[c]	24 (12)
Number (%) current smokers	31 (26)	19 (24)	0.74[c]	50 (25)
Number (%) subjects consuming alcohol	35 (29)	52 (65)	0.001[c]	87 (44)
Number (%) girls on oral contraceptives	49 (41)	—	—	—
Dietary habits				
Median (IQR) meat (servings/month)	30 (20–30)	30 (20–30)	0.19[d]	30 (20–30)
Median (IQR) fish (servings/month)	3 (1–8)	3 (3–8)	0.44[d]	3 (1–8)
Median (IQR) milk (servings/month)	30 (8–30)	30 (8–60)	0.56[d]	30 (8–30)
Mean (SD) animal fat intake (g/day)	63.6 (26.3)	67.9 (22.6)	0.22[a]	65.3 (2.5)
Geographic location				
Number (%) residents of Peer (rural control area)	60 (50)	40 (50)	—	100 (50)
Number (%) residents of Wilrijk (waste incinerator)	21 (18)	21 (25)	—	42 (21)
Number (%) residents of Hoboken (nonferrous smelter)	39 (32)	19 (25)	0.22[c]	58 (29)

[a]Data that are normally distributed are presented as arithmetic means (SD) and were compared by Student's *t*-test. [b]Because of missing information, statistical analyses were performed in 119 girls and 77 boys. [c]Numbers (%) are compared by a χ^2 test. [d]Data that are not normally distributed are presented as medians (IQR) and were compared by Wilcoxon's rank test.

consumed locally produced diary products (30.1% vs. 12.1%; $p = 0.02$), meat (11.9% vs. 0.0%; $p = 0.07$), and vegetables (38% vs. 12%; $p = 0.002$).

Determinants of serum PCBs. The serum concentration of the lower chlorinated PCB congeners 28, 52, and 101 were all below their respective limits of detection (0.16, 0.21, and 0.18 nmol/L). The marker PCB congener profile (PCBs 138, 153, and 180) did not differ across the three areas. PCB congener 153 represented the major fraction (46%) of the combined marker PCBs (congeners 138, 153, and 180), whereas congeners 138 and 180 each accounted for 27% of the total. The correlations between the serum concentrations of PCB congeners 138, 153, and 180 ranged from 0.80 to 0.93 (all $p < 0.001$).

The geometric mean of the summed PCBs (138, 153, and 180) was higher in boys than in girls (1.67 nmol/L vs. 1.02 nmol/L; $p < 0.001$), and the corresponding PCB values standardized per gram of total serum lipids were 377 vs. 210 pmol ($p < 0.001$; Figure 1). The concentrations of the serum PCBs did not differ between smokers and nonsmokers (1.22 nmol/L vs. 1.28 nmol/L; $p = 0.56$). Single regression analysis (Table 2) showed that in boys as well as girls the combined serum concentrations of congeners 138, 153, and 180 (expressed per liter of serum) were negatively correlated with anthropometric variables. In girls, serum PCB concentration correlated positively with milk consumption, intake of fat, the duration of breast-feeding, and the concentrations of serum triglycerides and cholesterol, whereas in boys it correlated positively with fat intake and serum triglycerides, and marginally ($p = 0.06$) with the duration of breast-feeding. In both girls and boys, serum PCB concentrations were negatively associated with body fat content and positively with residence in Wilrijk (Table 2).

The sex-related differences in the serum PCB concentration disappeared after allowing for calculated body fat content (1.28 in boys vs. 1.21 nmol/L in girls; $p = 0.61$). In further analysis, we applied stepwise multiple regression analysis in boys and girls combined, allowing for body fat content. The serum concentration of PCBs was independently and inversely associated with the body fat content but rose with the serum concentrations of total cholesterol and serum triglycerides, weeks of breast-feeding, animal fat intake, and residence in Wilrijk (Table 3). However, in subjects living in Wilrijk, we found no associations between serum PCB concentration and the consumption of local produce. All variables together explained 54.7% of the variation, with 43.9% from host factors and 10.8% from environmental factors.

Determinants of serum CALUX TEQ. The geometric mean of the serum TEQ values did not differ according to smoking status (0.15 TEQ ng/L in smokers vs. 0.16 ng/L in nonsmokers; $p = 0.55$) or sex (Figure 1) (0.16 TEQ ng/L in boys vs. 0.14 TEQ ng/L in girls; $p = 0.41$). The TEQ values in boys and girls standardized to 1 g of total serum lipids were 36.4 and 30.8 pg TEQ ($p = 0.10$), respectively.

In both single and multiple regressions, the serum TEQ values (expressed per liter of serum) decreased by 19% per centimeter increase in triceps skinfold thickness and were about 29% higher in subjects living in Hoboken compared with the residents of the two other areas (Table 4). Adjusted for triceps skinfold thickness, subjects living in Hoboken ($n = 58$) who consumed vegetables from their own garden ($n = 7$) had a higher serum TEQ value (0.34 vs. 0.19 TEQ ng/L; $p = 0.08$). In contrast to PCBs, serum TEQ values were not significantly associated with serum total cholesterol and triglycerides. Triceps skinfold thickness and residential area explained together 9% of the serum TEQ variance.

The following variables did not enter any of the regression models both for serum PCBs and for TEQ: time between the last meal and blood sampling, birth weight, maternal age at birth, birth order, weeks of gestation, smoking, alcohol intake, the frequencies of intake of meat and fish, and socioeconomic status of the parents. In an additional analysis, the associations were maintained (serum lipids not offered in the set of independent variables) when we expressed serum PCBs and TEQ per gram of fat instead of per liter of serum.

Discussion

In our study we identified determinants of serum concentrations of PCBs and TEQ in older teenagers. The PCB congeners 138, 153, and 180 usually account for 40–60% of the total PCB body burden (*19*). In our study, the lower chlorinated PCBs (congeners 28, 52, and 101) were below the limit of detection. As result of the lower degree of chlorination, the lower chlorinated congeners are in general hydroxylated in reactions catalyzed by cytochrome P450, whereas the higher chlorinated PCBs are resistant to biotransformation (*20*). The concentration of lower chlorinated PCBs in serum reflects recent exposure rather than body burden (*1*). Detection of these lower chlorinated compounds would have required much more serum volume. We expressed dioxinlike compounds in TEQs that represent the potency to activate the AhR. Some of the PCB congeners also act through binding to the AhR (*16*). Previous studies, in

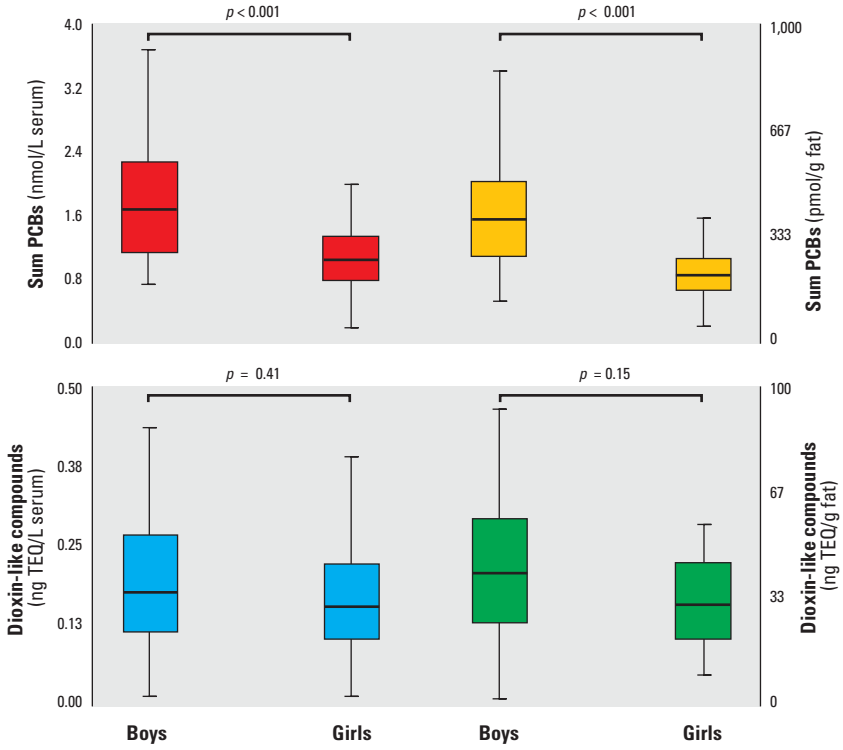

Figure 1. Box plots of serum PCBs (nmol/L and pmol/g serum fat) and serum dioxinlike compounds (TEQ ng/L and pg TEQ/g fat) in boys ($n = 79$) and girls ($n = 120$). Shown are the median (center line in each box), IQR (distance from top to bottom borders of each box), and the 5th and 95th percentiles (the bars at each end of the boxes).

which dioxin congeners were determined separately by using gas chromatography with high-resolution mass spectrometry, and in which the TEQ value was calculated by multiplying concentrations of individual dioxin congeners by their respective toxic equivalent factors (TEFs), should be interpreted cautiously, because TEFs for individual congeners are theoretical values based on various toxicologic end points obtained in different studies (*16*). Moreover, TEQs are usually assumed to be additive and not synergistic or antagonistic (*17*). The CALUX assay used in the present study is mechanistically based and thus measures all compounds in the serum that act via binding to the AhR.

In 1995, the median serum PCB levels in the general population of the United States ranged between 2 and 7 µg/L (~6–21 nmol/L) (*21*). In 286 German children 9–12 years of age, the mean serum concentration of the combined PCB congeners 138, 153, and 180 amounted to 181 ng/g lipids, which was higher than the mean vlaue in our total adolescent group (277 pmol/g ~ 1-3 ng/g lipids) (*22*). At the time of the present adolescent study, the same project consortium also collected blood samples in 200 healthy women (50–65 years old) from the same dwelling areas as the adolescents and used the same analytical methods for the quantification of CALUX TEQ and PCBs in 47 randomly constituted serum pools (25 for the Antwerp suburbs and 22 for the rural area) (*23*). The overall mean serum concentrations amounted to 36.0 pg TEQ/g fat for CALUX TEQ and to 365.4 ng/g fat (~983.3 pmol/g) for indicator PCBs (congeners 138, 153, and 180). Compared with the women's study, our adolescents showed rather similar mean serum TEQ values (30.8 and 36.4 pg TEQ/g fat in girls and boys, respectively), whereas the mean serum concentrations of indicator PCBs were much lower (210 and 377 pmol/g fat, respectively). The lower serum PCB concentration may be explained by the facts that in the Western world, exposure to PCBs has fallen since the 1970s (*24*) and, in modern communities, where life expectancy exceeds 70 years, serum PCBs in 16- to 18-year-old youths may reflect relatively recent exposure. The lower serum PCB concentration may thus represent a birth cohort effect. Hence, taking adolescents as study populations has advantages for studying secular trends in the serum concentration of PCAHs and for monitoring the effectiveness of environmental hygiene strategies aiming at the reduction of exposure to these compounds.

Host factors. In the present study, the host factors independently determining the serum concentrations of PCAHs are serum total cholesterol, serum triglycerides, and body fat content. These host factors explained 44% of the variance in serum PCBs and 4% of the variance in serum TEQ. Throughout life, PCAHs accumulate in adipose tissue, which represents the major storage site for these lipophilic compounds (*25*). The serum PCB concentrations found in this study were higher in boys than in girls. However, after adjustment for body fat content, the sex difference disappeared. In both single and multiple regressions, the serum levels of PCBs were negatively associated with body fat content and those of TEQ were negatively associated with skinfold thickness. A similar pattern was seen in younger children (*26*), but the opposite phenomenon was found in adults (*25,27*). Although the absolute body burden of PCAHs might be higher in obese than in lean adolescents, the finding of negative relationships between serum PCAHs and such anthropometric variables as body fat content and skinfold thickness might be linked to the growth spurt during early adolescence (*28,29*). In view of the assumption of a steady state between PCAH concentration in fat tissue and plasma, this would suggest the existence of a transient dilution effect for PCAHs in adipose tissue during growth. Because of the ubiquity of PCAHs in the environment and their high affinity for fat tissue, these compounds will continue to accumulate in adulthood (*21*), whereas body fat mass does not change much before age 60. For example, the 50th fat mass percentile of healthy subjects in 15–24 to 45–54 year age groups increases from 16% to 20.3% in males and from 26.3% to

Table 3. Correlates of serum PCBs[a] in multiple regression analysis (boys and girls combined, $n = 196$).

Independent variables	Estimate[b]	Partial R^2	p-Value
Host factors			
Body fat content[c] (> 1%)	−4.5 (−5.3–−3.7)	0.300	< 0.001
Total cholesterol (> 1 mmol/L)	11.3 (3.0–20.2)	0.023	0.002
Triglycerides (> 1 mmol/L)	30.9 (16.3–47.4)	0.116	< 0.001
Environmental factors			
Duration of breast-feeding (> 1 week)	1.1 (0.5–1.6)	0.044	< 0.001
Fat intake (> 1 g/day)	0.5 (0.3–0.7)	0.054	< 0.001
Residence in Wilrijk[d]	9.2 (3.0–18.8)	0.010	0.043

Significance and independent correlates of serum PCBs were identified by stepwise regression analysis. Each correlate in the table was therefore adjusted for the others. Variables considered for entry into the model were those reported in Table 1 and the calculated body fat content.
[a]Sum of congeners 138, 153, and 180 (volumetric units, log value). [b]Percentage change (95% CI) in serum PCB concentration associated with a 1-unit change in the independent variable. [c]Calculated according to the formula of Deurenberg et al. (*14*). [d]Compared with Peer and Hoboken.

Table 2. Correlates of serum PCBs[a] in single regression analysis by sex.

	Girls ($n = 120$)			Boys ($n = 79$)[b]		
Single regression model	Estimate[c]	R^2	p-Value	Estimate[c]	R^2	p-Value
Host factors						
Body weight (> 1 kg)	−1.2 (−2.1–−0.3)	0.066	0.007	−1.2 (−1.9–−0.4)	0.106	0.003
BMI (> 1 kg/m^2)	−4.7 (−7.3–−2.1)	0.091	0.001	−4.8 (−7.7–−1.8)	0.114	0.002
Triceps skinfold (> 1 cm)	−22.4 (−31.9–−11.6)	0.110	< 0.001	−27.7 (−38.7–−14.8)	0.162	< 0.001
Waist-to-hip ratio (> 0.1)	−0.8 (−1.0–−0.1)	0.050	0.047	−0.7 (−1.0–1.8)	0.01	0.31
Body fat content (> 1%)[d]	−3.8 (−6.0–−1.6)	0.085	0.001	−3.8 (−6.3–−1.4)	0.108	0.003
Total serum cholesterol (> 1 mmol/L)	23.2 (10.2–37.6)	0.103	< 0.001	6.6 (−5.3–20.0)	0.015	0.29
Serum triglycerides (> 1 mmol/L)	42.8 (19.9–70.2)	0.119	< 0.001	25.0 (5.1–48.6)	0.076	0.014
Environmental factors						
Duration of breast-feeding (> 1 week)[e]	1.4 (0.5–2.3)	0.080	0.002	0.8 (0.0–1.6)	0.046	0.06
Milk consumption (> 1 serving/month)	0.5 (0.1–0.9)	0.043	0.023	0.3 (−0.2–0.7)	0.016	0.27
Fat intake (> 1 g/day)	0.5 (0.2–0.9)	0.091	< 0.001	0.5 (0.1–0.9)	0.064	0.024
Residence in Wilrijk[f]	14.9 (−0.8–33.2)	0.028	0.06	15.6 (0.5–33.0)	0.054	0.046

All characteristics listed in Table 1 and the calculated body fat content were tested as explanatory variables, but results are given only for those variables that were significantly correlated with the serum PCB concentration in at least one sex.
[a]Sum of congeners 138, 153, and 180 (volumetric units, log value). [b]PCB measurement missing in one boy. [c]Percentage change (95% CI) in serum PCB concentration associated with a 1-unit change in the independent variables. [d]Calculated according to the formula of Deurenberg et al. (*14*). [e]Because of missing information, statistical analyses were performed in 119 girls and 77 boys. [f]Compared with Peer and Hoboken.

27.9% in females (*30*). In adulthood, the cumulative effect of PCAHs could outweigh the dilution effect of fat mass, whereas in childhood fat mass could cause a dilution effect. The latter may explain why the unadjusted serum PCB levels were much lower in girls than in boys (Figure 1), because the body fat content of girls was about double that of boys. In the course of life, the negative relation between serum PCAHs and body fat content in youths could switch to a positive one in adults. The intake of fat may increase both the serum PCB concentration (*1*) and the body fat content (*28*) and may outweigh the dilution effect of body fat content as observed in adolescents.

Besides accumulation in the adipose tissue, PCAHs preferentially bind to lipid components in the plasma, and the lipid fraction has been shown to be a predominant PCB carrier in plasma (*19*). In the present study, the serum concentration of PCBs correlated positively with the levels of triglycerides or total cholesterol in serum. The conclusions drawn from the multiple regression analysis, however, were not affected by the mode of expression of the serum PCAH concentrations: neither per gram of total serum lipids nor per liter of serum after allowing for serum lipids in multiple regression. In addition, the time interval between breakfast and the collection of the blood sample in the morning did not predict the serum concentrations of lipids, PCBs, or dioxinlike compounds. A typical breakfast in Belgium is light and usually consists of a few slices of bread and/or some cereals.

The findings above point out that host factors may bias between-group comparisons and that they should be taken into account when describing relations between internal PCAH exposure and health effects. Therefore, in epidemiologic studies, ensuring that host factors are as similar as possible for the exposed and reference groups is of great importance, but they should be preferentially allowed for in multivariate analysis.

Environmental factors. Because PCAHs are persistent in the environment, people may be exposed to them through their diet. The assumption is often made that diet represents the main route of exposure to PCBs and dioxins. Dairy products are major sources of PCBs and dioxinlike compounds (*31*). In accordance with studies in The Netherlands (*31*), we found in single-regression analysis significant and positive correlations between the serum concentration of PCBs and the intake of milk products or total fat. In our regression models, we did not find frequencies of fish and meat consumption to be predictors of the serum PCB or TEQ concentrations, most likely because of the limited sample size and possibly also because we could not specify the kind of meat or the species of fish (*32*). Other possible reasons for the lack of association with the consumption of meat and fish are that we obtained the information on dietary factors only for the year preceding the study and that the food pattern of youngsters is known to be more variable over time than that of adults.

Throughout life, PCAHs accumulate in the mother's body. During pregnancy these chemicals transfer from the mother to her fetus through the placenta (*33*), and in newborns and infants their body burden of PCAHs increases with maternal age, the duration of gestation, and breast-feeding (*8,33*). The higher chlorinated PCBs and dioxins have half-lives of 14 years (*31*) and 7 years (*34*), respectively. The level of PCAHs in human milk increases with maternal age but declines with the number of births (*8*). In the present study, we found that breast-feeding, after more than 15 years, was still a significant determinant of the serum concentration of higher PCB congeners. Although breast-feeding is likely to contribute to the child's body burden of PCBs, this should not discourage mothers from breast-feeding their babies because of its overall beneficial effects on general development of the infant (*7,35*) and its trophic properties for counteracting hypocalcemia (*35*), allergic diseases (*36*), infections (*36*), and vitamin deficiencies (*35,36*).

Comparison between locations after adjustment for the significant covariables showed that the serum PCB concentration was 9.2% higher in adolescents living in Wilrijk (waste incinerators) whereas the serum TEQ value was 29% higher in adolescents living in Hoboken (nonferrous smelter). The different geographical pattern might be linked to differences in sources of PCAHs between the two suburbs. The metallurgic industry in Hoboken claims to be a world leader in recycling of precious metal-bearing materials (250,000 tons/year), among them autocatalysts, electrical/electronic scrap, and photographic products (*37*). Such industries may release to the atmosphere dioxins that are formed during the smelting and refining processes that recover precious and other valuable metals from the waste, which usually contains chlorine-based plastics (*12,38*). The higher serum TEQ values in Hoboken are in line with the increased environmental dioxin pollution in the nonferrous smelter area at the time of the study (> 27 pg TEQ/m^2 deposition per day compared with a background value of ≤ 6.8 pg TEQ/m^2) (*12*). In addition, we found also a link between serum TEQ values and the consumption of locally grown vegetables. Less straightforward is the situation in Wilrijk, where we found only higher serum PCB concentrations in the adolescents despite historical records of increased environmental dioxin pollution (*11*). At municipal waste incinerator sites, increased emission of PCBs may originate from wood preservatives, contaminated oil, and older electrical equipment present in the waste to be handled (*6,39*) and/or may be formed during the combustion process (*5*). The release of PCAHs in the atmosphere due to waste incinerator activity can contaminate the food chain with PCBs and dioxinlike compounds (*40,41*). To our surprise, we could not demonstrate any link between the consumption of locally produced foodstuffs and the serum PCB or TEQ concentrations in the adolescents of Wilrijk. Such associations might have been blurred by the facts that the incinerators had been shut down for almost 2 years at the time of our study in 1999, and that the half-life of dioxins in serum is on average half that of PCBs. The combination of these two factors might have resulted in an accelerated washout of dioxins, because the source of its production and emission was no longer in operation. However, as in our study, higher serum PCB concentrations have been found in 10-year-old children who lived in the neighborhood of a waste incinerator in Germany (*42*). In general, the atmospheric levels of PCBs appear to be higher in urban

Table 4. Correlates of the serum TEQ level in single and multiple regression analysis (girls and boys combined, $n = 200$).

Independent variables	Estimate[a]	Partial R^2	*p*-Value
Single regression model			
Host factors			
Triceps skinfold (> 1 cm)	−18.5 (−29.9–−5.4)	0.040	0.008
Environmental factor			
Residence in Hoboken[b]	28.5 (10.6–49.1)	0.052	< 0.001
Multiple regression model			
Host factors			
Triceps skinfold (> 1 cm)	−18.8 (−29.7–−6.3)	0.040	0.006
Environmental factor			
Residence in Hoboken[b]	28.5 (10.7–49.1)	0.051	0.001

All characteristics listed in Table 1 and the calculated body fat content were tested as explanatory variables, but results were given only for those variables that were significantly correlated with serum TEQ.
[a]Percentage change (95% CI) in serum TEQ (volumetric units, log value), associated with a 1-unit change in the independent variable. [b]Compared with Peer and Wilrijk.

than in rural areas (*39*). For example, in 1996 the atmospheric concentrations of PCBs measured at urban and rural locations in Baltimore were 0.38–3.36 and 0.02–0.34 ng/m^3, respectively (*43*). It is thus not surprising that PCB uptake from inhalation of contaminated ambient air may lead to serum PCB concentrations that exceed the mean background in serum (*44*). Unfortunately, the lack of historical PCB measurements in the environment of the Wilrijk area prevents us from making causal inferences as to the environmental compartment(s) that would significantly influence the serum PCB concentrations in the Wilrijk adolescents.

Conclusions

This study surprisingly showed that the duration of breast-feeding is still a significant predictor of the serum concentration of PCBs at 16–18 years of age. In addition, in 16–18-year-old adolescents the serum concentration of PCBs increased with higher concentrations of serum lipids, whereas it declined with the body fat content. The latter probably explains the higher concentration of serum PCBs in boys. We found host factors to be the most substantial determinants of serum PCBs. They should be taken into account in studies dealing with the contributions of environmental exposure to the body burden of PCBs and adverse health effects of these compounds. We found the measurement of marker PCBs and TEQ values in the serum of the adolescents sensitive enough to pick up geographical differences in environmental exposure to these chemicals.

References and Notes

1. Lauwerys RR, Hoet P. Industrial Chemical Exposure: Guidelines for Biological Monitoring. Boca Raton, FL:Lewis Publishers CRC Press, 2001.
2. van Larebeke N, Hens L, Schepens P, Covaci A, Baeyens J, Everaert K, Bernheim JL, Vlietinck R, De Poorter G. The Belgian PCB and dioxin incident of January–June 1999: exposure data and potential impact on health. Environ Health Perspect 109:265–273 (2001).
3. Muto H, Takizawa Y. Dioxins in cigarette smoke. Arch Environ Health 44:171–174 (1989).
4. Lackmann G, Angerer J, Tollner U. Parental smoking and neonatal serum levels of polychlorinated biphenyls and hexachlorobenzene. Pediatr Res 47:598–601 (2000).
5. Stach J, Pekarek V, Grabic R, Lojkasek M, Pacakova V. Dechlorination of polychlorinated biphenyls, dibenzo-*p*-dioxins and dibenzofurans on fly ash. Chemosphere 41:1881–1887 (2000).
6. Rowat SC. Incinerator toxic emissions: a brief summary of human health effects with a note on regulatory control. Med Hypotheses 52:389–396 (1999).
7. Koopman-Esseboom C, Weisglas-Kuperus N, De Ridder MA, Van der Paauw CG, Tuinstra LG, Sauer PJ. Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. Pediatrics 97:700–706. (1996).
8. Lanting CI, Fidler V, Huisman V, Boersma ER. Determinants of polychlorinated biphenyl levels in plasma from 42-month-old children. Arch Environ Contam Toxicol 35:135–139 (1998).
9. Staessen JA, Nawrot T, Den Hond E, Thijs L, Fagard R, Hoppenbrouwers K, Koppen G, Nelen V, Schoeters G, Vanderschueren D, et al., for the Environment and Health Study group. Renal function, cytogenetic measurements and sexual development in adolescents in relation to environmental pollutants: a feasibility study of biomarkers. Lancet 357:1660–1669 (2001).
10. Roels HA, Buchet JP, Lauwerys RR, Bruaux P, Claeys-Thoreau F, Lafontaine A, Verduyn G. Exposure to lead by the oral and the pulmonary routes of children living in the vicinity of a primary lead smelter. Environ Res 22:81–94 (1980).
11. Belgium. VITO. Studie van de gezondheidsaspecten en gezondheidsrisico's ten gevolge van de milieuverontreiniging in de Neerlandwijk te Wilrijk. Studie uitgevoerd in opdracht van het Ministerie van de Vlaamse Gemeenschap, Departement Gezondheidsbeleid. 1998/TOX/R/0097 Mol, Belgium: Vlaams Instituut voor Technologisch Onderzoek, 1998.
12. Vlaamse Milieumaatschappij. Statistisch nieuws. Meetresultaten dioxinedeposities 2000 VMM. Available: http:// www.vmm.be/vmm/dioxines-28-9-00.doc [cited 12 October 2001].
13. Breedveld BC, Hammink J, van Oosten HM. Nederlandse Voedingsmiddelentabel. Voorlichtingsbureau voor de Voeding. Den Haag, The Netherlands: Toegepast Natuurwetenschappelijk Onderzoek, 1996.
14. Deurenberg P, Weststrate JA, Seidell JC. Body mass index as a measure of body fatness: age- and sex-specific prediction formulas. Brit J Nutr 65:105–114 (1991).
15. Fastabend A. Bestimmung umweltbedingter Konzentrationen von PCB und Organochlorpestiziden in Blut mit GC-ECD unter Anwendung optimierter Probenvorbereitungsverfahren. [PhD thesis]. Clausthal, Germany:University of Clausthal, 2000.
16. Murk AJ, Legler J, Denison MS, Giesy JP, van de Guchte C, Brouwer A. Chemical-activated luciferase gene expression (CALUX): a novel *in vitro* bioassay for Ah receptor active compounds in sediments and pore water. Fundam Appl Toxicol 33:149–160 (1996).
17. Murk AJ, Leonards PE, van Hattum B, Luit R, van der Weiden ME, Mit M. The CALUX assay adapted and validated for measuring TCDD equivalents in blood plasma. Environ Toxicol Chem 16:1583–1589 (1997).
18. Murk AJ, Leonards PE, van Hattum B, Luit R, van der Weiden ME, Smit M. Application of biomarkers for exposure and effect of polyhalogenated aromatic hydrocarbons in naturally exposed European otters (*Lutra lutra*). Environ Toxicol Pharmacol 6:91–102 (1998).
19. Phillips DL, Pirkle JL, Burse VW, Bernert JT Jr, Henderson LO, Needham LL. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. Arch Environ Contam Toxicol 18:495–500 (1989).
20. Safe S. Toxicology, structure–function relationship, and human and environmental health impacts of polychlorinated biphenyls: progress and problems. Environ Health Perspect 100:259–268 (1992).
21. Kimbrough RD. Polychlorinated biphenyls (PCBs) and human health: an update. Crit Rev Toxicol 25:133–163 (1995).
22. Wuthe J, Piechotowski I, Papke O, Zier B, Gabrio T, Kramer D, Kouros B, Schwenk M, Pfaff G. First data on background levels on non-ortho and mono-ortho PCBs in blood of residents from southern Germany. Chemosphere 32:567–574 (1996).
23. Koppen G, Covaci A, Van Cleuvenbergen R, Schepens P, Winneke G, Nelen V, Schoeters G. Comparison of CALUX-TEQ values with PCB and PCDD/F measurements in human serum of the Flanders Environmental and Health Study (FLEHS). Toxicol Lett 123:59–67 (2001).
24. Brouwer A, Ahlborg UG, van Leeuwen F, Feeley MM. Report of the WHO working group on the assessment of health risks for human infants from exposure to PCDDs, PCDFs and PCBs. Chemosphere 37:1627–1643 (1998).
25. Duarte-Davidson R, Jones KC. Polychlorinated biphenyls (PCBs) in the UK population: estimated intake, exposure and body burden. Sci Tot Environ 151:131–152 (1994).
26. Ryan JJ, Hsu CC, Boyle MJ, Guo YL. Blood serum levels of PCDFs and PCBs in Yu-Cheng children perinatally exposed to a toxic rice oil. Chemosphere 28:1263–1278 (1994).
27. Falk C, Hanrahan L, Anderson HA, Kanarek MS, Draheim L, Needham L, Patterson D. Body burden levels of dioxin, furans, and PCBs among frequent consumers of Great Lakes sport fish. The Great Lakes Consortium. Environ Res 80:S19-S25 (1999).
28. Forbes GB. Human Body Composition: Growth, Aging, Nutrition, and Activity. New York:Springer-Verlag, 1987.
29. van der Molen GW, Kooijman SA, Slob W. A generic toxicokinetic model for persistent lipophilic compounds in humans: an application to TCDD. Fundam Appl Toxicol 31:83–94 (1996).
30. Kyle UG, Genton L, Slosman DO, Pichard C. Fat-free and fat mass percentiles in 5225 healthy subjects aged 15 to 98 years. Nutrition 17:534–541 (2001).
31. Patandin S, Dagnellie PC, Mulder PG, Op de Coul E, Van der Veen JE, Weisglas-Kuperus N, Sauer PJ. Dietary exposure to polychlorinated biphenyls and dioxins from infancy until adulthood; a comparison between breast-feeding, toddler, and long-term exposure. Environ Health Perspect 107:45–51 (1999).
32. Fürst P, Beck H, Theelen R. Assessment of human intake of PCDDs and PCDFs from different environmental sources. Toxic Substances J 12:133–150 (1992).
33. Lackman GM, Angerer J, Salzberger U, Tollner U. Influence of maternal age and duration of pregnancy on serum concentrations of polychlorinated biphenyls and hexachlorobenzene in full-term neonates. Biol Neonate 76:214–219 (1999).
34. Pirkle JL, Wolfe WH, Patterson DG, Needham LL, Michalek JE, Miner JC, Peterson MR, Phillips DL. Estimates of the half-life of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in Vietnam Veterans of Operation Ranch Hand. J Toxicol Environ Health 27:165–171 (1989).
35. Gordon N. Nutrition and cognitive funcion. Brain Dev 19:165–170 (1997).
36. Hoppu U, Kalliomaki M, Laiho K, Isolauri E. Breast milk-immunomodulatory signals against allergic diseases. Allergy Suppl 67:23–26 (2001).
37. Boghe D. Electronic scrap: a growing resource. Metal Bulletin Monthly, June 2001, pp. 21–24.
38. Buekens A, Stieglitz L, Hell K, Huang H, Segers P. Dioxins from thermal and metallurgical processes: recent studies for the iron and steel industry. Chemosphere 42:729–735 (2001).
39. ATSDR. Toxicological Profile of Polychlorinated Biphenyls. Atlanta, GA:Agency for Toxic Substances and Disease Registry, 2000.
40. Lovett A, Foxal CD, Creaser CS. PCB and PCDD/DF congeners in locally grown fruit and vegetable samples in Wales and England. Chemosphere 34:1421–1436 (1997).
41. Lovett A, Foxal CD, Creaser CS. PCB and PCDD/DF concentrations in egg and poultry meat samples from known urban and rural locations in Wales and England. Chemosphere 37:1671–1685 (1998).
42. Holdke B, Karmaus W, Kruse H. Körperlast an polychlorierten Biphenylen im Vollblut bei 7–10 jährigen Kindern in der Umgebung einer Sonderabfallverbrennungsanlage [Body burden of polychlorinated biphenyl compounds in whole blood of 7–10-year-old children in the area of a hazardous waste incineration facility]. Gesundheitswesen 60:505–512 (1998).
43. Offenberg JH, Baker JE. Influence of Baltimore's urban atmosphere on organic contaminants over the northern Chesapeake Bay. J Air Waste Manage Assoc 49:959–965 (1999).
44. Neisel F, von Manikowsky S, Schumann M, Feindt W, Hoppe HW, Melchiors U. Humanes Biomonitoring auf Polychlorierte Biphenyle bei 130 in einer Grundschule exponierten Personen [Human biomonitoring of polychlorinated biphenyls in 130 exposed elementary school children]. Gesundheitswesen 3:137–149 (1999).