Polychlorinated dibenzo-p-dioxins, furans, and biphenyls (PCDDs/PCDFs and PCBs) in breast milk and early childhood growth and IGF1

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Abstract

Experimental studies have shown that dioxin-like chemicals may interfere with aspects of the endocrine system including growth. However, human background population studies are, however, scarce. We aimed to investigate whether early exposure of healthy infants to dioxin-like chemicals was associated with changes in early childhood growth and serum IGF1. In 418 maternal breast milk samples of Danish children (born 1997–2001) from a longitudinal cohort, we measured polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls (pg or ng/g lipid) and calculated total toxic equivalent (total TEQ). SDS and SDS changes over time (Δ SDS) were calculated for height, weight, BMI, and skinfold fat percentage at 0, 3, 18, and 36 months of age. Serum IGF1 was measured at 3 months. We adjusted for confounders using multivariate regression analysis. Estimates (in parentheses) correspond to a fivefold increase in total TEQ. TEQ levels in breast milk increased significantly with maternal age and fish consumption and decreased with maternal birth year, parity, and smoking. Total TEQ was associated with lower fat percentage (-0.45 s.d., CI: -0.89 ; -0.04), non-significantly with lower weight and length at 0 months, accelerated early height growth (increased Δ SDS) (Δ SDS 0–18 months: $+0.77$ s.d., CI: 0.34 ; 1.19) and early weight increase (Δ SDS 0–18: $+0.52$ s.d., CI: 0.03 ; 1.00), and increased IGF1 serum levels at 3 months ($+13.9$ ng/ml, CI: 2.3 ; 25.5). Environmental exposure to dioxin-like chemicals was associated with being skinny at birth and with higher infant levels of circulating IGF1 as well as accelerated early childhood growth (rapid catch-up growth).

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Introduction

Studies of populations accidentally exposed to high amounts of dioxin-like chemicals as well as experimental

animal studies have shown that dioxin-like chemicals may interfere with the endocrine system resulting in deleterious effects on reproductive development and growth (Aoki 2001, Mocarelli *et al.* 2008, Nishijo *et al.* 2012). Epidemiological studies of possible effects of background levels of these chemicals are, however, scarce (White & Birnbaum 2009).

Humans are exposed to dioxins and polychlorinated biphenyls (PCBs) throughout life mainly via food of animal origin. The exposure levels generally decrease. Major sources of the compounds used to be poorly controlled urban waste incineration, but improvement of burning processes in modern good-quality incinerators (high incineration temperature >1000 °C) has dramatically reduced dioxin emission from this source. Levels are, however, still relatively high in many European countries (Ulaszewska *et al.* 2011). These chemicals have long half-lives and bio-accumulate; thus, the body burden

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usually increases with age (Linden 2010). Women reduce their body burden with childbearing and breast-feeding. First-born children, therefore, receive a higher load of these chemicals in early life (Ulaszewska *et al.* 2011).

Factors predicting early childhood growth patterns are currently of increasing interest in epidemiological studies, as both low birth weight and rapid growth in early childhood are associated with adiposity in adolescents, earlier pubertal maturation (Ong *et al.* 2009) and are strong predictors of development of obesity and diseases in adults (Monteiro & Victora 2005, Gluckman *et al.* 2008). Maternal smoking in pregnancy, early weaning, and primiparity are factors known so far to predict rapid early childhood growth (Chrestani *et al.* 2013). Other environmental factors may also play a role, but to our knowledge, no studies have so far investigated the role of background environmental exposure to chemicals such as dioxins and dioxin-like chemicals on early growth patterns.

Thus, we aimed to study associations between levels of exposure to dioxins, PCBs, and IGF1 levels and growth in early childhood. We hypothesized that background exposures to these chemicals might affect both birth weights and subsequent growth patterns.

Materials and methods

In the longitudinal 'Copenhagen Mother Child Cohort of Growth and Reproduction' with recruitment from 1997 to 2001 ($n=2098$) (Chellakooty *et al.* 2006), mothers were asked to collect breast milk samples for analysis of environmental chemicals. Breast milk samples ($n=417$) were randomly selected among samples with a sufficient volume, belonging to children who preferably attended the clinical child examinations at 0, 3, 18, and 36 months of age.

The 0-month measurements took place at birth or a few days hereafter, before discharge from the maternity ward. In case of preterm birth, the examination took place around the expected date of delivery and the 3- and 18-month examinations were also corrected for prematurity.

As an estimate of the pre- and early postnatal exposure to dioxins and dioxin-like chemicals, non-dioxin-like PCBs, flame retardants, and PFCs, the milk samples were analyzed for seven dioxin- (PCDD), ten furan- (PCDF), 12 dioxin-like PCB- (dl-PCB), six non-dioxin-like PCB- (ndl-PCB), and eight polybrominated diphenyl ether (PBDE) congeners, as well as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS).

The study was conducted according to the Helsinki II Declaration and was approved by the local ethics committee (KF 01-030/97/KF 01276357) and the Danish Data protection Agency (1997-1200-074). The families gave their informed written consent to the study. Other aspects of this study have previously been published (Boisen *et al.* 2004, Chellakooty *et al.* 2006, Kai *et al.* 2006, Main *et al.* 2006).

Clinical examinations

Height was measured using a wall-mounted stadiometer to the nearest mm (Holtain Ltd, Crymch, UK). Weight was measured

to the nearest 0.1 kg using electronic scales (Seca Delta, Model 707, Seca, Hamburg, Germany and Bisco Model PERS 200, Bisco, Farum, Denmark). BMI was calculated as weight (kg) divided by height squared (m^2).

Skinfolds were measured at four anatomical sites (triceps, subscapular, suprailiac, and biceps) with a Harpenden calliper (John Bull, British Indicators Ltd, West Sussex, UK) with a precision of 0.1 mm after allowing the jaws to close for ~ 2 s (Rodriguez *et al.* 2005). Fat percentage was calculated using Slaughters equation (for girls: $(1.33 (\text{triceps} + \text{subscapular}) - 0.013 (\text{triceps} + \text{subscapular})^2 - 2.5)$ and for boys: $(1.21 (\text{triceps} + \text{subscapular}) - 0.008 (\text{triceps} + \text{subscapular})^2 - 1.7)$ (Slaughter *et al.* 1988).

All physicians in the study participated in workshops to assure and maintain standardization. All anthropometrical measurements were measured in triplicate and means were used for tables and analyses.

Age- and gender-specific SDSs for weight, height, BMI, and skinfold fat percentage were calculated using the cohort as reference. Catch-up growth was defined as a positive change in SDS (ΔSDS) ≥ 0.67 s.d. for weight and height respectively (Ong *et al.* 2000, Chrestani *et al.* 2013), and catch-down growth was defined as $\Delta\text{SDS} \leq -0.67$ s.d. (Ong *et al.* 2004). Non-fasting blood samples were drawn at 3 months.

Hormonal analysis

The 3-month blood samples were analyzed for IGF1 with a validated RIA in our own laboratory (Juul *et al.* 1994). In brief, IGF1 was used as radioligand in the assay after acid-ethanol extraction and cryoprecipitation of serum samples to remove interfering IGF binding proteins. The intra- and interassay coefficients of variation (CV) were ≤ 10.3 and $\leq 9.4\%$ respectively. Limit of detection was 21 ng/ml.

Milk samples and chemical analysis

Samples were collected between first and third postnatal month. Mothers were given oral and written information to feed the baby first and then sample milk aliquots by manual expression into a glass container. The milk was frozen in glass bottles in household freezers until they were delivered at the 3-month examination, where they were stored at -20°C until analysis as described previously (Main *et al.* 2006).

The chemical analyses were performed (blinded to knowledge about mothers/children) by the Laboratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA), Nantes-Atlantic National College of Veterinary Medicine, Food Science and Engineering (ONIRIS) Nantes, France. The methodologies applied to isolate, detect, and quantify persistent organic pollutants including dioxins (17 PCDD/F congeners) and polychlorobiphenyls (12 dioxin-like and six non-dioxin-like congeners) (Antignac *et al.* 2006, Costera *et al.* 2006) as well as brominated flame retardants (eight PBDE congeners) have been described earlier (Cariou *et al.* 2005, Antignac *et al.* 2009).

Before extraction, ^{13}C -labeled congeners were added to each sample for quantification according to the isotopic dilution method. Breast milk samples were first submitted to

a liquid/liquid extraction with pentane. Resulting extracts were weighed to measure fat content and reconstituted in hexane for further sample clean up. Then, three purification steps were performed, using successively acid silica, florisil, and celite/carbon columns. PCDD/F, PCB, and PBDE measurements were performed by gas chromatography (Agilent 7890A) coupled to high-resolution mass spectrometry on electromagnetic sector instruments (JEOL MS 700D or 800D), using a 10 000 resolution and the single ion monitoring (SIM) acquisition mode. All these methods were fully validated according to current European criteria in the field of regular control of foodstuff of animal origin and accredited according to the ISO 17025 standard. Recoveries were typically in the 80–120% range, and the method's global extended uncertainties were lower than 20%.

Toxic equivalence factor and toxic equivalent quantity

The toxicity of dioxins, furans, and PCBs is expressed as toxic equivalence factor (TEF), determined by the affinity with the aryl hydrocarbon (Ah) receptor in comparison to the most toxic congener 2,3,7,8-TCDD (TEF=1). The total toxicity of a mixture of dioxins and dioxin-like compounds can be expressed as the toxic equivalent quantity (TEQ). The TEQ for each congener is the product of concentration and the TEF of the individual congener. The total TEQ was calculated as the sum of all: $\text{Total TEQ} = \sum(\text{Conc}_{\text{PCDD}_{1-7}} \times \text{TEF}_{\text{PCDD}_{1-7}}) + \sum(\text{PCDF}_{1-10} \times \text{TEF}_{1-10}) + \sum(\text{PCB}_{1-12} \times \text{TEF}_{1-12}) + \sum(\text{PCB}_{\text{cop}_{1-6}} \times \text{TEF}_{1-6})$ (Van den Berg *et al.* 2006).

Questionnaire data

At enrollment in pregnancy, the women answered questionnaires concerning former pregnancies, height, weight, height of child's father, lifestyle, and socioeconomic status. Missing data on height of parents were supplemented at later child examinations. Target SDS was calculated based on mean of mother and father height +6.5 cm for boys and −6.5 cm for girls (Andersen *et al.* 1982) (data missing for 56 parents). Mothers' pre-pregnancy BMI was categorized as <20, 20–25, or >25. Socioeconomic status was based on parental education and occupation according to national standards (Hansen 1982). The social class (1=high to 5=low) of the highest ranking parent living with the child was used. Weight for gestational age (WGA) was expressed as the deviation of birth weight for a given gestational age (in % from the expected) (Marsal *et al.* 1996). Appropriate weight for gestational age (AGA) was defined as a WGA between −22 and +22% (−2 and +2 s.d.). Parity was categorized as one (primiparous mother) or two (1–4 earlier child births).

Mothers were asked whether they smoked, and if yes, how many cigarettes per day. The following categories were used for analyses: 3: smoking >10 cigarettes, 2: smoking 5–10 cigarettes, 1: smoking <5 cigarettes, 0: non-smoking (or stopped before pregnancy).

Mothers were asked how often they had fish for main course, on bread or in salads. For analyses, maternal fish intake during pregnancy was categorized as no/seldom or more than once a month.

At 3 months, the mothers were asked whether they had introduced other food items than breast milk (all children included in this study was breastfed, but some also received formula feeding). This was categorized as only breast milk: yes/no.

During the examinations at 3, 18, and 36 months of age, mothers were asked whether they had stopped breast-feeding (missing data in 52), and if yes, in which week *post partum*. Time of weaning was categorized as before/after (</≥) 6 months.

Statistical analysis

We analyzed the data by applying a linear machine learning classifier approach, the Partial Least Square (PLS), which is particularly suited when there are many predicting variables and there is multicollinearity among these. The analyses were performed using Simca-P.10.5 (Umetrics, Inc., Umea, Sweden) adjusted for confounders (same as later analyses). Contribution plots (score mode) were obtained, where the y-axis represents the PLS coefficients (scaled and centered). This approach describes the relationship between weight/height and all congeners. The contribution plots display which chemical positively or negatively contribute to the model. Hereafter, we used TEQs in all further analyses. Total TEQ was log-transformed to normalize residuals. We used log5 transformation (as opposed to log10) in order to give effect estimates corresponding to a fivefold (instead of tenfold) increase in total TEQ, which was considered relevant, given the distribution of total TEQs in the population (Fig. 1). For descriptive statistics (differences in TEQs between categorical variables), *t*-test and univariate regression analysis (≥3 categories) were used.

Associations between total TEQs and growth outcomes were analyzed with linear/multiple regression analysis using three strategies/models: i) all children, unadjusted, ii) all children,

Figure 1 Distribution of total toxic equivalent (total TEQ 2005) of PCDDs, PCDFs, and dioxin-like PCBs in breast milk from mothers of 417 healthy Danish children.

adjusted for relevant confounders, and iii) adjusted analyses for children of non-smoking women only.

Furthermore, we added analyses for samples from primiparous women only, and including AGA, term (≥ 37 gestational weeks) children only.

All adjusted models included the precise age at examination. For examinations at 0 and 3 months, we adjusted in addition for the total number of days post-conception to correct for gestational length. Fat percent of the breast milk was considered as a potential confounding factor, although it showed to have no impact on growth outcomes. It was not included in final models as it also did not have impact on size of estimates. Maternal smoking in pregnancy, maternal BMI,

target height SDS, and weaning were also considered potential confounding factors. Factors with a $> 10\%$ impact on estimates were included in final models.

Statistically significant results * were defined as $P < 0.05$, but associations that were near significant ($P \leq 0.1$) were also marked (*).

Results

The distribution of total TEQ levels in the Danish breast milk samples is shown in Fig. 1. Population characteristics and concentrations of PCDDs, PCDFs, and dioxin-like PCBs expressed as total TEQ are shown in Table 1.

Table 1 Population characteristics and concentrations of polychlorinated dibenzo-p-dioxins (PCDDs)/polychlorinated dibenzofurans (PCDFs), dioxin-like polychlorinated biphenyls (dl PCBs) expressed as toxic equivalents^a (TEQ 2005) in Danish milk samples.

	Total TEQ (PCDD/F+dl-PCB)					P value ^b
	Count (%)	Mean	Min	Max	Median	
Total	417 (100)	21.2	4.9	114.1	20.2	
Sex						
Boys	218 (52.3)	21.7	4.9	114.1	20.6	0.2
Girls	199 (47.7)	20.8	8.6	73.5	19.6	
Parity*						
1	277 (66.4)	22.3	8.9	114.1	20.9	<0.005
2–5	140 (33.6)	19.2	4.9	47.7	18.0	
Maternal fish intake*						
No/seldom	63 (16.1)	17.8	8.6	30.8	16.6	<0.005
> 1/month	329 (83.9)	21.6	4.9	114.1	20.3	
Mothers' age at child birth (years)*						
<25	25 (6.0)	16.3	8.6	30.8	15.8	<0.005
25–30	164 (39.3)	20.2	9.0	95.7	19.7	
30–35	161 (38.6)	22.4	4.9	114.1	19.8	
>35	67 (16.1)	22.8	9.1	36.9	22.9	
Mother pre-pregnancy BMI (kg/m ²)						
<20	64 (16.6)	20.3	8.6	73.5	18.5	>0.1
20–25	243 (63.3)	21.4	4.9	114.1	20.4	
>25	77 (20.0)	19.7	5.5	47.7	18.5	
Social class*						
1–2	267 (65.6)	22.0	4.9	114.1	20.6	<0.05
3–5	140 (34.4)	19.8	5.5	47.7	19.1	
Maternal cigarette smoking in pregnancy*						
No	368 (88.2)	21.7	5.5	114.1	20.6	<0.05
<5	14 (3.4)	17.1	9.1	31.1	18.0	
5–10	24 (5.8)	18.9	4.9	36.9	18.0	
>10	11 (2.6)	16.7	8.6	24.0	16.1	
Exclusively breastfed at 3 months						
No	76 (18.3)	22.4	8.9	114.1	20.8	>0.2
Yes	340 (81.7)	20.9	4.9	95.7	20.0	
Time of weaning (months)						
<6	80 (22.3)	20.9	9.9	47.7	20.0	>0.2
≥ 6	279 (77.7)	21.7	8.6	114.1	20.4	
Height 0–18 Δ SDS						
< -0.67	84 (22.3)	19.0	8.6	38.6	18.4	<0.01
≥ -0.67 , <0.67	181 (48.0)	21.9	9.0	114.1	20.8	
≥ 0.67	112 (29.7)	22.7	4.9	95.7	21.3	
Weight 0–18 Δ SDS						
< -0.67	88 (23.5)	19.5	8.6	38.6	19.1	<0.01
≥ -0.67 , <0.67	176 (47.1)	21.5	4.9	73.5	20.5	
≥ 0.67	110 (29.4)	23.4	9.1	114.1	21.3	

* $P < 0.05$.

^aBased on 2005 TEF values Van den Berg (2006). ^bDifferences in TEQs between categorical variables were tested with *t*-test and by univariate regression analysis (≥ 3 categories) (log-transformed TEQs).

Figure 2 Chemical contribution plot for weight Δ SDS 0–18 (adjusted for maternal smoking in pregnancy, weaning before/after 6 months, and precise age (gestational age + age at examination at 0 months and precise age at 18 months)).

Total TEQ levels decreased with increasing maternal year of birth independently of maternal age. The significantly lower TEQ levels with lower social class disappeared when adjusting for fish-eating habits (more women with high socioeconomic status were fish eaters). Lower TEQ levels in smokers were attenuated, but still significant, when adjusting for fish-eating habits of the women.

Figures 2 and 3 show which individual congeners contribute most to accelerated growth between 0 and 18 months in a multivariate analysis. The analysis of all congeners together with confounders showed an association with changes of weight and height SDS from birth to 18 months of age ($r=0.27$, $r=0.31$ respectively for models with three PLS components). The combined

Figure 3 Chemical contribution plot for height Δ SDS 0–18 (adjusted for maternal smoking in pregnancy, weaning before/after 6 months, precise age (gestational age + age at examination at 0 months, and precise age at 18 months), and target height SDS).

exposure pattern of dioxins and dioxin-like chemicals positively affected height and weight gain in infants, while most PBDEs, PBBs, and PFCs did not, or had (non-significant) negative associations.

Unadjusted and adjusted mean changes in growth parameters associated with a fivefold increase in total TEQ are shown in Table 2. Total TEQ levels were not associated with changes in gestational age (+0.1 day, CI: -5.3; 5.5) or WGA (-2.2%, CI: -7.8; 3.3). TEQ levels were also not associated with changes in BMI/fat% from skinfold (3, 18, and 36 months, Δ SDS 0-3, 0-18, or 0-36 months) (data not shown).

Analyses restricted to samples from primiparous mothers slightly reduced estimates (height Δ SDS 0-18: +0.51 s.d., CI: -0.02; 1.04 ($n=199$) and weight Δ SDS 0-18: +0.39 s.d., CI: -0.18; 0.97 ($n=236$)), whereas analyses restricted to term, AGA children increased estimates (height Δ SDS 0-18: +0.91 s.d., CI: 0.47; 1.35 ($n=270$) and weight Δ SDS 0-18: +0.67 s.d., CI: 0.17; 1.16 ($n=308$)).

Maternal breast milk of children who experienced rapid catch-up growth (≥ 0.67 s.d.) for weight or height between 0 and 18 months had significantly higher total TEQ than of children without catch-up (12.2%, CI: 4.5; 20.8).

Discussion

In this large study of Danish infants, we found that levels of exposure to environmental dioxin-like chemicals were associated with accelerated infant height and weight gain

and increased IGF1 concentrations at 3 months. Maternal breast milk of children with catch-up growth contained higher concentrations of dioxin-like chemicals. In addition, dioxin-like chemicals were associated with lower fat percentage and a non-significant tendency to lower weight at 0 months. To our knowledge, this paper is the first to show associations to rapid infant growth. Our findings are supported by a Taiwanese study that reported marginally higher IGF1 and height in 5-year-old children with high placental dioxin levels (TEQ PCDD/PCDFs > 15) compared with those with lower dioxin levels (TEQ PCDD/PCDFs < 15) (Su *et al.* 2010).

Primiparity has consistently been reported to be associated with rapid infant growth (Ong *et al.* 2002, Karaolis-Danckert *et al.* 2009, Chrestani *et al.* 2013). We did not adjust for parity in our analysis, as parity is an important predictor of chemical levels as the maternal level of persistent chemicals declines with parity and breast-feeding. However, analyses on samples from primiparous women alone did not change results. Thus, our data indicate that exposure to environmental chemicals contribute to the previously found association between primiparity and catch-up growth. If exposure to persistent chemicals causes catch-up growth, this might to some extent explain why first-born children grow faster in early life than their siblings.

We found non-significant negative associations between TEQs and length/weight at birth. This is in line with other studies with comparable exposure levels that have reported negative effects of dioxin-like chemicals on birth weight (Patandin *et al.* 1998, Tajimi *et al.* 2005, Konishi *et al.* 2009).

Table 2 Mean change (B and 95% CI) in growth parameters in 417 children associated with a fivefold increase in total TEQ^a.

	Model 1: unadjusted		Model 2: adjusted for relevant confounders ^b		Model 3: adjusted ^c in children of non-smoking mothers $n=368$	
	B	95% CI	B	95% CI	B	95% CI
Weight SDS 0 months (s.d.)	-0.35	-0.80; 0.10	-0.31(*)	-0.66; 0.05	-0.38(*)	-0.76; 0.00
Weight SDS 3 months (s.d.)	0.03	-0.37; 0.42	-0.03	-0.42; 0.37	0.03	-0.47; 0.41
Weight SDS 18 months (s.d.)	0.32	-0.12; 0.76	0.26	-0.20; 0.72	0.40	-0.10; 0.90
Weight SDS 36 months (s.d.)	0.15	-0.32; 0.63	0.12	-0.37; 0.62	0.14	-0.41; 0.70
Skinfold fat % SDS 0 months (s.d.)	-0.53*	-0.99; -0.08	-0.46*	-0.89; -0.04	-0.52*	-1.08; -0.03
Skinfold fat % SDS 3 months (s.d.)	-0.32	-0.78; 0.15	-0.25	-0.69; 0.20	-0.07	-0.56; 0.43
Skinfold fat % SDS 36 months (s.d.)	0.02	-0.44; 0.48	-0.00	-0.47; 0.47	-0.07	-0.48; 0.62
Δ Weight SDS 0-3 months (s.d.)	0.39(*)	-0.07; 0.84	0.22	-0.13; 0.58	0.30	-0.08; 0.68
Δ Weight SDS 0-18 months (s.d.)	0.72*	0.19; 1.25	0.52*	0.03; 1.00	0.69*	0.18; 1.21
Δ Weight SDS 0-36 months (s.d.)	0.52(*)	-0.03; 1.06	0.39	-0.12; 0.91	0.41	-0.15; 0.96
Length SDS 0 months (s.d.)	-0.22	-0.63; 0.20	-0.24	-0.57; 0.09	-0.34(*)	-0.70; 0.01
Length SDS 3 months (s.d.)	0.32(*)	-0.06; 0.70	0.16	-0.20; 0.52	0.09	-0.31; 0.49
Height SDS 18 months (s.d.)	0.56*	0.14; 0.98	0.52*	0.10; 0.94	0.48*	0.02; 0.95
Height SDS 36 months (s.d.)	0.37(*)	-0.09; 0.83	0.46(*)	-0.01; 0.93	0.32	-0.21; 0.85
Δ Height SDS 0-3 months (s.d.)	0.55*	0.15; 0.94	0.37*	0.11; 0.64	0.40*	0.11; 0.69
Δ Height SDS 0-18 months (s.d.)	0.91*	0.44; 1.37	0.77*	0.34; 1.19	0.79*	0.33; 1.26
Δ Height SDS 0-36 months (s.d.)	0.67*	0.17; 0.18	0.55*	0.08; 1.03	0.52*	0.00; 1.04
IGF1 at 3 months (ng/ml)	13.7*	2.1; 25.4	13.9*	2.3; 25.5	13.9*	0.9; 26.9

* $P < 0.05$ (bold cells); (*) $P \leq 0.1$.

^aBased on TEF values from van den Berg (2006). ^bAll: precise age at examination (gestational age + examination age for 0 and 3 months). All height measures adjusted for target height SDS. All outcomes at 18 and 36 months adjusted for weaning ≤ 6 months. Weight, height, and skinfold fat % SDS at 0 months and Δ weight/height SDS from 0 to 3, 18, 36 months adjusted also for maternal smoking. ^cSame adjustments as above except for maternal smoking in pregnancy.

Women with higher socioeconomic status had significantly higher TEQ levels, which was explained by differences in fish-eating habits. Non-smoking women also had significantly higher TEQ levels than smoking women. This is in accordance with recent findings of lower levels of dioxins, furans, and PCBs among smokers (Jain & Wang 2011). In our study, some (but not all) of this difference could be attributed to differences in fish-eating habits. Another explanation could be a faster decay of chemicals in smokers than in non- and ex-smokers, as previously reported among workers occupationally exposed to dioxins (Flesch-Janys *et al.* 1996). Such findings suggest that lifestyle patterns may significantly modulate the impact of environmental exposures on health parameters, supporting the need for sophisticated statistical models to study potential associations.

We measured chemicals in breast milk as a biomarker of maternal/prenatal exposure, as these samples could easily be collected non-invasively and the chemicals have a long half-life *in vivo*. Levels in breast milk correlate with levels in maternal fat tissue, blood, and cord blood (Needham *et al.* 2002, Wittsiepe *et al.* 2007, Nakamura *et al.* 2008). The size of our population was relatively large compared with other studies, which enabled us to include a number of relevant confounders into the analyses. As an example, we checked that the found associations were not explained by confounding by fat content of the milk. In general, estimates from adjusted and unadjusted analyses did not differ much. Neither did analyses including subgroups of the population such as only non-smoking mothers or children born at term and being AGA. This supports that our observation is a true finding. We, however, cannot rule out residual confounding. As an example, other classes of (not measured) persistent chemicals correlated with PCDD/F or PCB might also have contributed to the found associations.

Total TEQs have been used in other studies concerning endocrine and growth outcomes (Tajimi *et al.* 2005, Everett & Thompson 2012, Rennert *et al.* 2012). However, total TEQs/TEFs are not based on endocrine toxicity/growth outcomes but are derived from binding to the Ah receptor. Thus, we also assessed the contribution of single congeners in a joint analysis (PLS model). Several dioxin-like congeners contributed significantly to a positive association with accelerated growth in early childhood, especially those with the highest TEFs. The Ah receptor is an essential component of the adaptive xenobiotic stress system, which recognizes toxic xenobiotics and triggers their elimination. However, it has a wider physiological relevance, as it is also involved in metabolism and the central regulation of energy balance (Barouki *et al.* 2012). This is illustrated by the dramatic reduction of body weight (wasting syndrome) seen in rats and other laboratory animals after both pre- and postnatal dioxin exposure

(Linden *et al.* 2010). We speculate that the catch-up growth associated with dioxin-like chemicals might at least partly be explained by a compensatory mechanism following intrauterine growth restriction. Fivefold increases in toxicity (which are relevant given the distribution of TEQs in our data) corresponded to a clinically significant change in growth curves for height and weight SDS.

Our findings of this possible link between dioxins and rapid infant growth may be of concern as accelerated growth is associated with increased waist circumference, blood pressure (Tzoulaki *et al.* 2010), risk of obesity (Monteiro & Victora 2005), and risk of cardiovascular disease (Gluckman *et al.* 2008). Furthermore, persistent chemicals are associated with increased risk of adult diabetes in both cross-sectional and longitudinal studies (Taylor *et al.* 2013). Accelerated growth in early childhood may thus represent an intermediate factor between chemical exposures and such increased risk of adult disease.

As velocity of weight gain in early childhood (before 2 years) has also been associated with earlier onset of both pubertal growth spurt, attainment of peak height velocity, and menarche (Karaolis-Danckert *et al.* 2009, Ong *et al.* 2009), children with higher exposure to these chemicals may also experience earlier puberty. In line with this, a German study recently reported an association between dioxins in cord blood and breast milk and serum levels of DHEAS at 6–8 years of age (Rennert *et al.* 2013).

In conclusion, we found that early exposure to dioxins and dioxin-like chemicals was associated with accelerated early childhood growth in height and weight – a growth pattern that is known to be associated with increased risk of adult obesity and disease.

Discussion from meeting

Åke Bergmann (Stockholm, Sweden): You concentrated on the evaluation of dioxins and TEQs (total toxic equivalency factor of dioxins) which you showed to increase the growth of length in infants, but you omitted to discuss the reverse effect on growth induced by the PBDEs (flame retardant polybrominated diphenyl ethers). How does this influence your conclusions?

Christine Wohlfahrt-Veje (Copenhagen, Denmark): There was a weak (non-significant) negative association between PBDEs and growth. However, when we adjust for PBDEs in our analysis, it has no impact on effect estimates for total TEQs and hence it does not change our conclusions.

Richard Ivell (Dummerstorf, Germany): Did you control for ethnicity? Different ethnic groups have different growth tables.

Christine Wohlfahrt-Veje: All the children in this cohort are Danish Caucasians.

Julie Boberg (Søborg, Denmark): At what age were the breast milk samples taken?

Christine Wohlfahrt-Veje: We did not want to collect milk until breast-feeding was established; therefore, the first few days of lactation were not sampled. The mothers collected milk thereafter and submitted the samples at the 3-month checkup.

Julie Boberg: At this time, the child is in an early rapid growth period. It is possible that rapid growth was not caused by dioxins but that the most rapidly growing children require more milk and deplete their mothers' fat stores to a greater extent, thereby receiving more dioxins and PCBs. Alternatively, mothers of rapidly growing children might have to eat more in order to produce sufficient milk, and high dietary dioxins/PCBs could increase levels in the milk.

Christine Wohlfahrt-Veje: The concentrations of chemicals are expressed in pg/g lipid, and we have adjusted for the level of fat in the milk. Our results are not affected by taking lipid content into account.

Jérôme Ruzzin (Bergen, Norway): Have you data on milk consumption? The rapid growing babies possibly drink much more milk than the slow growers. If the mother's milk is high in dioxins, the babies are not necessarily highly exposed, depending on the amount consumed.

Christine Wohlfahrt-Veje: It is difficult to measure how much milk a baby is receiving but I do not think that is important. All the babies were breast fed and we documented when supplementary feeding was initiated and for how long breast-feeding continued. Adjusting for these made no difference. We can assume that the levels of compounds in the milk are a proxy for intrauterine exposure because breast milk dioxins are associated with levels in maternal and cord blood.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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