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Prenatal dioxin exposure and neurocognitive development in Hong Kong 11-year-old children

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ABSTRACT

Background and objectives: *In utero* exposure to dioxins and related compounds have been associated with adverse neurocognitive development in infants. It is unclear whether neurodevelopmental deficits persist to childhood. We assessed the association of prenatal dioxin exposure with neurocognitive function in 11-year-old children, and to test whether the association is modified by duration of breast-feeding.

Methods: In this prospective study of 161 children born in Hong Kong in 2002, prenatal dioxin exposure was proxied by the dioxin toxicity equivalence (TEQ) in breast milk collected during the early postnatal period as determined by the Chemical-Activated LUciferase gene eXpression (CALUX) bioassay. We used multivariate linear regression analyses to assess the association of prenatal dioxin exposure with the performance on the Wechsler Intelligence Scale for Children-IV, Hong Kong, the Hong Kong List Learning Test, the Tests for Everyday Attention for Children and the Grooved Pegboard Test, adjusting for child's sex, mother's place of birth, mother's habitual seafood consumption, mother's age at delivery and socioeconomic position.

Results: Measures of neurocognitive and intellectual function, including full-scale IQ, fine motor coordination, verbal and non-verbal reasoning, learning ability and attention at 11 years old did not show significant variations with prenatal dioxin exposures (proxied by CALUX-TEQ total dioxin load in early breast milk). None of these associations varied by breastfeeding duration or sex.

Conclusions: Neurocongitive function, as measured with psychological tests, in 11-year-old children was not associated with prenatal dioxin exposure to background levels of dioxins in the 2000s in Hong Kong.

1. Introduction

Dioxins (polychlorinated dibenzo-para-dioxins and polychlorinated dibenzofurans, or PCDD/Fs) and dioxin-like polychlorinated biphenyls (PCBs) are a group of structurally related organic pollutants which persist in the environment, and bioaccumulate in animal sources of food and human tissues (IARC, 1997). Most people are exposed to extremely low background concentrations of dioxins, however the potential harm of dioxins is of particular concern in growing fetuses and breastfed infants (Birnbaum, 1995). Transplacental (Schecter et al., 1990) and lactational (O'Leary et al., 1970) dioxin transfer, even at background concentrations could be substantial in fetuses and infants in view of their limited capacity to metabolize organic compounds and their low body weight (Weiss et al., 2004). Dioxins and PCBs are neurotoxic (Brouwer et al., 1995), especially to fetuses and infants who are undergoing rapid neurodevelopmental changes.

There are still unanswered questions concerning the impact of prenatal exposure to low levels of dioxins on neurodevelopment. First, prospective cohort studies in the 1980s that reported subtle adverse effects of background dioxin exposure on psychomotor development in infancy mainly studied PCBs (Gladen et al., 1988; Huisman et al., 1995). The few observational studies that assessed neurodevelopmental impact of prenatal exposures to background dioxins reported mixed findings. In the 1990s, poorer motor skills

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were observed in six-year-old children from a Dutch cohort who had higher prenatal dioxin exposure, proxied by Toxic Equivalence (TEQ) of dioxin and PCBs in breast milk (Vreugdenhil et al., 2002). In a recent Vietnamese study with much lower dioxin TEQ levels in breast milk (average PCDD/F-TEQ 12.5 pg/g fat) the investigators observed an association between dioxin TEQ level in breast milk and deficits in fine motor function at 4 months of age (Tai et al., 2013). However, in recent studies in Germany and Japan, where levels of prenatal dioxin (PCDD/Fs) TEQ exposure (respectively 11 pg/g fat in breast milk and 11.9 pg/g fat in maternal blood) were similarly low, no association between prenatal dioxin exposure and Bayley Scales of infant development was found (Wilhelm et al., 2008; Nakajima et al., 2006). Second, it is still unclear whether PCB-related neurocognitive deficits, if any, observed in infancy persist or diminish in childhood. While poorer intellectual function and attention by prenatal PCB exposure was still detected at 9-11 years in the Dutch (Vreugdenhil et al., 2004b; Vreugdenhil et al., 2004a), Michigan and Oswego cohorts (Jacobson and Jacobson, 1996; 2003; Stewart et al., 2008), prenatal PCB exposure-related deficits in neurocognitive function observed in infancy were no longer detected at older ages in the German Dusseldorf (Winneke et al., 2005) and North Carolina (Gladen and Rogan, 1991) cohorts. Third, although earlier studies concluded that the observed adverse effects of dioxins, including delays in psychomotor development (Koopman-Esseboom et al., 1996), neurodevelopment (Huisman et al., 1995) and intellectual function (Jacobson and Jacobson, 1996) have been associated with prenatal, rather than lactational, dioxin and/or dioxin-like PCB exposure, it is still inconclusive as to whether children who are breastfed longer are protected from the adverse neurocognitive consequence of prenatal dioxin exposure (Patandin et al., 1999; Jacobson and Jacobson 2002). Unlike in western societies, Hong Kong, a recently developed migrant population in the south of China, has little social patterning of breastfeeding – with both less educated mothers migrating from the Chinese Mainland and higher educated local born mothers being more likely to breastfeed longer. Given higher maternal socio-economic position is usually associated with both breastfeeding practice and more intellectual stimuli in most developed settings, a population with little social patterning of breastfeeding and lower breastfeeding rates, such as in Hong Kong, would be ideal for teasing out the effects of breastfeeding when assessing the impact of prenatal dioxin exposure on neurocognitive outcomes.

In 2002, we took part in the World Health Organization (WHO) coordinated dioxin exposure study where dioxin content in pooled breast milk samples collected from postpartum mothers from different countries were assessed (Hedley et al., 2006). The WHO study concluded that the average dioxin content in humans, measured as WHO-TEQs in Hong Kong were the highest among the five participating Asia-Pacific countries, but lower than the levels for at least half of the participating European countries. In addition to the WHO protocol which determined the mass and toxicity of 17 dioxin congeners and 12 dioxin-like PCB congeners in pooled milk samples, we estimated dioxin content in individual breast milk samples using a bioassay (Nelson et al., 2006; Hui et al., 2007). The bioassay determined total dioxin toxicity in early breast milk samples, which served as a surrogate of mother's dioxin body load and provided an estimate of the prenatal dioxin exposure. Our study objective was to assess the children of this cohort to determine whether levels of prenatal dioxin exposure in Hong Kong have long-term neurodevelopmental impact.

2. Methods

2.1. Participants and study design

Participants (n=161) were recruited from a cohort of 316 children born in Hong Kong in 2002 to mothers who participated in the WHO survey on human dioxins levels (Hedley et al., 2006). The 2002 study followed the protocol of the 2002/03 WHO dioxins exposure study with 316 first time mothers with recent singleton births recruited from 16 well baby clinics. Early breast milk samples were collected from the mothers at 2–6 weeks postpartum for determining the participating mothers' dioxin body load. We included partially breastfeeding mothers due to the low exclusive breastfeeding rate in the early postnatal period in our population.

2.2. Ethical approval

The study was approved by the Institutional Review Board of the University of Hong Kong/ Hospital Authority Hong Kong West Cluster, the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee and the ethics committee in the City University of Hong Kong. Informed written consent from the participating mothers and assent from their children were obtained.

2.3. Prenatal dioxin exposure

Prenatal dioxin exposure was proxied by maternal dioxin body load soon after delivery, as reflected by the dioxin content in breast milk collected at 2-6 weeks postpartum. The dioxin content in individual breast milk samples was measured by the Chemical-Activated LUciferase gene eXpression (CALUX) bioassay, which reported CALUX-TEQs, i.e. toxicity through the aryl hydrocarbon (Ah-) receptor pathway, benchmarked against the most toxic dioxin congener, 2, 3, 7, 8 tetra-chlorodibenzo-para- dioxin (TCDD). Of the original 161 participating mothers we successfully re-contacted in this follow-up, individual CALUX-TEQ in breast milk was not determined in 33 of them and were estimated using multiple imputation, based on a flexible additive regression model with predictive mean matching (Schafer, 1999) incorporating data on CALUX-TEQ, pooled dioxin contents, ages of both child and mother when breast milk was collected, birth weight, gestational age, sex, mother's smoking, mothers' place of birth, mother's fish consumption during pregnancy, parents' education attainment, household income and interactions of interest (prenatal dioxin exposure and breastfeeding duration) and the outcome measures of neurocognitive functions (Moons et al., 2006). Maternal CALUX-TEQ was imputed for 20% of the children who were included in this study.

The bioassay was performed by the BioDetection Systems b.v. in the Netherlands which we have described in detail previously (Nelson et al., 2006). In brief, this bioassay comprises a genetically modified H4IIE rat hepatoma cell-line, incorporating the firefly luciferase gene coupled to cytosolic aryl hydrocarbon receptors (Ah-receptor) as a reporter gene for the presence of dioxins and dioxin-like compounds. Dioxins bind to Ah-receptors and induce the transcription of the recombinant gene, which produces luciferase and emits light. The amount of light produced is proportional to the amount of ligand-Ah-receptor binding, which is expected to be directly proportional to the toxicity mediated by the Ah-receptor.

The sample was thawed just before extraction. Fat-soluble compounds, such as poly-aromatic hydrocarbons, chlorinated and brominated aromatic hydrocarbons, were extracted with hexane: diethylether (97:3) and fat content was then determined gravimetrically. All fat extracted from the samples was used for clean-