# Prenatal exposure to dioxins and subsequent neurocognitive and developmental function in Hong Kong Chinese children

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**KEY MESSAGES**

1. There was no significant difference in the neurocognitive functions in terms of full-scale IQ, fine motor coordination, verbal and non-verbal reasoning, memory, learning, and attention in 11-year-old children by levels of prenatal dioxin exposure proxied by maternal dioxin body load soon after delivery.
2. There was no detectable deficit in neurocognitive function in older children even at the high-end of prenatal exposure to background dioxins.
3. Nonetheless, it is recognised that growing foetuses are vulnerable to the harmful effects of environmental pollutants. Continued efforts should be directed towards identifying and controlling environmental sources of these substances in Hong Kong and Mainland China.

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## Introduction

Dioxins and polychlorinated biphenyls (PCBs) are a group of structurally related organic pollutants in the environment and in animal sources of food and human tissues. High levels of dioxins and PCBs are neurotoxic.[1] The impact of transplacental and lactational transfers of very low level of dioxins and PCBs on neurocognitive function in infants and children is of particular concern. Early prospective cohort studies have reported subtle adverse effects of prenatal exposure to dioxins and dioxin-related compounds on psychomotor development in infancy. However, findings were mixed on whether these neurocognitive deficits in infancy persist[2] or diminish in childhood. Given the endocrine disrupting effect of dioxins, assessment of other health impacts from prenatal dioxin exposure is needed, including growth, timing of puberty, immunity, reproductive health, and metabolic health.

To assess any potential impact of prenatal dioxin exposure on cognitive ability and intellectual function, we recalled the mothers who participated in a study on dioxin in breast milk in Hong Kong in 2002[3] to prospectively follow up their children at the age of 11 years. We also assessed associations of prenatal dioxin exposure with other health markers.

## Methods

The 2002 study followed the protocol of the 2002/03 World Health Organization dioxins exposure study.[3] A total of 316 first-time mothers with recent singleton births were recruited from 16 well baby clinics. Of them, 161 were followed up for medium-term (at 11 years old) consequences of prenatal dioxin exposure. This study was approved by the Institutional Review Board of The University of Hong Kong/ Hospital Authority Hong Kong West Cluster. We obtained informed consent from the participating mothers and children.

Prenatal dioxin exposure was proxied by maternal dioxin body load soon after delivery (dioxin content in breast milk collected at 2 to 6 weeks postpartum). The dioxin content in individual breast milk samples was measured by the dioxin responsive chemical-activated luciferase gene expression (CALUX) bioassay, which reported toxic equivalents (TEQs) benchmarked against the most toxic dioxin congener, as maternal CALUX-TEQs.[4] The bioassay was performed by BioDetection Systems b.v. in the Netherlands.

The Wechsler Intelligence Scale for Children, Fourth Edition (Hong Kong) (WISC-IV-(HK)), the Hong Kong List Learning Test, the Tests for Everyday Attention for Children (TEA-Ch) and the Grooved Pegboard Test were administrated to the children to measure a wide range of neurocognitive domains, including full-scale IQ, fine motor coordination, verbal and non-verbal reasoning, learning and attention. Neuropsychological assessments were performed by clinical psychologist trainees or senior research assistants blinded to the children's dioxin

exposure. A clinical psychologist was responsible for ensuring assessment quality and interpreting the assessment results. All assessments were carried out in a classroom setting except for nine which were carried out at participants' home at the family's request.

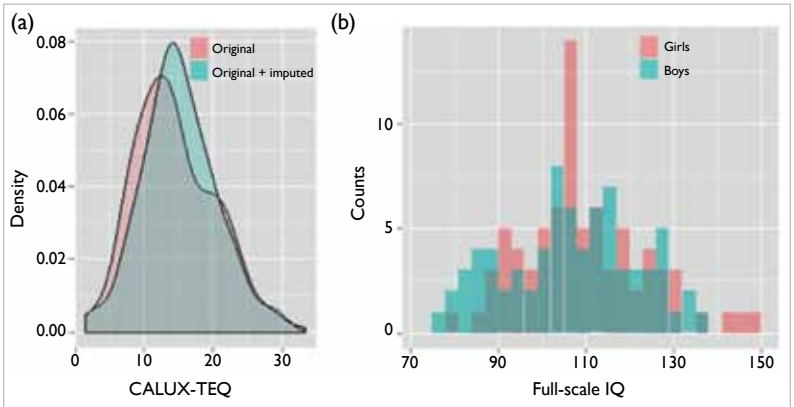


FIG 1. Distribution of (a) maternal dioxin responsive chemical-activated luciferase gene expression – toxic equivalents (CALUX-TEQ) with and without imputed data and (b) full-scale IQ by sex.

TABLE 1. Characteristics by maternal dioxin responsive chemical-activated luciferase gene expression – toxic equivalents (CALUX-TEQ)

| | Quartiles of maternal CALUX-TEQ* | | | |
|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th |
| CALUX-TEQ, pg/g fat | 8.6 | 13.4 | 16.1 | 22.5 |
| Boys, % | 52 | 55 | 47 | 43 |
| Birth weight, kg | 3.1 | 3.2 | 3.2 | 3.2 |
| Gestational age, weeks | 39.2 | 39.5 | 39.4 | 39.4 |
| Mother's age at delivery, years | 29.2 | 30.7 | 30.0 | 31.8 |
| Having a Hong Kong born mother, % | 65 | 70 | 57 | 66 |
| Having at least one sibling, % | 67 | 51 | 56 | 45 |
| Father's education, % | | | | |
| Junior high or lower | 28 | 22 | 21 | 18 |
| Senior high | 29 | 39 | 35 | 37 |
| Tertiary or higher | 43 | 40 | 43 | 45 |
| Mother's education, % | | | | |
| Junior high or lower | 10 | 8.9 | 14 | 15 |
| Senior high | 55 | 52 | 64 | 51 |
| Tertiary or higher | 34 | 40 | 22 | 34 |
| Household monthly income, % | | | | |
| <HKD25 000 | 48 | 32 | 43 | 38 |
| ≥HKD25 000 | 51 | 68 | 56 | 61 |
| Age at breast milk sampling, weeks | 4.2 | 4.1 | 4.4 | 4.4 |
| Exclusively breastfed at sampling, % | 54 | 60 | 50 | 50 |
| Breastfeeding duration, weeks | 6.8 | 9.6 | 8.1 | 7.1 |

* Data are presented as mean or %

Weight, height, body fat percentage, and blood pressure were measured by trained research assistants. Self-reported Tanner stages on breast/genital development at the time of assessment and age of menarche for girls were collected. Participating mothers also reported other information of the children, including behaviour and emotional problems (using Child Behaviour Checklist), history of serious infections and autoimmune diseases, habitual fish consumption, duration of breastfeeding, previous experience with cognitive function tests, and previous diagnosis of cognitive function deficits.

Multivariate linear regression was used to assess the association of prenatal dioxin exposure with each neurocognitive or health endpoint. Whether the associations varied by the duration or exclusivity of breastfeeding was assessed from the significance of interaction term. We adjusted for potential confounding factors that were associated with CALUX-TEQ level, including child's sex, place of birth, age at delivery, habitual seafood consumption of the mother, markers of family socio-economic position (education and income of parents), and child's age at assessment.

In 33 participating mothers who only had dioxin levels (as WHO-TEQ) in pooled breast milk but not individual CALUX-TEQ in breast milk determined in 2002, multiple imputation was used to predict their maternal CALUX-TEQ, based on a flexible additive regression model with predictive mean matching incorporating data on CALUX-TEQ, pooled WHO-TEQ, factors associated with maternal dioxin body load, interactions of interest (ie, prenatal dioxin exposure and breastfeeding duration), and outcome measures. Results from 10 imputed datasets were summarised into single estimates with confidence intervals adjusted for missing data uncertainty. A complete case analysis was also performed.

## Results

Of 316 mothers in the 2002 dioxin exposure study, 161 mother-and-child pairs participated in the follow-up study. Loss to follow-up was mainly due to loss of contact (n=101), particularly among new immigrant mothers who had lower education level. Included participants and the whole cohort were comparable in terms of distribution of maternal CALUX-TEQ (14.9±6.0 vs 14.5±5.8 pg/g fat, Fig 1a) and maternal age. The maternal CALUX-TEQ level was not associated with education attainment or household income before or after imputation.

The mean age of the children at follow-up was 11.3±0.3 years. Full-scale IQ obtained from the WISC-IV (HK) was normally distributed (Fig 1b). One child previously diagnosed as being mentally retarded was included in the data analyses but was excluded from the plots. On average children with

higher full-scale IQ from WISC-IV-HK also had a higher learning score in Hong Kong List Learning test (indicating a greater ability in learning and better verbal memory), lower scores in the TEA-Ch (indicating a greater ability to focus), and a shorter time taken for the Grooved Pegboard Test (indicating a better visual-motor coordination) [Table 1]. Girls had higher scores in verbal comprehension index and processing speed index and almost all scores in the Hong Kong List Learning tests, whereas boys had higher scores in the block design in WISC-IV (HK). Children of higher educated mothers had a higher perceptual reasoning index and a higher score in matrix reasoning but not a higher full-scale IQ or better performance in other tests.

The plots of mean test scores by quartiles of maternal CALUX-TEQ showed no sign of threshold effects or low-dose effects of maternal CALUX-TEQ on markers of neurocognitive functions (Fig 2). Maternal CALUX-TEQ, either as a continuous variable or categorical variable, were not associated with the performance in the four tests, after adjusting for child's sex, mother's age at delivery, mother's place of birth, mother's habitual seafood consumption, parents' education, household income, and age at assessment (Table 2). Maternal CALUX-TEQ were not associated with the markers of metabolic health (body mass index, % body fat and blood pressure), behaviour outcomes (attention and internalising and externalising behaviour), earlier pubertal development (reaching stage II for boys or stage III for girls and menarche earlier than 12 years for girls), presence of allergy, autoimmune disease, and history of serious infections (data not shown). Similar results were obtained using complete case analysis with 128 children without imputed data (data not shown).

Both breastfeeding duration (mean, 7.8 weeks; range, 2–24 weeks) and exclusivity at the time of sampling (54%) were not associated with any neurocognitive function indicated in the four tests. None of the associations of maternal CALUX-TEQ with neurocognitive or health endpoints varied by breastfeeding duration or exclusivity (data not shown).

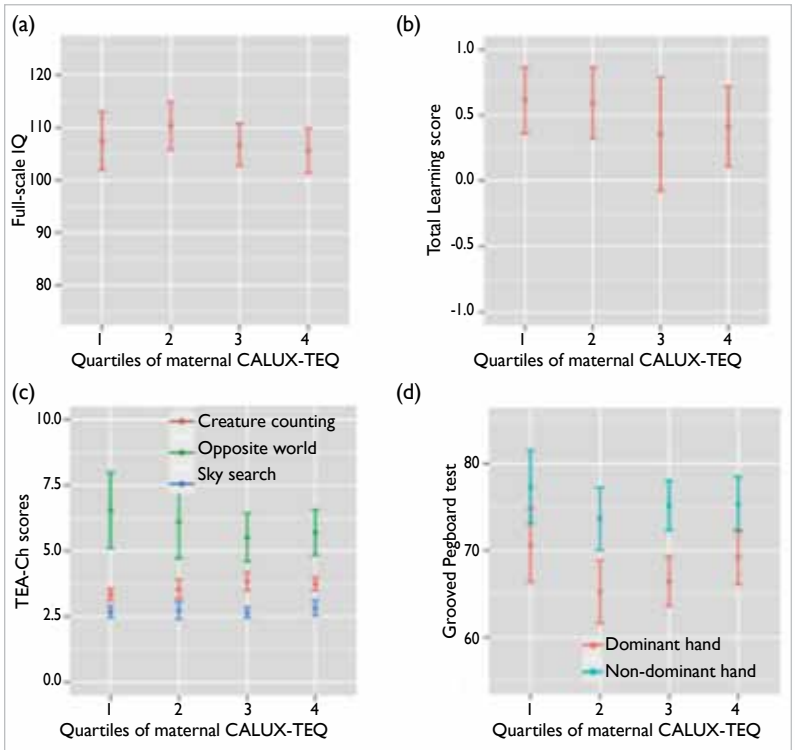


FIG 2. Mean scores of (a) the Wechsler Intelligence Scale for Children, Fourth Edition (Hong Kong) [WISC-IV-HK], (b) the Hong Kong List Learning Test, (c) the Tests for Everyday Attention for Children (TEA-Ch), and (d) the Grooved Pegboard test by quartiles of maternal dioxin responsive chemical-activated luciferase gene expression – toxic equivalents (CALUX-TEQ).

## Discussion

We did not observe any significant difference in the neurocognitive functions in terms of full-scale IQ, fine motor coordination, verbal and non-verbal reasoning, memory, learning, and attention in 11 years old children by levels of prenatal dioxin exposure. There was no detectable deficit in neurocognitive function in 11-year-old children even at the high-end of prenatal exposure to background dioxins.

In-utero exposure to dioxins and related compounds has been reported to be associated with adverse neurocognitive development in infants. It is not clear whether such deficits in psychomotor abilities and cognitive function persist to childhood. There are only few studies on children born after 2000s; both positive and null associations of prenatal dioxin or PCB exposure have been reported with markers of neurodevelopment in infants. Our 2002 birth cohort reported that prenatal background dioxin exposure was not associated with child neurocognitive function at 11 years, regardless of breastfeeding duration. Associations of prenatal PCB exposure with poorer intellectual function and deficit in attention have been observed at 11-year-olds in the Michigan cohort and at 9-year-olds in the Dutch cohort, particularly among children with a shorter breastfeeding duration or less optimal home environments. Compared with these two cohorts, our cohort had a much lower PCBs levels in the breast milk samples and probably lower in-utero dioxin exposure. The lack of association in our study was consistent with the similar IQ in children with middle to low PCB exposure in the Michigan study. The US Collaborative Perinatal Project, with a lower mean maternal serum PCBs (2.8 ng/mL) than that of the Michigan mothers (6 ng/mL),

TABLE 2. Association of prenatal dioxin exposure with neurocognitive function at 11 years old

| Test | n | Mean±SD | β (95% CI) adjusted for confounders | | P for interaction | |
|---|---|---|---|---|---|---|
| | | | CALUX-TEQ | CALUX-TEQ at 4th quartile* | Breastfeeding duration | Breastfeeding exclusivity† |
| Wechsler Intelligence Scale for Children, Fourth Edition (Hong Kong) | | | | | | |
| Full-scale IQ | 152 | 107±15 | -0.1 (-0.6 to 0.4) | -1.3 (-7.6 to 4.9) | 0.75 | 0.95 |
| Verbal comprehension index | 160 | 107±17 | -0.2 (-0.8 to 0.3) | -1.8 (-8.5 to 4.8) | 0.99 | 0.64 |
| Working memory index | 153 | 105±15 | -0.0 (-0.5 to 0.5) | -0.4 (-6.3 to 5.5) | 0.53 | 0.48 |
| Perceptual reasoning index | 161 | 108±14 | 0.0 (-0.4 to 0.5) | -1.0 (-6.8 to 4.0) | 0.99 | 0.61 |
| Processing speed index | 161 | 101±16 | 0.0 (-0.6 to 0.6) | 0.4 (-6.8 to 7.6) | 0.65 | 0.99 |
| Score (scaled) from subtest | | | | | | |
| Similarities (verbal reasoning) | 152 | 11.7±3.2 | -0.04 (-0.15 to 0.07) | -0.7 (-2.0 to 0.6) | 0.52 | 0.48 |
| Block design (visual abstract ability) | 161 | 11.1±3.0 | 0.01 (-0.09 to 0.11) | -0.3 (-1.5 to 0.9) | 0.32 | 0.42 |
| Matrix reasoning (spatial reasoning ability) | 161 | 11.4±3.1 | -0.01 (-0.11 to 0.09) | -0.4 (-1.6 to 0.9) | 0.52 | 0.61 |
| Cancellation (visual-perceptual speed) | 152 | 11.0±3.1 | 0.00 (-0.10 to 0.11) | 0.2 (-1.2 to 1.5) | 0.85 | 0.97 |
| The Hong Kong List Learning Test (z-score) | | | | | | |
| Learning | 161 | 0.47 | -0.01 (-0.04 to 0.03) | 0.0 (-0.4 to 0.4) | 0.90 | 0.69 |
| Short delay recall difference | 161 | 0.60 | 0.01 (-0.03 to 0.04) | 0.2 (-0.3 to 0.7) | 0.67 | 0.88 |
| Long delay recall difference | 161 | 0.76 | -0.00 (-0.04 to 0.03) | 0.2 (-0.3 to 0.7) | 0.87 | 0.77 |
| Trials 1-3 intrusion errors | 160 | -0.19 | 0.02 (-0.02 to 0.06) | 0.2 (-0.3 to 0.7) | 0.84 | 0.34 |
| Trials 1-3 preservation errors | 161 | 0.06 | 0.01 (-0.03 to 0.04) | 0.2 (-0.2 to 0.7) | 0.96 | 0.88 |
| Test for everyday attention for children | | | | | | |
| Creature counting | 159 | 3.6±1.0 | -0.05 (-0.18 to 0.08) | -0.2 (-1.9 to 1.6) | 0.32 | 0.45 |
| Opposite world | 161 | 6.1±4.0 | 0.02 (-0.01 to 0.05) | 0.1 (-0.3 to 0.5) | 0.94 | 0.39 |
| Sky search | 161 | 2.8±0.9 | 0.00 (-0.03 to 0.04) | 0.1 (-0.3 to 0.5) | 0.68 | 0.79 |
| Grooved Pegboard Test, seconds | | | | | | |
| Dominant hand | 161 | 68±13 | -0.2 (-0.7 to 0.3) | 0.3 (-5.1 to 5.6) | 0.60 | 0.84 |
| Non-dominant hand | 161 | 76±14 | -0.2 (-0.7 to 0.3) | -0.0 (-6.0 to 6.0) | 0.59 | 0.99 |

* Compared with children with maternal CALUX-TEQ of 1st to 3rd quartiles

† When breast milk sample was collected

similarly reported no association of prenatal PCB with neurodevelopment at both infancy and childhood. In addition, the North Carolina cohort, the New York Oswego cohort, and the German Dusseldorf cohort reported a diminishing effect of PCBs on neurodevelopment in infancy to childhood. Such 'transient' neurotoxic effect may be due to compensatory mechanisms by intellectual stimuli from the environment or endogenous functional recovery.[5] The lack of association in our study could be partly due to such compensatory mechanisms, although we were unable to confirm it owing to unknown neurocognitive function at younger ages.

The endocrine disrupting effect of dioxins has been reported to be associated with disrupted reproductive health, altered immune systems, and increased diabetes risks. However, we did not observe any difference in related endpoints by prenatal dioxin exposure. The impact of prenatal exposure to low background levels of dioxins may only induce small changes, if any, to markers of metabolic, reproductive, and immunological health, as compared with other factors such as diet, stress, and genetic susceptibility. A larger sample size that includes subgroups with higher exposure to dioxins, together with adjustment of confounding factors, is required to detect such subtle endocrine disrupting effects.

There are limitations to this study. Only 51% of the original cohort were included. However, the distribution of maternal CALUX-TEQ of those included was similar to the whole cohort. The bias secondary to loss of follow-up should be small. In addition, the sample size was small, which allowed an 80% power to detect an effect size of 0.45 between groups (equivalent to 6.6 full-scale IQ points). However, this is similar to what was detected in the Michigan study. Furthermore, we did not have

cord blood dioxin content. However, dioxin in early breast milk highly correlates with cord blood dioxin and provides a proxy for prenatal dioxin exposure. Finally, only 20% of maternal CALUX-TEQ were imputed; however, the complete case analysis obtained similar results.

## Conclusion

Prenatal exposure to background levels of dioxins is not associated with any deficit in neurocognitive function in terms of intelligence, learning, memory, and attention at 11 years of age. Although this null association is reassuring, it is still important to recognise that growing foetuses are vulnerable to the harmful effects of environmental pollutants. Continued and enhanced efforts should be directed towards identifying and controlling environmental sources of these substances.

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### References

1. Birnbaum LS. Developmental effects of dioxins. Environ Health Perspect 1995;103(Suppl 7):89-94.
2. Jacobson JL, Jacobson SW. Evidence for PCBs as neurodevelopmental toxicants in humans. Neurotoxicology 1997;18:415-24.
3. Hedley AJ, Wong TW, Hui LL, Malisch R, Nelson EA. Breast milk dioxins in Hong Kong and Pearl River Delta. Environ Health Perspect 2006;114:202-8.
4. Nelson EA, Hui LL, Wong TW, Hedley AJ. Demographic and lifestyle factors associated with dioxin-like activity (CALUX-TEQ) in human breast milk in Hong Kong. Environ Sci Technol 2006;40:1432-8.
5. Stewart PW, Reihman J, Lonky EI, Darvill TJ, Pagano J. Cognitive development in preschool children prenatally exposed to PCBs and MeHg. Neurotoxicol Teratol 2003;25:11-22.