

Effects of dioxin exposure on gaze behaviour in 3-year-old children in Vietnam

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Introduction

Bien Hoa airbase is the largest former US airbase in southern Vietnam, and was a storage and preparation site for herbicides sprayed from military airplanes during Operation Ranch Hand (1961–1971). Environmental and physiological levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in that area are still elevated four decades after the spraying ended^{1,2}. In our previous survey, TCDD levels in breast milk were approximately 4–5 times higher in primiparous women and 7–8 times higher in multiparous women in the Bien Hoa area than in women in unsprayed areas².

Birth cohort studies in dioxin-contaminated areas of Vietnam and the Netherlands have reported that perinatal dioxin exposure affected neurodevelopment, increasing rates of autistic traits³ and underdevelopment of visual cognition^{4,5}. Eye-tracking analysis can be used to examine gaze behaviour in children with autism who rarely fix their gaze on human faces^{6,7}, especially the eye areas^{7,8}, suggesting that eye tracking can assist in identifying autistic traits early. In the present study, we examined 3-year-old children residing near the Bien Hoa airbase whose mothers had high TCDD levels in breast milk, to assess the effects of perinatal dioxin exposure on gaze behaviour.

Methods

Study subjects

In 2012, a total of 210 mother-and-newborn pairs living in 10 communes near Bien Hoa airbase were enrolled in the birth cohort study² and followed 3 years later to assess the children's neurodevelopment. One hundred and sixty-three children from that cohort were included in the present survey (79.5% participation rate). We excluded 20 children who could not complete the gaze test for lack of attention or for whom we could not obtain data such as maternal education and age, family income, parity categories (primiparae or multiparae), number of weeks of gestation, birth weight, or precise age. The analysis therefore included 143 children. Levels of TCDD and toxic equivalency (TEQ) values of polychlorinated dibenzodioxins and polychlorinated dibenzofurans (TEQ-PCDDs/Fs) in maternal breast milk collected in the previous survey², 1 month after birth, were used as markers of perinatal exposure. Table 1 lists maternal and child characteristics.

We also measured behaviour associated with autism spectrum disorder using the Autism Spectrum Rating Scales (ASRS, MHS Inc., North Tonawanda, NY, USA) by interviewing the children's caregivers. Seven children (four boys and three girls) were suspected of elevated autistic traits because their total ASRS scores were ≥ 60 , indicating high risk (ASRS Technical Manual). The Bayley Scales of Infant and Toddler Development, third edition (Bayley-III) were used to evaluate children's neurodevelopment across three domains: cognition, language, and motor skills.

Gaze behaviour test

Tobii Studio (Tobii Technology AB, Danderyd, Sweden) was used to assess gaze behaviour. Children were seated comfortably next to their caregivers in front of a computer screen. The distance between children's eyes and the screen was approximately 60 cm and unobstructed. Ten static pictures containing various numbers of children's faces were used as a stimulus, with 3 seconds for each picture. Prior to the test, children had to calibrate the picture successfully using mapped points.

Analysis of the duration of fixation

The total duration of fixation on the faces in the pictures and on each picture as a whole was measured using Tobii software (default I-VT filter, Tobii Studio). The percentage of total duration of fixation on faces was defined as the ratio of the total duration of fixation on faces areas in ten pictures divided by the total duration (in seconds) of fixation for ten pictures, multiplied by 100. Children, whose total duration of fixation was less than 1.02 seconds (5th percentile) because of lack of attention, were excluded from the analysis.

Results and discussion

The adjusted mean percentage of the total duration of fixation on faces was compared between children exposed to high and low levels of TCDD and TEQ-PCDDs/Fs, with the cutoff values of the 75th percentile set at 3.6 pg/g lipid for TCDD and 12.7 pg-TEQ/g lipid for TEQ-PCDDs/Fs after controlling for confounding factors using a general linear model. Table 2 displays the findings. Although no significant differences were found in boys with high and low exposure to TCDD, the adjusted mean percentage of the total duration of fixation on faces (%) in the high-TCDD group was significantly lower [$F(1, 53) = 10.47$, $P = 0.002$, $\eta^2 = 0.165$] in girls, with 26.2% (standard error: 5.3) in the high-exposure group and 47.0% (standard error: 3.3) in the low-exposure group. Even after excluding children who were suspected to have autistic traits, the adjusted mean percentage of the total duration of fixation on faces was significantly lower among girls in the high-TCDD group [$F(1, 49) = 7.19$, $P = 0.010$, $\eta^2 = 0.128$] than among girls in the low-TCDD group (Table 2). There was no significant difference in the adjusted mean percentage of the total duration of fixation on faces between children in the high- and low-TEQ-PCDDs/Fs groups in either sex.

To assess whether a dose-response relationship existed between levels of TCDD exposure and performance on the gaze behaviour test, the children were divided into three groups according to the TCDD levels measured in maternal breast milk shortly after birth. The low-exposure group consisted of children whose mothers had < 3.6 pg/g lipid TCDD (75th percentile); the moderate-exposure group consisted of children whose mothers had 3.6–

Table 1 Maternal and child characteristics (N = 143)

Subject	Characteristics	Unit	Mean (SD), N[%]
Mothers	Age	years	28.6 (4.6)
	Education	years	10.6 (2.3)
	Monthly income	million VND	9.7(13.2)
	Parity (%primiparae)	N [%]	54 [37.8]
	Children	Gender (rate of boys)	N [%]
Children	Gestational period	week	39.0 (1.3)
	Birth weight	g	3292 (416)
	Age at examination	months	37.5 (0.68)
	Suspected autistic trait*	N [%]	7 [5.0]
	Breast milk	2,3,7,8-TetraCDD#	pg/g lipid
PCDDs/Fs-TEQ#		pg-TEQ/g-lipid	9.6 (1.73)

*Suspected autistic traits: a total Autism Spectrum Rating Scales score ≥ 60 .
#Geometrical mean and geometrical standard. VND, Vietnam dong

7.1 pg/g lipid TCDD, and the high-exposure group consisted of children whose mothers had ≥ 7.1 pg/g lipid TCDD (90th percentile) (Fig. 1). In boys (Fig. 1A), the high-TCDD group had a shorter duration of fixation on faces than the moderate- and low-TCDD groups, but without reaching statistical significance. In girls (Fig. 1B), the adjusted mean percentage of the total duration of fixation on faces decreased significantly as TCDD levels increased [F(2, 52) = 5.24, $P < 0.05$, $\eta^2 = 0.168$].

Table 2 Adjusted mean percentage of the total duration of fixation on faces in children exposed to high and low levels of TCDD and TEQ-PCDDs/Fs

	Low exposure				High exposure				<i>P</i> -value
	N	adj. Mean	95%CI Lower Upper		N	adj. Mean	95%CI Lower Upper		
Boys									
TCDD	59	50.0	44.8	55.2	17	45.6	35.6	55.5	0.450
TEQ-PCDDs/Fs	58	48.8	43.4	54.1	18	49.9	40.1	59.7	0.845
<i>Children without suspected autistic traits</i>									
TCDD	55	48.7	43.3	54.1	15	42.0	31.5	52.6	0.271
Girls									
TCDD	43	47.0	40.4	53.6	18	26.2	15.7	36.8	0.002
TEQ-PCDDs/Fs	44	42.5	35.5	49.5	17	36.7	25.2	48.2	0.395
<i>Children without suspected autistic traits</i>									
TCDD	41	47.6	40.7	54.5	16	29.1	17.6	40.6	0.010

Bottom cut-off values are 3.6 pg/g lipid for TCDD and 12.7 pg-TEQ/g lipid for TEQ-PCDD/Fs. Analytical covariates included maternal age and education, weeks of gestation, family income, birth weight, and child's age.

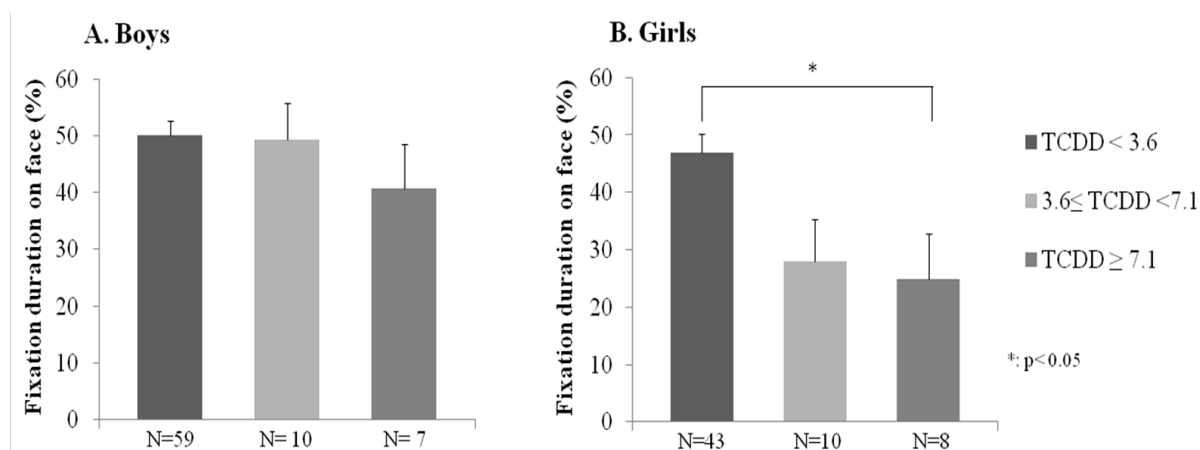


Fig. 1 Adjusted mean percentage of the total duration of fixation on faces, stratified by levels of exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

* $P < 0.05$ compared with high- (TCDD ≥ 7.1 pg/g lipid) and low- (TCDD < 3.6) exposure groups.

These findings suggest that high perinatal exposure to TCDD may alter children's social behaviour, particularly in girls. Observing faces and making eye contact aid neurodevelopment by improving learning ability and social skills⁹. Studies that investigated gaze behaviour in children with autism reported atypical behaviour with fewer

fixations on the whole face^{7,8} compared with gaze behaviour in children with typical development. In the present study, however, even after excluding children with suspected autistic traits, the duration of fixation on faces remained significantly lower in girls in the high-TCDD group. We also found lower Bayley cognitive scores at borderline significance ($P = 0.052$) in girls in the high-TCDD group. This indicates that perinatal TCDD exposure may affect cognitive development, including visual cognitive development associated with gaze behaviour, which is not directly related to autistic traits. To our knowledge, there are no reports of the effects of dioxin on gaze behaviour in children or adults. Prenatal exposure to background levels of one congener of polychlorinated biphenyls (PCBs), number 118, was reported to influence the fixation pattern during observation of biological motion in Japanese infants aged 4 months, without an association between exposure level and neurodevelopmental and social relation scores¹⁰. These findings suggest that PCB exposure even at background levels may impair infant socio-cognitive development, which has been reported in children with autism¹¹. Neuronal development in brain areas related to cognitive processing of social information may therefore be sensitive to the toxicity of PCBs and dioxins, and gaze behaviour tests may be a valid tool for detecting such impairments in infants and young children. In our study, however, the effects of TCDD were sex-specific, and atypical gaze behaviour was noted only in girls, who generally show greater interest than boys in faces¹². Follow-up to examine cognitive abilities, including face recognition, is warranted in Vietnamese children residing in dioxin-contaminated areas.

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