The CHECH study: A prospective pregnancy cohort study on CChemical exposure and children’s health in Tianjin, China

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ABSTRACT

The CHEChemical Exposure and Children’s Health (CHECH) study is an ongoing pregnancy cohort study in Tianjin, China. This paper describes the background, aim and the study design, which can be followed by future researchers to design and conduct similar studies. The abundance and the potential adverse health outcomes of endocrine disrupting chemicals (EDCs) is concerning. More notably, developing fetuses and infants are more vulnerable to EDCs exposure. The CHECH study aims to investigate the importance of early life exposure to multiple EDCs (phthalates and their metabolites, bisphenol A and their substitutes, perfluorinated compounds and poly brominated diphenyl ethers) for multiple health outcomes in Chinese children, namely sexual development, neurodevelopment, metabolism and growth, as well as asthma and allergy. A total of 2238 pregnant women were recruited in Tianjin from May 2017 to April 2021 with a response rate of 90%. Among these women, 2255 children were born with available information, including 47 pairs of twins. Urine samples were collected from pregnant women and children, while air and dust samples were obtained from the home environment during pregnancy and infancy periods. Information on children’s health was gathered through physical examinations and questionnaires. The CHECH study, which collected exposure information and health outcomes at multiple time points, will contribute to the understanding of prenatal exposure to EDCs and their impact on children’s health, thereby facilitating the development of risk assessments aimed at reducing exposure and associated health risks.

Introduction

Chronic health outcomes such as asthma, allergy, obesity and diabetes are on the rise among children (Judson, 2004) and have become a major public health problem. Exposure to environmental toxic pollutants is a suspected cause of such outcomes. The abundance and potential adverse health outcomes of endocrine disrupting chemicals (EDCs) is concerning. Endocrine disruptors are exogenous substances that interfere with the process of hormone action in human body (Diamanti-Kandarakis et al., 2009). They are commonly used in consumer products and materials, such as phthalates as plasticizers in polyvinyl chloride (PVC) flooring and children’s toys, bisphenol A (BPA) in polycarbonate plastics, perfluorinated compounds (PFCs) in stain-resistant products, and poly brominated diphenyl ethers (PBDEs) as flame retardants in electrical appliances and furniture (Bornehag et al., 2012; Tang et al., 2019). These substances can leak into the environment, causing environmental pollution and human exposure. They have been detected in the air (Huang et al., 2020; Shoeib et al., 2011), in settled dust (Zhang et al., 2020) and on various surfaces (Huang et al., 2022) in indoor environment. Moreover, EDCs or their metabolites have been detected in human fluids, such as urine (Bornehag et al., 2021; Zhao et al., 2022), blood (Björvång et al., 2020; Shu et al., 2018; Wikström et al., 2020) and even amniotic fluid (Jensen et al., 2012). The abundant sources of EDCs result...
in persistent human exposure through ingestion, inhalation and dermal absorption (Beko et al., 2013) with a wide spectrum of adverse health outcomes.

At present, many studies have found the adverse effects of EDCs on human health. Studies have shown that phthalate exposure not only elevates the risk of asthma and allergy in children (Bornehag and Nandberg, 2010) but also in adults (Hoppin et al., 2013). Exposure to BPA may be associated with diabetes, obesity, severe coronary artery stenosis, and possibly affect human reproductive health (Rezg et al., 2014). PBDEs may have adverse effect on neurodevelopment and thyroid function (Lim et al., 2008). Studies have also found that PFCs may have neurotoxicity, immunotoxicity and reproductive toxicity (Lau et al., 2007). In recent years, mixed EDCs exposure has been found to be risk to children’s cognitive function (Tanner et al., 2020), weight gain trajectory (Svensson et al., 2021) and sexual development (Bornehag et al., 2019).

More notably, developing fetuses and infants are more vulnerable to EDCs exposure due to (a) critical windows of vulnerability when organs and systems grow rapidly (Grandjean et al., 2009), (b) immature metabolism (Grandjean et al., 2008) and (c) greater intake of harmful substances relative to their body size (Vrijheid et al., 2012). Exposure to EDCs may not follow a traditional dose-response curve, but may have adverse health effects even at extremely low levels of exposure, especially when such exposure occurs during critical developmental periods (Diamanti-Kandarakis et al., 2009). Moreover, there is often a time lag between exposure and health effects. Health outcomes of early life exposure may not appear immediately, but later in life (Diamanti-Kandarakis et al., 2009).

Since the early 20th century, cohort studies focusing on early life exposure to EDCs and children’s health have been established in western countries. The Columbia Center for Children’s Environmental Health (CCCEH) cohort recruited 727 pregnant women from 1998 to 2006 to investigate the health effects of prenatal exposure to environmental toxic pollutants (Perera et al., 2003). The EDEN (Etude des Déterminants pré et post nataux du développement dela santé de l’Enfant) Cohort in France was established in 2003 and recruited 2002 pregnant women to study the prenatal and early postnatal determinants of child health and development (Heude et al., 2016). The Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SEMLA) study, initiated in 2007, enrolled a total of 2582 pregnant women with the aim of investigating the importance of early life exposure to environmental toxicants (Bornehag et al., 2012). The Canadian Healthy Infant Longitudinal Development (CHILD) Study, which recruited 3624 pregnant women from 2008 to 2012, was designed to explore the interactions of environmental and genes and provide information on the developmental pathways for childhood allergy and asthma (Takaro et al., 2015). The Infant Development and the Environment Study (TIDES) focused on examining the association between prenatal phthalate exposure and infant genital morphology by enrolling 969 pregnant women from 2010 to 2012 (Swan et al., 2015).

The above cohort studies were set up over a decade ago, during a period of rapid technological advances. As the identification and prohibition of hazards EDCs took place, substitutes emerged and exposure levels changed (Bornehag et al., 2015). Moreover, the Chinese population exhibits different genes, environments and lifestyles, which may contribute to disparities in exposure and outcomes. While, there are limited studies on early life exposure to EDCs and children’s health in China.

To address the research gap, the CHemical Exposure and Children’s Health in China (CHECH) study has been designed to increase the understanding of early life EDCs exposure to health outcomes in Chinese children, such as birth outcomes, asthma and allergies, obesity and development.

Methods

The CHECH study is an ongoing pregnancy cohort study in Tianjin, China. Initiated in 2017, it aimed to recruit 2300 mother-child pairs and longitudinally tracked children from fetal development, birth until school age. The CHECH study consisted of two phases, baseline sampling and follow-up visits. The baseline sampling phase involved the collection of samples and information starting from the enrollment of pregnant women until their children reached 1 year of age. The follow-up visits phase was planned with health examinations and questionnaires from birth until the age of 7 years.

Study area and participants

The study area and collaboration

The CHECH study was conducted jointly by Tianjin University and Tianjin Women and Children’s Health Center. Tianjin Women and Children’s Health Center was responsible for training and overall planning of Women and Children’s Health Centers in each district and community hospitals, as well as recruitment, sample collection and follow-up visits. Tianjin University took charge of sample transportation, storage and analysis, as well as home inspections. The study was carried out in Tianjin, situated approximately 130 kilometers away from Beijing, the capital of China. Tianjin covers an area of 11,866.45 square kilometers and has a resident population of 13.63 million (https://://stats.tj.gov.cn/). It consists of 16 districts, including 6 central urban districts, 4 surrounding urban districts and 6 suburbs. The study was conducted within six central urban districts (Hedong district, Nankai district, Hebei district, Hexi district and Hongqiao district) and two surrounding urban districts (Jinnan district and Dongli district). Fig. 1 shows the geographical location of Tianjin alongside the study area highlighted.

Recruitment

The majority of pregnant women in Tianjin visited community hospitals to establish pregnancy records. At this point, the community hospital doctors who had received unified training introduced the study to the pregnant women and asked for their willingness to participate. The inclusion criteria were maternal age between 18 and 45 years, gestational period within 15 weeks, planning to deliver and reside in Tianjin.

Phase 1 – baseline sampling

The baseline sampling phase included the collection of dust samples (using pizza boxes) from maternal bedroom during pregnancy, maternal urine samples in the first trimester (under 16 weeks) and third trimester (after 28 weeks), dust samples (using pizza boxes) from children’s bedroom and samples (using filter socks and vacuum cleaner, Zhao et al., 2021) from home inspection. Information on potential confounding factors for proper statistical modelling were obtained through pregnancy questionnaires. The questionnaire surveys, as well as urine sample collections, were conducted during routine physical examinations at the hospital. Dust samples (using pizza boxes) were collected at residential buildings with assistance of parents. Fig. 2 shows the process of baseline sampling.

Biological sampling

Morning urine samples from pregnant women were collected at the hospital on the day of the first and third trimester examination. Urine samples from children were collected by their parents at the age of 1 year and sent to the hospital. To minimize potential background contamination (e.g., phthalates), the 75 mL glasses were thoroughly washed, dried and provided to mothers for collecting at least 20 mL of urine per sample. Mothers were instructed to avoid plastic materials during urine collection. Subsequently, urine samples were brought back...
to the laboratory and divided into two 10 mL glass vials, which were then stored in a freezer at -20 °C until analysis.

Environmental sampling

Each participating family in the study was provided two pizza boxes along with instructions to collect dust themselves from their bedrooms. The first pizza box was given to the pregnant woman on the day of the first trimester examination. It was brought home, opened and placed on a table or shelf above the floor in the mother’s bedroom to collect dust. The box was closed after 15-16 weeks and brought back to the hospital on the day of the third trimester examination. At the same time, they were provided a second pizza box, which was opened after delivery and placed above the floor in the child’s bedroom, closed and returned to the hospital six months later. The boxes returned by participants were transferred to lab within a week, and dust samples were sucked from the boxes into filter socks using a vacuum cleaner equipped with self-made mouthpieces. Dust samples were wrapped with aluminum foil, labeled and stored in a refrigerator at -20 °C until analysis.

Home inspections

In order to identify sources of EDCs and examine the influence of environmental parameters on EDC concentrations, a subgroup of 297 pregnant women in the study were invited to do home inspections. The home inspections were conducted twice, once during pregnancy and again before the child’s first birthday, both in winter. During home inspection we measured indoor air temperature, relative humidity, concentrations of carbon dioxide and volatile organic compounds (VOCs) for two weeks in each home. Additionally, dust samples were collected from floor, mattress and table/shelf surfaces in mother/child’s bedroom. We also surveyed building characteristics and home environment. For the first inspection, dust samples were obtained from the mother’s bedroom while for the second inspection they were collected from the child’s bedroom. Portable indoor air quality monitors (AZ® 7798) were placed in each room to continuously monitor temperature, relative humidity and carbon dioxide concentrations over a period of two weeks. Three dust samples were collected using filter socks and vacuum cleaner (Zhao et al., 2021), subsequently stored in a refrigerator at -20 °C until analysis. Passive sampling method was used to measure the concentrations of VOCs. A Tenax adsorbent tube was fixed at a height of 1.8~2 m in the bedroom using an L-shape bracket. The sampling duration lasted for 2 weeks. VOCs were analyzed using thermal desorption followed by gas chromatography-mass spectrometry, and chromatograms were saved for subsequent analysis. Home inspectors recorded building characteristics and home environment including the type of residence, decoration and number of toys. Mothers provided the frequency of using consumer products in the questionnaire. Moreover, maternal health problems (e.g., sick building syndrome symptoms) and perceived indoor air quality were investigated using questionnaires during home inspections. The questionnaire can be found in the Supplementary Materials.
Questionnaires

Three questionnaires (see Supplementary Materials) were used during pregnancy to obtain information about the pregnant women and their families. The three questionnaires were completed during hospital visits in the first trimester (under 16 weeks), second trimester (16–28 weeks), and third trimester (after 28 weeks). The first questionnaire included basic demographic information (i.e., age, occupation, level of education, income, etc.), history of disease such as allergies of family members, lifestyle (i.e., smoking habits, pet ownership and dietary habits) and residential environment including materials of walls and floors, heating and ventilation system, moisture and odor problems in the home. Data regarding the ease of conception was also collected. The second questionnaire focused on the dietary habits, health and medication usage of pregnant women. The third questionnaire collected information from pregnant women on lifestyle, supplement intake, exposure to hazardous substances, health status and medication usage.

Phase II – follow-up visits

Physical examinations and questionnaires were used to obtain information on children’s health and development (Fig. 3). Four health domains are under investigation: sexual development, neurodevelopment, metabolism and growth, as well as asthma and allergy. Follow-up assessments were conducted three times during the first year of life (i.e., at six weeks, six months and one year of age), followed by annual evaluations until the age of 7 years. Parents completed a questionnaire at each follow-up visit. Questionnaire at 6 weeks of infants focused on feeding patterns, sleep status, as well as maternal and child’s health. At 6 months, the child’s feeding, sleep, pet ownership, smoking and the health of mother and child were asked. The children’s development, food intake and health status were recorded on the questionnaire at the age of 1 year. Since then, the children’s development and health conditions were investigated by questionnaire during annual follow-up visits.

Sexual development

Different aspects of sexual development were assessed, including anogenital distance (AGD) measurements in boys, puberty onset and status assessment, anthropometric measurements focusing on sexual development, play behavior analysis and 2D:4D digit ratio examination. AGD was measured at around 10 months, while other assessments were planned to be conducted at 7 years old.

AGD serves as an indicator of androgen exposure during foetal development in utero and functions as a biomarker of testicular dysgenesis syndrome (TDS) (Thankamony et al., 2014). One hundred and two (102) boys aged 7 to 12 months (the median was 10 months) were invited to measure AGD. Two types of AGD were measured: the distance from the center of the anus to the anterior base of the penis and from the center of the anus to the junction of the wrinkled scrotum and smooth perineal skin. The method for AGD measurement were described elsewhere (Sathyarayana et al., 2015). In brief, the infant was lying on a flat surface with his legs in a frog leg position back at a 60–90° from his torso. The examiner stood in front of the infant and performed three measurements for each type of AGD. The coefficient of variation and mean value was calculated for further analysis based on three measurements. Important co-factors were measured and recorded, including age, weight, length and head circumference.

The onset and status of puberty were intended to be assessed through questionnaires distributed to parents when their child was 7 years old, inquiring about the presence and timing of pubic hair development in their child. Parents of girls were specifically asked about the occurrence and timing of menarche, as well as development of breast. Anthropometric measurements, including length, weight, BMI, hip circumference, were planned to be taken during the visit at the age of 7 years, with a focus on prepubertal overweight and obesity. The Preschool Activities Inventory (PSAI), a validated instrument used to assess sexually dimorphic play behavior, was designed to be employed to compute scores in order to evaluate the play behavior of children (Swan et al., 2010). The 2D:4D digit ratio was planned to be measured using a calliper. The right hand digits were placed flat on the edge of a table, with the palm positioned at an angle of 100–120° relative to the fingers.

Neurodevelopment

Children’s development such as movement and language were investigated in the questionnaire at the age of 1 year. Maternal reports were utilized to document the developmental milestones achieved by infants, including assisted sitting, independent sitting, crawling, supported standing, independent standing, supported walking, and independent walking. Parents also reported the age at which various language-related milestones occurred: being amused, babbling during interactions to adults, uttering words like “dada” and “mama”, imitating parental pronunciation, consciously calling out to mom and dad, speaking single words, and articulating two or three-word phrases consecutively. At two years of age, neurodevelopment was assessed in all children using Age and Stage Questionnaires, Third Edition (ASQ-3) (Squire et al., 2009), a validated developmental screening tool designed for children aged 1 to 66 months. This comprehensive assessment evaluated five key domains of development, including communication, gross motor skills, fine motor skills, problem-solving abilities, and personal-social interactions. Healthcare doctors used simple tools, such as picture book, cubes, buttons, etc. to evaluate children’s abilities and asked parents about children’s behaviors at home. The results showed abnormal development should be transferred to professional doctors.

Metabolism and growth

Birth outcomes (delivery mode, birth length, weight and APGAR (Activity, Pulse, Grimace, Appearance and Respiration) score) and physical examination records were available in the maternal and child health system. Beginning at the age of 1 year, height, weight and hip circumference were measured at each follow-up visit to assess

![Fig. 3. follow-ups visit process.](image-url)
overweight and obesity in children.

**Asthma and allergy**

Family history of allergies was investigated in the first trimester questionnaire. At each follow-up, questionnaires were used to determine whether children were diagnosed with asthma, rhinitis, and eczema. The specific questions on asthma and allergy were similar to those in ISAAC study (Asher et al., 1995). The guardian was asked in the questionnaire whether their child had eczema, asthma and rhinitis symptoms or had been diagnosed by a doctor. If the answer was yes, additional inquiries were made regarding the age of onset and whether any form of treatment had been administered.

**Ethical approval**

This study was ethically approved by Tianjin Women and Children’s Health Center, China (approval number: 201706012–1-GZ1). Written informed consent was obtained from pregnant women who voluntarily agreed to participate in this research.

**Results and discussion**

From May 2017 to April 2021, 2487 pregnant women who met the inclusion criteria were invited, and 2238 of them were recruited, with a participation rate of 90 %. Fig. 4 shows the process of recruitment and data collection during pregnancy. So far, all prenatal samples have been collected and the biobank has been established. Out of the 297 pregnant women invited for home inspection, a total of 277 households participated in the assessment during pregnancy. A total of 1408 (62.9 %) dust samples, 2003 (89.5 %) urine samples and 2227 (99.5 %) questionnaires were collected during the first trimester. In the second trimester, 1895 pregnant women completed the questionnaire, with a response rate of 84.7 %. We obtained 1237 (55.3 %) urine samples and 1505 (67.2 %) questionnaires in the third trimester. According to a parallel Swedish SELMA study (Bornehag et al., 2012), a total sample size of 1000 was evaluated as providing adequate statistical power (e.g. 80 % power with 5 % significance), considering both gender-specific effects and mixture exposure validation. The limited response rate may have caused selection bias, leading to differences between those lost to follow-up and those not lost to follow-up. However, considering our prospective design and the objective of investigating the association between early life exposure to EDCs and disease in children, these disparities in characteristics did not obviously affect our analysis.

The demographic information of pregnant women and their families was collected using questionnaires and presented in Table 1.

Among the 2238 pregnant women included in the study, a total of 21 experienced miscarried or stillbirths, while 2264 children were successfully delivered. Among these births, there were 47 pairs of twins and 9 cases born outside of Tianjin who declined to provide birth information. The demographic information of newborn is shown in Table 2.

The CHECH study is a population-based pregnancy cohort study. We collected various exposure information at multiple time points in early life and planned to collect multiple health outcomes of children. Compared to cross-sectional and retrospective designs, the prospective design is ideal for enhancing causal inference (Vrijheid et al., 2012). In addition, the information obtained from the CHECH study can be used to establish a comprehensive “full chain model” that encompasses information on EDCs. This model enables the tracking of such pollutants from their sources through environmental exposure to human intake and, ultimately, the evaluation of potential health effects (Bornehag et al., 2012; Preece et al., 2021, 2022a, 2022b). The full chain model will enhance comprehension of EDCs and facilitate the development of risk assessment aimed at reducing exposure and associated health risks.

In China, there are several studies focusing on the impact of early life environmental exposure on children’s health, including the prospective Tongji birth cohort study (Xia et al., 2016), the Laizhou Wan Birth Cohort (LWBC) study (Xie et al., 2013) and the Ma’anshan Birth Cohort (MABC) study (Zhu et al., 2020). The CHECH study is similar in size to most of the cohort studies established in China. These studies focused on environmental factors including metal, parabens, bisphenols, PBDEs and phthalates. The difference between these cohort studies and our CHECH study is that we collected both environmental (dust) and biological (urine) samples, making it possible to associate environmental and human exposures. The EDCs examined in our study include phthalates, bisphenols, PBDEs and PBDEs, the difference between theses cohort studies and our CHECH study is that we collected both environmental (dust) and biological (urine) samples, making it possible to associate environmental and human exposures. The EDCs examined in our study include phthalates, bisphenols, PBDEs and PBDEs. The inclusion of multiple EDCs enhances our ability to evaluate the risk associated with exposure to mixtures.

**Table 1**

| Demographic information of pregnant women and their families, n = 2227. | N | Percentage%
|---|---|---
| Maternal education level | | |
| Junior high school and lower | 152 | 6.8 |
| High school | 231 | 10.4 |
| College/university and higher | 1844 | 82.8 |
| Father’s education level | | |
| Junior high school and lower | 193 | 8.7 |
| High school | 292 | 13.1 |
| College/university and higher | 1742 | 78.2 |
| Smoking mother after pregnancy | | |
| Yes | 26 | 1.2 |
| No | 2201 | 98.8 |
| Smoking father after pregnancy | | |
| Yes | 815 | 36.6 |
| No | 1412 | 63.4 |
| Allergy history in the family | | |
| Yes | 315 | 14.1 |
| No | 1912 | 85.9 |

* The child’s mother/father/sibling has had or is suffering from allergies.

**Table 2**

| Demographic information of newborn, n = 2255. | | |
|---|---|---
| Gender, N (%) | | |
| Male | 1185 (52.4) |
| Female | 1070 (47.3) |
| Gestational age (weeks), (mean ± SD) | 38.76 ± 1.56 |
| Weight (g), (mean ± SD) | 3339 ± 477.5 |
| Height (cm), (mean ± SD) | 49.92 ± 2.48 |
| Twins, (pairs) | 47 |
| Low birth weight (<2500 g), N (%) | 85 (3.8) |
| Macroglossia (>4000 g), N (%) | 149 (6.6) |
| Preterm birth (before 37 weeks), N (%) | 136 (6.0) |
exposure to EDCs on children’s health, there is limited literature on their study design and methodology, especially in China. The novelty and strength of this paper lies in its presentation of the study design and procedures employed in the CHECH study, providing valuable insights into key considerations when researchers designing similar investigations. The detailed sampling methods for environmental samples and biological samples, along with the health examination protocols outlined in this study, served as a guide for researchers conducting studies.

Our study is limited by the fact that we conducted home inspections only in winter, rather than throughout all four seasons, which may not be representative enough. Future studies are encouraged to conduct home inspections across all four seasons.

Conclusion

This paper presented the study design and detailed methodology of a pregnancy cohort study, named the Chemical Exposure and Children’s Health (CHECH) study, providing a valuable guide for future researchers to design and conduct similar studies. The CHECH study, which collected exposure information and health outcomes at multiple time points, will contribute to the understanding of prenatal exposure to EDCs and their impact on children’s health, thereby facilitating the development of risk assessments aimed at reducing exposure and associated health risks.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found in the online version, at doi:10.1016/j.heha.2023.100084.

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