Japan Environment and Children's Study

(JECS)

Study Protocol (ver. 1.4)

National Institute for Environmental Studies
National Centre for Japan Environment and Children's Study

Statement of Compliance

This Study shall be conducted in accordance with its Protocol reviewed and approved by the institutional review boards (IRBs), complying with the Ethical Guidelines for Epidemiological Research (MEXT and MHLW) as well as the Ethical Guidelines for Analytical Research on the Human Genome/Genes (MEXT, MHLW and METI). The Principal Investigator shall assure that no deviation from or change to the Protocol will take place without prior approval from the IRBs, except when necessary to eliminate any immediate hazard(s) to the Study participants. Adjunct Study protocols shall be prepared by Regional Centres and subject to review and approval by an IRB to which each Regional Centre must report. When a new protocol is approved or the existing protocol is changed, the Regional Centres promptly shall report to the Steering Committee.

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List of Abbreviations

IRB	Institutional Review Board
JECS	Japan Environment and Children's Study
MEXT	Ministry of Education, Culture, Sports, Science and Technology
MHLW	Ministry of Health, Labour and Welfare
METI	Ministry of Economics, Trade and Industry
NIES	National Institute for Environmental Studies
MOP	Manual of Procedure/Miscellaneous Operation Procedure
MOE	Ministry of the Environment
SOP	Standard operating procedure

Overview

In March 2010, Ministry of the Environment (MOE) published a conceptual plan for the Japan Environment and Children's Study (JECS) that drafts a nation-wide birth cohort study on children's health and development. The goal of the JECS is to identify environmental factors that affect children's health and development during the foetal period and/or in early childhood, in order to facilitate development of a better environmental risk management system. Specifically, the JECS focuses on the effect of children's exposure to chemical substances. JECS examines hypotheses regarding to associations between environmental factors and children's adverse health outcomes in several different domains: Reproduction/pregnancy complications, congenital anomalies, neuropsychiatric disorders, immune system deficits/allergic responses and metabolic/endocrine system dysfunctions. JECS assesses the level of children's environmental exposures and measures variables that are relevant to health outcomes. Additionally, the JECS also examines possible covariates and confounders including physical and social environment, genetic factors and behaviours.

The JECS is operated in cooperation among several research institutions. The National Centre for JECS or Programme Office, which is situated in the National Institute for Environmental Studies (NIES), takes a directive role for the JECS, such as preparing various standard operating procedures (SOPs), managing data and storing biological samples. The Medical Support Centre, which resides in the National Centre for Child Health and Development, supports the Programme Office with its medical expertise. The Programme Office and Medical Support Centre cooperate together with 15 Regional Centres that are located in universities and other research institutions. Regional Centres select their own study areas (i.e., a single or multiple administrative districts), taking account of their number of births, regional representativeness and level of potential environmental exposures. The Regional Centres are responsible for recruiting and maintaining study participants and gathering data in their selected study areas at each study phase.

The prospective participants of the JECS are 100,000 children and their parents who live in designated study areas. The JECS aims to recruit expecting mothers who have lived in the study areas and are expected to stay there for the next three years from January 2011. The children born to these mothers are followed until they reach 13 years of age. The JECS study is planned to continue until 2032, allowing five years of data analysis after the completion of data collection.

The JECS consists of: 1) Main Study that includes all the participants recruited; 2) Sub-Cohort Study with 5,000 participants randomly extracted from the Main Study; 3) Pilot Study that examines validity and feasibility of study protocols before they are applied to the Main Study and 4) Adjunct Studies conducted by each or any combination of JECS organisation(s) using extramural funding targeting all or a part of the Main Study participants, which must be approved by MOE. In the Main Study, extensive biological sample collections are performed at a variety of time points. The

biological samples include blood and urine from mothers during pregnancy and at birth; cord blood at delivery; hair and breast milk from mothers at one month visits; hair from children at one month, and if accessible, blood of fathers. Blood and urine samples from selected children are collected in the Sub-Cohort Study. Concentrations of different chemical agents in these collected biospecimens are measured, in order to estimate the degree of chemical exposures of the study participants. The biospecimens are also utilized to assess biomarkers of the health outcomes such as allergies as well as to conduct genetic analyses. Questionnaire surveys and physical examinations are also conducted in order to enhance the assessment for environmental exposure, health outcomes and covariates/confounders.

Amendment: The accident of the Fukushima Daiichi Nuclear Power Plant happened in March 2011. The study area of Fukushima Regional Centre is expanded due to the increasing national concern over the impact of radioactivity on health.

1 Organisation

1.1 Principal Investigator

The Principal Investigator of the JECS is the Director of the National Centre for Japan Environment and Children's Study.

1.2 Programme Office

The Programme Office is established in the National Institute for Environmental Studies as National Centre for Japan Environment and Children's Study (JECS). The Programme Office takes a directive role for the JECS, including preparing various SOPs; accumulating and maintaining data collected by Regional Centres; storing and handling biological and environmental specimens and performing chemical analyses on the specimen. The Programme Office also provides administrative and technical support for Regional Centres and is responsible for risk management and public communication.

1.3 Medical Support Centre

Medical Support Centre is established in the National Centre for Child Health and Development. The Medical Support Centre provides the Programme Office with medical expertise, specifically, by developing/selecting health outcome measures; standardising the measurement procedures; preparing manuals for the measurements and training personnel who are responsible for gathering data of health outcome variables at each Regional Centre. The Medical Support Centre provides Regional Centres with advices on medical issues.

1.4 Regional Centres

The Regional Centres are responsible for recruiting study participants in respective study areas; collecting data from the participants at each study phase and collaborating with local governments and local health care providers (defined as "cooperating health care providers"). As a part of recruitment and data collection, the Regional Centres directly contact the study participants in order to inform them the study protocols; obtain the written consent from them; register the participants; collect biospecimens; gather the information from medical records and carry out questionnaire surveys and environmental measurements.

The following list presents 15 Regional, their designated study areas, planned sample sizes and corresponding organisations (

Table 1).

Table 1: Regional Centres, study areas, planned sample sizes and corresponding organisations

Regional Centres	Study Area	Planned Sample Size	Organisation
Hokkaido	Sapporo (Kita-ku and Toyohira-ku), Asahikawa, part of Kitami, Oketo, Kunneppu , Tsubetsu and Bihoro	8,250	Hokkaido University Sapporo Medical University Asahikawa Medical University The Japanese Red Cross, Hokkaido College of Nursing
Miyagi	Kesennuma, Minamisanriku, Ishinomaki, Onagawa, Osaki, Wakuya, Misato, Kami, Shikama, Kurihara, Tome, Iwanuma, Watari and Yamamoto	9,900	Tohoku University
Fukushima	Fukushima Prefecture	15,900	Fukushima Medical University
Chiba	Kamogawa, Minamiboso, Tateyama, Kyonan, Katsuura, Isumi, Onjuku, Ootaki, Kisarazu, Sodegaura, Futtsu, Kimitsu, Ichimiya and Chiba (Midori-ku)	6,400	Chiba University
Kanagawa	Yokohama (Kanazawa-ku), Yamato and Odawara	6,650	Yokohama City University
Koshin	Kofu, Chuo, Koshu, Yamanashi, Fujiyoshida, Ina, Komagane, Tatsuno, Minowa, Iijima, Minamiminowa, Nakagawa and Miyada	7,250	University of Yamanashi Shinshu University
Toyama	Toyama, Kurobe, Uozu, Namerikawa, Asahi and Nyuzen	5,700	University of Toyama
Aichi	Ichinomiya and Nagoya (Kita-ku)	5,850	Nagoya City University
Kyoto	Kyoto (Sakyou-ku and Kita-ku), Kizugawa and Nagahama	3,850	Kyoto University Doshisha University
Osaka	Kishiwada, Kaizuka, Kumatori,	8,000	Osaka University

	Izumisano, Tajiri, Sennan, Hannan, Misaki and Izumi		Osaka Medical Centre Research Institute for Maternal and Child Health
Hyogo	Amagasaki	5,600	Hyogo College of Medicine
Tottori	Yonago, Sakaiminato, Daisen, Houki, Nanbu, Kofu, Hino, Nichi-nan and Hiezu	3,000	Tottori University
Kochi	Kochi, Nankoku, Shimanto, Yusuhara, Kohnan, Kami, Sukumo, Tosashimizu, Kuroshio, Ohtsuki and Mihara	7,000	Kochi University
Fukuoka	Kitakyushu (Yahatanishi-ku) and Fukuoka (Higashi-ku)	7,600	University of Occupational and Environmental Health
			Kyushu University
South Kyushu/ Okinawa	Minamata, Tsunagi, Ashikita, Am-akusa, Reihoku, Kami-Amakusa, Hitoyoshi, Nishiki, Asagiri, Taragi, Yunomae, Mizukami, Sagara, Itsuki, Yamae, Kuma, Nobeoka and Miyakojima	5,750	Kumamoto University
			University of Miyazaki
			University of the Ryukyu

1.5 Steering Committee and Advisory Committees

The Steering Committee is established by the Programme Office and formed by representatives from the Programme Office, Medical Support Centre, Regional Centres and the MOE. The Principal Investigator of the JECS chairs the Steering Committee. The Steering Committee is the highest decision making body of the JECS. Under the Steering Committee, subcommittees, such as advisory committees, the ethics committee and liaison committees are organized as appropriate. The Steering Committee receives advices and recommendations from the Project Evaluation Committee externally established by the MOE that monitors the operation of the JECS from scientific and ethical perspectives.

2 Background

There has been a growing concern regarding the effects that environmental pollution posed to children, especially the vulnerability of children to harmful substances in the environment, throughout the world. In 1997, the Miami Declaration on Children's Environmental Health was adopted at the G8 Environment Ministers' Meeting in Miami. In late 1990's, Denmark, Norway and the United States commenced large-scale epidemiological studies on approximately 100,000 children to investigate the effects of environmental factors on children's health and development. In 2009, children's environmental health was highlighted again at the G8 Environment Ministers' Meeting held in Syracuse, Italy, where ministers agreed to cooperate in scientific research to push this movement forward.

Meanwhile, in August 2006, the MOE held a conference on children's environmental health. The conference specifically focused on children's vulnerability to environmental hazards. According to the report from the conference, children tend to have a unique pattern of chemical exposure due to their physical characteristics (e.g., metabolic disposition and toxicokinetics) and behavioural characteristics (e.g., staying close to floor, consuming more fruit and milk compared to adults). The report also indicated that children are possibly more vulnerable to environmental hazards than adults because of their immature physiological and biochemical function. These emphasize the importance of investigating effects of environmental hazards on children.

To evaluate the impact of environmental factors on human health, a great deal of research has been performed using laboratory animals. However, the findings of such studies can not always be extrapolated to humans due to the difference in physiological and morphological features. In contrast, epidemiological research enables us to directly observe the effects of environmental factors on humans. (Report of the Conference on Epidemiological Study of Children's Environmental Health, March 2008).

In April 2008, MOE organized the Expert Group on the Epidemiological Research for Children's Environmental Health (later converted to JECS Working Group) and started planning a new nation-wide epidemiological study. After small pilot studies were conducted at several locations to examine the appropriateness and feasibility of the study protocols, in March 2010, the Working Group published a conceptual plan for a large-scale birth cohort study covering all areas of Japan. This JECS Protocol is developed in accordance with the draft conceptual plan.

3 Study Objectives

The primary objective of the JECS is to reveal environmental factors that affect children's health and development. Specifically, the JECS aims to evaluate the effects of exposure to chemical substances during the foetal stage and/or in early childhood on children's health and development, which would eventually lead to better environmental risk assessment and management system. Children show a rapid and considerable development during foetal period and early and middle childhood, and as a result, their health status frequently changes. Additionally, exposure to certain environmental factors during foetal period and childhood may affect their health. JECS is designed as a prospective birth-cohort study that follows participating children from foetal period until they reach 13 years of age.

JECS holds five major domains of research hypotheses (Table 2). In order to test these hypotheses, in addition to chemical exposures, various possible confounders and modifiers (such as physical, genetic, social and lifestyle related factors) are measured through environmental measurement and questionnaire survey.

Table 2: Research hypotheses

Pregnancy/reproduction	・ Parents' (mother and father) exposure to chemical substances in the environment affects the sex ratio of their newborn. ・ Exposure to chemical substances in the environment causes abnormal pregnancy. ・ Exposure to chemical substances in the environment causes abnormal development of foetuses and neonates.
Congenital anomalies	・ Exposure to chemical substances in the environment is related to the incidence of congenital anomalies. ・ Congenital anomalies are caused by the combined effects of genetic susceptibility and exposure to chemical substances in the environment.
Neuropsychiatric development	・ Exposure to chemical substances in the environment during foetal period and/or in early childhood, either alone or in combination with genetic susceptibility, is related to later diagnoses of developmental disorders and/or other neuropsychiatric disorders. ・ Exposure to chemical substances in the environment during foetal period and/or in early childhood, either alone or in combination with genetic susceptibility, is related to later quality of

	neuropsychiatric development and development of neuropsychiatric symptoms.
Immune system/allergy	・ Exposure to chemical substances in the environment during foetal period and/or in early childhood is related to later development of allergic disease.
Metabolism/endocrine system	・ Exposure to chemical substances in the environment during foetal period and/or in early childhood is related to later development of obesity, insulin resistance and type 2 diabetes mellitus. ・ Exposure to chemical substances in the environment during foetal period and/or in early childhood is associated with later bone mass and bone density. ・ Exposure to chemical substances in the environment during foetal period and/or in early childhood influences physical growth. ・ Exposure to chemical substances in the environment during foetal period and/or in early childhood is related to later degree of sexual maturation and sex differentiation of the brain. ・ Exposure to chemical substances in the environment during foetal period and/or in early childhood has a significant impact on later thyroid function.

Regarding to paediatric cancers, no hypothesis is proposed in JECS since the cohort size of 100,000 is not considered large enough to secure the sufficient number of cases for statistical examination of the relationship between exposure to environmental factors and development of paediatric cancers. However, JECS collects the cancer data and participates in the International Childhood Cancer Cohort Consortium (I4C) that aims to pool multiple cohort data of childhood cancers for further analyses.

4 Study Areas and Participants

4.1 Selection of Study Areas

Each Regional Centre selects its own study areas. The study area of the JECS is defined as geographical areas where participating pregnant women reside. A study area consists of one or several local administrative units (e.g., city and town). Each Regional Centre selects one or more study area(s) on the basis of the number of births, regional representativeness and level of potential exposures to environmental pollutants.

4.2 Selection of Study Participants

The participants are pregnant women who meet all of the inclusion criteria but not the exclusion criteria and their children. The children's fathers are registered as participants only when the mothers (or children after birth) participate in the study.

4.2.1 Inclusion criteria

1) A pregnant woman whose expected delivery date must be between 1 August 2011 and 31 March 2014
2) A pregnant woman must reside in one of the study areas selected by Regional Centres at the time of the recruitment and be expected to reside continually in Japan for the foreseeable future
3) A pregnant woman must visit a cooperating health care providers selected by a Regional Centre or local government offices to obtain a Mother-Child Health Handbook in a study area during the recruiting period

4.2.2 Exclusion criteria

1) A pregnant woman does not consent to participate in the study
2) A pregnant woman shows difficulty in comprehending the study procedures or filling out the questionnaires without support
3) A pregnant woman is reportedly not accessible at the time of delivery (e.g. a woman who plans to give birth outside the study area)

4.3 Method of Recruitment

The recruiting period is from January 2011 to March 2014. However, the recruitment for the participating child's father is attempted even after March 2014 until his child completes one-month check-up after birth.

Regional Centres working together with cooperating health care providers are responsible for recruiting participants. The participants (pregnant women, their partners and children) are selected so that they can represent the population residing in the study area. Either or both of the following two recruitment methods are applied.

4.3.1 Recruitment through cooperating health care providers

Regional Centres request cooperation from all of the health care providers that pregnant women living in the study areas possibly visit for prenatal examination and/or delivery. All the health care providers that have agreed on this study will be designated as cooperating health care providers. All the pregnant women living in the study areas who visit the cooperating health care providers are asked to participate in the study.

4.3.2 Recruitment through local government offices

In cooperation with local governments, when pregnant women living in the study areas apply for the Mother and Child Health Handbook (an official booklet given complimentary to all expecting mothers in Japan when they get pregnant in order to receive municipal services for pregnancy, delivery and childcare) at the local government offices, Regional Centres provide them with the information about the JECS and ask for their participation. When the pregnant woman shows interest in the study, field staff from the Regional Centres ask her which health care provider she would visit for prenatal care and delivery. If the health care provider is designated as a cooperating health care provider, the pregnant woman is asked to participate in the study. If possible, informed consent is obtained at the same time when the Mother and Child Health Handbook is issued at a local government office.

The JECS aims to recruit more than 50% of all pregnant mothers who reside in the study areas during the recruitment period.

4.4 Early Termination of Participation

Effort of following participants shall be terminated when the following incidents happen to the participating children:

1) Miscarriage
2) Stillbirth
3) Death of participating children

At these times, the children's parents are also excluded from the study. The corresponding Regional Centres need to collect necessary information about the termination and resister them before termination.

5 Study Design

5.1 Main Study

The Main Study is conducted with all the participants recruited by all the Regional Centres, and its content is nationally unified.

5.2 Sub-Cohort Study

The participants of the Sub-Cohort Study are randomly extracted from those of the Main Study at all the Regional Centres. The Sub-Cohort Study includes extended outcome and exposure assessments that are practically difficult to be administered in the Main Study because of its size. The written consent to the protocol of the Sub-Cohort Study is obtained separately from that of the Main Study. The sample size of the Sub-Cohort Study is 5,000 or 5% of the Main Study.

5.3 Adjunct Study

Adjunct Studies can be proposed and conducted by the Programme Office, Medical Support Centre, Regional Centres, or any combination of them using extramural funding. Adjunct Studies may include either all or a part of the Main Study participants. Proposals for Adjunct Studies need to be approved by MOE, ensuring that they do not interfere with the conduct of the Main Study and Sub-Cohort Study. The proposal of an Adjunct Study is submitted to the Steering Committee in advance of MOE's review.

6 Assessment/Measurement of Health Outcomes and Exposure

This section describes procedures for the assessment of health outcomes as well as environmental and genetic factors, and other related factors.

6.1 Assessment of Health Outcomes

On the following list are the outcome variables measured in the Main Study and the Sub-Cohort Study. JECS sets up SOPs that illustrate the outcomes measurement methods and procedures. Priority health outcomes are listed in Table 3.

Table 3: Priority health outcomes

Pregnancy/reproduction	Sex ratio, abnormal pregnancy, miscarriage, stillbirth, preterm delivery, birth weight, physical development after birth (e.g., motor function, kidney function, and lung function)
Congenital anomalies	Hypospadias, cryptorchidism, cleft lip and palate, intestinal atresia, ventricular septal defect, chromosome aberration
Neuropsychiatric developmental disorders	Developmental delay or deviation (mental retardation and other cognitive difficulties), autism spectrum disorder, learning disorder (LD), attention deficit hyperactivity disorder (ADHD), mental disorders (e.g., gender identity disorder), and other symptoms and behavioural characteristics
Immune system disorders	Food allergy, atopic dermatitis, asthma, allergic rhinitis, Kawasaki disease
Metabolic and endocrine system disorders	Abnormal glucose tolerance, obesity, effects on reproductive organs, genital dysplasia, sex differentiation of the brain
Childhood tumours	Leukaemia, brain tumours

6.2 Exposure Measurement

6.2.1 Biomonitoring

Analysing chemical substances and their metabolites in biospecimens is a major instrument for JECS exposure measurements besides questionnaire and modelling (ambient air and house dust measurement for Sub-Cohort Study). Chemical substances for evaluation are selected from the substances that easily accumulate in the human body; those that are known to easily pass through the placenta; those that children are often exposed to; and those that are of great public concern. To identify critical windows of exposure, biological specimens (e.g. blood and urine) are collected from mothers twice dur-

ing the pregnancy (early and mid–late). Umbilical cord blood samples are also collected at the time of birth. Additionally, breast milk samples are collected during lactation period. Hair samples from mothers and children are also collected and analysed specifically for detecting mercury. Since previous studies have indicated that fathers' exposure to chemical substances has certain impacts on their children's health, blood samples are collected from fathers when accessible. The chemical substances planned to be analysed for are listed in Appendix.

6.2.2 Ambient Measurement and Modelling

Besides biomonitoring, direct environmental measurements and modelling are used for air pollutant, indoor contaminants and radioactivity.

6.2.3 Genetic Analyses

JECS understands the importance of phenotypic difference both for exposure and health effect variation. The detailed study protocol (including analysis procedures) for genetic analyses will be prepared in future and reviewed for approval by IRBs. Participants will be informed the protocol and their consent will be re-taken before conducting genetic analysis.

6.2.4 Covariates and Potential Confounders

A series of questionnaires are used to measure covariates and potential confounders including demographic variables (e.g. residential address, education, employment, house-hold income), lifestyle factors (e.g. stress level, diet, smoking and drinking habits, exercise, sleep), physical environment (e.g., heat, ionizing radiation, housing condition, and neighbourhood), social/psychological factors (e.g., personality, social support), medical history (including pregnancy history) and medical history of family members.

6.3 Study Schedule

Table 4 shows the overall schedule of the Main Study and Sub-Cohort Study. The JECS is planned to continue until 2032, allowing five years for data analysis after all the participating children reach 13 years of age.

During their pregnancy, the data of the enrolled mothers are collected three times, once for each trimester (Table 3). After the mothers give birth, questionnaires are sent out to them every 6 months, taking account of the speed of the children's growth and development. Meanwhile, the in-

formation filled in the Mother and Child Health Handbook is transcribed to gather additional data regarding the children's growth and development.

Table 4: Study Milestones

Data collection Timing	Data collection method (Main Study)	Data collection method (Sub Study)
At recruitment (first trimester)	Medical record Questionnaire Biospecimen (Mother: blood (30 ml) and urine (50 ml), Father: blood (30 ml))	
Second and third trimester	Questionnaire Biospecimen (Mother: blood (30 ml) and urine (50 ml))	
At delivery	Medical record Biospecimen (Child: Umbilical cord blood (20–35 ml))	
Within a few days after birth (during hospitalization)	Biospecimen (Mother: blood (20 ml), hair (2 mg), Child: dried blood spot)	
1 month old	Medical record Questionnaire Biospecimen (Mother: breast milk (20 ml); Child: hair (2 mg))	
6 months old	Questionnaire	
1 year old	Questionnaire	
1.5 years old	Questionnaire	Environmental measurements
2 years old	Questionnaire	Developmental test Medical check (blood test, skin examination, etc.)
2.5 years old	Questionnaire	

3 years old	Questionnaire Mother and Child Health Handbook transcription	Environmental measurements
3.5 years old	Questionnaire	
4 years old	Questionnaire	Developmental test Medical check (blood test, skin examination, etc.)
4.5–5.5 years old	Questionnaire (once every 6 months)	
6 years old	Questionnaire Paediatric examination Body measurement (height, weight, etc.) Biospecimen (Child: urine (50 ml) and possibly blood (under consideration)) Mother and Child Health Handbook transcription	Developmental/Neuropsychological test and/or interviews (at 6, 8, 10 and 12 years old) Medical checks (blood test, skin examination, etc.; at 6,8,10 and 12 years old) Environmental measurement (once or twice, under consideration)
6.5–11.5 years old	Questionnaire (once every 6 months) School medical records	
12 years old	Questionnaire Paediatric examination Physical measurement (body height, body weight, etc.) Biospecimen (Child: urine (50 ml) and possibly blood (under consideration)) School medical records	

Note: The planned analytical parameters for biospecimens are presented in the Appendix. As necessary, additional data collection shall be conducted to gather participants' information about several specific diseases in more detail, referring to their medical record and/or school record. Questionnaires are filled in by the child's mother, except for at the recruitment at which they are filled by both father and mother, separately.

6.4 Following Participants

The participating children are followed until they reach the age of 13 years. The target retention rate is 80% or greater. Each Regional Centre is responsible for gathering data from the participants whom it

recruited, even when the participants move outside of their study areas in the middle of the study. However, if their participant moves to an area covered by another Regional Centre, the corresponding Regional Centre shall take over the role of following this participant, continuing plausible parts of the study. If their participant becomes out of reach through phone call or mailing in the middle of the study, the responsible Regional Centre needs to make every possible effort (e.g., accessing the registration data of the local government) to continue to follow the participant. If the participant is found to be completely inaccessible after every effort is made, she/he is considered to be dropped out from the study.

Information about child's birth and the date of birth is gathered through medical record at the time of delivery. The information regarding pregnancy and delivery (e.g., duration of pregnancy, birth weight) is confirmed through referral of the Mother and Child Health Handbook. When a child's birth record is missing or a child is stillborn, the information is collected through the reference of the Resident Registry and/or the report of Vital Statistics in cooperating with national and local governments. These official records are also utilized when participants (children, mothers, and fathers) decease, in order to verify their deaths and find out the reason of the deaths.

Changes in the residence of participants is informed through direct notifications from participants or returned mails. When participants become unreachable, the Resident Registry is referred to identify their current residential place.

The changes in demographic status of the participants, such as marital status of the mothers/fathers (divorce, remarriage, becoming a widow) and legal guardian of the participating children, are notified by participants directly and confirmed by reference of the Resident Registry as necessary.

7 Ethics and Protection of Participants' Rights and Information

7.1 Institutional Review Board (IRB)

The study's protocol and procedure for handling the collected individual data including biospecimens complies with the Ethical Guidelines for Epidemiological Research published by MEXT and MHLW. If genetic analyses are conducted as a part of the study, they should also comply with the Ethical Guidelines for Analytical Research on the Human Genome/Genes established by METI, MEXT and MHLW. The protocols of the Main Study and Sub-Cohort Study are submitted to the Review Committee for Epidemiological Studies (organised by the Ministry of Environment Ethics Committee) and the Ethical Committee for Medical Studies within the National Institute for Environmental Studies, to obtain their approval. Subsequently, the protocols are reviewed by each Regional Centre's IRB.

Organisations that conduct the Adjunct Studies are responsible for obtaining approvals from each IRB, the results of which are reported to the Steering Committee of the JECS.

7.2 Data Management System

All necessary measures will be taken to ensure that study participants' privacy and confidentiality is protected in accordance to the guidelines presented in the section 6.1.

All the collected data are stored in an electronic data management system (DMS) maintained by the Programme Office. The DMS is located in the facility with physical and technical safety management system complying with the Information Security Policy of Ministry of the Environment (ver. 4, August 2010). The following data are separated with each other for their storage: participants' personally identifiable information; de-identified or coded data collected through questionnaires, testing, home visits and laboratory analyses; and the de-coding table that are used to link the de-identified/coded data with personally identifiable information. Each Regional Centre has a privilege of accessing to all the data it has collected. The Programme Office is responsible for modifying and deleting the stored data when necessary. To prevent personally identifiable information from unintentional disclosure, the access to the DMS is limited to selected and trained personnel within the Programme Office, Medical Support Centre and Regional Centres. The rooms that host DMS terminals are maintained locked, allowing only the designated personnel to enter the rooms. The printed documents (e.g., consent forms) containing personally identifiable information are stored in lockable cabinets until the end of the study period.

At the Programme Office, Medical Support Centre, and Regional Centres, system administrators of personally identifiable information are appointed from among those who have a profession-related confidentiality obligation but are not participating in the JECS. Personnel who are given rights to access personally identifiable information at the Programme Office, Medical Support Centre,

and Regional Centres have to submit a written agreement regarding protection of personally identifiable information to the system administrator of the respective institution.

7.3 Informed Consent

The participation of the children is proceeded preferably after their parents with custody (both father and mother or mother if she has sole custody) fully understand the contents of the study. The agreement on study participation of children and mother is obtained from pregnant mothers. For their partners (fathers), the consent from them is also obtained after providing them with the information about the JECS study protocol. The information collection from mother or father is is carried out after receiving consent from them respectively.

When they reach an appropriate age, participating children will be provided with the opportunities of being explained about the contents of the study using verbal expressions that are easily understandable for children. Further discussion is required on the necessity of obtaining written informed consent from participating children, specifically when using an invasive data collection method, such as blood sampling, is planned to be used. Carrying out such an invasive data collection method is permitted only after obtaining an approval from the Ministry of Environment Review Committee for Epidemiological Studies.

7.3.1 Informed Consent Procedure

The Regional Centre staff explain about the study to the participants on the face-to-face basis and obtain written consent to the study protocol. The staff who are allowed to take this role are those who have completed the mandatory training and are designated as a research coordinator (RC) of the JECS. The training is held by the Programme Office. The RCs hold a licensure/certificate of an occupation with legal obligation of confidentiality (e.g. doctors, nurses, midwives, etc.) or sign a nondisclosure agreement with the director of the institution they belong to. All Regional Centres use the same informed consent form provided by the Programme Office.

The RCs explain every element of the informed consent form to the participants face-to-face using easily understandable verbal expressions. The consent document must be signed by the participant after they fully understand all of the following aspects of the study and agree upon them:

a) Purpose of the study

b) Methods of the study

c) Eligibility for the participation

d) Duration of the study

e) Potential benefits

f) Potential risks and discomforts

g) Assurance of privacy protection and confidentiality of records and data

h) Usage and storage of collected data

i) Potential outcomes of the study

j) Voluntary participation and withdrawal

k) Compensation

l) Contact information

When any new research procedures are added to the study protocol (e.g., blood collection from children), the participants are informed and a new consent are obtained from the legal guardians of the participating children. When the children become mature enough to understand the verbal explanation of the new procedure, they will also be informed about the study procedure. The informed consent documents are duplicated and maintained by both the participants and the Regional Centres until the end of the study.

7.3.2 Participant withdrawal

When a withdrawal request is received from a participant, the request is shared with the Programme Office and the responsible Regional Centre. Withdraw procedure must be formally proceeded by obtaining a written form of withdrawal document from the participating children or their legal guardian. Participant's survey responses, clinical and biochemical data, and biological specimens are appropriately processed or discarded according to the participant's/legal guardian's request. Upon completion of the withdrawal procedure, the participant or the legal guardian is informed by the written from.

7.4 Long Term Storage of Biospecimens and Data

The Programme Office maintains a part of biospecimens including blood, cord blood, urine, breast milk, and hair collected from the participating mothers, their children, and the children's fathers, for additional analyses (e.g., gene analyses) that will be planned and conducted in future. The biospecimens are stored in a long-term storage facility located in the NIES.

All the data and biospecimen collected from the participants are stored until 2032, 5 years after completion of the data collection. When JECS is determined to be continued beyond 2032, duration of the data storage will also be extended as long as the study lasts. The possible extension of data and biospecimen storage period is also documented in the consent form. The data and biospecimen are stored and maintained in the condition of anonymous yet traceable.

Specific rules and procedures are set to provide the collected data and biospecimen to researchers/research institutions that plan to use them for other studies. The special sub-committee es-

tablished in the Steering Committee examines each application and determines whether the data/biospecimen should be provided. Due to the fact that the collected biospecimens are precious and limited, the sub-committee evaluates each study proposal on strict standards, such as the degree of contribution of the study to the JECS, and determines which study should have priority to receive the biospecimens. The data/biospecimens will be provided not only to researchers/research institutions who are members of JECS research group, but also to those who are not, after being anonymized by removing personally identifiable information from the original data.

Additionally, the MOE plans to provide data/biospecimens after the completion of the JECS study, establishing data archives and biospecimen bank. All of these possible future plans are included in the consent form.

7.5 Genetic Analyses and Counselling

When genetic analyses are determined to be conducted, the procedure of disclosing their outcomes will be reviewed by NIES IRB. When the outcome is shared with the participants, qualified physicians specializing in clinical genetics or certified genetic counsellors shall be appointed as supervisors in charge.

7.6 Information Protection and Communication

Researchers and staff involved in the JECS make every effort to protect its participants from any risks and prevent them from suffering from any disadvantages caused by participation of the study.

The result derived from the analyses of questionnaires and biological specimens are actively shared with the participants upon their agreement in the consent form.

When the JECS uncovers clinically relevant but unexpected findings, the Steering Committee sets up a sub-committee to examine and determine the contents of the finding that should be reported to the participants and the reporting procedure.

8 Sample size

The number of the participants for the Main Study is 100,000 (Table 5). This number is considered sufficient to evaluate the effect of an environmental variable with the relative risk 1.3 on development of a disease/health outcome with prevalence rate of approximately 10% (e.g., infantile obesity and allergic disease), with sufficient statistical power when both outcome variable and environmental variable are coded binary (presence/absence). This number of participants also allows us to

test the effect of environmental variables with the relative risk 2.0 or greater on development of disease/health outcome variable with prevalence rate of 1.0 % or less.

The number of participants for the Sub-Cohort Study is set as 5,000, which is sufficient to test hypotheses regarding to the association between environmental variables and diseases with high prevalence rate, such as obesity and allergic diseases.

Table 5: Sample sizes necessary to test hypotheses statistically (Conditions: significance level = 5%, statistical power = 80%, relative risk=2.0, statistical test = adjusted Chi-square test (one-sided))

Name of disease	Prevalence	Number per 100,000	The percentage of individuals with a high level of exposure to a certain chemical substance				
			1%	3%	5%	10%	25%
Obesity	10%	10,000	8,100	***28,34***	***1,780***	***1,010***	***580***
Atopic dermatitis (5 years old)	3.8%	3,770	23,200	8,101	***5,080***	***2,860***	***1,632***
ADHD (5 years old)	3%	3,000	29,600	10,367	6,500	***3,660***	***2,088***
Asthma (5 years old)	2.4%	2,400	37,300	13,034	8,200	***4,610***	***2,624***
Cryptorchidism	0.7%	700	130,600	45,634	28,680	16,110	9,164
Down's syndrome	0.1%	100	921,100	321,667	202,160	113,510	64,536
Hypospadias	0.05%	50	1,843,400	643,700	404,580	227,150	129,140
Type 1 diabetes mellitus	0.001%	1	92,221,800	32,203,934	20,240,500	11,363,740	6,460,364

9 Statistical Analysis

As outcome variables and exposures are measured at several different waves, methods applicable to longitudinal datasets are used for statistical analyses. Nested case-control and case-cohort approaches are also used. The outcome variables (Y) shall include presence/absence of disease, the onset of disease (time to event), and variables composed based on the responses to the questionnaires, while the explanatory variable (X) shall include the exposures and confounders.

9.1 Variables by Single Measurement

When analysing the relationship between the outcome variable (Y) and the explanatory variable (X) both of which are measured only once, the increased onset rate of the diseases shall be calculated by performing a regression analysis, controlling confounders related to the outcome variable (e.g. presence/absence of onset, duration, time to event, etc.). The examples of outcome variables (Y) measured only once include those collected at the time of delivery (e.g. birth weight, gender, congenital anomalies). The examples of the exposure factors (X) measured only once are chemical substances collected during pregnancy and those contained in umbilical cord blood.

9.2 Variables by Multiple Measurements

When planned analyses include the outcome variables (Y) that are measured at multiple times (e.g., presence/absence of a certain symptom, variables associated with neuropsychiatric development), the participant's intra-individual variation shall be taken into consideration during their analyses. Multiple measurements of an outcome variable enable quantification of the exposure effect at each time point and estimation of the growth curve of study participants for the target variable.

9.3 Multiple measurement of explanatory variable (X)

When planned analyses include the exposure measurements (X) that are measured at multiple times, the change in exposures and intra-individual measurement errors shall be incorporated in statistical models using for their analysis.

10 Procedure Manuals

The procedures of measurement, analyses, data collection and management, and quality assurance of measurements of outcome and exposure variables are described in separate standard operating procedures (SOPs). The SOPs stipulate the following: Methods for measurement/analysis, methods for data/sample collection, methods for training personnel responsible for data collection, methods for assuring quality of the study, and methods for auditing conducted to ensure compliance with the study protocol. The SOPs also addresses the methods of data coding/input, the methods of identifying coding/input errors, electronic software and hardware used for data management and the methods of han-

dling biospecimens and environmental samples (i.e., methods of transportation, preservation and disposal). Regional Centres and cooperating health care provides may create their own SOPs for other specific tasks.

11 Publication of the Study Progress and Results

Each Regional Centre periodically reports the progress of the study to the Steering Committee, while the Programme Office updates the storage status of the collected biospecimens and data. The Programme Office then submits an annual study progress report to the MOE each year, which is made available to the public.

The results derived from the study are published in international peer reviewed scientific journals as well as shared with the study participants through JECS website. The detailed methods for publication of the study results are described in a separate SOP.

12 Reporting to the IRB and the Project Evaluation Committee

During the study period, the annual study progress reports are submitted to the Review Committee for Epidemiological Research in the MOE. The Committee reviews the report and provides the Programme Office with feedback from an ethical perspective. Any changes made in the study protocol must be notified to the MOE and reviewed and approved by the Committee.

The annual study progress reports, including change in study protocol and procedure, are also submitted to the Project Evaluation Committee established in the MOE and modified in accordance with their advice/guidance.

13 Research Funding

The JECS are funded directly by the MOE. For Adjunct Studies, extramural funding, such as research grants provided by government ministries and agencies (including the MOE) as well as private sectors, is acquired. The principal investigators of the Adjunct Studies are required to promptly report any conflicts of interest generated among the study group members and agencies providing research funds for the Adjunct Study to the Steering Committee.

14 Intellectual Property

JECS allows researchers to apply for a patent for invention produced during the course of the study. The researcher who has produced the invention is required to apply for a patent together with the

Principal Investigator of the JECS and all the other researchers who have been involved in the invention and belong to the Programme Office, Medical Support Centre or Regional Centres. Rules for patents application based on any materials and biospecimens provided outside to the JECS are stipulated elsewhere.

Appendix1: Analytical parameters measured through collection of biospecimen

1. Blood

(1) Exposures

Lead, Cadmium
Total mercury, methyl mercury
Heavy metals
Polychlorinated biphenyl (PCBs): typical isomers 4–7 species
Hydroxylated PCBs: typical isomer
Polybrominated diphenyl ethers (PeBDE, OBDE, etc.)
Dioxins (PCDDs/PCDFs 17 species, Co-PCB (DL-PCB) 12 species)
Hexachlorobenzene (HCB), Pentachlorobenzene (PeCB)
Chlordane analogues (cis-, trans-chlordane, cis-, trans-nonachlor, oxychlordane)
DDT, DDE, etc.
Drin-agricultural chemicals, e.g., dieldrin
Heptachlor analogues (cis-, trans-Heptachlorepoxide)
Hexachlorocyclohexane (α, β, γ, δ-HCH)
Mirex
Chlordecone
Toxaphene
Hexabromocyclododecane (HBCD)
Organic fluorides (PFOA, PFOS, PFCAs (C6, 9-12), PFASs (C6, C10))

(2) Health outcomes

Glycohemoglobin A1c (HbA1c)
Specific IgEs
Total IgE
Red blood cell count, white blood cell count, differential white blood count, haemoglobin, haematocrit, platelet, mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC)
LDL cholesterol
Total cholesterol
Free cholesterol
Triglyceride
HDL cholesterol
Total protein, Albumin

Phospholipid
Folic acid
25(OH) vitamin D
Alkaline phosphatase (ALP)
RLP-cholesterol
Luteinizing hormone (LH)
Follicle-stimulating hormone (FSH)
Oestradiol
Prolactin
Testosterone
Free testosterone
Dehydroepiandrosterone sulfate (DHEA-S)
Androstenedione
Adiponectin
Resistin
Inhibin
Transferrin
Ferritin
Retinol
Tocopherol
Thyroid stimulating hormone (TSH)
Free-thyroxine (Free-T4)
Various specific antibodies
Anti-thyroid peroxidase antibody (TPOAb)
Anti‑thyroglobulin antibody (TgAb)
Leptin
Creatinine
C-reactive protein (CRP)

2. Urine

(1) Exposures

Arsenic compounds sorted by chemical form ((III), (V), arsenobetaine, methylarsenic acid, di-methylarsenic acid, trimethyarsine oxide, etc.)
iodine, perchloric acid, nitrate nitrogen, etc.
Organophosphate pesticide metabolites (Dimethyl phosphate (DMP), Diethyl phosphate (DEP),

Dimethyl thiophosphate (DMTP), Diethylthiophosphate (DETP), etc.)
3-Methyl-4-nitrophenol (Fenitrothion metabolite), Para-nitrophenol (Parathion metabolite)
Methamidophos (Acephate metabolite)
Pyrethroid metabolites (Phenoxybenzoic acids (PBA), 2,2-dimethylcyclopropane-1-carboxylic acids (DCCA))
Ethylenethiourea (ETU), etc.
Imidacloprid metabolites (6-Chloronicotinic acid), Acetamiprid metabolites, etc.
Pentachlorophenol (PCP), Chlorophenol compounds (atrazine, bentazon, diuron, bromobutide and debrominated body, Glyphosate)
Flutolanil, Carpropamid, Iprodione, Flusulfamide
Nitro musks (Musk xyene, Musk ketone)
Cyclic musks (HHCB (Galaxolide), AHTN (Tonalide), ADBI (Celestolide), AHMI (Phantolide), DPMI (Cashmeran), ATII (Traseolide))
Phthalate metabolites (8–10 species including mono-(2-ethylhexyl) phthalate)
Bisphenol A, Tetrabromobisphenol A, Bisphenol F, Nonylphenol, etc.
Parabens (methyl-, ethyl-, propyl-, butyl-, benzyl-hydroxybenzoate, etc.)
Triclosan
Benzophenone
DEET (*N*,*N*-diethyl-3-methylbenzamide)
Aromatic hydrocarbons and their degradants/metabolites (1-OH-Pyrene, 1-,2/9-,3-,3-OH-Phenanthrene, etc.)
Cotinine, Thiocyanate
Dichlorobenzene
Plant oestrogen
Caffeine
Pyridine
Acrylamide
Tributoxyethyl phosphate (TBEP), Tributyl phosphate (TBP)
8-Hydroxydeoxyguanosine (8-OHdG), 8-isoprostane

(2) Health outcomes

Creatinine
Specific gravity
N-acetyl-beta-D-glucosaminidase (NAG), β2-microglobulin

3. Breast milk

(1) Exposures

Iodine, perchloric acid, nitrate nitrogen, etc.
Dioxins (PCDDs/PCDFs 17 species, Co-PCBs (DL-PCBs) 12 species)
PCBs: typical isomers 4–7 species
Hydroxylated PCBs: typical isomer
Hexachlorobenzene (HCB), Pentachlorobenzene (PeCB)
Chlordane analogues (cis-, trans-chlordane, cis-, trans-nonachlor, oxychlordane)
DDT, DDE, etc.
Drin-agricultural chemicals, e.g., dieldrin
Heptachlor analogues (cis-, trans-Heptachlorepoxide)
Hexachlorocyclohexane (α, β, γ, δ-HCH)
Mirex
Chlordecone
Toxaphene
Polybrominated diphenyl ethers (PeBDEs, OBDEs, etc.)
Polybrominated biphenyls (HBBs, PeBBs, etc.)
Phthalate metabolites (8–10 species including mono(2-ethylhexyl) phthalate)

4. Filter paper blood sample

(1) Health outcome

Thyroid stimulating hormone (TSH)

5. Hair

(1) Exposures

Total mercury

Appendix 2. Variables collected at each phase

Timing	**Method**	**Variables**		
		Outcome	**Exposure**	**Other variables**
First pregnancy trimester	Questionnaire filled in by primary care physician			Expected delivery date, height, weight, pregnancy status, pregnancy complication, delivery history, history of infertility treatment
	Prenatal check-up			Height, weight, blood pressure
	Mother-report questionnaire		Diet, occupation, other environmental exposures	Marital status, family member, pregnancy and delivery history, medical history, medication history, DV K6, SF-8, IPAQ Smoking, occupation, diet, drinking
	Maternal blood	Allergen-specific IgE, Total IgE	Chemical substances with longer half-lives	Complete blood count, TP, Alb, Hb_{A1c}, TC, LDL-C, cholesterol, TG, HDL-C, phospholipids
	Maternal urine		Chemical substances with shorter half-lives	Cotinine, thiocyanate, creatinine, specific gravity of urine
	Father-report questionnaire		Diet, occupation, other environmental exposures	Height, weight, medical history, medication history K6, SF-8, AQ10 Smoking, occupation, diet, drinking

	Paternal blood	Total IgE	Chemical substances with longer half-lives	TP, Alb, TC, LDL-C, cholesterol, TG, HDL-C, phospholipids
Second–third pregnancy trimester	Prenatal check-up			Weight, blood pressure, 50gGCT
	Mother-report questionnaire		Diet, built environment, occupation, other environmental exposures	K6, SF-8, IPAQ, AQ10 Stressful events, DV Smoking, occupation, built environment Diet, drinking, eating habits, supplement Education, income, social support
	Maternal blood		Metals, chemical substances with longer half-lives	TP, Alb, TC, cholesterol, TG, phospholipids, folic acid
	Maternal urine		Chemical substances with shorter half-lives	Creatinine, specific gravity of urine
At delivery	Questionnaire filled in by primary care physician	Multiple birth, miscarriage, stillbirth, somatometry, sex of child, labour complication, neonatal jaundice, neonatal complication, congenital anomaly		Weight, blood glucose level, mode of delivery (including painless delivery), information during pregnancy (infection, medication, physical/mental disease, nutrition counseling)
	Umbilical cord blood	Total IgE	Metals, chemical substances with longer half-lives	TP, Alb, TC, cholesterol, TG, phospholipids
Within a few days after birth	Maternal blood		Metals, chemical substances with shorter half-lives	TP, Alb, TC, LDL- C, cholesterol, TG, phospholipids
	Dried blood spot	TSH	Chemical substances with longer half-lives	
	Maternal hair		Hg	
Age one	Questionnaire	Puerperium history,		

month	filled in by primary physician	somatometry, prolonged jaundice, congenital anomaly		
	Questionnaire	Physical symptoms (e.g., fever), child growth, neuropsychiatric development, allergy		Kangaroo care, crying, sleep, Attachment scale, postpartum depression Smoking, Drinking
	Breast milk		Chemical substances with longer half-lives	
	Child hair		Hg	
Age 6 month	Questionnaire	Medical history, growth, neuropsychiatric development, allergy	Food allergen	Family relations, postpartum depression, parents' health status, partner's participation in parenting, lactation, baby food, binding scale, sleep, vaccination
Age 1	Questionnaire	Medical history, growth, neuropsychiatric development, allergy	Food allergen	Occupation, parents' health status, lactation, baby food, crying, sleep, parenting environment, TV/PDA exposure, social relationships, vaccination, health-related events
Age 1.5	Questionnaire	Medical history, growth, neuropsychiatric development, allergy	Food allergen	Occupation, parents' health status, lactation, baby food, crying, sleep, parenting environment, TV/PDA exposure, social relationships, vaccination, health-related events
	Sub-Cohort Study		Indoor/outdoor air quality (VOCs, NOx, SO_2, O_3, PM), house dust	

			(metals, POPs, pesticides, phthalates,…), dwelling observation	
Age 2	Questionnaire	Medical history, growth, neuropsychiatric development, allergy	Food allergen	Occupation, parents' health status, lactation, baby food, crying, sleep, parenting environment, TV/PDA exposure, social relationships, vaccination, health-related events
	Sub-Cohort Study	Neuropsychiatric development test, paediatrician's examination, blood test (IgE, IgG, IgA, TSH, fT4, 25(OH)D)		
Age 2.5	Questionnaire	Medical history, growth, neuropsychiatric development, allergy		Family relations, parents' health status, parenting stress, social bond, TV/PDA exposure, exercise, health-related events
Age 3	Questionnaire	Medical history, growth, neuropsychiatric development, allergy	Indoor chemical exposure, allergen (dust mites, etc.)	Smoking, parents' health status, socio-economic status, oral cavity, skin condition, defecation, urination, sleep, lifestyle, residential environment, parenting environment, TV/PDA exposure, social relationships, health-related events
	Mother–Child Handbook tran-	Height, weight, head circumference, chest		Pregnancy history, vaccination, dental history

	scription	circumference, growth curve, neuropsychiatric development		
	Sub-Cohort Study		Indoor/outdoor air quality (VOCs, NOx, SO_2, O_3, PM), dwelling observation	
Age 3.5	Questionnaire	Growth curve, neuropsychiatric development		Family relations, parents' health status, occupation, parenting stress, parenting attitude, partner's participation in parenting
Age 4	Questionnaire	Medical history, growth, neuropsychiatric development, allergy		Parents' health status, delactation, drinking, parenting environment, oral cavity, skin condition, defecation, urination, temperament, TV/PDA exposure, social relationships, health-related events
	Sub-Cohort Study	Neuropsychiatric development test, paediatrician's examination, blood test (IgE, IgG, IgA, TSH, fT4, 25(OH)D)		
Age 4.5–6	Questionnaire	Medical history, growth, neuropsychiatric development, allergy		Parents' health status, drinking, parenting environment, oral cavity, skin condition, defecation, urination, temperament, TV/PDA exposure, social relationships, health-related

				events

Appendix 3. Instruments

1. High priority outcome measurements (Category A)

	6 m	1.0 y	1.5 y	2.0 y	2.5 y	3.0 y	3.5 y	4.0 y	4.5 y	5.0 y	5.5 y	6.0 y	6 y+
Congenital anomalies	Med. hist.	Med. hist.		Med. hist. + MRT								Med. hist. + MRT	
Neuropsychiatric development	ASQ, sleep	ASQ, sleep	ASQ, sleep	ASQ, epilepsy (med. hist. + MRT)	ASQ, mod. ES-SENCE-Q	ASQ, sleep, epilepsy (med. hist. + MRT)	ASQ, SRS-P	ASQ, epilepsy (med. hist. + MRT)	ASQ, mod. ES-SENCE-Q	ASQ, SRS, SDQ, epilepsy (med. hist. + MRT)		ADHD-RS, sleep, epilepsy (med. hist. + MRT)	LD (TBD), ADHD-RS, sleep, develop-ment, epilepsy, PSAI
Immune system	ISAAC, food al-lergy, Kawasaki disease (med. hist. + MRT)	ISAAC, food al-lergy, Kawasaki disease (med. hist. + MRT)	ISAAC, food al-lergy, Kawasaki disease (med. hist. + MRT)	ISAAC, food al-lergy, Kawasaki disease (med. hist. + MRT)		ISAAC, food al-lergy, Kawasaki disease (med. hist. + MRT)		ISAAC, food al-lergy, Kawasaki disease (med. hist. + MRT)		ISAAC, food al-lergy, Kawasaki disease (med. hist. + MRT)		ISAAC, food al-lergy, Kawasaki disease (med. hist. + MRT)	ISAAC, food al-lergy, Kawasaki disease (med. hist. + MRT)
Metabo-lism/endocrine sys-tem	Growth	Growth, med. hist.	Growth, med. hist.	Growth, med. hist., MRT	Growth, med. hist.	Growth, med. hist.	Growth, med. hist.	Growth, med. hist.	Growth	Growth, med. hist.	Growth	Growth, med. hist., MRT,	Growth, med. hist., puberty

	6 m	1.0 y	1.5 y	2.0 y	2.5 y	3.0 y	3.5 y	4.0 y	4.5 y	5.0 y	5.5 y	6.0 y	6 y+
												puberty, body measure-ment	
Others (death, cancer, infections, …)	Med. hist., Cancer (MRT)	Med. hist., resident registry, Cancer (MRT)	Med. hist.	Med. hist., resident registry, Cancer (MRT)		Med. hist., resident registry, Cancer (MRT)		Med. hist., resident registry, Cancer (MRT)		Med. hist., resident registry, Cancer (MRT)		Med. hist., resident registry, Cancer (MRT)	Med. hist., resident registry, Cancer (MRT)
Transcriptions						MCH						MCH	School records

Abbreviations: MRT, medical record transcription; ADHD-RS, Attention Deficit Hyperactivity Disorder Rating Scale; ASQ, Ages & Stages Questionnaires; ISAAC, International Study of Asthma and Allergies in Childhood; PSAI, Pre-School Activity Inventory; SRS, Social Responsiveness Scale; SRS-P, Social Responsiveness Scale Preschool version; SDQ, Strengths and Difficulties Questionnaire; MCH, Mother–Child Handbook

2. Questionnaire instruments by outcomes and exposures

	6 m	1.0 y	1.5 y	2.0 y	2.5 y	3.0 y	3.5 y	4.0 y	4.5 y	5.0 y	5.5 y	6.0 y	6 y +
Health outcomes (Category A)													
Congenital anoma-lies	Med. hist.	Med. hist.		Med. hist. + MRT								Med. hist. + MRT	
Neuropsychiatric development													
Epilepsy		Med. hist.		Med. hist. + MRT		Med. hist. + MRT		Med. hist. + MRT		Med. hist. + MRT		Med. hist. + MRT	Med. hist. + MRT
ASD							SRS-P			SRS			
LD													TBD
ADHD										SDQ		ADHD-RS	ADHD-RS at age 9
Gender Identity Disorder													PSAI
Developmental milestones	ASQ	ASQ	ASQ	ASQ	ASQ, mod. ESSENCE-Q	ASQ	ASQ	ASQ	ASQ, mod. ESSENCE-Q	ASQ			
Sleep	IHQ	IHQ	IHQ			IHQ			IHQ			IHQ	IHQ
Immune system													
Kawasaki disease	Med. hist. + MRT	Med. hist. + MRT	Med. hist. + MRT	Med. hist. + MRT		Med. hist. + MRT		Med. hist. + MRT		Med. hist. + MRT		Med. hist. + MRT	Med. hist. + MRT

	6 m	1.0 y	1.5 y	2.0 y	2.5 y	3.0 y	3.5 y	4.0 y	4.5 y	5.0 y	5.5 y	6.0 y	6 y +
Asthma		ISAAC	ISAAC	ISAAC		ISAAC		ISAAC		ISAAC		ISAAC	ISAAC
Atopic dermatitis	ISAAC	ISAAC	ISAAC	ISAAC		ISAAC		ISAAC		ISAAC		ISAAC	ISAAC
Allergic rhinitis				ISAAC		ISAAC		ISAAC		ISAAC		ISAAC	ISAAC
Food allergy	IHQ	IHQ	IHQ	IHQ		IHQ		IHQ		IHQ		IHQ	IHQ
Food protein induced enterocolitis		Med. hist.	Med. hist.										
Metabolism/endocrine system													
Obesity	IHQ	IHQ	IHQ	IHQ	IHQ	IHQ	IHQ	IHQ	IHQ	IHQ	IHQ	IHQ, measurement	IHQ, school records
Growth curve	IHQ	IHQ	IHQ	IHQ	IHQ	IHQ, MHC	IHQ	IHQ	IHQ	IHQ	IHQ	IHQ, measurement, MHC	IHQ, school records
Diabetes		Med. hist.										Med. hist.	Med. hist.
Thyroid function		Med. hist.		Med. hist. + MRT		Med. hist.		Med. hist.		Med. hist.		Med. hist. + MRT	Med. hist.
Puberty		Med. hist.		Med. hist. + MRT		Med. hist.		Med. hist.		Med. hist.		Med. hist. + MRT, exam	Med. hist., exam
Other outcomes													

	6 m	1.0 y	1.5 y	2.0 y	2.5 y	3.0 y	3.5 y	4.0 y	4.5 y	5.0 y	5.5 y	6.0 y	6 y +
Death		Resident registry		Resident registry		Resident registry		Resident registry		Resident registry		Resident registry	Resident registry
Cancer	Med. hist. + MRT	Med. hist. + MRT		Med. hist. + MRT		Med. hist. + MRT		Med. hist. + MRT		Med. hist. + MRT		Med. hist. + MRT	Med. hist. + MRT
Infection	Med. hist.	Med. hist.	Med. hist.	Med. hist.		Med. hist.		Med. hist.		Med. hist.		Med. hist.	Med. hist.
Exposure													
Chemicals			IHQ (household chemicals)			IHQ (household chemicals)			IHQ (household chemicals, diet)			IHQ (household chemicals), HBM	IHQ (household chemicals), HBM
Indoor air pollutants			IHQ			IHQ			IHQ			IHQ	IHQ
Air pollutants	Model	Model	IHQ, model	Model	Model	IHQ, model	Model	Model	IHQ, model	Model	Model	IHQ, model	IHQ, model
Noise	Model	Model	IHQ, model	Model	Model	IHQ, model	Model	Model	IHQ, model	Model	Model	IHQ, model	IHQ, model
Other pollutants			IHQ, public data			IHQ, public data			IHQ, public data			IHQ, public data	IHQ, public data
Allergen			IHQ			IHQ			IHQ			IHQ	IHQ
Covariates/confounders													
Parents													

	6 m	1.0 y	1.5 y	2.0 y	2.5 y	3.0 y	3.5 y	4.0 y	4.5 y	5.0 y	5.5 y	6.0 y	6 y +
Nationality	IHQ												
Height and weight	IHQ				IHQ				IHQ				IHQ
Family	IHQ		IHQ		IHQ		IHQ		IHQ		IHQ		IHQ
Marital status	IHQ												
Smoking			IHQ			IHQ			IHQ			IHQ	IHQ
Drinking			IHQ			IHQ			IHQ			IHQ	IHQ
Occupation		IHQ					IHQ				IHQ		IHQ
Health degree	Self rated		Self rated		SF-8		Self rated		Self rated		SF-8		Self rated
Anxiety/depression	EPDS	K6						K6					K6
Attachment	Bonding scale	Bonding scale											
Mental stress	IHQ		IHQ		IHQ		IHQ		IHQ		IHQ		IHQ
Household income						IHQ							IHQ
Community support					IHQ								IHQ
Partners' participa-tion in childcare	IHQ			IHQ			IHQ			IHQ			
Childcare		IHQ	PSI	IHQ	PSI	IHQ	PSI	IHQ		PSI		IHQ	PSI at age 7
Children													
Height, weight, head circumference	IHQ	IHQ	IHQ	IHQ	IHQ	IHQ	IHQ	IHQ	IHQ	IHQ	IHQ	IHQ	IHQ

	6 m	1.0 y	1.5 y	2.0 y	2.5 y	3.0 y	3.5 y	4.0 y	4.5 y	5.0 y	5.5 y	6.0 y	6 y +
Family	IHQ		IHQ		IHQ		IHQ		IHQ		IHQ		IHQ
Passive smoking			IHQ			IHQ			IHQ			IHQ, HBM	IHQ, HBM
Delactation	IHQ	IHQ	IHQ	IHQ				IHQ				IHQ	
Diet	IHQ	IHQ	IHQ	IHQ					FFQ				FFQ
Cry		IHQ											
Sleep	IHQ	IHQ	IHQ			IHQ			IHQ			IHQ	IHQ
Temperament								IHQ					
Vision/hearing									IHQ				School record
Oral/skin condition	IHQ		IHQ	IHQ		IHQ		IHQ		IHQ		IHQ	School record
Defecation/urination						IHQ, ROME-III		IHQ, ROME-III		IHQ, ROME-III		IHQ, ROME-III	IHQ, ROME-III
Built environment			IHQ			IHQ			IHQ			IHQ	IHQ
Childcare environ-ment		IHQ		IHQ		IHQ		IHQ		IHQ		IHQ	IHQ
TV/PDA exposure		IHQ		IHQ	IHQ	IHQ		IHQ		IHQ		IHQ	IHQ
Social life	IHQ	IHQ		IHQ		IHQ		IHQ		IHQ		IHQ	IHQ
Vaccination	IHQ	IHQ		IHQ								IHQ	IHQ
Health related events		IHQ	IHQ	IHQ	IHQ	IHQ		IHQ					
Exercise					IHQ				IHQ				IHQ,

	6 m	1.0 y	1.5 y	2.0 y	2.5 y	3.0 y	3.5 y	4.0 y	4.5 y	5.0 y	5.5 y	6.0 y	6 y +
													School record
Medication													TBD

Abbreviations: MRT, medical record transcription; Abbreviations: Abbreviations: ADHD-RS, Attention Deficit Hyperactivity Disorder Rating Scale; ASQ, Ages & Stages Questionnaires; EPDS, Edinburgh Postnatal Depression Scale; ISAAC, International Study of Asthma and Allergies in Childhood; PSAI, Pre-School Activity Inventory; PSI, Parenting Stress Index; SRS, Social Responsiveness Scale; SRS-P, Social Responsiveness Scale Preschool version; SDQ, Strengths and Difficulties Questionnaire; MCH, Mother–Child Handbook; IHQ, in-house developed questionnaire; FFQ, food frequency questionnaire; TBD, to be determined