Japan Environment & Children's Study

(JECS)

Sub-Cohort Study Protocol (ver. 1.01)

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

1. Sub-Cohort Study

Japan Environment and Children's Study (JECS) is a nation-wide birth cohort study which aims to identify environmental factors that affect children's health and development. The main part of the JECS (Main Study) aims to recruit 100,000 mother–child pairs and collect data from all the participants. The Main Study utilises questionnaire (including medical record transcription) and modelling for exposure and health outcome measurements. For some exposure and outcome measurements, home visit and person-to-person examination is required. Such include indoor air quality, dust chemistry, neurodevelopmental tests and paediatric examination. Those measurements are particularly expensive so that they cannot be applied to 100,000 participants. Thus, those extended exposure and outcome measurements are performed for a subgroup randomly selected from the Main Study, which is called as Sub-Cohort Study.

Sub-Cohort Study is planned to conduct with 5,000 participants who are randomly extracted from all the participants of the Main Study. The participants are recruited from all the Regional Centres. Sub-Cohort Study employs face-to-face assessment of neuropsychiatric development, body measurement, paediatrician's examination, blood/urine collection for clinical test and chemical analysis, and home visit (ambient air measurement and dust collection).

2. Objectives

Sub-Cohort Study is designed to collect extended health outcome information and exposure data that are not measured in the Main Study. It employs not only a cross-sectional approach, but also a longitudinal scheme, utilizing unique characteristics of a birth-cohort study. Sub-Cohort Study follows its participants, selected from the Main Study, until the end of the study. This is to examine the onset of diseases as well as change in severity of their symptoms, scores of developmental test and biomarkers, as results of exposure to environmental factors. Additionally, by comparing the results of the Sub-Cohort Study and those of the Main Study in which most of the data are collected through questionnaire, the validity of questionnaires is evaluated.

The outcome variables assessed specifically in the Sub-Cohort Study include following: developmental stage, allergic sensitization, thyroid function, and physical growth. The variables (e.g., Vitamin D) that potentially mediate the association between the environment/diet and child development are also measured in the Sub-Cohort Study.

3. Participants

3.1. Number of participants

The number of participants of the Sub-Cohort Study is planned to be 5,000. This number is determined to enable us to test hypotheses regarding to diseases with a high prevalence (e.g., obesity, allergic disease), with sufficient statistical power.

3.2. Prospective participants

Prospective participants are the participants (children) of the Main Study who were born after 1 April 2013 and were permitted to continuously participate in the Sub-Cohort Study through written consent obtained from their mother or legal guardian. To be a participant of the Sub-Cohort Study, all the following data should be available.

- All the questionnaire data and medical record collected from first trimester to age 6 months
- Biospecimens (except for umbilical cord blood) collected at first trimester, second and third trimester and delivery

3.3. Sampling and recruitment of participants

From the participants of the Main Study meeting the criteria for being a participant of the Sub-Cohort Study, the Programme Office randomly extracts children and creates recruiting lists for each Regional Centre at a regular interval using the data management system and distributes the list to each Regional Centre. The total number of the children on the recruiting list is determined taking account of the proportion of the prospective participants who would agree for participation at each Regional Centre, which is estimated based on the results of recruitment of participants for the Main Study. The number of the children extracted for the list who reside in the study area of each Regional Centre is proportional to the number of the participants of the Main Study in the area. If a Regional Centre has several study areas geographically separated with each other, recruiting lists are created separately for each of the study areas.

Each Regional Centre sends a document including information about the Sub-Cohort Study to the parents of the children on the lists. Then, the Regional Centre calls to the participants' mothers to confirm that they plan to continue to live in the study area until their children reach age 4. Only when the child and his/her parent (or a legal guardian) are considered to be unlikely to move out of the study area, the parent is provided detailed information and asked to her child's participation in the Sub-Cohort Study. For each child on the recruiting list, the Regional Centre follows this procedure in order, until the number of the children reaches predetermined number. If the number of the children with participation agreement is not sufficient even after all the children on the recruiting

list are contacted, the Programme Office repeats the sampling procedure to create an additional recruiting list.

At the initial home visit for environmental measurements (i.e., on the first day of data collection for the Sub-Cohort Study), before the data collection begins, the participant' mother (or legal guardian) is asked to review the information of the Sub-Cohort Study and sign the consent form.

4. Methods

4.1. Overall data-collection schedule

Outcome variables are measured through a developmental test and a medical examination when the participants become age 2 and 4, while environmental measurements is conducted when they become approximately age 1.5 and 3. Environmental measurements at age 1.5 and 3 are completed before measurement of outcome variables at age 2 and 4, respectively. Participants' urine samples are collected at the timing when outcome variables are measured at age 4.

It is planned to collect health outcome information through neuropsychiatric developmental test and medical examination at age 6, 8, 10 and 12. During this period (between age 6-12), environmental measurements are also planned to be conducted once or twice.

4.2. Measurement of outcome variables

4.2.1. Developmental test

To assess outcome variables in the neuropsychiatric development domain, developmental/cognitive tests are administered individually to the participants. For the developmental test used at age 2 and 4, Kyoto Scale of Psychological Development is used, as it has been most widely used in clinical settings in Japan. The personnel who carry out this test is trained and certificated by the Programme Office. The testing batteries administered at age 6 or later have not yet been determined, but will be discussed and determined at the Steering Committee.

4.2.2. Medical examination

Medical examinations are conducted by paediatricians at age 2 and 4 years. The medical examinations consist of measurement of height, weight, pulse, respiratory rate, blood pressure and body temperature; visual examination of head and neck, chest, abdomen, back, external genitals and skin (using UK working party criteria); neuromotor developmental test and phlebotomy. It is planned to conduct nitric oxide measurement in children's breath and spirometry after 6 years of age.

For the phlebotomy, a specific procedure is adopted in order to minimise children's and their parents/guardians' physical pain and mental distress. Local anaesthetic is used if parents/guardians permit it. Parents/guardians are asked to stay in the room throughout the medical examination when they agree. In addition to the paediatrician, co-medical support members stay in the room during the phlebotomy to distract the children to reduce their fear and anxiety. Paediatricians and co-medical staff receive a specific training to provide care for the children before/during/after the blood drawing. The total amount of blood collected from 2 and 4 years old is 4 ml.

The variables specifically measured through medical are described in the following sections.

1) Immune system disorders/allergy

   Nonspecific IgE, specific IgE, IgG, IgA of inhalant and food antigens

2) Metabolism/endocrine system

   Thyroid stimulating hormone (TSH), free thyroxine (fT4), 25(OH) Vitamin D and body measurement

4.3. Evaluation of environmental exposure

Children's environmental exposures are measured the ambient environmental measurements and biomonitoring. Environmental measurements include indoor and outdoor volatile organic compounds (VOCs) by passive diffusion samplers, particulate matter by gravimetric determination and house dust collection. Dwelling observation is also conducted to observe possible environmental hazards inside and around the children's home. Those are all performed by trained and certificated field staff of each Regional Centre. Biospecimens collected during the medical examination are tested for clinical biochemistry and aliquoted into cryovials for later chemical analysis.

4.3.1. Mite allergen/Endotoxin

Mites and endotoxin are measured in dust collected from the mattress/futon that children regularly use for sleep. The field staff vacuum a specific area (50 cm x 1 m) of the children's mattress/futon using a specified handy-cleaner with a designated filter for 2 minutes.

4.3.2. Heavy metals/non-volatile organic compounds

Heavy metals (e.g., lead, cadmium) and organic compounds (e.g., PCBs, PBDEs, agricultural chemicals, phthalates) are measured in house dust in vacuum cleaner bags. Participants are asked to install the specified vacuum cleaner bag and collect dust in their usual manners for a month. Those who use vacuum cleaners without bags (e.g., cyclonic vacuum cleaners) are asked to collect dust for

one month and transfer the dust into plastic bags provided by the field staff. Priority chemicals are measured in biospecimens.

4.3.3. Volatile organic compounds

Volatile organic compounds (VOCs) are sampled by passive diffusion samplers and analysed by gas chromatography or liquid chromatography mass spectrometer. VOCs measured include formaldehyde, acetaldehyde, toluene, ethylbenzene, xylene, styrene, and *p*-dichlorobenzene. Nitrogen oxides (NOx), and sulphur oxides (SOx) are also measured. Samplers are installed in the room in which children spends the most of the time. The samplers are also placed outside the house. VOCs are determined as average concentrations of seven days.

4.3.4. Particulate matters

Particulate matters (PMs) are collected by an active pump operated intermittently for seven days (5 min pumping and 30 min resting). PMs are collected in the same places where volatile organic compounds are collected (both indoor and outdoor). $PM_{2.5}$ and PM_{10} are measured separately by a gravimetric method.

4.3.5. Noise

A geographic noise model is constructed to estimate environmental noise that each participant is exposed to. In order to assess average sound insulation of participants' houses, noise levels are measured inside and outside of the selected participants' houses which are likely to be exposed to traffic, railroad and/or aircraft noises. The houses and timing of measurements are determined by the noise model.

4.3.6. Dwelling observation

Room temperature and humidity are recorded during the VOC sampling. The field staff conduct observations using the dwelling observation sheet. They also fill the sheet collecting the information about commodity (e.g., insecticide and air fresheners) and the type and amount of chemical substances of daily use.

5. Reporting results to the participants

All the collected individual data including results of developmental tests, medical examinations, biomonitoring and environmental measurements are reported to the corresponded participant. The results are not reported to individuals who refuse to receive the data. Special teams are organised within the Programme Office, Medical Support Centre and each Regional Centre to answer

questions from the participants who have received the report.