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Biological Monitoring of Insecticide Exposure during Toddler Years as a Critical Period for Brain Development

Michihiro KAMIJIMA

Nagoya City University Graduate School of Medical Sciences

1 Kawasumi, Mizuho-cho, Mizuho-ku,

Nagoya 467-8601, JAPAN

Tel: +81-52-853-8171 / Fax: +81-52-859-1228

E-mail: kamijima@med.nagoya-cu.ac.jp

In cooperation with: Nagoya University Graduate School of Medicine

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Insecticides are environmental chemicals widely used in agricultural and public health settings and in individual households. In 2010, it was reported that children (aged 8−15 years) with higher concentrations of urinary isecticide metabolites were more likely to be diagnosed with attention deficit/hyperactivity disorder (Bouchard *et al.*, 2010). Thereafter, epidemiological studies focusing on possible links between insecticide exposure and neurodevelopment in children have attracted more global attention. After birth, the brain develops rapidly during the infant and toddler periods and the developing brain is more susceptible to neurotoxicants. In addition, insecticide exposure doses per body weight are likely higher in children. Therefore, exposure measurements during the infant and toddler periods are indispensable to epidemiological studies investigating the relationship between exposure to insecticides and pediatric neurodevelopment.

Biological monitoring (biomonitoring) is an effective approach to precisely quantifying individual exposure levels. However, such exposure during the critical window of brain development has rarely been addressed in epidemiological studies since urine collection, necessary for exposure assessment of chemicals with short biological half-lives, is not feasible outside clinical settings for children too young to control urination. Urine collection bags devised primarily for clinical purposes enable researchers to collect intact urine from non-toilet-trained children; however, they are not ideal research tools due to inconveniences such as possible urine leakage and skin irritation caused by the adhesive used to attach the collection bag to the child.

One potential approach to overcoming these drawbacks is to extract urine from diapers for measuring urinary insecticide markers. This requires minimal parental effort for urine collection compared with the use of urine collection bags. Therefore, the present study has mainly aimed to establish methods of measuring exposure to insecticides acting on the nervous system, using urine extracted from disposable diapers after use, collected from more than 1,000 participants of the Japan Environment and Children's Study (JECS), and to clarify the urinary concentration ranges of chemicals derived from three insecticide lines, i.e., organophosphates, pyrethroids and neonicotinoids.

Figure 1 illustrates the concept of exposure assessment using urine taken from diapered children. The urine is extracted with acetone from absorbents incorporated in the diapers. To assess the level of absorption of the insecticide metabolites into the absorbent of the diapers, cross-validation tests were performed using concentrations measured from urine samples spiked with calibration standards and those from the same urine samples which were poured into and subsequently extracted from the diaper absorbent. The metabolites' absorption into the diapers was quantified to calculate the urinary metabolite concentrations.

Methods of collecting urine from diapers and measuring organophosphate and pyrethroid metabolites and neonicotinoid compounds with an ultra-high sensitive chromatograph and mass spectrometry were successfully developed, making it possible to measure the chemicals in the urine of diapered children. A method developed for organophosphate insecticide metabolites has already been reported (Oya *et al.*, 2017). That method was then applied to the urine samples collected in the present study. Diapers after use or first morning voids were collected from JECS participants in the Aichi regional subcohort when they reached 1.5 (1,077 children) and three years of age (1,671 children) from June 2015 through May 2018, and the concentration ranges of their urinary markers were clarified. At least one dialkylphosphate (DAP, a common urinary metabolite of organophosphate insecticides) was detected from all the study participants in both the 1.5- and three-year-old children groups. The detected ranges of total urinary DAP concentrations in these age groups were 4.7–8033 nmol/L and 4.6–8228 nmol/L, respectively. To our knowledge, this is the first study to examine insecticide exposure levels in Japanese children using urine extracted from used disposable diapers. At present reference values for insecticide-derived urinary markers have yet to be set in Japan. The results could be used to determine such values in the Japanese toddler population. In addition, the relationship between the marker concentrations and health and development outcomes can be analyzed in future studies as adjunct studies of JECS when the outcome information becomes available. In the present study, some associations between the urinary marker levels and possible exposure routes were also suggested. It was also clearly shown that urinary marker concentrations fluctuated throughout urine collection seasons, and that use of indoor pesticides and consciousness of dietary habits were possibly associated with exposure levels. Furthermore, the urine samples collected in this study can be used in the future as well to investigate the possible effects of chemicals other than these insecticides on children's health and development after establishing methods to measure the biomarkers of interest. The present study could thus contribute to the improvement of JECS and better environmental policy.

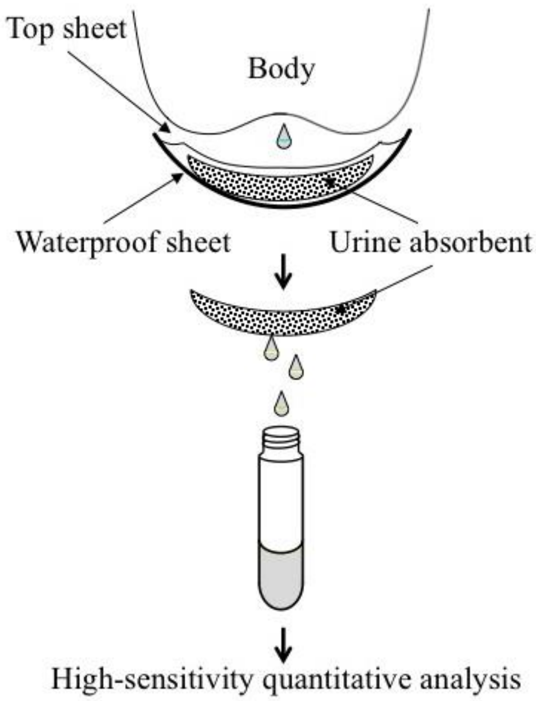

Fig. 1 Analysis of insecticide metabolites in urine extracted from disposable diapers.

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