Title: Risk assessment for Thai residents exposed to microplastics

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Purposes: To understand the human health effects of airborne MPs and to assess the associated risks.

Specific Objectives

1.To characterized airborne microplastics in ambient air

2.To assess individual exposure to airborne MPs by determining MPs concentration in individual exposure and in urine.

3.To analyzed and interpreted transcriptomic and metabolomic data in blood and urine samples

4.To identify toxic, predicted effects and evaluate their risk assessments

Methods:

Study locations: The study area for this project consists of high-traffic site (Outdoor), medium-traffic site (Outdoor) and low traffic site (Indoor) in Thailand. Bangkok was selected as the target area for this study because it has been dealing with traffic problems for long decades.

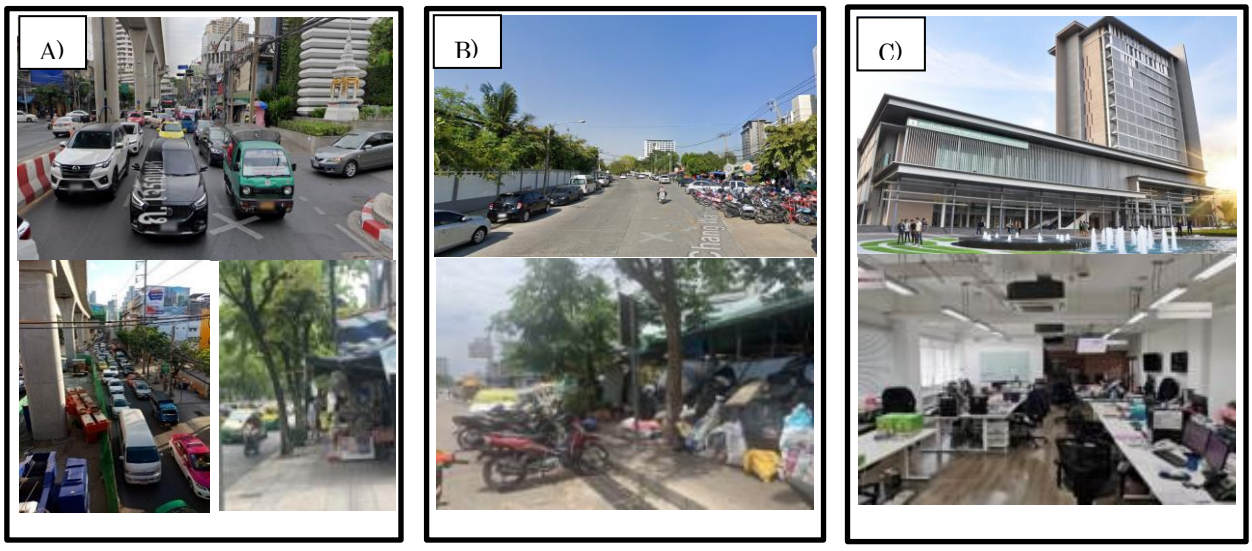

Figure 1 Location of the study area in Bangkok, Thailand

A) High traffic congested area (Outdoor) Taksin, Bangkok, Thailand

B) Medium traffic congested area (Outdoor) Laksi, Bangkok, Thailand

C) Control site (Indoor) Laksi, Bangkok, Thailand

Subject recruitment

A total 120 subjects (healthy and non-smoker) were recruited from 40 and 41 subjects residing nearby roadside at Taksin and Laksi area, respectively as well as 39 subjects from reference site. Subjects recruited from roadside areas were represented as a high and medium traffic-exposed group, while subjects at reference site were a low traffic-exposed group. All of subjects were requested to provide information in the questionnaire and signed consent form. The approval of the research was accepted by Thai ethics committee.

Sample collection

1. Air sample collection

Air samples were collected using air sample pump connected with cascade impactor. CF-300 Aluminium foil filters (25 mm diameter) were placed in each stage (Stage A >2.5 μm, stage B 2.5-1 μm, stage C 1-0.5 μm, and stage D 0.5-0.25 μm) of cascade air sampler. Ambient air (n=27) and personal air (n=120) sampling were collected at the flow rate approximately 9.00 m^3/min for 6-8 hours. After collection, all filters were kept in desiccator for one day to remove field humidity. Each filter was weighed to determine particle mass (METTLER TOLEDO microbalances). Then, all filters were individually taken in Analyslide™ Petri Dishes and kept in a desiccator until analysis. The mass was calculated to particles concentration in ambient and personal air samples as follow:

$$\text{Particle concentration } (\frac{\mu g}{m^3}) = \frac{\text{Particles mass } (\mu g)}{\text{Time (min)x Air flow rate}(\frac{m^3}{min})}$$

2. Biological sample collection

2.1 Urine

Urine samples were collected, in the morning prior to start working and afternoon approximately 8 hours after finish working. Urine samples were collected in 50 mL in a sterile polypropylene tube with screw cap, and keep in 4°C of ice box during transport form field to laboratory and stored at -80 °C until analysis.

2.2 Whole blood and plasma

Whole blood and plasma were obtained by sterilized venipuncture. Each

sample was pipetted into clean vials and transported to the lab on dry ice and stored at -80°C until analysis.

2.2.1 RNA from whole blood

Samples of 3 ml of whole blood were transferred to Tempus™ Blood RNA Tube for transcriptomic analysis. RNA extraction was performed by using RNeasy Mini Kit (Qiagen, Germany). RNA level was quantified by ND-1000 Nanodrop (Thermo Fisher Scientific, Waltham, MA, USA).

2.2.2 Plasma

Samples of 3 mL of whole blood were transferred to separated polypropylene tubes and added 5% Ethylene Diamine Tetra Acetic acid (EDTA) as anticoagulant agent. Next, all samples were centrifuged at 2,000 g at 20 °C for 10 min, and the supernatant (Plasma) was reserved for metabolomic analysis, plastic additives and other chemical compounds.

Result

This study consisted of three study locations including high-traffic exposure area (Taksin, Bangkok, Thailand), medium-traffic exposure area (Laksi, Bangkok, Thailand) and low traffic-exposure area (Laksi, Bangkok, Thailand). The collected area was conducted in indoor area in the same location at Laksi. Ambient air samples were collected in high-, medium- and low-traffic locations. Personal air sample and urine sample were collected from high traffic-exposed (n=40), medium traffic-exposed (n=41) and low traffic-exposed subjects (n=39).

Demographic characteristic

A total of 120 healthy volunteers were enrolled in this study. The low traffic-exposed group is the subject working in indoor area, which consists of 19 (49%) males and 20 (51%) females with a median age of 43 years (ranging from 26 to 60 years). While the medium and high traffic-exposed group are the subjects working at the roadside area where are located in traffic-congested areas. The medium and high traffic-exposure group consists of 19 males (46%), 19 males (47.5%) and 22 females (54%), 21 females (52.5%), with a median age of 51 years (ranging from 21 to 59 years), 47.5 years (ranging from 18 to 60 years), respectively, which gender and age were not significant difference among the study groups. There was a significant difference in education level, occupation and working period in three groups, with the majority of subjects in medium-and high-traffic areas being street vendors (36.5%, 55%), and security guard (41.5%, 12.5%), and the majority of education levels being elementary school (27%, 25%), and high school (41%, 45%), respectively. The majority of subjects in low-

traffic areas was officers (58.5%), with bachelor's degrees or higher being the most common educational level.

Characterization of particulate matter (PM) in ambient and personal air samples

1. **Determination of concentration of particulate matter (PM) in ambient air samples**

This study conducted to determine PM concentration in ambient air samples in various sites among high-, medium-and low-traffic exposure. The mean concentration of ambient PM collected from high traffic- areas was significantly higher ($p<0.01$) than those of medium-, low-traffic areas by approximately 1.3-fold and 2.5-fold (8.64 vs 15.97 $\mu g/m^3$ and 8.64 vs 21.17 $\mu g/m^3$) respectively

2. **Determination of concentration of particulate matter (PM) in personal air samples**

The increased of PM in ambient air at high-traffic congested area, humans can directly and continuously inhale these PM into the body. Personal air were used to determine the individual exposure to PM in ambient air. The study was conducted in various sites in high-, medium- and low-traffic exposure. The concentration of personal PM exposure from high- and medium-traffic exposed subjects (29.15 $\mu g/m^3$ and 17.85 $\mu g/m^3$, respectively) were significantly higher than those of low-exposed subjects (6.49 $\mu g/m^3$) by approximately 3.68-fold and 2.67-fold, respectively.

Future challenges

This year, my study will be focused on transcriptomic and metabolomic analysis. The RNA-Seq data will be analyzed using the CLC Genomic Workbench program. I plan to use prominent databases such as KEGG, GO, and DiseaseComps, in addition to bioinformatics tools like ClusterProfiler, Cytoscape, and IPA, for transcription factor, pathway, and disease enrichment analyses.

The metabolome, including urinary metabolites, will be analyzed with liquid chromatography-quadrupole time-of-flight mass spectrometry (LC/Q-TOF-MS). In addition, plasma will also be used for plastic additives analysis. Differences in concentrations of toxic chemicals among the study sites will be statistically examined. Thus, this analysis will help predict phenotypic effects caused by disruption of biological information networks.

Furthermore, DIABLO (Data Integration Analysis for Biomarker

discovery using a Latent component method for Omics studies) will be performed using a dataset of all quantitative values with pollutant concentrations. These analyses will enable to identify toxic chemicals that account for predicted effects and to assess their risk. Finally, my ultimate goal is to prepare our research for publication, with the intention of submitting it to international journals with high impact factors.