Title: Risk assessment for Thai residents exposed to microplastics

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

Specific Objectives

1.To characterize microplastics in ambient air from Bangkok

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

3.To analyze and interpret transcriptomic and metabolomic data in blood and urine samples

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

Methods:

Study locations and subject recruitment:

This study was conducted in Bangkok, Thailand, with 120 healthy, non-smoking participants selected from three areas with different levels of traffic exposure: high-traffic and medium-traffic roadside areas (Taksin and Laksi), and a low-traffic site (Indoor). Participants completed questionnaires and provided their consent, and the study received approval from the Thai ethics committee.

Sample collection (Air, Urine and Blood)

Ambient and personal air sampling was conducted from March to May 2023 using a cascade impactor with CF-300 aluminum foil filters (25 mm) at 4 stages ranging from >2.5 to 0.25 µm. Air samples (ambient: n=27, personal: n=120) were collected at 9.00 m^3/min for 6-8 hours. Blood and urine samples were collected at the end of the air-sampling period and stored at −80 °C until analysis.

Analysis of MPs using micro–Fourier Transform Infrared Spectroscopy (µFTIR)

MPs were identified using µFT-IR spectroscopy with a spotlight 200i microscope (PerkinElmer, Japan), scanning within 4000–600 cm at 4–8 scans. Around 50-60% of the filter was analyzed, recording MPs' size, shape, color, and

transparency. Particles were photographed before spectral analysis in ATR mode, with some spectra smoothed and adjusted for clarity.

Analysis of MPs using Py-GC-MS/MS

The filters were placed in sample cups (Frontier Laboratories' Eco-cup LF) and directly injected into the Py-GC-MS/MS. MPs were measured using a Frontier Lab Pyrolyzer™ (EGA/PY-3030D) with an Auto-Shot Sampler™ (AS-1020E) connected to a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph with an Ultra ALLOY capillary column (30 m x 0.25 mm I.D x 0.5 µm film). The system was paired with a Thermo Scientific™ Orbitrap™ Exploris GC mass spectrometer. Pyrolyzer temperature programs were optimized per the supplier's guidelines. Detected polymers included Polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Acrylonitrile Butadiene Styrene (ABS), Styrene-Butadiene (SBR), Polymethyl Methacrylate (PMMA), Polycarbonate (PC), Polyvinyl Chloride (PVC), Polyurethane (PU), Polyethylene Terephthalate (PET), Nylon-6 (N-6), and Nylon-6,6 (N-66).

RNA isolation, next-generation sequencing, and data analysis

Total RNA was extracted from whole blood using the RNeasy Mini Kit (Qiagen Germany) and quantified with a Nanodrop ND-1000 (Thermo Fisher Scientific, USA). Purity (A260/A280: 2.20–1.80, A260/230: >1.0) and RNA integrity (RIN >7.0) were confirmed using an Agilent 2100 Bioanalyzer (Agilent, USA). RNA sequencing was performed by Rhelixa Inc. (Tokyo, Japan) on the Illumina NovaSeq 6000 platform, generating 150 bp paired-end reads (average 26.7 million reads per sample). Data were trimmed and mapped to the human reference genome using CLC Genomics Workbench® 21.

Results:

Exposure to ambient MPs in traffic-congested areas was assessed by the direct measurement of MPs concentration in ambient air. Personal exposure to MPs can be assessed by measuring individual air exposure to MPs and the concentration of MPs in urine. The potential health risk from MPs exposure was assessed by using integration of transcriptomic data and metabolomic data.

Demographic characteristic

This study involved 120 healthy volunteers divided into three groups based on traffic exposure: low, medium, and high. The low traffic-exposure group, consisting mainly of indoor workers (officers), had a median age of 43 years and

higher education levels, with fewer passive smokers but a higher rate of respiratory allergies. The medium and high traffic-exposure groups, composed mainly of street vendors and security guards, had a median age of 51 and 47.5 years, respectively, with more passive smokers but fewer respiratory allergies. Significant differences in education, occupation, and working hours were found between the groups, although environmental issues in their residences, grilled food consumption, and plastic bottle use were not significantly different.

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

The mean concentration of ambient PM collected from high traffic-area was significantly higher ($p<0.01$) than those of medium- and low-traffic areas by approximately 1.3-fold and 2.5-fold (21.74 vs 18.41 μg/m^3 and 21.74 vs 8.64 μg/m^3) respectively. Personal air samples were used to determine the individual exposure to PM through ambient air. The concentration of personal PM exposure from high- and medium-traffic exposure subjects (29.15 μg/m^3 and 17.85 μg/m^3, respectively) were significantly higher than those of low-traffic exposure subjects (6.49 μg/m^3) by approximately 3.68-fold and 2.67-fold, respectively.

Characterization of atmospheric MPs in ambient and personal air samples using micro-FTIR

A total of MPs particles in the study areas were detected, with sizes ranging from 50 to 350 μm. The MPs were characterized in the forms of fibers, fragments, and films, displaying thread-like shapes, irregular shapes, and transparent plastic sheets, respectively. Ambient and personal air samples detected various MP polymers, including Nylon, PMMA, polyester, LDPE, PE, PET, PS, PVC, and PP. Among the shapes detected in the air samples, fibers and fragments were the predominant forms.

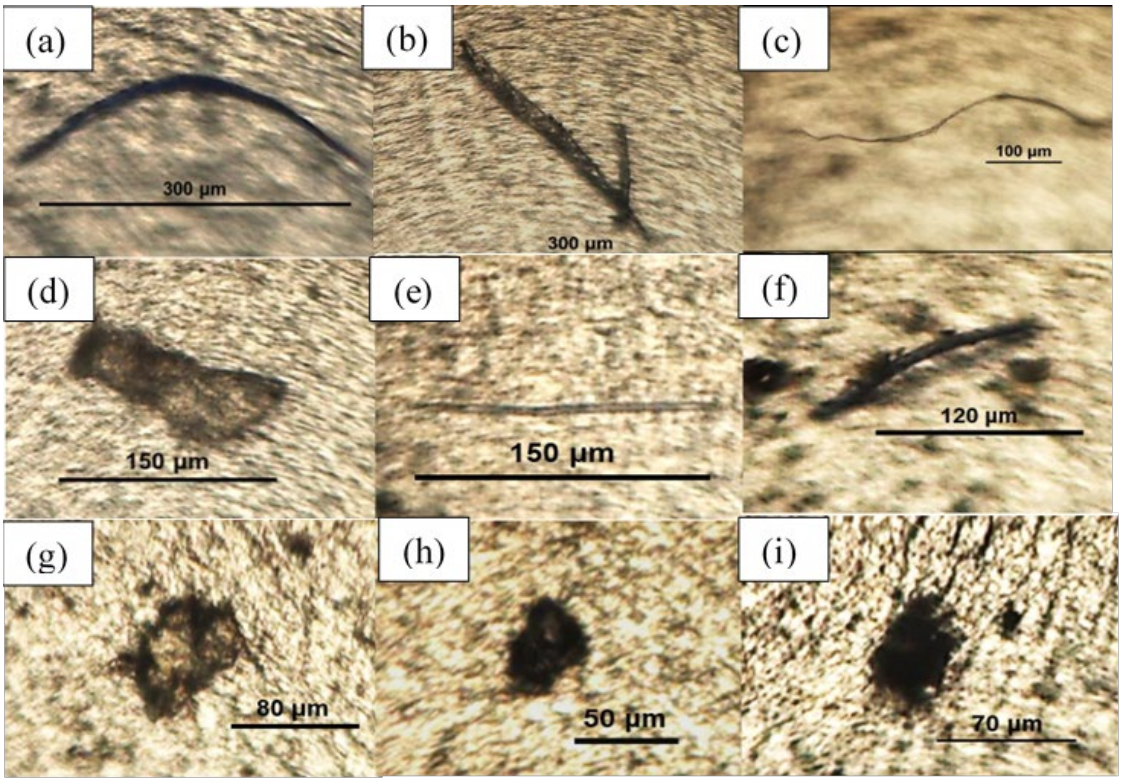

Figure 1 MP polymers image by micro-FT-IR. Nylon (a), PMMA (b), Polyester (c), LDPE (d), PE (e), PET (f), PS (g), PVC (h), and PP(i)

Assessment of MPs in the air samples from individual residents

The ambient concentration of total MPs in high- and medium-traffic areas was higher than that in low-traffic areas (1.59, 1.32, and 0.89 µg/m3, respectively). Human exposure to MPs was assessed as determining the concentration of MPs in individual exposure. In agreement with ambient MPs, high- and medium-exposed subjects had a significantly increased exposure to MPs, compared to the low-exposed subjects (2.53, 1.33, and 0.86 µg/m3, $p<0.001$, respectively).

Assessment of MPs in the urine samples from individual residents

The urine sample was collected to determine the biomarker of MPs exposure. Total MPs in urine samples were higher in high- and medium-traffic exposed groups than those in low traffic exposed group. In the morning urine samples, the mean concentration of MPs in the urine of the high- and medium-exposed subjects was higher than that of the low-exposed subjects by approximately 1.8-fold ($p <0.01$) and 1.15-fold ($p <0.05$), respectively. The mean concentration of MPs in the afternoon urine of the high- and medium-exposed subjects was significantly higher than that of the low-exposed subjects by approximately 2.6-fold ($p<0.01$) and 1.8-fold respectively. With creatinine-

adjusted concentration, there was no significant difference in the concentration of MPs detected in morning urine among the groups, a significant difference was observed in afternoon urine among the groups.

Analysis of the blood plasma transcriptome from individual residents

The RNA-Seq data from female blood plasma samples was analyzed using the CLC Genomic Workbench program. A total of 191 (73 up- and 118 down-regulated), 133 (23 up- and 110 down-regulated), and 27 (13 up- and 14 down-regulated) genes were detected when comparing high- to low-traffic exposure groups, medium- to low-traffic exposure groups, and high- to medium-traffic exposure groups, respectively. Pathway analysis of these DEGs, using KEGG and GO databases, revealed significant enrichment in the pathways of erythrocyte differentiation, gas transport, and blood microparticles. The association between contaminants and transcriptome using class coinertia analysis (CIA) in R (ade4 package) showed relatively low separation among sample groups, possibly due to the small sample size, consisting solely of females and healthy individuals.

Future Challenges:

I will continue my work by focusing on analyzing transcriptomic data from both male and female samples. Increasing the number of samples for RNA analysis will help generate more reliable data and improve confidence in the results. I plan to use databases such as KEGG, GO, and DiseaseComps, along with bioinformatics tools like ClusterProfiler, Cytoscape, and IPA (Ingenuity Pathway Analysis) for transcription factor, pathway, and disease enrichment analyses. Not only have I analyzed MPs in urine, but I also plan to analyze phenolic compounds in urine using Liquid Chromatography with tandem mass spectrometry (LC-MS/MS). Furthermore, I will investigate the metabolome, including urinary metabolites, using liquid chromatography-quadrupole time-of-flight mass spectrometry (LC/Q-TOF-MS).

I will conduct DIABLO (Data Integration Analysis for Biomarker Discovery using a Latent Component Method for Omics Studies) on a dataset that includes all quantitative values along with pollutant concentrations. These analyses will help identify toxic chemicals responsible for the predicted effects and evaluate their associated risks.

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