Targeted Metabolomics Induced by Microplastics

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Contributor: Kuan-Lu Wu , Wan-Rou Liao , Wei-Chen Lin , Sung-Fang Chen

It has been reported that microplastics (MPs) are present in several organs in different species, including the liver, intestines, kidney, and other organs, where they have the potential to cause detrimental effects such as disrupting endocrine regulation, neurotoxicity, and reproductive toxicity. Therefore, metabolomics studies have attracted considerable attention from researchers who are eager to reveal the mechanism behind the biomarkers that have been discovered by serum chemistry tests, histological tests, or polymerase chain reaction (PCR) analyses. Metabolomics studies can be achieved in two ways, namely targeted and untargeted. Targeted metabolomics detects defined groups of metabolites, providing quantitative results with a high degree of sensitivity and data quality. The advantage of targeted metabolomics is that a complicated data process is not required, but that relatively limited information can be obtained in a single analysis.

microplastics LC-MS metabolomics toxicity

1. Data Acquisition Methods for Targeted Metabolomics

Targeted metabolomics concerned with the measurement of specific metabolites within a given metabolome. Single reaction monitoring (SRM) and multiple reaction monitoring (MRM) by liquid chromatography-tandem triple quadrupole mass spectrometry (LC-QqQ-MS) are two of the most commonly used data acquisition modes in targeted metabolomics. In SRM, the first quadrupole (Q1) selects and transmits a precursor ion into the second quadrupole (q2), also known as the collision chamber, where it undergoes further fragmentation. The predefined product ions are transmitted to the third quadrupole (Q3) and reach the detector for *m/z* analysis [1]. While SRM is a more selective method as it only monitors one fixed transition, MRM over multiple mass windows rapidly and records the intensities of those product ions simultaneously, enabling the acquisition of a more comprehensive information (**Figure 1a**). Because of the two selection steps, the specificity of SRM/MRM is ensured, making this approach an ideal method for quantitative analysis. Moreover, the quantitation analysis can be achieved by the peak area of the extracted ion chromatogram directly. Therefore, the data processing method of targeted metabolomics is much easier than that of untargeted metabolomics.

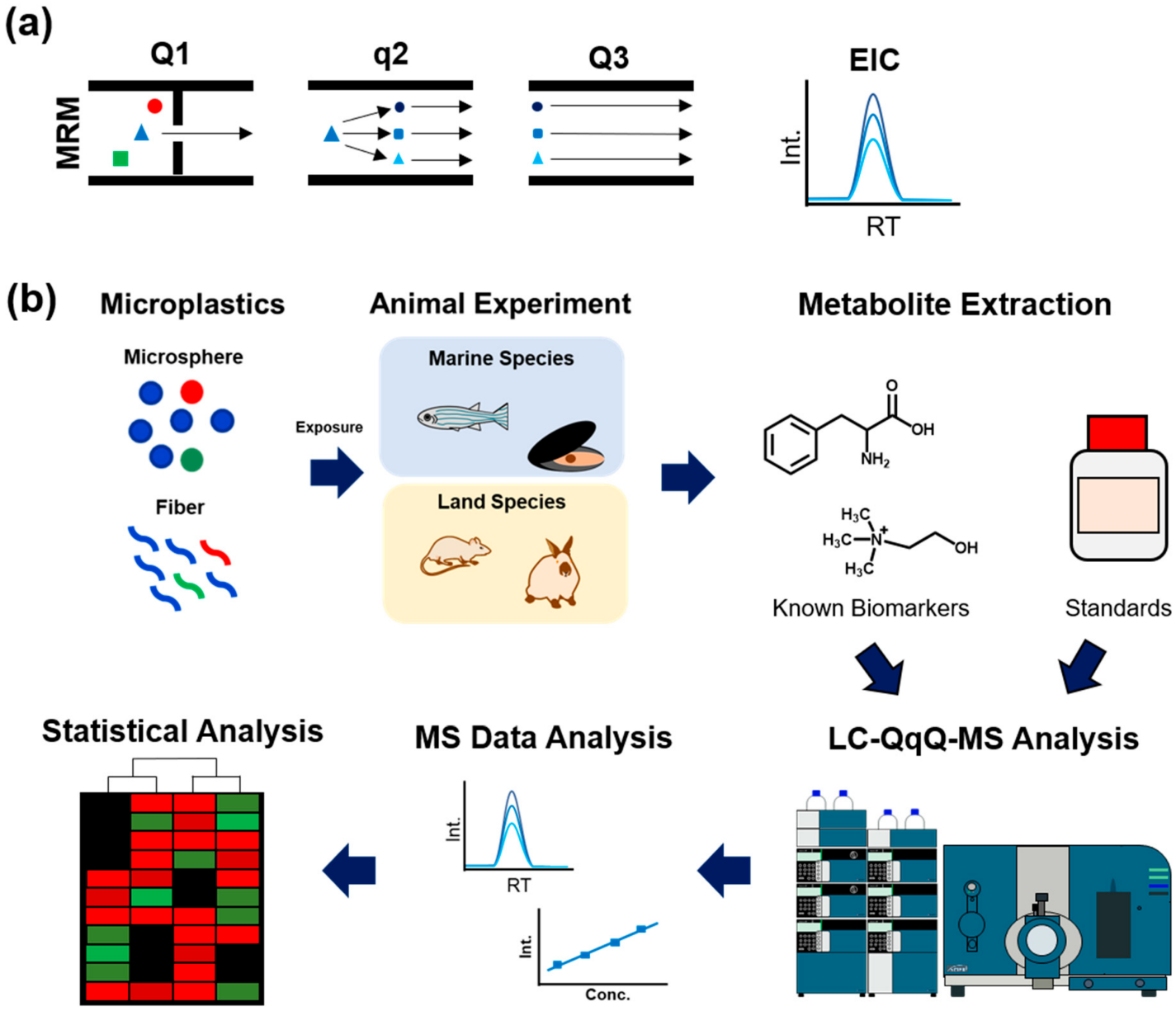

Figure 1. (**a**) Introduction to MRM, the dominant data acquisition mode in targeted metabolomics. (**b**) The general workflow of the MPs-induced targeted metabolomics.

In targeted metabolomics strategies, SRM or MRM often targets the specific signals of the product ions from the predefined metabolites. The peak area of these signals is used to accurately and precisely determine the concentration and relative abundancies of the selected small numbers of endogenous metabolites that are pre-known and expected [2]. One of the most challenging parts of targeted metabolomics is to select the target metabolite that could be disrupt by the MPs. For the reason outlined above, researchers select the metabolite group of interest in advance by pre-known knowledge, for example, serum chemistry tests [3] and an untargeted metabolomics pre-analysis [4]. **Figure 1b** demonstrates the general workflow for targeted metabolomics.

2. Targeted Metabolomics Studies of Aquatic Species

MPs are clearly shaping and affecting aquatic ecosystems and research on the effects of aquatic pollutants aquatic systems is now a priority. The zebrafish (*Danio rerio*) and the perch (*Perca fluviatilis*), two fresh water fish species, were exposed to two different sizes of polyethylene (PE) over a period of 21 days by dispersing the PE-MPs into the fish tank [5]. The PE-MPs in the tissue were characterized by Fourier transform infrared spectroscopy, finding that PE-MPs with 10–45 µm diameters accumulated in the liver tissue and PE-MPs with 106–125 µm accumulated in the gills of both species. The metabolomics analysis was achieved by selecting 32 metabolites, including amino acids, carbohydrates, and those involved in nucleic acid metabolism. These metabolites were detected by amide hydrophilic interaction chromatography (HILIC) coupled with QqQ-MS. The result shows that the levels of metabolites related to nucleic acid metabolism were increased in the perch gills, whereas the metabolites the aromatic and amino acids decreased significantly. The level of choline was the only one that increased in both exposures in the perch liver.

Another study proposed that the exposure to 10 µm polystyrene (PS)-MPs dominantly affects neurotoxicity leading to the dysregulation of the cholinergic agents, dopaminergic, and GABAergic neurotransmission systems in developing zebrafish embryos. This could potentially cause seizurogenic effects [6]. PS-MPs were also found to have a certain degree of effect on the zebrafish heart, causing a significant decrease in both heart function and swimming competence. Moreover, indices of enhanced levels of oxidative stress and metabolic adjustments were observed in the hearts of zebrafish embryos [7]. It was observed that the Kreb's cycles related metabolites, including pyruvic acid and acetyl-carnitine, were increased, while the levels of carnitine, succinic acid, α-ketoglutaric, and amino acids were reduced.

3. Targeted Metabolomics Studies of Terrestrial Species

MPs in soil pose environmental risks, potentially adversely impacting terrestrial animals. By analyzing the metabolites present in soil organisms, the approach provides direct and accurate reflects of their physiological responses to soils that are contaminated with PE. Among 485 metabolites identified by untargeted metabolomics strategies in earthworm (*Lumbricus terrestris*) intestines, two differential metabolites were quantified via LC-QqQ-MS. L-phenylalanine and succinic acid levels are decreased, which indicates a reduced energy supply and production and are used as potential biomarkers for evaluating toxic effects [4]. PS-MPs, also one of the primary environmental MPs pollutants, have been found to cause adverse impact on plant growth [8]. The results showed that PS-MPs were absorbed by, and accumulated in, barley plants, leading to the limitation of rootlet development. The concentrations of these two important plant hormones, indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA), were significantly decreased in leaves of this species. A similar trend was found for the roots of 3-indolepropionic acid (IPA) and IBA. To investigate the effects of PS-MPs on animals, male mice were exposed to 5 µm of pristine and fluorescent polystyrene for a period of six weeks [9]. This research demonstrated that PS-MPs were present in the guts of the mice and that these particles could disrupt the gut barrier functions and reduce intestinal mucus secretion. In addition, the presence of succinyl acetone, 11 amino acids, and 25 carnitine derivatives in the serum was detected, indicating that MPs caused metabolic disorders in amino acid metabolism and bile acid metabolism. In another study, male mice were administered an oral gavage of 5 µm PS-MPs for a

period of 33 days. The level of aspartate aminotransferase (AST) in serum was increased, and 7-ketolithocholic acid (7-ketoLCA) and taurocholic acid (TCA) in fecal samples were decreased [3]. Overall, these studies suggest that PS-MPs could post a threat to both plants and animals by disturbing their metabolic functions. **Table 1** lists the experimental conditions for this targeted metabolomics research.

Table 1. The MPs, model, dose, and selected biomarkers of the studies cited above for MPs-induced targeted metabolomics.

MPs	Model; Tissue; Sample Numbers	Exposure Dose	Selected Biomarkers	Ref
PS (<5 μm)	Mice (*Mus musculus*); serum and fecal; *n* = 10	0.1 mg/day	Aspartate aminotransferase (AST) 7-Ketolithocholic acid (7-ketoLCA) Taurocholic acid (TCA)	[3]
PS (<0.5 mm)	Barley (*Hordeum vulgare*); leaves and roots; *n* = 3	2 mg/L	Indole-3-acetic acid (IAA) Indole-3-butyric acid (IBA) 3-Indolepropionic acid (IPA)	[4]
PE (10–45 and 106–125 μm)	Perch (*Perca fluviatilis*) and zebrafish (*Danio rerio*); gills and liver; *n* = 10	10 mg/g	Aromatic Amino acids Choline	[5]
PS (<0.5 mm)	ICR mice (*Mus musculus*); gut; *n* = 8	100 μg/L 1000 μg/L	Succinyl acetone, 11 Amino acids 25 Carnitines	[6]
PS (10 μm)	Zebrafish (*Danio rerio*); embryos; *n* = 30	0 particles/mL 500 particles/mL 5000 particles/mL 50,000 particles/mL	Choline Betaine Dopamine 3-Methoxytyramine γ-Aminobutyric acid	[7]
PS (3–12 μm)	Zebrafish (*Danio rerio*); heart; *n* = 7	In vivo: 10 mg/g Ex vivo: 26 and 260 mg/L	Pyruvic acid Acetylcarnitine Carnitine Succinic acid α-Ketoglutaric Amino acids	[8]
PE (< 5 mm)	Earthworm (*Amynthas corticis*); intestines; *n* = 10	Earth sample	L-phenylalanine Succinic acid	[9]

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