separations

MDPI

Review

A Review of Liquid Chromatography-Mass Spectrometry Strategies for the Analyses of Metabolomics Induced by Microplastics

Kuan-Lu Wu, Wan-Rou Liao, Wei-Chen Lin and Sung-Fang Chen *

Department of Chemistry, National Taiwan Normal University, Taipei 11677, Taiwan
* Correspondence: sfchen@ntnu.edu.tw; Tel.: +886-2-77496180

Abstract: Microplastics (MPs) (microscopic plastic particles) are defined as plastic fragments in the range of 1 to 5 mm. They are invisible and usually too small to be seen by humans but pollution by MPs has become an issue due to the rising use of plastic products. Pollution of microscopic plastics has gained international attention in recent years and has become an important issue in the field of environmental protection, food safety, and human health. Numerous studies have reported that MPs have the potential for causing detrimental effects in various species. The focus of this mini review was on LC-MS-based metabolomics research into this issue by targeted and untargeted approaches. We also summarized biomarkers for assessing toxicity in land and aquatic species that are induced by MPs with different sizes and shapes, type of monomer, and the dose. Based on previous research results, MPs have the potential for affecting energy metabolism and the immune system, chronic inflammation, and neurotransmitter disorders in a wide variety of species. These biomarkers discovered by metabolomics are consistent with other methods, showing the reliability of LC-MS-based metabolomics. Further research is highly anticipated to explore other toxicity effects that are induced by MPs.

Keywords: microplastics; LC-MS; metabolomics; toxicity

Citation: Wu, K.-L.; Liao, W.-R.; Lin, W.-C.; Chen, S.-F. A Review of Liquid Chromatography-Mass Spectrometry Strategies for the Analyses of Metabolomics Induced by Microplastics. *Separations* **2023**, *10*, 257. https://doi.org/10.3390/separations10040257

Academic Editors: Maria Nieddu and Elena Baralla

Received: 9 March 2023
Revised: 3 April 2023
Accepted: 13 April 2023
Published: 16 April 2023

1. Introduction

Microplastics (MPs) are plastic fragments with diameters or lengths of less than 5 mm. They are mostly released from larger suspended plastic products via photo- and thermal degradation [1]. Due to the rapid increase in the use of plastic products (approximately 380 million tons of plastics are produced each year) [2], MPs can now be classified as emerging ubiquitous pollutants, and have gained international attention in the recent year [3]. Moreover, MPs are reported to be widely spread over numerous ecosystems, including air [4], soil [5], and the oceans [6]. Because of their small size, they are easily ingested by living creatures, which leads to bioaccumulation [7]. This results in MPs from contaminated seafood, salt, and packing materials being part of our food [8].

It has been reported that MPs are present in several organs in different species, including the liver [9], intestines [10], kidney [11], and other organs, where they have the potential to cause detrimental effects such as disrupting endocrine regulation [12], neurotoxicity [13], and reproductive toxicity [14]. 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 [15], histological tests [16], or polymerase chain reaction (PCR) analyses [17]. Metabolomics can reflect a phenotype in much more direct ways compared with transcriptomics and proteomics [18]. It is used to systematically analyze small molecule metabolites and to interpret the effects of the disturbance by the level (or ratio) of metabolomics after a pathway analysis using bioinformatic tools (ex: KEGG) [19]. Since metabolomics has been a powerful tool for elucidating the

toxicity of environmental pollutants [20], many reviews have mentioned that metabolomics has advanced the knowledge of MPs-related toxicology [21].

A variety of analytical techniques, including nuclear magnetic resonance (NMR) [22], spectroscopy, and liquid chromatography (LC) [23] or gas chromatography (GC) [24] coupled with mass spectrometry (MS), have been used to evaluate MPs-induced metabolomics. Although NMR spectroscopy is often used in metabolomics studies, the extensive sample preparation process makes it a less convenient method [25]. Mass spectrometry is a popular choice for metabolomics research due to its high selectivity and superior sensitivity. MS also provides several different acquisition modes to meet the requirements for an analysis. A comprehensive metabolomics profile can be readily obtained via chromatography coupled with MS. Gas chromatography-tandem mass spectrometry (GC-MS), another approach to such studies, can be used to detect volatile metabolites, which narrows the coverage of global profiling. Liquid chromatography-tandem mass spectrometry (LC-MS) is also suitable for identifying relatively polar compounds and is now being used in this field of research.

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 [26]. 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. Untargeted metabolomics, on the other hand, provides global screening results from a more comprehensive perspective [27]. However, compound identification and data treatment make it much more difficult in comparison with targeted metabolomics. Herein, this review summarized both targeted and untargeted metabolomics research for different species/models that are exposed to different MPs with various doses, sizes, and shapes. Disorder metabolic biomarkers and pathways were also summarized. This information could provide aid to future research directed at evaluating the detrimental effects on humans caused by MPs in food products.

2. Targeted Metabolomics

2.1. 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 [28]. 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.

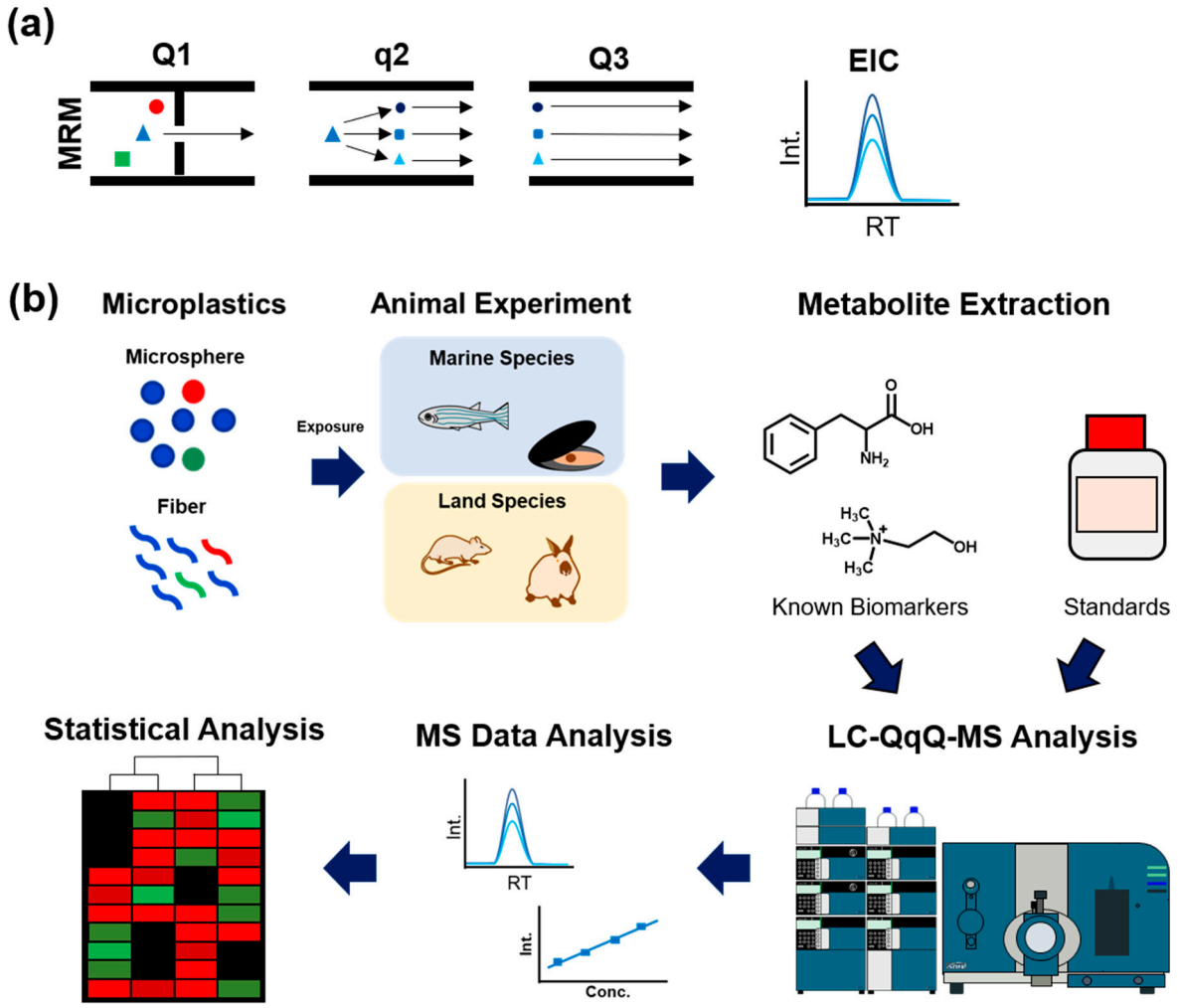

Figure 1. (**a**) Introduction to MRM, the dominant data acquisition mode in targeted metabolomics. (**b**) The general workflow of the MPs-induced targeted metabolomics.

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 [29]. 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 [30] and an untargeted metabolomics pre-analysis [31]. Figure 1b demonstrates the general workflow for targeted metabolomics.

2.2. 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 [32]. 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 [33]. 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 [34]. 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.

2.3. 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 [31]. PS-MPs, also one of the primary environmental MPs pollutants, have been found to cause adverse impact on plant growth [35]. 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 [36]. 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 [30]. 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)	[30]
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)	[31]
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	[32]
PS (<0.5 mm)	ICR mice (*Mus musculus*); gut; *n* = 8	100 μg/L 1000 μg/L	Succinyl acetone, 11 Amino acids 25 Carnitines	[33]
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	[34]
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	[35]
PE (< 5 mm)	Earthworm (*Amynthas corticis*); intestines; *n* = 10	Earth sample	L-phenylalanine Succinic acid	[36]

3. Untargeted Metabolomics

3.1. Data Acquisition Methods of Untargeted Metabolomics

Untargeted metabolomics is the profiling of large numbers of endogenous small molecules metabolites [37]. Figure 2b demonstrates the general workflow that is used in untargeted metabolomics. It is a powerful tool for discovering differential levels of metabolites from multiple pathways in one analysis. Liquid chromatography tandem high-resolution mass spectrometry (LC-HRMS), including quadrupole time-of-flight (Q/TOF) and quadrupole orbitrap (Q/Orbitrap), are the most commonly applied methods for untargeted metabolomics studies [38].

Among recent studies on metabolomics that are disturbed by MP, two types of data acquisition modes were used, namely the full scan and data dependent acquisition (DDA) modes. Figure 2a demonstrates the principle for the two data acquisition modes. A full scan provides accurate mass and retention times with high feature coverage [39]. The main drawback to this method is that an MS2 spectrum cannot be obtained in a single analysis. DDA can compensate for this drawback by acquiring the MS2 spectrum of the co-eluted product ions with a high intensity. As a consequence, the DDA mode has the capability for more accurate compound identification by sacrificing the feature coverage. The DDA mode has been the most frequently-applied scan mode in untargeted metabolomics studies. Unlike SRM/MRM in targeted metabolomics, little information can be obtained from a total ion chromatogram (TIC) in a full scan and DDA (for example, Figure 3). The most challenging aspect for untargeted metabolomics is data interpretation.

3.2. Untargeted Metabolomics Studies of Aquatic Species

Microplastics are widely distributed in various environments and their presence is already ubiquitous in the ocean system [40] where they pose a threat to aquatic creatures. Untargeted metabolomics research has been conducted on a wide variety of aquatic species. Among them, the zebrafish is one of the most commonly-used species [10]. In recent research, the metabolomics change in the larval of zebrafish that had been exposed to PE-MPs was analyzed by LC-Q/Orbitrap-MS using DDA [23]. The results showed that the levels of 59 phospholipids (glycerol phospholipids, lysophosphatidylinositol, lysophosphatidylserine, etc.) related substances were significantly altered. This indicates that cell membrane formation, cell proliferation, and cell differentiation were affected. Furthermore, exposure to PE-MPs could lead to chronic inflammation. Similar results for phospholipid dysregulating in zebrafish were also reported in another article [41]. In addition, the researchers compared the damage caused by PE-MPs and polyethersulfone (PES) MPs, and the findings indicated that PES-MPs could have a detrimental effect on the production of steroid hormones and norepinephrine, a neurotransmitter of the phenolic compounds produced in the synthesis of PES.

The exposure of PE-MPs has also been examined in farmed tilapia (*Oreochromis niloticus*) [42]. The metabolites were profiled by LC-Q/Orbitrap-MS. In total, 327 and 153 metabolites were identified in the positive and negative modes in electrospray ionization by matching the MS/MS to the database. The metabolites that were altered by dietary exposure to PE-MPs were also identified. According to the following KEGG pathway analysis of the differential metabolites acquired from statistical analysis, alanine, aspartate and glutamate metabolism, purine/pyrimidine metabolism, glycerophospholipid metabolism, and aminoacyl-tRNA biosynthesis were significantly enriched.

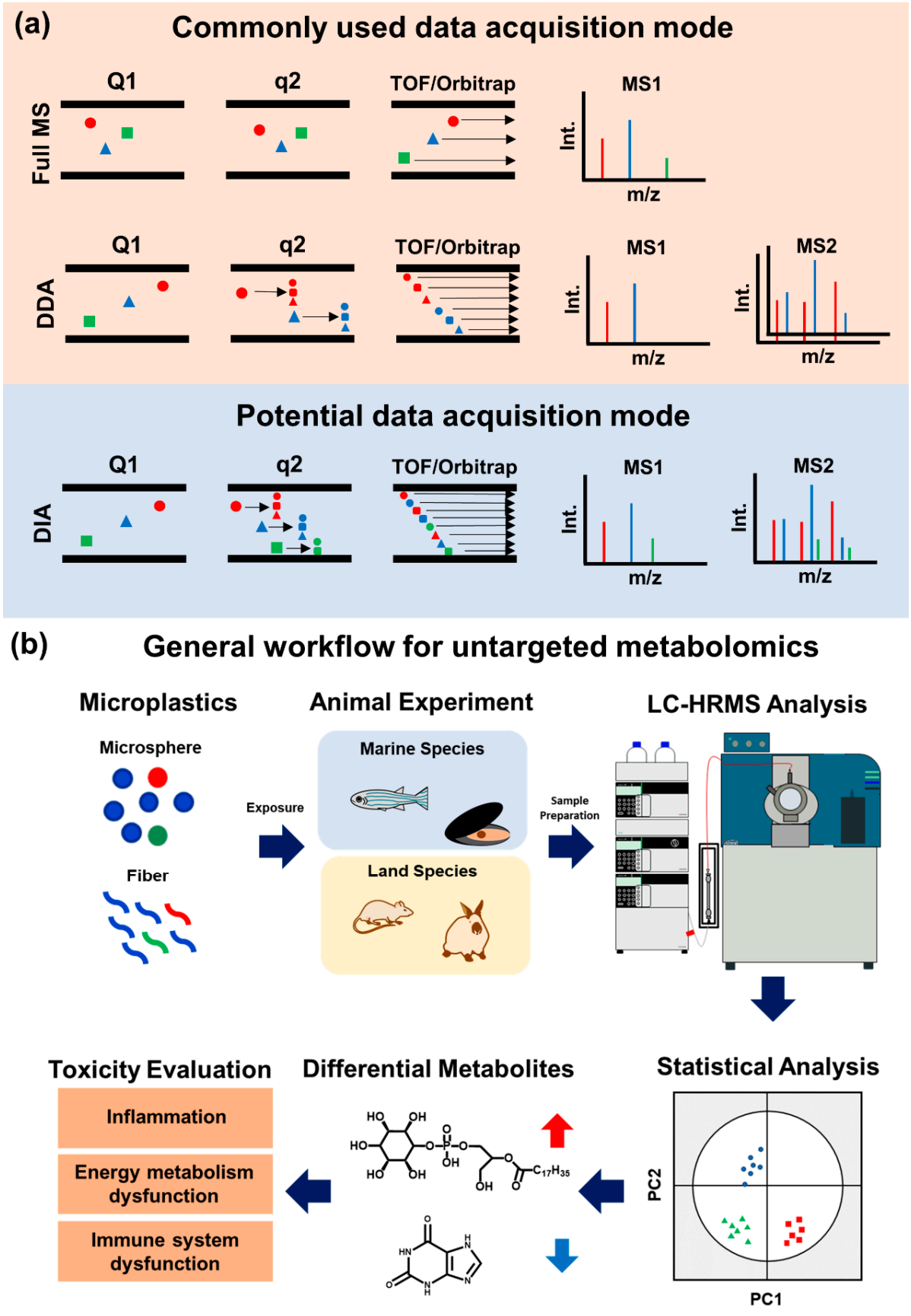

Figure 2. (**a**) Commonly used data acquisition modes (full scan and DDA) and the potential data acquisition mode. (**b**) The general workflow of the MPs-induced untargeted metabolomics.

PS is another common MP that has a detrimental effect on aquatic species [43]. The metabolomics disturbed by PS-MPs in goldfish (*Carassius auratus*) were profiled by LC-MS [44]. After a pathway analysis, the results indicated that the levels of amino acid metabolism, pyrimidine metabolism, neurotransmitters, and ATP-binding cassette (ABC) transporters were altered. Similar results were reported for the swordtail fish (*Xiphophorus helleri*) [45]. The research profiled the metabolites in the liver tissue of swordtail fish using LC-Q/TOF-MS with DDA. Amino acid metabolism and ABC transporters were disturbed by the PS-MPs. Immune-related fatty acids (linoleic acid and arachidonate) were also affected.

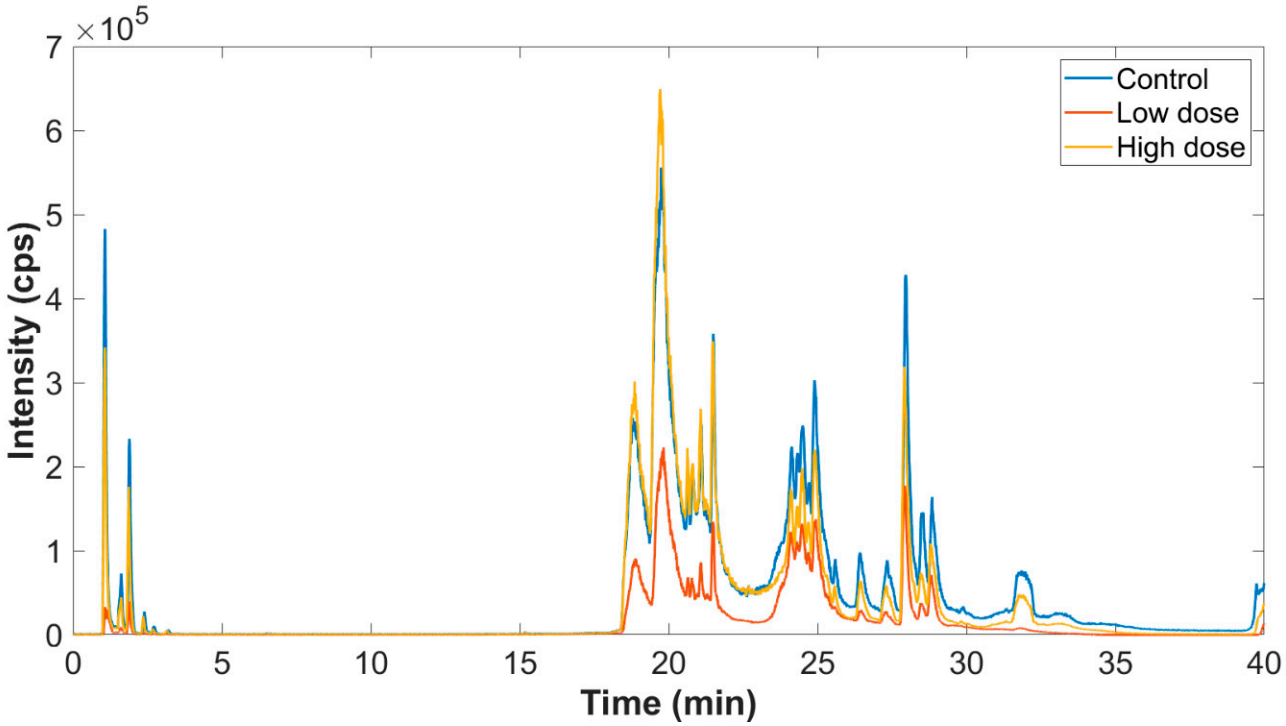

Figure 3. A total ion chromatogram (TIC) of metabolites extracted from the mouse liver tissue exposed to PS-MPs.

Aquatic species such as shellfish are also commonly used as models for exposure to MPs [46]. Amine/phenol metabolites in marine mussels (Mytilus coruscus) disturbed by PS-MPs were profiled using dansyl-chloride chemical isotope labeling methods using LC-Q/TOF-MS under the full scan mode [47]. The chemical isotope labeling method not only allows the comparison of different groups achieved in a single injection but also enhances the retention ability of polar metabolites in reversed-phase (RP) liquid chromatography. In this case, five identified metabolites followed the same pattern as the concentrations of the MPs were increased. The increase in the levels of imidazoleacetic acids, 3,4-dihydroxyphenylpropanoic acid, 3-(2,3-dihydroxyphenyl) propanoic acid, and 1,3-diaminopropane could indicate disordered amino acid metabolism (histidine, tyrosine, phenylalanine, and alanine metabolism). Increased levels of imidazoleacetic acid and 4-pyridoxic acids are also an indicator of oxidative stress. This article also showed that after seven days (without exposure to PS-MPs exposure), these metabolites generally returned to the level of the control group. In another study, oysters (*Crassostrea gigas*) were exposed to irregular MPs derived from two polymer types—PE and PET (polyethylene terephthalate). Similar to the aforementioned research on fish species, PE and PET exposure could lead to changes in energy metabolism and inflammation responses [48].

Aquatic arthropods are also vulnerable to exposure to MPs. The shrimp *Litopenaeus vannamei*, a global distributed specie, was also used as the model and the toxicology effect of five types of MPs (PE, PS, PVC (Polyvinylchloride), PTFE (Polytetrafluoroethylene), and PP (Polypropylene)) was examined [49]. These MPs caused variations in hemolymph metabolites, especially in amino acids and alpha-linoleic acid metabolism, and 28 metabolites were altered in the MPs-treated group. The variation of these metabolites in the shrimp's hemolymph could indicate that detrimental effects, such as tumorigenesis, oxidative stress, and immune homeostasis, were triggered by the exposure to the MPs mentioned above. Another study reported on the effects of MPs on the metabolic processes in *Daphnia magna* but the corresponding molecular toxicity mechanisms remain unclear [50]. In the 20 μm and 30 μm PE-MPs exposure group, the relative quantity of 88 and 91 metabolites were changed, respectively. Phosphatidylcholine was downregulated and phosphatidylethanolamine, mainly phospholipids, was upregulated in the 20 μm PE-MPs group. Regarding the 30 μm PE-MPs group, the downregulation of amino acid metabolites, including L-glutamine, L-glutamate and malic acid, shows that larger particle sizes can exert a greater interference on energy metabolism.

3.3. Untargeted Metabolomics Studies of Terrestrial Species

Pollution by MPs has also been recognized as an environmental threat and a health hazard to humans [51]. Mice are commonly used as the model for evaluating the toxicity of MPs accumulation in terrestrial mammals and human species [9]. In previous research, ICR mice (*Mus musculus*) were exposed to PS-MPs with diameters lower than 1 μm [52]. To mimic the uptake dose for humans (assuming that an adult drinks one teabag daily), the concentration of exposure to MPs was set at 1.43 × 108 items/kg body weight/day. The metabolites in livers were screened via the DDA mode using an LC-Q/orbitrap-MS to reveal the extent of disturbance on gut-liver axis. The findings reported in this study showed that the crosstalk between the gut and liver ultimately led to insulin resistance and even diabetes. Another study reported on the research profiles of metabolites in chicken liver [53]. In that study, glutamate and glutamine synthesis were found to be upregulated, and the formation of abnormal metabolites was induced by PS-MPs that affect the brain through the liver–brain axis. Table 2 lists the experimental conditions and the results of the untargeted metabolomics research.

3.4. Novel Data Acquisition of Untargeted Metabolomics

Aside from the full scan and DDA modes, data-independent acquisition [3], including all-ion fragmentation (AIF), MS^E, and sequential window acquisition of all theoretical fragment-ion spectra (SWATH), etc., has been used in LC-MS-based metabolomics research [54]. As with the DDA mode, DIA can simultaneously acquire MS1 and MS2 data. Moreover, its ability to obtain MS2 data for all precursor ions is quite high. This capability allows DIA to provide unbiased MS2 data for those ions with low intensity and quantitative data with a better linear range [55]. Although DIA has not been applied to assess metabolomics that are disrupted by exposure to MPs, it is a promising untargeted metabolomics strategy for revealing the mechanism responsible for the toxicity of MPs.

Table 2. The MPs, model, method, dose, and results for the studies cited for the MPs-induced untargeted metabolomics.

MPs	Modell; Tissue; Sample Numbers	Analysis Method (LC-MS)	Exposure Dose	Results	Ref
PE (1–4 μm)	Zebrafish (*Danio rerio*); Larval; *n* = 5	LC-Q/Orbitrap-MS	0 μg/L 10 μg/L 100 μg/L 1000 μg/L	(1) ESI [8]: 72 metabolites were significantly changed, 20 increased; 52 decreased. (2) ESI (+): 96 metabolites were significantly changed, 40 increased; 56 decreased. (3) 59 phospholipids total difference: LPA, LPC, LPE, LPG, LPI, LPS, PC, PE, PS; 57 decreased, 2 increased (LPI 20:5 and PC 18:3e/3:0).	[23]
PE and PES (100 μm)	Zebrafish (*Danio rerio*); whole fish; *n* = 9	LC-Q/TOF-MS	0 mg/L 1 mg/L PE 1 mg/L PES	(1) PE-exposure: lipid metabolism, fatty acid metabolism, vitamin metabolism, TCA cycle, and amino acid metabolism are perturbated. (2) PES-exposure: lipid metabolism, fatty acid metabolism, vitamin metabolism, TCA cycle, and amino acid metabolism are adversely affected. (3) Vitamin B1 metabolism and porphyrin metabolism were identified in both PE and PES-treated groups.	[41]
PE (50 and 125 μm)	Nile tilapia (*Oreochromis niloticus*); liver; *n* = 9	LC-Q/Orbitrap-MS	1–3 weeks: 5% body weight 4–9 weeks: 3% body weight	(1) 327 positive and 153 negative metabolites were identified (2) Ingesting smaller MPs mainly affects multiple classes of biomolecules such as amino acid, purine, pyrimidine, glycerophospholipid, and aminoacyl-tRNA. (3) Ingesting larger MPs mainly affects amino acid and fatty acid metabolism.	[42]

Table 2. *Cont.*

MPs	Modell; Tissue; Sample Numbers	Analysis Method (LC-MS)	Exposure Dose	Results	Ref
PS (500 nm and 30 μm)	Goldfish (*Carassius auratus*); brain; $n = 5$	-	0 mg/L 500 nm PS: 0.26 mg/L 0.69 mg/L 30 μm PS: 0.26 mg/L 0.69 mg/L	(1) 230 and 265 significantly different metabolites were detected in Nano_high and Micro_high groups. (2) 27 and 30 KEGG pathways were significantly altered in Nano_high and Micro_high groups. (3) Metabolites detected in the MP exposure groups were closely related to metabolism and signal transduction, including "Metabolic pathways" (ko01100), "cAMP signaling pathway" (ko04024), and "Taste transduction" (ko04742).	[44]
PS (1 μm)	Swordtail fish (*Xiphophorus helleri*); liver; $n = 6$	LC-Q/TOF-MS	0 particles/L 10^6 particles/L 10^7 particles/L	(1) In group 10^6 particles, 5780 metabolites up-regulated, 6621 metabolites down-regulated, and acquired 37 significantly differential metabolites. (2) In group 10^7 particles, 6262 metabolites up-regulated, 6139 metabolites down-regulated, and acquired 103 significantly differential metabolites.	[45]
PS (2 μm)	Marine mussels (*Mytilus coruscus*); hemolymph; $n = 6$	LC-Q/TOF-MS(CIL methods)	0 particles/L 10 particles/L 10^4 particles/L 10^6 particles/L	(1) 163 metabolites positively identified and 318 putatively identified with high-confidence. (2) Micro-PS caused significant changes in the metabolism of tyrosine, phenylalanine, histidine, beta-alanine, and vitamin B6 metabolism in the hemolymph of mussels, and also affected the antioxidant and immune parameters.	[47]

Table 2. *Cont.*

MPs	Modell; Tissue; Sample Numbers	Analysis Method (LC-MS)	Exposure Dose	Results	Ref
PE (10.7–93.9 μm) PET (9.0–56.0 μm)	Oysters (*Crassostrea gigas*); digestive gland; *n* = 8	LC-Q/Orbitrap-MS	0 μg/L 10 μg/L PE 1000 μg/L PE 10 μg/L PET 1000 μg/L PET	(1) The toxic order of the four treatment groups was PEL < PETL < PEH < PETH, indicating that PET is more toxic to oysters with prolonged exposure of a given concentration. (2) Among these differential metabolites, 57 were obtained in all four treatments, 4 differential metabolites were shared between PEL and PEH groups and 26 differential metabolites were shared between PETL and PETH groups.	[48]
PE (6–18 μm) PTFE (1–8 μm) PP (1.77–18 μm) PS (100–200 μm) PVC (1–13 μm)	White shrimp (*Litopenaeus vannamei*); hemolymph; *n* = 6	LC-Q/Orbitrap-MS	1.0 mg/L	(1) Differential metabolites: PVC included 34 upregulated and 90 downregulated, PTFE included 96 upregulated and 54 downregulated, PP included 38 upregulated and 60 downregulated, PS included 50 upregulated and 88 downregulated, PE included 42 upregulated and 83 downregulated. (2) 28 Differential metabolites were shared between the five MP-treated groups.	[49]
PE (20 and 30 μm)	*Daphnia magna*; whole; *n* = 6	LC-Q/Orbitrap-MS	0 mg/L 20 mg/L 60 mg/L	(1) MPs-20 caused significant changes in 12 metabolic pathways, totaling 45 metabolites, whereas MPs-30 caused significant changes in 25 metabolic pathways, totaling 93 metabolites. (2) MPs-20 mainly affected amino acid metabolism, digestive system, cancers: overview, and lipid metabolism pathways. (3) MPs-30 mainly affected amino acid metabolism, digestive system, and cancers: overview pathways.	[50]

Table 2. *Cont.*

MPs	Modell; Tissue; Sample Numbers	Analysis Method (LC-MS)	Exposure Dose	Results	Ref
PS (1 µm)	ICR mice (*Mus musculus*); liver; $n = 8$	LC-Q/Orbitrap-MS	$4.5 \times 10^4 - 4.3 \times 10^6$ particles/day *	(1) 353 metabolites were identified from 5974 metabolite features extracted from the raw data acquired in positive- and negative-ionization modes. (2) I3A, PAGly, betaine, choline, and carnitine metabolites were significantly perturbed, and this perturbation was accompanied by changes in the abundance of associated microbiota. (3) Modulating the gut microbiota may represent a new way for preventing health risks associated with microplastics.	[52]
PS (2 µm)	Chicken (*Gallus gallus domesticus*); liver; $n = 6$	LC-Q/TOF-MS	0 mg/L 1 mg/L 10 mg/L 100 mg/L	(1) Liver metabolism disorders and increased glutamine and glutamate synthesis in chicken. (2) Excessive glutamine and glutamate enter the cerebellar tissue through the broken blood-brain barrier, triggering autophagy-dependent ferroptosis and apoptosis.	[53]

* The dietary human intake for the presence of microplastics in a single teabag.

4. Conclusions

MPs pollution and the resulting toxicity have become a topic of great interest. Microplastics are commonly found in plankton aquatic life and have also been found to persist throughout the food chain. This mini-review summarized metabolomics studies in aquatic and terrestrial species using LC-MS techniques. Among the targeted approaches, MRM in LC-QqQ-MS is the most commonly used, while DDA in conjunction with LC-HRMS is the most frequently applied approach in untargeted metabolomics. Based on these results, microplastics could alter energy metabolism and the immune system and cause chronic inflammation and neurotransmitter disorder in a wide variety of species. Further research is expected to explore other currently-unknown toxicity effects induced by MPs. Regarding techniques, the DIA mode in LC-HRMS can be practically applied to the field. It is a convenient and efficient process and represents an attractive alternative for use in metabolomics research.

Author Contributions: Conceptualization and writing—original draft, K.-L.W. and W.-R.L.Wan-Rou Liao; visualization, K.-L.W. and W.-R.L. Wei-Chen Lin; writing—review and editing, S.-F.C. and W.-R.L.Wan-Rou Liao; review and project administrator, S.-F.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Acknowledgments: This research was supported by grants (111-2113-M-003 -012) from the National Science and Technology Council of Taiwan, Republic of China.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Fotopoulou, K.N.; Karapanagioti, H.K. Degradation of various plastics in the environment. *Hazard. Chem. Assoc. Plast. Mar. Environ.* **2019**, *78*, 71–92.
2. Tsuchimoto, I.; Kajikawa, Y. Recycling of Plastic Waste: A Systematic Review Using Bibliometric Analysis. *Sustainability* **2022**, *14*, 16340.
3. Hale, R.C.; Seeley, M.E.; La Guardia, M.J.; Mai, L.; Zeng, E.Y. A global perspective on microplastics. *J. Geophys. Res. Ocean.* **2020**, *125*, e2018JC014719. [CrossRef]
4. Yao, Y.; Glamoclija, M.; Murphy, A.; Gao, Y. Characterization of microplastics in indoor and ambient air in northern New Jersey. *Environ. Res.* **2022**, *207*, 112142. [PubMed]
5. Tian, L.; Jinjin, C.; Ji, R.; Ma, Y.; Yu, X. Microplastics in agricultural soils: Sources, effects, and their fate. *Curr. Opin. Environ. Sci. Health* **2022**, *25*, 100311.
6. Sun, Q.; Li, J.; Wang, C.; Chen, A.; You, Y.; Yang, S.; Liu, H.; Jiang, G.; Wu, Y.; Li, Y. Research progress on distribution, sources, identification, toxicity, and biodegradation of microplastics in the ocean, freshwater, and soil environment. *Front. Environ. Sci. Eng.* **2022**, *16*, 1.
7. Li, Z.; Chao, M.; He, X.; Lan, X.; Tian, C.; Feng, C.; Shen, Z. Microplastic bioaccumulation in estuary-caught fishery resource. *Environ. Pollut.* **2022**, *306*, 119392. [CrossRef]
8. Kwon, J.-H.; Kim, J.-W.; Pham, T.D.; Tarafdar, A.; Hong, S.; Chun, S.-H.; Lee, S.-H.; Kang, D.-Y.; Kim, J.-Y.; Kim, S.-B. Microplastics in food: A review on analytical methods and challenges. *Int. J. Environ. Res. Public Health* **2020**, *17*, 6710. [CrossRef]
9. Lu, Y.; Zhang, Y.; Deng, Y.; Jiang, W.; Zhao, Y.; Geng, J.; Ding, L.; Ren, H. Uptake and accumulation of polystyrene microplastics in zebrafish (Danio rerio) and toxic effects in liver. *Environ. Sci. Technol.* **2016**, *50*, 4054–4060. [CrossRef]
10. Qiao, R.; Deng, Y.; Zhang, S.; Wolosker, M.B.; Zhu, Q.; Ren, H.; Zhang, Y. Accumulation of different shapes of microplastics initiates intestinal injury and gut microbiota dysbiosis in the gut of zebrafish. *Chemosphere* **2019**, *236*, 124334.
11. Meng, X.; Zhang, J.; Wang, W.; Gonzalez-Gil, G.; Vrouwenvelder, J.S.; Li, Z. Effects of nano-and microplastics on kidney: Physicochemical properties, bioaccumulation, oxidative stress and immunoreaction. *Chemosphere* **2022**, *288*, 132631. [CrossRef] [PubMed]
12. Han, Y.; Shi, W.; Tang, Y.; Zhou, W.; Sun, H.; Zhang, J.; Yan, M.; Hu, L.; Liu, G. Microplastics and bisphenol A hamper gonadal development of whiteleg shrimp (*Litopenaeus vannamei*) by interfering with metabolism and disrupting hormone regulation. *Sci. Total Environ.* **2022**, *810*, 152354.

13. Kim, J.-H.; Yu, Y.-B.; Choi, J.-H. Toxic effects on bioaccumulation, hematological parameters, oxidative stress, immune responses and neurotoxicity in fish exposed to microplastics: A review. *J. Hazard. Mater.* **2021**, *413*, 125423. [PubMed]
14. Liu, Z.; Zhuan, Q.; Zhang, L.; Meng, L.; Fu, X.; Hou, Y. Polystyrene microplastics induced female reproductive toxicity in mice. *J. Hazard. Mater.* **2022**, *424*, 127629. [PubMed]
15. Lee, S.; Kang, K.-K.; Sung, S.-E.; Choi, J.-H.; Sung, M.; Seong, K.-Y.; Lee, S.; Yang, S.Y.; Seo, M.-S.; Kim, K. Toxicity study and quantitative evaluation of polyethylene microplastics in ICR mice. *Polymers* **2022**, *14*, 402. [CrossRef]
16. Deng, Y.; Zhang, Y.; Lemos, B.; Ren, H. Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. *Sci. Rep.* **2017**, *7*, 46687. [PubMed]
17. Shin, H.; Jeong, C.-B. Metabolism deficiency and oxidative stress induced by plastic particles in the rotifer Brachionus plicatilis: Common and distinct phenotypic and transcriptomic responses to nano-and microplastics. *Mar. Pollut. Bull.* **2022**, *182*, 113981. [CrossRef]
18. Feussner, I.; Polle, A. What the transcriptome does not tell—Proteomics and metabolomics are closer to the plants' patho-phenotype. *Curr. Opin. Plant Biol.* **2015**, *26*, 26–31. [CrossRef]
19. Kanehisa, M.; Araki, M.; Goto, S.; Hattori, M.; Hirakawa, M.; Itoh, M.; Katayama, T.; Kawashima, S.; Okuda, S.; Tokimatsu, T. KEGG for linking genomes to life and the environment. *Nucleic Acids Res.* **2007**, *36*, D480–D484. [CrossRef]
20. Liu, L.; Wu, Q.; Miao, X.; Fan, T.; Meng, Z.; Chen, X.; Zhu, W. Study on toxicity effects of environmental pollutants based on metabolomics: A review. *Chemosphere* **2022**, *286*, 131815.
21. Bhagat, J.; Zhang, L.; Nishimura, N.; Shimada, Y. Application of omics approaches for assessing microplastic and nanoplastic toxicity in fish and seafood species. *Trends Anal. Chem.* **2022**, *154*, 116674. [CrossRef]
22. Cappello, T.; De Marco, G.; Conti, G.O.; Giannetto, A.; Ferrante, M.; Mauceri, A.; Maisano, M. Time-dependent metabolic disorders induced by short-term exposure to polystyrene microplastics in the Mediterranean mussel *Mytilus galloprovincialis*. *Ecotoxicol. Environ. Saf.* **2021**, *209*, 111780. [CrossRef] [PubMed]
23. Zhao, Y.; Qin, Z.; Huang, Z.; Bao, Z.; Luo, T.; Jin, Y. Effects of polyethylene microplastics on the microbiome and metabolism in larval zebrafish. *Environ. Pollut.* **2021**, *282*, 117039. [CrossRef]
24. An, Y.; Hong, S.; Kim, Y.; Kim, M.; Choi, B.; Won, E.-J.; Shin, K.-H. Trophic transfer of persistent toxic substances through a coastal food web in Ulsan Bay, South Korea: Application of compound-specific isotope analysis of nitrogen in amino acids. *Environ. Pollut.* **2020**, *266*, 115160. [CrossRef] [PubMed]
25. Fang, Z.-Z.; Gonzalez, F.J. LC–MS-based metabolomics: An update. *Arch. Toxicol.* **2014**, *88*, 1491–1502. [CrossRef]
26. Roberts, L.D.; Souza, A.L.; Gerszten, R.E.; Clish, C.B. Targeted metabolomics. *Curr. Protoc. Mol. Biol.* **2012**, *98*, 30.2.1–30.2.24. [CrossRef]
27. Vinayavekhin, N.; Saghatelian, A. Untargeted metabolomics. *Curr. Protoc. Mol. Biol.* **2010**, *90*, 30.1.1–30.1.24.
28. Kondrat, R.W.; McClusky, G.A.; Cooks, R.G. Multiple reaction monitoring in mass spectrometry/mass spectrometry for direct analysis of complex mixtures. *Anal. Chem.* **1978**, *50*, 2017–2021. [CrossRef]
29. Griffiths, W.J.; Koal, T.; Wang, Y.; Kohl, M.; Enot, D.P.; Deigner, H.P. Targeted metabolomics for biomarker discovery. *Angew. Chem. Int. Ed.* **2010**, *49*, 5426–5445. [CrossRef]
30. Jiang, P.; Yuan, G.-h.; Jiang, B.-r.; Zhang, J.-y.; Wang, Y.-q.; Lv, H.-j.; Zhang, Z.; Wu, J.-l.; Wu, Q.; Li, L. Effects of microplastics (MPs) and tributyltin (TBT) alone and in combination on bile acids and gut microbiota crosstalk in mice. *Ecotoxicol. Environ. Saf.* **2021**, *220*, 112345. [CrossRef]
31. Jiang, X.; Yang, Y.; Wang, Q.; Liu, N.; Li, M. Seasonal variations and feedback from microplastics and cadmium on soil organisms in agricultural fields. *Environ. Int.* **2022**, *161*, 107096. [CrossRef] [PubMed]
32. Bobori, D.C.; Dimitriadi, A.; Feidantsis, K.; Samiotaki, A.; Fafouti, D.; Sampsonidis, I.; Kalogiannis, S.; Kastrinaki, G.; Lambropoulou, D.A.; Kyzas, G.Z. Differentiation in the expression of toxic effects of polyethylene-microplastics on two freshwater fish species: Size matters. *Sci. Total Environ.* **2022**, *830*, 154603. [CrossRef]
33. Jeong, S.; Jang, S.; Kim, S.S.; Bae, M.A.; Shin, J.; Lee, K.-B.; Kim, K.-T. Size-dependent seizurogenic effect of polystyrene microplastics in zebrafish embryos. *J. Hazard. Mater.* **2022**, *439*, 129616. [CrossRef]
34. Dimitriadi, A.; Papaefthimiou, C.; Genizegkini, E.; Sampsonidis, I.; Kalogiannis, S.; Feidantsis, K.; Bobori, D.C.; Kastrinaki, G.; Koumoundouros, G.; Lambropoulou, D.A. Adverse effects polystyrene microplastics exert on zebrafish heart–Molecular to individual level. *J. Hazard. Mater.* **2021**, *416*, 125969. [CrossRef]
35. Li, S.; Wang, T.; Guo, J.; Dong, Y.; Wang, Z.; Gong, L.; Li, X. Polystyrene microplastics disturb the redox homeostasis, carbohydrate metabolism and phytohormone regulatory network in barley. *J. Hazard. Mater.* **2021**, *415*, 125614. [CrossRef] [PubMed]
36. Jin, Y.; Lu, L.; Tu, W.; Luo, T.; Fu, Z. Impacts of polystyrene microplastic on the gut barrier, microbiota and metabolism of mice. *Sci. Total Environ.* **2019**, *649*, 308–317. [CrossRef] [PubMed]
37. Alonso, A.; Marsal, S.; Julià, A. Analytical methods in untargeted metabolomics: State of the art in 2015. *Front. Bioeng. Biotechnol.* **2015**, *3*, 23. [PubMed]
38. Ivanisevic, J.; Want, E.J. From samples to insights into metabolism: Uncovering biologically relevant information in LC-HRMS metabolomics data. *Metabolites* **2019**, *9*, 308. [CrossRef]
39. Guo, J.; Huan, T. Comparison of full-scan, data-dependent, and data-independent acquisition modes in liquid chromatography–mass spectrometry based untargeted metabolomics. *Anal. Chem.* **2020**, *92*, 8072–8080. [CrossRef] [PubMed]

40. Yang, H.; Chen, G.; Wang, J. Microplastics in the marine environment: Sources, fates, impacts and microbial degradation. *Toxics* **2021**, *9*, 41. [CrossRef]
41. Medriano, C.A.; Bae, S. Acute exposure to microplastics induces metabolic disturbances and gut dysbiosis in adult zebrafish (*Danio rerio*). *Ecotoxicol. Environ. Saf.* **2022**, *245*, 114125.
42. Lu, X.; Zhang, J.-X.; Zhang, L.; Wu, D.; Tian, J.; Yu, L.-J.; He, L.; Zhong, S.; Du, H.; Deng, D.-F. Comprehensive understanding the impacts of dietary exposure to polyethylene microplastics on genetically improved farmed tilapia (*Oreochromis niloticus*): Tracking from growth, microbiota, metabolism to gene expressions. *Sci. Total Environ.* **2022**, *841*, 156571. [CrossRef] [PubMed]
43. Zhang, X.; Wen, K.; Ding, D.; Liu, J.; Lei, Z.; Chen, X.; Ye, G.; Zhang, J.; Shen, H.; Yan, C. Size-dependent adverse effects of microplastics on intestinal microbiota and metabolic homeostasis in the marine medaka (*Oryzias melastigma*). *Environ. Int.* **2021**, *151*, 106452. [CrossRef] [PubMed]
44. Shi, W.; Sun, S.; Han, Y.; Tang, Y.; Zhou, W.; Du, X.; Liu, G. Microplastics impair olfactory-mediated behaviors of goldfish Carassius auratus. *J. Hazard. Mater.* **2021**, *409*, 125016. [CrossRef] [PubMed]
45. Zhang, Y.-K.; Yang, B.-K.; Zhang, C.-N.; Xu, S.-X.; Sun, P. Effects of polystyrene microplastics acute exposure in the liver of swordtail fish (*Xiphophorus helleri*) revealed by LC-MS metabolomics. *Sci. Total Environ.* **2022**, *850*, 157772. [CrossRef] [PubMed]
46. Sussarellu, R.; Suquet, M.; Thomas, Y.; Lambert, C.; Fabioux, C.; Pernet, M.E.J.; Le Goïc, N.; Quillien, V.; Mingant, C.; Epelboin, Y.; et al. Oyster reproduction is affected by exposure to polystyrene microplastics. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 2430–2435. [CrossRef]
47. Huang, W.; Wang, X.; Chen, D.; Xu, E.G.; Luo, X.; Zeng, J.; Huan, T.; Li, L.; Wang, Y. Toxicity mechanisms of polystyrene microplastics in marine mussels revealed by high-coverage quantitative metabolomics using chemical isotope labeling liquid chromatography mass spectrometry. *J. Hazard. Mater.* **2021**, *417*, 126003. [CrossRef]
48. Teng, J.; Zhao, J.; Zhu, X.; Shan, E.; Zhang, C.; Zhang, W.; Wang, Q. Toxic effects of exposure to microplastics with environmentally relevant shapes and concentrations: Accumulation, energy metabolism and tissue damage in oyster *Crassostrea gigas*. *Environ. Pollut.* **2021**, *269*, 116169. [CrossRef]
49. Duan, Y.; Xiong, D.; Wang, Y.; Zhang, Z.; Li, H.; Dong, H.; Zhang, J. Toxicological effects of microplastics in Litopenaeus vannamei as indicated by an integrated microbiome, proteomic and metabolomic approach. *Sci. Total Environ.* **2021**, *761*, 143311. [CrossRef]
50. Wang, P.; Li, Q.-Q.; Hui, J.; Xiang, Q.-Q.; Yan, H.; Chen, L.-Q. Metabolomics reveals the mechanism of polyethylene microplastic toxicity to *Daphnia magna*. *Chemosphere* **2022**, *307*, 135887. [CrossRef]
51. Blackburn, K.; Green, D. The potential effects of microplastics on human health: What is known and what is unknown. *Ambio* **2022**, *51*, 518–530. [CrossRef] [PubMed]
52. Shi, C.; Han, X.; Guo, W.; Wu, Q.; Yang, X.; Wang, Y.; Tang, G.; Wang, S.; Wang, Z.; Liu, Y.; et al. Disturbed Gut-Liver axis indicating oral exposure to polystyrene microplastic potentially increases the risk of insulin resistance. *Environ. Int.* **2022**, *164*, 107273. [CrossRef] [PubMed]
53. Yin, K.; Wang, D.; Zhao, H.; Wang, Y.; Zhang, Y.; Liu, Y.; Li, B.; Xing, M. Polystyrene microplastics up-regulates liver glutamine and glutamate synthesis and promotes autophagy-dependent ferroptosis and apoptosis in the cerebellum through the liver-brain axis. *Environ. Pollut.* **2022**, *307*, 119449. [CrossRef] [PubMed]
54. Wang, R.; Yin, Y.; Zhu, Z.-J. Advancing untargeted metabolomics using data-independent acquisition mass spectrometry technology. *Anal. Bioanal. Chem.* **2019**, *411*, 4349–4357. [CrossRef] [PubMed]
55. Zha, H.; Cai, Y.; Yin, Y.; Wang, Z.; Li, K.; Zhu, Z.-J. SWATHtoMRM: Development of High-Coverage Targeted Metabolomics Method Using SWATH Technology for Biomarker Discovery. *Anal. Chem.* **2018**, *90*, 4062–4070. [CrossRef] [PubMed]