

A snapshot of biomarkers of exposure for environmental monitoring

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1. Introduction

Biomarkers discovery is the last step in the metabolomics workflow after an appropriate experimental design, chemical analysis, data processing, and statistical analysis. According to the World Health Organization (WHO, 1993), the term “biomarker” is used in a broad sense to include almost any measurement reflecting an interaction between a biological system and an environmental agent, which may be chemical, physical, or biological. Particularly this chapter is focused on the study of organic compounds as chemical environmental stressors. Although many other definitions can be found in the literature, from a metabolomics point of view and henceforth, biomarker will be considered as any biological molecule found in blood, body fluids, or tissues of an organism which levels are altered due to an exposure (Lam, 2009). Therefore, biomarkers are used to indicate an exposure to a certain contaminant or the effects of this contaminant in an organism.

Metabolomics is a high-throughput semiquantitative approach that allows simultaneous detection of a set of metabolites. The metabolites' coverage depends, among others, on the type of analysis undertaken. Targeted metabolomics analysis covers a relatively small amount of metabolites, usually a list of predefined metabolites chosen with regard to the

biological sample analyzed, while nontargeted analysis aims to detect and identify as many metabolites as possible without a prior selection. As mentioned in Chapter 1, around 1500 metabolites are identified using nontargeted analysis and between 200 and 500 using targeted analysis. These figures clearly illustrated the big amount of data available and the higher potential of nontargeted metabolomics analysis for the discovery of biomarkers and profiling of the metabolome. The characterization of the metabolic profile also depends on the instrument, sample pretreatment, and statistical method carried out. Nontargeted analysis is usually undertaken with both nuclear magnetic resonance and high-resolution mass spectrometry (HRMS) coupled to liquid or gas chromatography. Once the metabolites, which levels significantly change due to an exposure to a chemical, are identified, they may be quantified as well, but this only happens in a small proportion compared to the entire set of metabolites identified. Changes in metabolites levels are linked to disruption of metabolic pathways where they are involved and ultimately to pathologies.

In this chapter, a revision of the biomarkers identified using metabolomics that significantly change due to an exposure to organic contaminants is presented. For this purpose, we have selected fish as model organism and liquid chromatography–mass spectrometry as instrumental technique, considering both metabolomics targeted and nontargeted analysis. Exposure under laboratory conditions and to a single contaminant was also chosen in order to narrow the variability of the data. Taken into account these conditions and the information reported along this book a literature research was done. After gathering all the papers published so far (until 2019), an analysis of the reported biomarkers, the effects shown, and the metabolic pathways disrupted was done in order to find out if there was any common trend. The type of molecules most altered due to exposure to organic contaminants and the metabolic pathways affected were studied, and the possibility of finding a potential “universal biomarker” of environmental quality was explored.

1.1 Metabolic profile of fish exposed to organic contaminants

Table 11.1 presents the information gathered in the literature research. A total of 15 articles have been included. They were published in the last 9 years, which indicates the novelty of metabolomics application in environmental field for biomarkers discovery. Besides, an increasing number of papers have been published since 2016, corresponding to an intensification of metabolomics popularity for analyzing biological samples in environmental monitoring. Different fish species have been used by the authors like *Odontesthes bonariensis* (Carriquiriborde et al., 2012), *Oncorhynchus mykiss* (Roszkowska et al., 2018), *Oryzias melastigma* (Lei et al., 2017), *Pimephales promelas* (Ekman et al., 2015), *Rutilus rutilus* (Flores-Valverde et al., 2010), *Solea senegalensis* (Alvarez-Muñoz

Table 11.1: Biomarkers reported in the literature, effects, and pathways disrupted, based on exposure to single compounds, metabolomics analysis with LC-MS, and fish as a model organism.

Type	Contaminant	Biomarkers	Effect observed	Pathway disrupted	Publication
PhAC	17 α -Ethinylestradiol	Hydroxyprogesterone	↓	Steroid biosynthesis	Flores-valverde et al. (2010)
		Androstenedione	↓	Steroid biosynthesis	
		11-Hydroxyandrostenedione	↓	Steroid biosynthesis	
		11-Ketotestosterone	↓	Steroid biosynthesis	
		Cortisol	↑	Glucocorticoid biosynthesis	
Pesticide	Cypermethrin	Cortisone	↑	Glucocorticoid biosynthesis	Carriquiriborde et al. (2012)
		Taurocholic acid (TCA) (C24)	↑	Bile acid metabolism	
		Taurotrihydroxycoprostanic acid (TTHCA) (C27)	↑	Bile acid metabolism	
		Taurodeoxycholic acid (TDCA) (C26)	↑	Bile acid metabolism	
		Bilirubin (C33)	↑	Bile acid metabolism	
Surfactant	Alcohol-polyethoxylated	Taurocholic acid (TCA) (c24bile acids)	↑	Bile acid metabolism	Álvarez-Muñoz et al. (2014)
		Hydroxytaurocholic acid (C24 acid)	↑	Bile acid metabolism	
		Scymnol sulfate (C27 bile alcohol)	↑	Bile acid metabolism	
		Cyprinolsulfate (C27 bile alcohol)	↑	Bile acid metabolism	
		Cortisol	↓	Glucocorticoid biosynthesis	
		Tetrahydrocortisone	↓	Glucocorticoid biosynthesis	
		Glycerophosphatidylcholine (PC) (16:0/hydroxy 18:1)	↑	Lipid homeostasis	
		LysoPC (14:0)	↓	Lipid homeostasis	
		PC (16:0/16:0)	↓	Lipid homeostasis	
		PC (18:0/18:1)	↓	Lipid homeostasis	
Plasticizer	Bisphenol A	Palmitoyl carnitine	↓	Lipid homeostasis	Ekman et al. (2015)
		Guanine	↑(M)	Purine degradation	
		Xanthine	↑(M)	Purine degradation	
		Hypoxanthine	↑(M)	Purine degradation	
		Guanosine	↑(M)	Purine degradation	
		Inosine	↑(M)	Purine degradation	
		Uridine	↑(M)	Pyrimidine metabolism	
		Methionine	↑(M)	Glutathione metabolism	
		Proline	↑(F)	Arginine and proline metabolism	
		Uracil	↑(M)	Pyrimidine metabolism	
		Carnitine	↑(F)	ABC transporters	
		Glycolic acid	↓(F)		
		Hydroxynonanoic acid	↓(F)		
		O-phosphoethanolamine	↓(F)		

Continued

Table 11.1: Biomarkers reported in the literature, effects, and pathways disrupted, based on exposure to single compounds, metabolomics analysis with LC-MS, and fish as a model organism.—cont'd

Type	Contaminant	Biomarkers	Effect observed	Pathway disrupted	Publication
PAH	Benzo[a]anthracene Benz[a]anthracene-7,12-dione	Hydroxyphenyllactic acid	↑		Elie et al. (2015)
		Proline	↑	Arginine and proline metabolism	
		Threonine	↑	Glutathione metabolism	
		5-Oxoproline	↑	Glutathione metabolism	
		Isoleucine	↑	Protein degradation (starvation)	
		Lysine	↑	Carnitine metabolism	
		Methionine	↑	Glutathione metabolism	
		Serotonin	↑	Tryptophan metabolism	
		Phenylalanine	↑	Phenylalanine biosynthesis	
		Arginine	↑	Arginine metabolism	
		Tyrosine	↑	Phenylalanine, tyrosine biosynthesis	
		Tryptophan	↑	Phenylalanine metabolism	
		Cystathionine	↑	Glutathione metabolism	
		Serine	↑	Glutathione metabolism	
		N-arachidonoyl taurine	↑		
		S-adenosylhomocysteine	↑	Glutathione metabolism	
		S-adenosylmethionine	↑		
		Indole	↑	Tryptophan metabolism	
		Betaine	↑		
		Tyramine	↑	Phenylalanine, tyrosine biosynthesis	
		Pipecolic acid	↑		
		Indolelactic acid	↑		
		Dimethyl-L-arginine	↑		
		Phosphocreatine	↓		
		Propionylcarnitine	↑	Fatty acid metabolism	
		5-L-glutamyl-L-alanine	↑		
		L-threoninyl-L-glutamate	↑		
		Inosine monophosphate (IMP)	↑	Purine metabolism	
		Guanosine monophosphate (GMP)	↑	Purine metabolism	
		Adenosine diphosphate	↓	Purine metabolism	
		Phosphoenol pyruvate	↑		
		L-gamma-glutamyl-L-leucine	↑		

		E-(gamma-glutamyl)-lysine	↑	
		Adenosine monophosphate	↓	
		Guanosine 5'diphosphate	↓	Purine metabolism
		Adenosine diphosphate ribose	↓	Purine metabolism
		2-Hydroxycinnamic acid	↑	
		Phosphocholine	↓	Glycerophospholipid metabolism
		Docosahexaenoic acid	↓	Fatty acid metabolism
		D-fructose 1,6-bisphosphate	↓	
		D-glycerate-3-phosphate	↑	
		D-ribose-5-phosphate	↑	Purine metabolism
		6-Phosphogluconic acid	↑	
		P-coumaric acid	↑	
		Hypoxanthine	↑	Purine metabolism
		Xanthine	↑	
		Guanine	↓	Purine metabolism
		Dopamine	↑	Phenylalanine, tyrosine biosynthesis
		Cytosine	↑	
		Cytidine	↑	
		Inosine	↑	Purine metabolism
		Uridine	↑	
		Guanosine	↑	Purine metabolism
		Cytidine diphosphate	↓	
		Uridine diphosphate	↓	
		Nucleotide adenine dinucleotide	↓	
		Choline	↑	Glycine, serine, and threonine metabolism
		Citric acid	↑	Alanine, aspartate, and glutamate metabolism
		Glutathione	↑	Glutathione metabolism
		Uric acid	↑	Purine metabolism
		Allantoic acid	↑	
		Allantoin	↑	
		Niacinamide	↑	Nicotinate metabolism

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Table 11.1: Biomarkers reported in the literature, effects, and pathways disrupted, based on exposure to single compounds, metabolomics analysis with LC-MS, and fish as a model organism.—cont'd

Type	Contaminant	Biomarkers	Effect observed	Pathway disrupted	Publication
Pesticide	Chlorpyrifos	L-carnitine	↑	Fatty acid metabolism	Gómez-Canela et al. (2017)
		L-isoleucine	↑	Protein degradation (starvation)	
		L-leucine	↑		
		Taurine	↑	Neurotransmitter	
		γ-Aminobutyric acid (GABA)	↓	Neurotransmitter	
		L-glutamic acid	↓	Neurotransmitter	
		L-valine	↑	Protein degradation (starvation)	
		L-proline	↑	Arginine and proline metabolism	
		B-alanine	↓	Alanine, aspartate, and glutamate metabolism	
		Creatine	↑	Glycine, serine, and threonine metabolism	
		L-threonine	↑	Glutathione metabolism	
		5-Oxoproline	↑	Glutathione metabolism	
		L-glutamine	↓	Purine metabolism	
		L-histidine	↓		
		L-phenylalanine	↑	Phenylalanine metabolism	
		2-Ketobutyric acid	↓		
		N ₂ -succinyl-L-ornithine	↑		
		Tyramine	↑	Neurotransmitter	
		Propionylcarnitine	↑		
		N-acetylhistidine	↑		
		Phosphocreatine	↑		
		ADP ribose	↑		
		L-acetylcarnitine	↑	Fatty acid metabolism	
		N-acetylornithine	↓		
		Creatinine	↑		
		3-Dehydroxycarnitine	↓	Fatty acid metabolism	
		B-cyprinol sulfate	↑		
		Docosahexaenoic acid	↓	Fatty acid metabolism	
		Linoleic acid	↓	Fatty acid metabolism	
		Oleic acid	↓	Fatty acid metabolism	
		Palmitic acid	↓	Fatty acid metabolism	
		Myristoleic acid	↓	Fatty acid metabolism	

BFR	2,20,4,40-tetrabromodiphenyl-ether	Glycerol-3-phosphate	↑	Sugar metabolism (starvation)	Lei et al. (2017)
		Hypoxanthine	↓	Purine metabolism	
		Adenine	↓	Purine metabolism	
		Inosine	↓	Purine metabolism	
		Amp	↓	Purine metabolism	
		Inosinic acid	↓	Purine metabolism	
		ADP	↓		
		ATP	↑		
		Adenosine	↑		
		L-lactic acid	↓	Sugar metabolism (starvation)	
		Phosphoric acid	↑		
		Piperidine	↑		
		Glutathione	↓	Glutathione metabolism	
		Threonic acid	↓	Ascorbate and cofactor metabolism	
		Glucose-6-phosphate	↓	Carbon metabolism	
		D-maltose	↓	ABC transporters	
		Maltotriose	↑	ABC transporters	
		Dethiobiotin	↑	Biotin metabolism	
		Niacinamide	↑	Nicotinate metabolism	
		C-aminobutyric acid (GABA)	↑	Neurotransmitter	
		Lysine	↑	Carnitine metabolism	
		Glutamine	↑	Purine metabolism	
		Arginine	↑	Arginine metabolism	
		Glutamate	↑	Alanine, aspartate, and glutamate metabolism	
		Glycine	↑	Neurotransmitter	
		Threonine	↑	Glutathione metabolism	
		Valine	↑	Protein degradation (starvation)	
		Methionine	↑	Glutathione metabolism	
		Spermine	↑	Polyamine metabolism	
		3,4-Dihydroxymandelic acid	↑	Norepinephrine metabolism	
		Vanillylmandelic acid	↑	Norepinephrine and epinephrine metabolism	
		Acetylcholine	↑	Neurotransmitter	

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Table 11.1: Biomarkers reported in the literature, effects, and pathways disrupted, based on exposure to single compounds, metabolomics analysis with LC-MS, and fish as a model organism.—cont'd

Type	Contaminant	Biomarkers	Effect observed	Pathway disrupted	Publication
Plasticizer	Bisphenol A	Guanine	↓	Purine metabolism	Ortiz-Villanueva et al. (2018)
		L-proline	↓	Arginine and proline metabolism	
		L-threonine	↓	Glutathione metabolism	
		Taurine	↓	Primary bile acid biosynthesis	
		5-Oxo-D-proline	↑		
		Creatine	↓	Glycine, serine, and threonine metabolism	
		L-carnitine	↓	ABC transporters	
		L-phenylalanine	↑	Phenylalanine metabolism	
		Diazepinone riboside	↑		
		L-serine	↑	Glycine, serine, and threonine metabolism	
		L-valine	↑	Protein degradation (starvation)	
		Taurine	↓	Primary bile acid biosynthesis	
		L-leucine	↑		
		D-glucuronolactone	↑		
		6-Pyruvoyltetrahydropterin	↓	Folate biosynthesis	
		6-Lactoyltetrahydropterin	↓	Folate biosynthesis	
		Adenosine monophosphate (AMP)	↓	Purine metabolism	
		Inosine monophosphate (IMP)	↓	Purine metabolism	
		5-Aminopentanal	↓		
		2,4-Dichlorobenzoate	↑		
		5β-Cyprinolsulfate	↓	Primary bile acid biosynthesis	
		4-Guanidinobutanoic acid	↓		
		L-tyrosine	↑	Phenylalanine metabolism	
		Uracil	↓	Pyrimidine metabolism	
		4-Hydroxybutanoic acid	↑		
		Octanoic acid	↑		
		2-Oxo-3-hydroxy-4-phosphobutanoic acid	↓		
		Mesaconate	↓	Glyoxylate and dicarboxylate metabolism	
		Hypoxanthine	↓	Purine metabolism	
		L-glutamine	↑	Purine metabolism	

PFC	Perfluorooctanesulfonate	Lyso(PC) (22:6)	↑	Glycerophospholipid metabolism	Ortiz-Villanueva et al. (2018)
		Diacylglycerol (DG) (38:4)	↑		
		Phosphatidylcholine (PC) (32:1)	↑		
		PC(34:1)	↑	Purine metabolism	
		PC(36:5)	↑		
		PS(44:12)	↓		
		Phosphatidylserine (PS) (40:6)	↓		
		PS(44:12)	↓		
		Glyceryl 1-monostearate	↑		
		Inosine	↑		
		Uridine diphosphate-N-acetylglucosamine	↓		
		Choline	↑	Glycine, serine, and threonine metabolism	
		L-lactic acid	↓	Phenylalanine metabolism	
		Maleamic acid	↓		
		Benzoic acid	↑		
		Salicylic acid	↓		
		Citric acid	↓	Alanine, aspartate, and glutamate metabolism	
		Phosphoric acid	↓	Glutathione metabolism	
		Glutathione	↑		
		6-Succinoaminopurine	↓	Purine metabolism	
		Uric acid	↓		
		Retinal	↓	Arginine and proline metabolism	
		L-proline	↓		
		Creatine	↓	Glycine, serine, and threonine metabolism	
		L-tyrosine	↑	Phenylalanine metabolism	
		L-phenylalanine	↑	Phenylalanine metabolism	
		L-methionine	↓	Glutathione metabolism	
		L-asparagine	↓	Alanine, aspartate, and glutamate metabolism	
		D-glucuronolactone	↑	Purine metabolism	
		Guanosine monophosphate (GMP)	↓		
		Inosine monophosphate (IMP)	↓		

Continued

Table 11.1: Biomarkers reported in the literature, effects, and pathways disrupted, based on exposure to single compounds, metabolomics analysis with LC-MS, and fish as a model organism.—cont’d

Type	Contaminant	Biomarkers	Effect observed	Pathway disrupted	Publication
Biocide	Tributyltin	Adenosine monophosphate (AMP)	↓	Purine metabolism	Ortiz-Villanueva et al. (2018)
		Hypotaurine	↑		
		L-acetylaspartate	↓	Alanine, aspartate, and glutamate metabolism	
		5β-Cyprinolsulfate	↓	Primary bile acid biosynthesis	
		2-Oxo-3-hydroxy-4-phosphobutanoic acid	↓		
		Oxidized glutathione	↓		
		Inosine	↑	Purine metabolism	
		Uracil	↑	Pyrimidine metabolism	
		Neopterin	↑	Folate biosynthesis	
		Guanosine	↑	Purine metabolism	
		4-Coumarate	↑	Phenylalanine metabolism	
		2-Oxoglutarate	↓	Alanine, aspartate, and glutamate metabolism	
		Citric acid	↓	Alanine, aspartate, and glutamate metabolism	
		D-maltose	↑	ABC transporters	
		D-glucose 6-phosphate	↓	Carbon metabolism	
		D-maltose	↑	ABC transporters	
		Nicotinamide	↑		
		4-Aminohippuric acid	↑		
		Alanine	↓	Alanine, aspartate, and glutamate metabolism	
		L-valine	↓	Protein degradation (starvation)	
		Taurine	↓	Primary bile acid biosynthesis	
		5-Oxo-D-proline	↑		
		Creatine	↓	Glycine, serine, and threonine metabolism	
		L-glutamine	↑	Purine metabolism	
		L-methionine	↓	Glutathione metabolism	
		L-carnitine	↓	ABC transporters	
		L-tyrosine	↑	Phenylalanine metabolism	
		L-proline	↓	Arginine and proline metabolism	

		L-glutamate	↓	Alanine, aspartate, and glutamate metabolism	
		L-acetylcarnitine	↑	Fatty acid metabolism	
		6-Pyruvoyltetrahydropterin	↓		
		Adenosine monophosphate	↓		
		Guanosine monophosphate	↓	Purine metabolism	
		Hypotaurine	↑	Taurine and hypotaurine metabolism	
		5-Aminopentanoate	↓	Arginine and proline metabolism	
		B-Nitropropanoate	↓		
		5β-Cyprinolsulfate	↓	Primary bile acid biosynthesis	
		Methylhexadecanoic acid	↑		
		4-Hydroxybutanoic acid	↑		
		2-Oxo-3-hydroxy-4-phosphobutanoic acid	↓		
		4-Aminobutanoate	↓	Alanine, aspartate, and glutamate metabolism	
		Oxidized glutathione	↓		
		Dehydrodiconiferyl alcohol	↑		
		Estrone glucuronide	↑		
		Hypoxanthine	↓	Purine metabolism	
		LysoPC(18:1)	↑		
		LysoPC(18:0)	↑		
		PC(32:1)	↑		
		PC(36:5)	↑		
		PS(44:12)	↓		
		LysoPC(16:0)	↑		
		LysoPC(22:6)	↑		
		PS(40:6)	↓		
		Glyceryl 1-monostearate	↑		
		Neopterin	↑	Folate biosynthesis	
		Inosine	↑	Purine metabolism	
		Cytidine	↓	Pyrimidine metabolism	
		Uridine	↓	Pyrimidine metabolism	
		Uridine diphosphate-N- acetylglucosamine	↑		
		Maleamic acid	↓		
		6-Succinoaminopurine	↓		
		A-D-glucose	↑	Carbon metabolism	
		Maltotriose	↓	ABC transporters	

Continued

Table 11.1: Biomarkers reported in the literature, effects, and pathways disrupted, based on exposure to single compounds, metabolomics analysis with LC-MS, and fish as a model organism.—cont'd

Type	Contaminant	Biomarkers	Effect observed	Pathway disrupted	Publication
PhAC	Ibuprofen	N-undecanoylglycine	↑	Arginine and proline metabolism	Song et al. (2018)
		Histidinyl-histidine	↓		
		Temocaprilat	↑		
		Cysteineglutathione disulfide	↑		
		Deoxyguanosine	↓	Purine metabolism	
		L-cysteinyglycine disulfide	↑	Arginine and proline metabolism	
		N-a-acetylcitrulline	↓	Tyrosine metabolism	
		Gamma-glutamyl-beta-aminopropionitrile	↓		
		3,5-Diiodo-L-tyrosine	↓		
		Asparaginy-alanine	↓		
		(6S)-6-beta-hydroxy-1,4,5,6-tetrahydronicotinamide-adenine dinucleotide	↓	Alanine metabolism	
		Nicotinic acid adenine dinucleotide	↑		
		Benzoyl-coa	↓		
		ADP-ribose 20-phosphate	↓		
		Beta-alanyl-coa	↓		
		Dopamine glucuronide	↓	Purine metabolism	
		5β-Cyprinolsulfate	↓		
		Alpha-tetrasaccharide	↑		
		Stearoylcarnitine	↓		
		Phosphoribosyl formamido carboxamide	↑		
		Nadh	↓	Glutathione metabolism	
		(3S)-Hydroxy-tetracos-	↑		
		6,9,12,15,18,21-all-cis-hexaenoyl-coa	↑		
		Cholesterol ester (15:0)			
		Eicosadienoic acid	↑	Alanine metabolism	
		Leukotriene B4 ethanolamide	↓		
		Acetyl-coa	↓	Alanine metabolism	
		Oleamide	↓		
		(23S)-23,25-dihdroxy-24-oxovitamins	↑	Alanine metabolism	
		D3 23-(beta-glucuronide)	↑		

PCP	Oxybenzone	2,3-Diaminosalicylic acid	↓		
		Ceramide(d18:0/24:0)	↑		
		Phytosphingosine-1-P	↑		
		Secaloside C	↑		
		5,6,7,8-Tetrahydromethanopterin	↑		
		Adenine	↓	Purine metabolism	
		Glutathione	↓		
		Mesoporphyrin IX	↓		
		6-Succinoaminopurine	↓		
		Xanthylic acid	↑	Purine metabolism	
		D-erythro-Eritadenine	↓		
		Deoxycytidine	↓	Purine metabolism	
		1,4-Beta-D-Glucan	↑		
		Chitobiose	↓		
		3-Carboxy-1-hydroxypropylthiamine diphosphate	↓		
		Ophthalmic acid	↓		
		Vitamin D ₂ 3-glucuronide	↑		
		D-serine	↓	Glycine, serine, and threonine metabolism	Ziarrusta et al. (2018) ^a
		5-Oxo-L-proline	↓	Glutathione metabolism	
		Trans-2,3-dihydroxycinnamate	↑	Phenylalanine metabolism	
		3-(4-Hydroxyphenyl)pyruvate/2-hydroxy-3-(4-hydroxyphenyl)propenoate	↑	Phenylalanine metabolism	
		Phenylacetyl glycine	↑	Phenylalanine metabolism	
		Hippurate	↑	Phenylalanine metabolism	
		Nervonic acid	↑	Biosynthesis of unsaturated fatty acids	
		Docosahexaenoic acid	↑	Biosynthesis of unsaturated fatty acids	
		Docosadienoic acid	↑	Biosynthesis of unsaturated fatty acids	
		Γ-Linolenic acid/α-Linolenic acid/ Crepenynate	↑	Biosynthesis of unsaturated fatty acids	
		3,6-Nonadienal	↓	Biosynthesis of unsaturated fatty acids	
		Sn-glycerol 1-phosphate/sn-glycerol 3-phosphate	↑	Biosynthesis of unsaturated fatty acids	

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Table 11.1: Biomarkers reported in the literature, effects, and pathways disrupted, based on exposure to single compounds, metabolomics analysis with LC-MS, and fish as a model organism.—cont'd

Type	Contaminant	Biomarkers	Effect observed	Pathway disrupted	Publication
PhAC	Amitriptyline	Arginine	↓	Arginine metabolism	Ziarrusta et al. (2019) ^a
		Methionine	↑	Glutathione metabolism	
		Asparagine	↓	Alanine, aspartate, and glutamate metabolism	
		Glutamate	↓		
		N-formimino-l-glutamate	↑		
		C18:0 (stearoylcarnitine)	↑	Lipid metabolism	
		(9Me,4E,8E,10E-d19:3)	↓	Lipid metabolism	
		Ceramide (d18:1\ /24:1(15Z))	↓	Lipid metabolism	
		Phosphatidylethanolamine	↓	Lipid metabolism	
		Monoacylglyceride (MG)	↓	Lipid metabolism	
		C4	↑	Lipid metabolism	
		PC (c34:1)	↑	Lipid metabolism	
		PC (c36:3)	↑	Lipid metabolism	
		PC (c38:2)	↑	Lipid metabolism	
		LysoPC (16:0)	↓	Lipid metabolism	
		LysoPC (18:1)	↑	Lipid metabolism	
		SM (c18:0)	↓	Lipid metabolism	
		C17:1-cooh	↑	Lipid metabolism	
		C16:1-oh	↑	Lipid metabolism	
		C5	↑	Lipid metabolism	
		LysoPC 20:3	↓	Lipid metabolism	
		LysoPC 24:1	↓	Lipid metabolism	
		Phosphatidylcholine (PC) C30:2	↑	Lipid metabolism	
		PC c32:1	↑	Lipid metabolism	
		PC c32:2	↑	Lipid metabolism	
		PC c32:3	↑	Lipid metabolism	
		Sphingomyelin (SM) C18:1	↑	Lipid metabolism	
		Uric acid	↑	Purine metabolism	
		Pantothenate	↑		
		3-Deoxyvitamin D3	↓		

PhAC	Dydrogesterone	Glutathione oxidized	↑		Jiang et al. (2019) ^b
		Taurocholic acid	↑	Bile acid synthesis and metabolism	
		Taurohyodeoxycholic acid	↑	Bile acid synthesis and metabolism	
		Acetylcarnitine	↑	Fatty acid metabolism	
		Butyryl carnitine	↑		
		Hexanoylcarnitine	↑		
		Myristoylcarnitine	↑		
		Palmitoyl carnitine	↑	Lipid homeostasis	
		5-Oxo-ETE	↑	Fatty acid metabolism	
		Palmitoleic acid	↑	Fatty acid metabolism	
		A-linolenic Acid	↑	Fatty acid metabolism	
		Stearidonic acid	↑	Fatty acid metabolism	
		5-Hetre	↑	Fatty acid metabolism	
		5-Hete	↑	Fatty acid metabolism	
		Phosphocholine	↑	Lipid metabolism	
		PC(14:0/0:0)	↑	Lipid metabolism	
		PE(16:0/0:0)	↑	Lipid metabolism	
		PE(16:1(9z)/0:0)	↑	Lipid metabolism	
		PE(18:1(9z)/0:0)	↑	Lipid metabolism	
		PG(16:0/0:0)	↑	Lipid metabolism	
		Hypoxanthine	↑	Purine degradation	
		Inosine	↑	Purine degradation	
		Guanine	↑	Purine degradation	
		Adenosine monophosphate (AMP)	↑	Purine degradation	
		Inosine monophosphate (IMP)	↑	Purine degradation	
		Guanosine monophosphate (GMP)	↑	Purine degradation	
		Uridine monophosphate UMP	↑	Purine degradation	
		UDP-glucose	↑		
		UDP-glucuronic acid	↑		
		UDP-N-acetylglucosamine	↑		
		Palmitoyl taurine	↑		
		Pantothenic acid	↑		

Continued

Table 11.1: Biomarkers reported in the literature, effects, and pathways disrupted, based on exposure to single compounds, metabolomics analysis with LC-MS, and fish as a model organism.—cont'd

Type	Contaminant	Biomarkers	Effect observed	Pathway disrupted	Publication
PAH	Benzo[a]pyrene	L-proline	↑	Arginine and proline metabolism	Roszkowska et al. (2018) ^c
		L-tryptophan	↑	Tryptophan metabolism	
		N-methyl-α-aminoisobutyric acid	↑		
		L-valine	↑	Protein degradation (starvation)	
		Taurine	↑	Primary bile acid biosynthesis	
		L-leucine	↑		
		L-isoleucine	↑	Protein degradation (starvation)	
		L-glutamate	↑		
		L-acetylcarnitine	↑	Fatty acid metabolism	
		Cysteine acid	↑		
		Tricosanoylglycine	↑		
		Pristanoylglycine	↑		
		N1-methyl-4-pyridone-3-carboxamide	↑		
		N1-methyl-2-pyridone-5-carboxamide	↑		
		5-Aminopentanoic acid	↑		
		Betaine	↑		
		3-Phenylpropionylglycine	↑		
		N-acetyl-L-phenylalanine	↑		
		Phenylpropionylglycine	↑		
		L-4-hydroxyglutamate semialdehyde	↑		
		O-acetylserine	↑		
		N-acetylserine	↑		
		N-methyl-D-aspartic acid	↑		
		5-Oxoprolinate	↑		
		Leucyl-proline	↑		
		Isoleucyl-proline	↑		
		N-acryloylglycine	↑		
		Pc(38:8)	↑	Lipid metabolism	
		Erythronic acid	↑		
		Pyrroline hydroxycarboxylic acid	↑		
		Pyrrolidonecarboxylic acid	↑		
		Pyroglutamic acid	↑		
		Threonic acid	↑	Ascorbate and cofactor metabolism	
		1-Pyrroline-4-hydroxy-2-carboxylate	↑		

PCB	PCB95	Aspartate acid	↑	Aspartate metabolism	Xu et al. (2016)
		Glutamic acid	↑	Neurotransmitter	
		Serine	↑	Glycine, serine, and threonine metabolism	
		Histidine	↑		
		Threonine	↑	Glutathione metabolism	
		Arginine	↑	Arginine metabolism	
		Alanine	↑	Alanine, aspartate, and glutamate metabolism	
		Tyrosine	↑	Phenylalanine, tyrosine biosynthesis	
		Valine	↑	Protein degradation (starvation)	
		Methionine	↑	Glutathione metabolism	
		Phenylalanine	↑	Phenylalanine biosynthesis	
		Leucine	↑		
		Isoleucine	↑	Protein degradation (starvation)	
		Lysine	↑	Carnitine metabolism	
		Proline	↑	Arginine and proline metabolism	
		Cysteine	↑		
		Tryptophan	↑	Tryptophan metabolism	
		Taurine	↑	Primary bile acids biosynthesis	
		Glutamine	↑	Purine metabolism	
		γ-Aminobutyrate (GABA)	↑	Neurotransmitter	
		Inosine monophosphate (IMP)	↑	Purine metabolism	
		Pipecolic acid	↑		
		Betaine	↑		
		N-acetylmethionine	↑		
		Choline	↑	Glycine, serine, and threonine metabolism	
		Pyroglutamic acid	↑		

Continued

Table 11.1: Biomarkers reported in the literature, effects, and pathways disrupted, based on exposure to single compounds, metabolomics analysis with LC-MS, and fish as a model organism.—cont'd

Type	Contaminant	Biomarkers	Effect observed	Pathway disrupted	Publication
Pesticides	Permethrin	Phenylalanine	↓	Phenylalanine metabolism	Tufi et al. (2016)
		Tryptophan	↓	Phenylalanine metabolism	
		GABA	↑	Neurotransmitter	
		Acetylcholine	↓	Neurotransmitter	
		Choline	↓	Glycine, serine, and threonine metabolism	
	Diazinon-o-analog	Phenylalanine	↓	Phenylalanine metabolism	
		Tryptophan	↓	Phenylalanine metabolism	
		GABA	↑	Neurotransmitter	
		Acetylcholine	↓	Neurotransmitter	
		Choline	↓	Glycine, serine, and threonine metabolism	
	Aldicarb	Phenylalanine	↑	Phenylalanine metabolism	
		Tryptophan	↓	Phenylalanine metabolism	
		GABA	↑	Neurotransmitter	
		Acetylcholine	↓	Neurotransmitter	
		Choline	↑	Glycine, serine, and threonine metabolism	
	Pirimicarb	Phenylalanine	↑	Phenylalanine metabolism	
		Tryptophan	↑	Phenylalanine metabolism	
		GABA	↓	Neurotransmitter	
		Acetylcholine	↓	Neurotransmitter	
		Choline	↓	Glycine, serine, and threonine metabolism	
	Imidacloprid	Phenylalanine	↑	Phenylalanine metabolism	
		Tryptophan	↑	Phenylalanine metabolism	
		GABA	↓	Neurotransmitter	
		Acetylcholine	↓	Neurotransmitter	
		Choline	↓	Glycine, serine, and threonine metabolism	
	Diclorfos	Phenylalanine	↓	Phenylalanine metabolism	
		Tryptophan	↓	Phenylalanine metabolism	
		GABA	↑	Neurotransmitter	
		Acetylcholine	↓	Neurotransmitter	
		Choline	↓	Glycine, serine, and threonine metabolism	

	Chlorpyrifos	Phenylalanine	↑	Phenylalanine metabolism
		Tryptophan	↑	Phenylalanine metabolism
		GABA	↓	Neurotransmitter
		Acetylcholine	↓	Neurotransmitter
		Choline	↓	Glycine, serine, and threonine metabolism
	Carbaryl	Phenylalanine	↓	Phenylalanine metabolism
		Tryptophan	↓	Phenylalanine metabolism
		GABA	↓	Neurotransmitter
		Acetylcholine	↓	Neurotransmitter
		Choline	↓	Glycine, serine, and threonine metabolism

F, female; M, male.

^aFeatures with more than one potential identity not reported.

^bOnly biomarkers identified using LC-MS reported.

^cFeatures detected with high confidence level reported.

et al., 2014), and *Sparus aurata* (Ziarrusta et al., 2018, 2019), but the preferred one was *Danio rerio* (Elie et al., 2015; Gomez-Canela et al., 2017; Jiang et al., 2019; Ortiz-Villanueva et al., 2017, 2018; Song et al., 2018; Tufi et al., 2016; Xu et al., 2016). *D. rerio* is a popular model organism widely used in environmental research. It is a convenient and cost-effective species and provides conceptual insights into many aspects of vertebrate's biology, genetics, toxicology, and disease, besides its genome is sequenced (Segner, 2009).

Table 11.1 shows contaminant type and name, biomarkers reported, effect observed (increase or decrease of levels), pathway disrupted, and publication. Metabolomics studies carried out with fish exposed to different type of organic contaminants have been performed mainly with pesticides, with 11 compounds tested (cypermethrin, tributyltin, chlorpyrifos, permethrin, diazin-o-analog, aldicarb, pirimicarb, imidacloprid, diclorfos, carbaryl, and tributyltin (used as a pesticide additive in industrial and marine paints)). Followed by pharmaceuticals (PhACs) and polycyclic aromatic hydrocarbons (PAHs) with four and three compounds assessed, respectively (17 α -ethinylestradiol, ibuprofen, amitriptyline, and dydrogesterone, and benzo[a]pyrene, benzo[a]anthracene, and benz[a]anthracene-7,12-dione). Compounds belonging to other types of contaminants such as a plasticizer (bisphenol A), a brominated flame retardant (BFR) (2,20,4,40-tetrabromodiphenyl-ether), a polychlorinated biphenyl (PCB) (PCB95), a personal care product (PCP) (oxybenzone), a perfluorinated compound (PFC) (perfluorooctanesulfonate), and a surfactant (alcohol polyethoxylated) have been also evaluated. Therefore, a wide representation of contaminant types of general environmental concern has been studied in fish using metabolomics with the exception of nanomaterials. To the best of our knowledge, there is not any metabolomics work performed yet that aims to profile the metabolome of fish exposed to any kind of nanomaterial using HRMS.

Table 11.1 reports 504 biomarkers identified in fish which levels significantly changed due to a certain organic contaminant exposure. In 62% of them, the trend observed was an increase, while 37% showed a decrease in their levels. Hence, a rise in biomarkers' levels is the most common trend observed when fish is exposed to organic contaminants.

Regarding the type of biomarkers, based on the information presented in Table 11.1, amino acids and related compounds are the most abundant group (58%), followed by lipids (17%) (Fig. 11.1). Bile acids, nutrients, organic acids, sugars, and vitamins accounted with percentages that ranged between 2% and 5%. Molecules such as carbohydrates, acylglycerols, and synthetic metabolites were included in the "others" category (9%).

Amino acids are organic compounds that contain both an amine and a carboxyl group. According to the data shown in Table 11.1 and Fig. 11.1, they are the most sensitive type of molecule in fish, which levels change due to organic contaminant exposure. They have key roles such as building blocks of proteins and polypeptides, as well as precursors of hormones and low-molecular weight nitrogen substances (Wu, 2009). An optimal level of

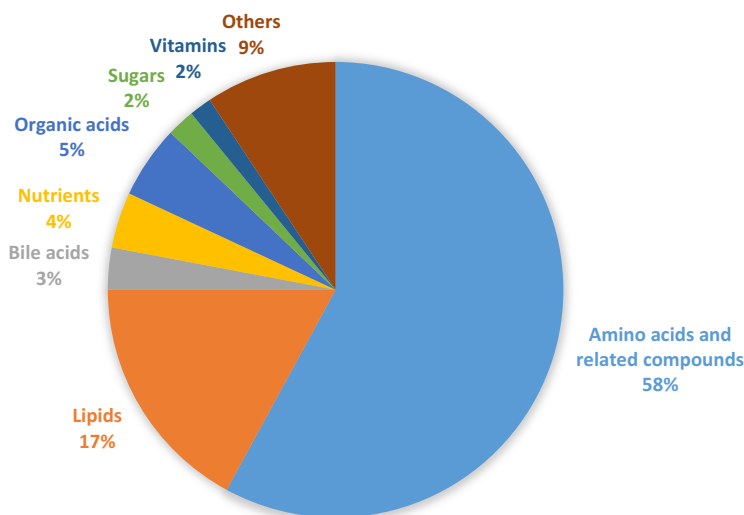


Figure 11.1

Nature of biomarkers most reported in fish exposed to organic contaminants using metabolomics and percentage of hit.

amino acids is crucial for maintaining organism homeostasis. An increase in their levels, as it is the tendency observed after contaminant exposure, may lead to neurological disorders, oxidative stress, and cardiovascular disease.

Lipids are also naturally occurring organic compounds that contain a long hydrocarbon chain (single or multiple) that may be saturated or unsaturated. They have a glycerol group bonded to the hydrocarbon chain and, depending on the type of lipid, to other molecule, for example, to a phosphate group in the case of phospholipids. Fatty acids are also considered a type of lipids; they contain carboxyl groups bonded to the hydrocarbon chain and serve as building blocks for other lipids (i.e., phospholipids). Consequently, they were included in the lipids category in Fig. 11.1. Lipids play important roles in energy storage, signaling, and they are structural components of cell membranes. An increase in their levels may lead to a disruption in cell membrane integrity with potential release of other cell components (Alvarez-Muñoz et al., 2014), oxidative stress (Zhao et al., 2015), and lipid storage disorders such as phospholipidosis (Xia et al., 2000).

2. Biomarkers' identity

Taking into consideration the two types of molecules more sensitive to contaminant exposure, amino acids and lipids, the identity of the specific biomarkers altered within these categories was studied. Table 11.2 presents amino acid and lipid identities together with the number of times that a specific biomarker was reported (considering data shown

Table 11.2: Amino acid and lipid identities together with the number of times that a specific biomarker was reported considering data shown in Table 11.1.

Biomarker	Times detected ^a	Tendency ^b
Phenylalanine	13	↑
γ-Aminobutyric acid (GABA)	10	—
Tryptophan	10	—
Proline	8	↑
Methionine	7	↑
Oxoproline	6	↑
Adenosine monophosphate (AMP)	6	↓
Glutathione	6	—
Hypoxanthine	6	—
Taurine	6	—
Valine	6	↑
Adenosine diphosphate (ADP)	5	↓
Glutamine	5	↑
Inosine monophosphate (IMP)	5	↑
Tyrosine	5	↑
Uridine diphosphate (UDP)	5	↑
Arginine	4	↑
Creatine	4	↓
Glutamate	4	—
Guanosine monophosphate (GMP)	4	—
Inosine	4	↑
Isoleucine	4	↑
Acetylcarnitine	4	↑
6-Succinoaminopurine	3	↓
Alanine	3	↓
Betaine	3	↑
Guanine	3	↑
Guanosine	3	↑
Carnitine	3	↓
Leucine	3	↑
Lysine	3	↑
Threonine	3	↑
Uridine	3	↑
6-Pyruvoyltetrahydropterin	2	↓
Adenine	2	↓
Cytidine	2	—
D-glucuronolactone	2	↑
Hypotaurine	2	↑
N1-methyl-2-pyridone-5-carboxamide	2	↑
N-acetylmethionine	2	—
Neopterin	2	↑
Phosphocreatine	2	—
Pipecolic acid	2	↑
Propionylcarnitine	2	↑
Serine	2	↑

Table 11.2: Amino acid and lipid identities together with the number of times that a specific biomarker was reported considering data shown in Table 11.1.—cont'd

Biomarker	Times detected ^a	Tendency ^b
Threonic acid	2	—
Tyramide	2	↑
Xanthine	2	↑
Phosphatidylcholine (PC)	16	↑
Lysophosphatidylcholine (PC)	10	↑
Phosphatidylserine (PS)	5	↓
2-Oxo-3-hydroxy-4-phosphobutanoic acid	3	↓
Docosahexaenoic acid	3	↓
4-Hydroxybutanoic acid	2	↑
Ceramide	2	—
Sphingomyelin	2	—
Linolenic acid	2	↑

The tendency observed in its effect is also shown as increase (↑), decrease (↓), or not clear trend (—).

^aAt least reported 2 times.

^bLevels increase (↑) or decrease (↓) in >50 times detected, (—) biomarker ↑ 50% of the times reported and ↓ in the other 50%; therefore, a trend was not established.

in Table 11.1), and the tendency observed in its effect. Only biomarkers that were reported at least twice are included in Table 11.2. The tendency was considered as increase (↑) or decrease (↓) when that was the effect shown in more than 50% of the observations. If an increase was observed in half of the observations and a decrease in the other half, a clear trend could not be established and a dash was assigned (—). Amino acids and lipids reported at least two times represented nearly half of the entire fish metabolome (48% according to Table 11.1). Concretely, their identities corresponded to 48 different amino acids and related compounds and 9 lipids (Table 11.2). Regarding the effect observed, the same increasing tendency as previously reported in Table 11.1 was detected (57% of the biomarkers increased, 21% decreased, and 22% not clear trend, Table 11.2).

The top five amino acids were held by phenylalanine, γ-aminobutyric acid (GABA), tryptophan, proline, and methionine, being the most detected amino acids with 13, 10, 10, 8, and 7 times detected, respectively. Phenylalanine, tryptophan, and methionine are essential amino acids, meaning that they cannot be synthesized from scratch by the organism and they must be supplied in the diet. Proline, although it is generally considered as nonessential, can be essential in some fish (Wu, 2009). GABA chemically speaking is an amino acid but the amino group is attached to the carbon atom at the position gamma instead of alpha; therefore, it is not incorporated into proteins. Regarding their functions, GABA acts as neurotransmitter in the central nervous system and reduces the activity of neurons or nerve cells. Both tryptophan and proline are functional amino acids that can regulate key metabolic pathways necessary for maintenance, growth, reproduction, and

immunity in organisms (Wu, 2009). Besides, proline and methionine are related to the antioxidant defense system and to oxidative stress disorders (Zhou et al., 2019). Phenylalanine is the precursor of the catecholamine neurotransmitters L-dopa, dopamine and epinephrine, and methionine a carnitine precursor (Ziarrusta et al., 2019).

In the lipids' groups a top three was established with phosphatidylcholine (PC), lysoPC, and phosphatidylserine (PS) as the most detected biomarkers (16, 10, and 5 times detected, respectively) (Table 11.2). They belong to the phospholipid class that is a major component of cell membranes due to its amphiphilic properties. Two hydrophobic fatty acid "tails" connected to a hydrophilic "head" by a glycerol molecule characterize their molecular structure. The head consists of a phosphate group that can be modified with an organic molecule such as choline, in the case of PC, or serine, for PS. LysoPC is derived from partial hydrolysis of PC by removing one of the fatty acids tails. PC and PS are major constituent of membranes and play an important role in membrane-mediated cell signaling.

3. Pathways disrupted

Once the fish metabolome is profiled and the identity of the biomarkers established, data interpretation, known as secondary analysis, is usually undertaken. The purpose of this analysis is to link the metabolites that significantly change with the metabolic pathways where they are involved in order to draw biological conclusions. This is known as pathway enrichment techniques, and they can be divided into three generations: over representation analysis, functional class scoring, and pathway topology (Khatri et al., 2012). Enrichment tools have been mainly developed for other "omics" technologies, such as genomics or proteomics, and suitable tools for metabolomics are still scarce (Marco-Ramell, 2018). The method identifies biological pathways that are enriched in a metabolites list more than would be expected by chance, by drawing from comprehensive databases like Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome, WikiPathways, and the Small Molecule Pathway Database (Picart-Armada et al., 2017). MetaboAnalyst is the tool generally used (Elie et al., 2015; Lei et al., 2017; Song et al., 2018; Xu et al., 2016), although manual checking in databases such as KEGG is also popular (Gomez-Canela et al., 2017; Ortiz-Villanueva et al., 2018; Ziarrusta et al., 2018). Manual checking is complex because the same metabolite can be involved in several pathways, for instance, according to KEGG L-Glutamine (C00064) is involved in 20 different pathways. Therefore, software such as MetaboAnalyst or Compound Discoverer, that has recently included this feature, is helpful.

According to the information presented in Table 11.1, 28 pathways were found to be disrupted in fish due to an exposure to organic contaminants. Fig. 11.2 shows the pathways with most compound hits and many of them are closely related. If a "disrupted pathway

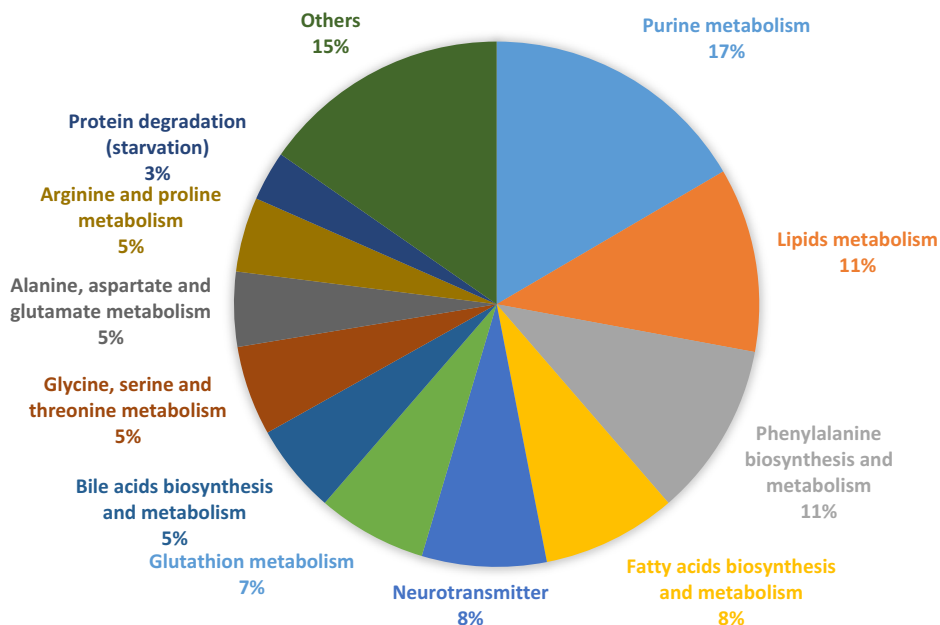


Figure 11.2

Pathways with most compound hits in fish exposed to organic contaminants.

ranking” is established based on the times that they have been pointed out, purine metabolism with 17% of the times holds the first position being the most altered metabolic route due to organic contaminant exposure in fish. In the second place are lipid metabolism and phenylalanine biosynthesis and metabolism with 11% of the times detected. Then, neurotransmitters and fatty acid biosynthesis and metabolism with 8% and glutathione metabolism with 7% hold the third position. In fourth place with 5% bile acid metabolism, glycine, serine, and threonine metabolism, alanine, aspartate, and glutamate metabolism, and arginine and proline metabolism. In fifth position, all the pathways that were pointed out less than a 3% are included, such as the ones related to protein degradation, sugar metabolism, steroid biosynthesis, etc., included in the “others” category.

Purine metabolism, also called purine nucleotide catabolism, may be a component of the homeostatic response of mitochondria to oxidative stress caused by organic contaminants (Elie et al., 2015). Phenylalanine biosynthesis and metabolism disruption in fish may be predictive of developmental neurobehavioral effects associated with contaminant exposure as it has been previously observed in rats and humans (Perera et al., 2012; Xia et al., 2011). Lipids and fatty acids play a major role as sources of energy for fish growing, reproduction, and movement. Besides, some functional lipids such as phospholipids are structural components of the double-layered surface of all cells (lipid bilayer) and disruption of their biosynthesis may affect membrane stability. Glutathione metabolism is

also crucial because it plays an important role in the detoxification of contaminants. The enzyme glutathione S-transferase (GST) catalyzes the conjugation of glutathione with xenobiotic compounds containing electrophilic centers. It is important for organisms to deal with this type of contaminants because they can react with macromolecules controlling cell growth such as DNA, RNA, and proteins (Hampel et al., 2016). Besides, many of these chemicals are carcinogenic.

4. Conclusions and future trends

More than 500 metabolites have been reported as potential biomarkers of organic contaminant exposure in fish by using metabolomics techniques. This entails a big amount of data difficult to handle and that generally is not fully exploited. In this chapter, a rational analysis of this information has been performed in order to subtract conclusions that may help to interpret data generated with metabolomics studies and serve as a guideline. The general tendency observed in 62% of the data was an increase in the levels of biomarkers. This suggests that a rise in biomarkers' body burden is the most common effect expected after organic contaminant exposure. Amino acids and lipids were the most sensitive groups of molecules altered, accounting for nearly half of the entire fish metabolome (48%). The top three amino acids and lipids which levels significantly changed due to contaminant exposure were phenylalanine, γ -aminobutyric acid (GABA), tryptophan, phosphatidylcholine, lysophosphatidylcholine, and phosphatidylserine. They may be proposed as potentials "universal biomarkers" of general organic contamination and environmental quality due to their higher sensitivity and nonspecific response to a certain chemical. To be fair, highly specific biomarkers are less abundant than nonspecific ones, but this may represent an advantage for a first screening of biological status and they can be used as an early warning tool of environmental quality. Later on, a more specific set of biomarkers may be applied in order to characterize exposure or effects of chemicals of concern. Biomarkers were related to metabolic pathways involved in order to draw biological conclusions. Twenty-eight pathways were altered due to contaminant exposure. A "pathway ranking" was established with purine metabolism being the most altered one, followed by lipids and phenylalanine metabolism.

In order to protect biological systems and maintain good environmental quality, it is necessary to study the overall biological effects of organic contaminant exposure. Metabolomics is a useful technique for this because it offers a good coverage of the organism metabolome and a snapshot of its biological status. Its popularity for analyzing biological samples in environmental monitoring is rapidly growing, and it will continue like this until the current challenges that scientist faced are achieved and it will become a routine tool for the assessment of environmental quality.

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