Metabolomics as a powerful tool to decipher the biological effects of environmental contaminants in humans

N. Bonvallot, Arthur David, Frédéric Chalmel, Cécile Chevrier, Sylvaine Cordier, Jean Pierre J. P. Cravedi, Daniel Zalko

Metabolomics as a powerful tool to decipher the biological effects of environmental contaminants in humans

Authors: Nathalie Bonvallot[1,2*], Arthur David[1,2], Frédéric Chalmel[1,3], Cécile Chevrier[1,3], Sylvaine Cordier[1,3], Jean-Pierre Cravedi[4], Daniel Zalko[4].

[1] INSERM UMR1085 IRSET, Rennes, France

[2] French School of Public Health (EHESP), Rennes, Sorbonne Paris Cité, France

[3] University of Rennes I, Rennes, France

[4] Toxalim, Université de Toulouse, INRA, INP-ENVT, INP-EI-Purpan, Université de Toulouse Paul Sabatier, Toulouse, France

* Corresponding author: Nathalie Bonvallot

E-mail: nathalie.bonvallot@ehesp.fr

EHESP School of Public Health, Avenue du Professeur Léon Bernard, CS74312, 35043 Rennes Cedex, France. Tel: +332 99 02 26 22. Fax: +332 99 02 26 75.

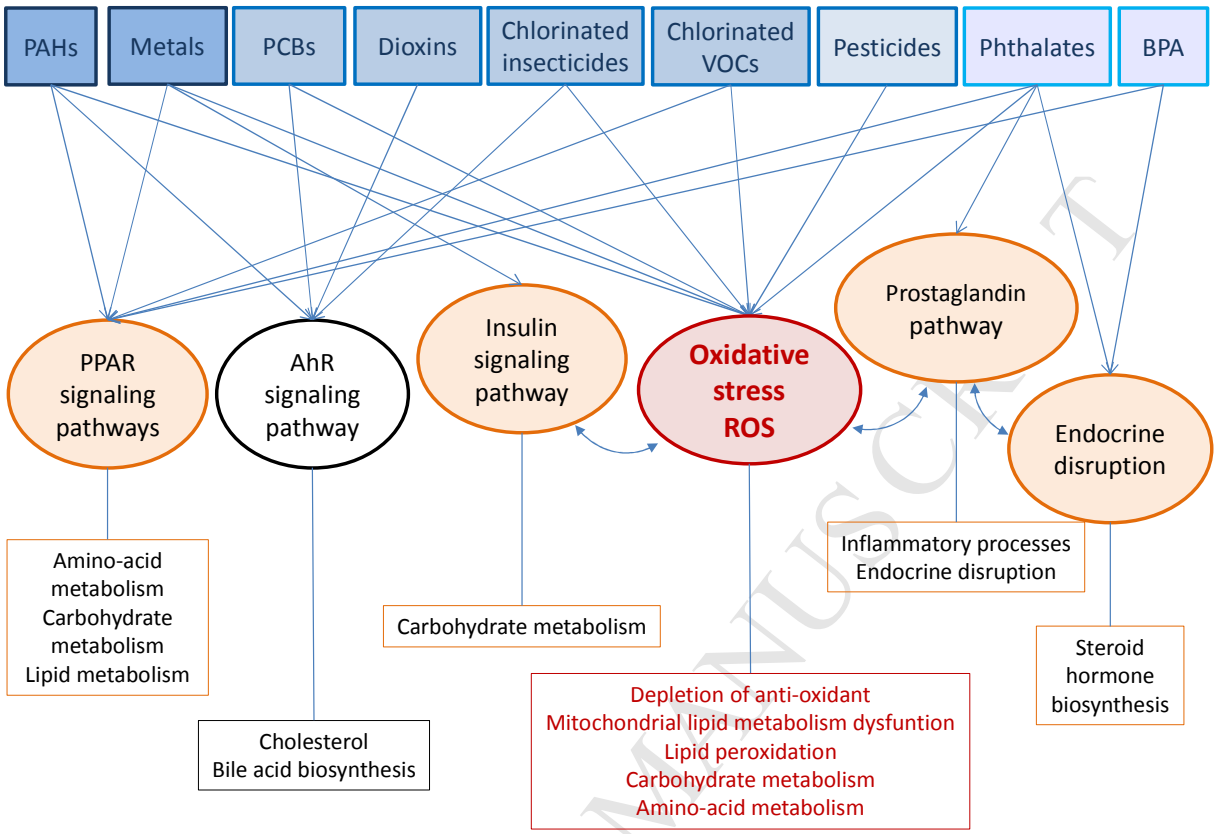
Key-words

metabolomics, environmental contaminants, biomarkers, human exposure

Declaration of competing financial interest

The authors declare they have no competing financial interests.

Graphical abstract



Abstract

Humans are exposed daily to a variety of environmental contaminants. For many of these, the associated health effects remain largely unexplored. There is growing evidence that untargeted metabolomics-based techniques could be used to identify simultaneously biomarkers of effect (i.e. disruption of endogenous metabolite profiles) and exposure (i.e. xenobiotic mixtures) in biological samples. This approach is expending quickly and has been used in several studies over the last 5 years to study biological effects of many environmental contaminants in humans. Hence, this review attempts to determine whether metabolomics might constitute a relevant tool to discover new early biomarkers of effects related to chemical exposure in humans. Published results suggest that common metabolic pathways are affected by specific groups of chemicals such as PAHs, metals, organochlorine compounds, pesticides, and endocrine disrupting plasticizers. Remarkably, disruption of cellular signaling pathways associated with oxidative stress may be primary drivers for further effects for all studied chemicals. In the future, the introduction of more sensitive analytical techniques to perform untargeted profiling of biological samples seems to be a good option to improve the coverage of the xeno-metabolome and to provide more relevant information on both chemical exposure and metabolic signatures of environmental contaminants. Some current challenges are finally discussed.

Introduction

Humans are exposed to a large variety of environmental contaminants through diet, water, air or direct skin contact. Exposure modalities rely on lifestyle, place of residence, occupation, food habits and air quality. Deciphering the potential health effects resulting from exposure to chemical mixtures humans are really exposed is extremely difficult and needs the combination of different approaches including epidemiology, toxicology or analytical chemistry. Metabolomics is increasingly used in toxicology, opening new perspectives to study changes at the metabolome level associated with chemical exposure. These holistic approaches combined with chemometrics allow to profile biological samples and simultaneously detect thousands of endogenous and exogenous small molecules (usually below 1000 Da) involved in fundamental biological processes (development, growth, and homeostasis). Metabolic fingerprints allow to seek for a discrimination between sub-populations according to exposure conditions. Beyond that, current developments of metabolomics aim to investigate the link between exposure and health issues, define markers of toxicity, and explore adverse outcome pathways (AOP) (1). Metabolomics account for the ultimate step in the cellular response (2). Of particular interest is the fact that metabolomics can be based on biological matrices whose collection uses non-invasive or minimally invasive methods (i.e. urine, blood, saliva). So far, most of the metabolomic studies carried out in human have aimed to identify biomarkers in clinical research (3–6). In toxicology, it was recently suggested that metabolomics may provide data of interest for the identification of both exposure biomarkers and mechanisms of toxicity but the relevancy of animal-to-human extrapolation remain a key challenge that needs to be explored (7). This review examines whether metabolomics may be considered as a relevant tool to unveil unidentified early biomarkers of effects related to chemical exposure directly in human. The search covered the period until October, 2017. Using the Web Of Knowledge™ and the Pubmed websites

(http://webofknowledge.com; http://www.ncbi.nlm.nih.gov/pubmed), publications were selected by searching in the field "topic" for: ((metabolomics OR metabonomics) AND environmental AND exposure). Publications were selected in line with the following criteria: applying non targeted metabolomics, addressing human environmental exposure, any kind (occupational or not, proximity to polluted areas, dietary exposure…); collecting human biological samples (urine, blood, saliva…). Publications focusing of human behavioral / recreational exposure (alcohol, tobacco) or exclusively solely relying on animal's exposure were not considered (laboratory mammals, fishes, invertebrates, crustaceans).

Description of covered populations and metabolomics approaches used

The search identified 22 publications that applied metabolomics to biological fluids of human populations environmentally exposed to contaminants (Table 1). Sixteen publications (8–23) studied the general population, including – for 7 of them – specifically vulnerable individuals: pregnant women, children or elder persons (9,12,14,20–23). The other studies concerned occupational exposure (24–29). Most studies were related to heavy metals: n=10, chlorinated persistent organic compounds (POPs): n=6 and polycyclic aromatic hydrocarbons (PAHs): n=3, followed by particulate matter (fine particles and PM2.5), pesticides, phthalates, bisphenol A (BPA) and trichloroethylene (TCE). For the general population, the proximity of a polluted area was studied in half of the cases (foundries, coking plants, waste incinerators, petrochemical complexes, agricultural areas, contaminated soils). For workers, all studies suggested an association between specific professional activities and occupational exposure (foundry and metals, herbicide production and dioxins, cleaning processes factories and TCE, boiler makers and welding fumes + PM). Except for pesticide exposure, which was estimated from geographic information systems (GIS) (data on agricultural activities matched with

residence), all studies quantified exposure either by using external (personal air exposure measurements at work shift) or internal measurements (biomonitoring based on blood or urine analyses). Only one study used both GIS, external measurements and biomarkers quantification (16). Metabolic profiles were generated from urine and/or blood using chemometrics analyses generally based on mass spectrometry or NMR, associated with Principal Component Analysis (PCA) and Partial Least Discriminant Square Analysis (PLS-DA).

Metabolic features related to environmental exposures

A summary of the reported metabolic changes and suggested pathways is presented in Table 2.

Metals and Polycyclic aromatic hydrocarbons (PAHs)

Reactive oxygen species (ROS) - mediated oxidative stress, and to a lesser extent, nitrosative stress, may be considered as one of the potential modes of action of metals and PAHs: all studies have shown changes in levels of metabolites indicating oxidation both for exposure to As, Cd, V, PAHs or to more complex mixtures (8,10–12,15,16,19,22–24): TCA-cycle disruption and amino-acid metabolism impairment evoke a mitochondrial dysfunction with insufficient energy production. Perturbation of lipid metabolism may also be due to oxidative or inflammatory processes (12,19,22,24). These modes of action have been related to liver and renal dysfunction, particularly for As and Cd (8,10,15,23). Modifications of cellular signaling may be considered as another mode of action, as it was suggested for V and PAHs which has an impact on PPAR and insulin signaling pathways leading to carbohydrate and amino-acid metabolism (16). Finally, endocrine disruption is one another mode of action suggested for As and Cd, with altered steroid hormone profiles (10,11).

Welding fumes are complex mixtures containing various types of gases (carbon monoxide, nitrogen oxides) and particulate matter composed of heavy metals, depending on the welding technique. Metabolites that are modified in exposed workers are involved in carbohydrate metabolism, amino-acid metabolism, redox pathways, and urea metabolism (25), which confirms the oxidative stress and inflammatory processes already suggested in populations exposed to heavy metals. Only three unsaturated fatty acids were found to be significantly reduced (26) but this is consistent with an impact on signaling pathway respectively involved in lipid regulation and apoptosis (PPAR and caspases), suggesting a potential risk to develop chronic diseases such as obesity, diabetes, atherosclerosis or cancer. In (25), the distinctive metabolic pattern of exposed workers compared to controls was masked when smokers were included, suggesting similar responses between welding fumes and smoking. This has been confirmed in (30), which study showed modifications both in anti-oxidant defense, amino-acid metabolism, and lipid regulation, relevant with the development of tobacco specific diseases (atherosclerosis, cancer).

Persistent organic pollutants (POPs)

Metabolomics studies that have investigated POPs exposure focused on dioxins and PCBs (13,18,20,27,28), and to a lesser extent, organochlorine pesticides (DDE, HCB and HCH) (14,18). Most of them showed dysregulations in cholesterol, steroid metabolism and bile acid biosynthesis which are consistent with AhR mediation and liver injury. Conjugated steroids and bile acids were considered as valuable biomarkers characterizing the exposure to several dioxins with various exposure scenarios (poisoning, acute/high occupational exposure, and chronic/low doses exposure in general population near a waste incinerator (13,28)). These biomarkers were also associated with prevalent type 2 diabetes in four Swedish cohorts (31), supporting associations between POPs and metabolic diseases. Surprisingly, (27) did not identify any significant metabolic feature after multiple testing correction. Impairment of lipid

metabolism (involving fatty acids, glycerophospholipids, sphingolipids and glycerolipids) was also demonstrated in populations exposed to PCBs, DDE and HCB (14,18,20). In case of PCBs exposure, glutathione pathway and amino-acid metabolism was also affected, suggesting an oxidative stress, inflammatory response and disruption of energy metabolism (20). This was recently confirmed in a Swedish cohort where serum ATP content was negatively associated with PCBs exposure in elderly individuals (32).

Chlorinated volatile organic compounds

Only one study investigated the metabolic pattern associated with TCE exposure in workers from cleaning process plants (29). The presence of numerous known and unknown chlorinated compounds and cysteine and methionine metabolites was in accordance with TCE detoxification in the liver (glutathione conjugation). The modification of bile acids biosynthesis may be a marker of both TCE glucuronidation and liver damage. Modifications in fatty acid metabolism and palmitoylcarnitine levels confirm this hypothesis by an alteration in PPAR signaling. Uric acid was related to the nephrotoxicity of TCE and alteration of purine catabolism to immunotoxic pathways (29). An oxidative stress was not suggested although modifications in amino-acid relate to TCA cycle remind to an imbalance in anti-oxidant defenses.

Pesticides

Only one study investigated the metabolic pattern associated with pesticides exposure (9). Few metabolites were found to be correlated with pesticide exposure after adjustment of confounding factors, likely because exposure was solely estimated from GIS and not further confirmed by individual pesticide measurements. Nevertheless, metabolome modifications suggest a protective mechanism linked with oxidative stress (impairment of TCA cycle, depletion of anti-oxidant defenses). This was supported by animal studies showing an

impairment of amino-acid metabolism, mitochondrial dysfunction, alteration of lipid regulation and glucose regulation imbalance, in association with organochlorine or organophosphorus alone or in mixture (7) or with a more complex pesticide mixture including herbicides, fungicides and insecticides (33).

Plasticizers

One study investigated the metabolic pattern associated with DEHP and DBP exposure (17). Authors suggested an oxidative stress induced by phthalates, due to changes in mitochondrial fatty acid beta-oxidation (modification of carnitine levels), amino-acids metabolism and antioxidant defenses (precursors of glutathione synthesis), based on the dysregulation of PPARs pathways. Another interesting mechanism concerns the disruption of prostaglandin pathways, which are largely involved in hormone regulation and inflammatory processes, and are consistent with the known endocrine disrupting properties of DEHP and DBP. Oxidative stress, lipid dysregulation, and impairment of amino-acid metabolism have also been suggested in mammals experiment (7).

The consequences of BPA exposure on the urinary metabolic profiles of children aged 7-9 were investigated (21). This study showed that the biosynthesis of steroid hormones and the metabolism of amino acids and glucose were affected in highly exposed children (highest quartile) compared to children with limited exposure (lowest quartile), mainly in females (21). These modifications observed in female may represent a risk of developing pathologies at an adult age (polycystic ovary syndrome, diabetes, metabolic syndrome or cardiovascular diseases). Other altered pathways (glucose) suggest an imbalance in energy homeostasis. These results are supported by data previously obtained in mice (34) and rats (35) showing that BPA exposure disrupts energy metabolism, glucose metabolism and amino acid metabolism. In these animals, alterations of neurotransmitters were also identified.

Discussion

Metabolomics technologies provide extensive information about phenotypic features of populations, by integrating upstream information given by transcriptomics and proteomics approaches. Their most important advantage in environmental health is their ability to detect early biological modifications, prior to more obvious signs of toxicity and adverse effects (8).

Metabolomics for identifying mechanisms of chemical toxicity

A growing body of literature over the last five years demonstrates that metabolic fingerprints can efficiently discriminate sub-populations characterized by distinctive exposure modalities, with extremely robust and valid multivariate statistic models. Discriminant metabolites provide valuable clues on the involved mechanisms. In this review we were able to distinguish four groups of chemicals characterized by common features, which may be used to explore or confirm adverse outcomes pathways (AOP) directly in humans:

- **Heavy metals and PAHs** have very similar metabolic features including modifications in anti-oxidant and anti-nitrosant processes, amino-acid metabolism, and lipid regulation.
- **Chlorinated compounds, persistent or not** (dioxins, PCBs, organochlorine insecticides and TCE) also display similar profiles, but involving more lipid classes – including cholesterol and sphingolipid metabolism – and bile acid biosynthesis, confirming the potential to induce chronic diseases such as atherosclerosis, diabetes or obesity.
- Metabolic changes induced by **phthalates** concern both anti-oxidant mechanisms (mitochondrial beta-oxidation, amino-acid metabolism) and the disruption of prostaglandin regulated pathway.

- **Bisphenol A** was found to alter predominantly the biosynthesis of steroid hormones and the metabolism of amino acids in children, mostly in females.
- Finally, for **pesticides**, available metabolomics studies only demonstrate (so far) an impact on oxidative stress pathways.

It is worth mentioning that some of the metabolites identified in available studies (such as serine, glutamate, glutathione, taurine, glycine, betaine, hippurate…) cannot be considered as specific biomarkers. In addition, disruption of cellular signaling pathways associated with oxidative stress seems to be primary drivers for further altered metabolic pathways including lipids, bile acids or amino-acids. These observations do not provide sufficient data for a relevant use in public health, and point out the need to provide simultaneously more quantitative exposure information. Finally, epidemiological study designs used (i.e. transversal rather than longitudinal) do not allow to ensure the predictive nature of biomarkers for further health effects.

Metabolomics and xeno-metabolomics: the sensitivity issue

Metabolomics aim at identifying in an unbiased manner biomarkers of effects and exposure that have so far remained unidentified. However, many xenobiotics and signaling compounds such as steroids are present at trace levels in biological matrices and most of the current platforms used to characterize the metabolome are not sensitive enough to detect these low abundant chemicals. To date, these platforms encompass NMR, GC-HRMS or LC-HRMS. However, the most widely used platform is LC-HRMS, typically utilizing an electrospray ionisation source. The popularity of LC-HRMS compared to other platforms can be explained by its increased sensitivity compared to NMR analysis and the soft ionisation process which allows structural elucidation. To illustrate this problem of sensitivity, out of the 22 studies considered here, only two were able to identify sensitive biomarkers of environmental exposure, urinary N-methyl-L-histidine was associated with Cd exposure and was considered

as a more sensitive biomarker of renal function than urinary N-acetyl-beta-D-glucosaminidase (U-NAG) (10); dodecadienyl-carnitine was considered as a reliable and sensitive biomarker for PAHs exposure since his level is associated with 1-hydroxyphenanthrene (12), although not very specific. To overcome this sensitivity issue, innovative analytical strategies (e.g., more selective sample preparation, miniaturized LC-ESI platforms to improve ionization efficiency, new HRMS such as ion mobility) as well as new bioinformatics tools are now being developed to improve the coverage of both the metabolome and the xeno-metabolome, namely endogenous metabolites and xenobiotic exposome (36–40). One study included in this review detected numerous metabolic features corresponding to xenobiotics (chlorinated chemicals) including unknown compounds (29). Another example includes the detection of many metabolites of five pesticides in urine samples from pregnant women by non-targeted UHPLC-HRMSn (41). Some of them may be considered as specific metabolites. It can be expected in the near future than the introduction of more sensitive analytical techniques for metabolomics will allow to better characterize the xeno-metabolome in order to provide more relevant information on both chemical exposure and biological signatures of xenobiotics. Furthermore, a more automated annotation work-flow with increased confidence in the annotations is needed to overcome the problems linked to unidentified features.

The importance of studying mixtures: some perspectives?

The hypothesis of a common metabolic feature involving oxidative stress and signaling pathway confirms the importance of carefully exploring mixture approaches. In the near future, the modeling of metabolomics data into metabolic networks should enable to tackle current limitations in the interpretation of the biological pathways modulated by chemicals, based on the list of discriminant metabolites. In this context, multi-omics data integration

involving not only the metabolome but also upstream information associating transcriptomic and proteomic data (when available) is desirable. Such an integrative approach provides global biological signatures to better categorize groups of chemicals both for environmental epidemiology and risk assessment. An innovative work is carrying out in order to integrate massive toxicogenomics data for classification of chemicals and prediction of toxicity. First results are promising with the identification of several clusters from a dataset comprised more than 500 chemicals for 7000 experimental conditions (42). Methodologies developed and provided knowledge will be made publicly available on the web (43).

Conclusion

A growing body of literature applying metabolomics in populations differently exposed to environmental contaminants was published last years. It shows that the application of this approach in the field of environmental health is still in its infancy, at a discovery stage. Metabolomics may become a powerful tool in the near future both in the identification of specific biomarkers related to mixtures, the characterization of exposures and the definition of adverse outcome pathways as it was suggested by (44) but it needs to consider several challenges, and particularly the acquisition of reproducible results in a prospective context (longitudinal designs) to obtain early biomarkers of disease and to address reverse causation; the validation of biomarkers in different groups of similarly exposed population (as it was done in (13)); and the characterization of the xeno-metabolome to provide more information on quantitative exposures (44).

References

1. Johnson CH, Ivanisevic J, Siuzdak G. Metabolomics: beyond biomarkers and towards mechanisms. Nat Rev Mol Cell Biol. 2016;17(7):451‑9.

2. Fiehn O. Metabolomics - the link between genotypes and phenotypes. Plant Mol Biol. 2002;48:151‐71.

3. Diaz SO, Pinto J, Graça G, Duarte IF, Barros AS, Galhano E, et al. Metabolic biomarkers of prenatal disorders: an exploratory NMR metabonomics study of 2nd trimester maternal urine and blood plasma. J Proteome Res. 2011;10(8):3732-42.

4. Kenny LC, Broadhurst DI, Dunn W, Brown M, North RA, McCowan L, et al. Robust early pregnancy prediction of later preeclampsia using metabolomic biomarkers. Hypertension. 2010;56(4):741‐9.

5. Mamas M, Dunn W, Neyses L, Goodacre R. The role of metabolites and metabolomics in clinically applicable biomarkers of disease. Arch Toxicol. 2011;85(1):5‐17.

6. Kim K. Urine metabolomics analysis for kidney cancer detection and biomarker discovery. Mol Cell Proteomics. 2009;8:558‐70.

7. Bonvallot N, Tremblay-Franco M, Chevrier C, Canlet C, Debrauwer L, Cravedi JP, et al. Potential input from metabolomics for exploring and understanding the links between environment and health. J Toxicol Environ Health, Part B-Crit Rev. 2014;17:21‐44.

8. Ellis J, Athersuch T, Thomas L, Teichert F, Perez-Trujillo M, Svendsen C, et al. Metabolic profiling detects early effects of environmental and lifestyle exposure to cadmium in a human population. BMC Med. 2012;10(1):61.

9. Bonvallot N, Tremblay-Franco M, Chevrier C, Canlet C, Warembourg C, Cravedi JP, et al. Metabolomics tools for describing complex pesticide exposure in pregnant women in Brittany (France). PLoS ONE. 2013;8(5):e64433.

306 10. Gao Y, Lu Y, Huang S, Gao L, Liang X, Wu Y, et al. Identifying early urinary
307 metabolic changes with long-term environmental exposure to cadmium by mass-
308 spectrometry-based metabolomics. Environ Sci Technol. 2014;48(11):6409□18.

309 11. Zhang J, Shen H, Xu W, Xia Y, Barr DB, Mu X, et al. Urinary metabolomics revealed
310 arsenic internal dose-related metabolic alterations: a proof-of-concept study in a Chinese male
311 cohort. Environ Sci Technol. 2014;48(20):12265□74.

312 12. Wang Z, Zheng Y, Zhao B, Zhang Y, Liu Z, Xu J, et al. Human metabolic responses
313 to chronic environmental polycyclic aromatic hydrocarbon exposure by a metabolomic
314 approach. J Proteome Res.;14(6):2583□93.

315 13. Jeanneret F, Tonoli D, Hochstrasser D, Saurat J-H, Sorg O, Boccard J, et al.
316 Evaluation and identification of dioxin exposure biomarkers in human urine by high-
317 resolution metabolomics, multivariate analysis and in vitro synthesis. Toxicol Lett.
318 2016;240(1):22–31.

319 14. Salihovic S, Ganna A, Fall T, Broeckling CD, Prenni JE, van Bavel B, et al. The
320 metabolic fingerprint of p,p'-DDE and HCB exposure in humans. Environ Int. 2016;88:60–6.

321 15. Xu Y, Wang J, Liang X, Gao Y, Chen W, Huang Q, et al. Urine metabolomics of
322 women from small villages exposed to high environmental cadmium levels. Environ Toxicol
323 Chem. 2016;35(5):1268–75.

324 16. Yuan T-H, Chung M-K, Lin C-Y, Chen S-T, Wu K-Y, Chan C-C. Metabolic profiling
325 of residents in the vicinity of a petrochemical complex. Sci Total Environ. 2016;548:260–9.

326 17. Zhang J, Liu L, Wang X, Huang Q, Tian M, Shen H. Low-level environmental
327 phthalate exposure associates with urine metabolome alteration in a Chinese male cohort.
328 Environ Sci Technol. 2016;50(11):5953–60.

18. Carrizo D, Chevallier OP, Woodside JV, Brennan SF, Cantwell MM, Cuskelly G, et al. Untargeted metabolomic analysis of human serum samples associated with exposure levels of Persistent organic pollutants indicate important perturbations in sphingolipids and glycerophospholipids levels. Chemosphere. 2017;168:731‐8.

19. Chen C-HS, Yuan T-H, Shie R-H, Wu K-Y, Chan C-C. Linking sources to early effects by profiling urine metabolome of residents living near oil refineries and coal-fired power plants. Environ Int. 2017;102:87‐96.

20. Eguchi A, Sakurai K, Watanabe M, Mori C. Exploration of potential biomarkers and related biological pathways for PCB exposure in maternal and cord serum: a pilot birth cohort study in Chiba, Japan. Environ Int. 2017;102:157‐64.

21. Khan A, Park H, Lee HA, Park B, Gwak HS, Lee H-R, et al. Elevated metabolites of steroidogenesis and amino acid metabolism in preadolescent female children with high urinary bisphenol A levels: a high-resolution metabolomics study. Toxicol Sci. 2017. DOI 10.1093.

22. Laine JE, Bailey KA, Olshan AF, Smeester L, Drobna Z, Styblo M, et al. Neonatal metabolomic profiles related to prenatal arsenic exposure. Environ Sci Technol. 2017;51(1):625‐33.

23. Li H, Wang M, Liang Q, Jin S, Sun X, Jiang Y, et al. Urinary metabolomics revealed arsenic exposure related to metabolic alterations in general Chinese pregnant women. J Chromatogr A. 2017;1479:145‐52.

24. Dudka I, Kossowska B, Senhadri H, Latajka R, Hajek J, Andrzejak R, et al. Metabonomic analysis of serum of workers occupationally exposed to arsenic, cadmium and lead for biomarker research: A preliminary study. Environ Int. 2014;68:71‐81.

25. Kuo CH, Wang KC, Tian TF, Tsai MH, Chiung YM, Hsiech CM, et al. Metabolomic characterization of laborers exposed to welding fumes. Chem Res Toxicol. 2012;25(3):676□86.

26. Wei Y, Wang Z, Chang C, Fan T, Su L, Chen F, et al. Global metabolomic profiling reveals an association of metal fume exposure and plasma unsaturated fatty acids. PLoS ONE. 2013;8(10):e77413.

27. Hosnijeh FS, Pechlivanis A, Keun HC, Portengen L, Bueno-de-Mesquita HB, Heederik D, et al. Serum metabolomic pertubations among workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Environ Mol Mutagen. 2013;54(7):558□65.

28. Jeanneret F, Boccard J, Badoud F, Sorg O, Tonoli D, Pelclova D, et al. Human urinary biomarkers of dioxin exposure: analysis by metabolomics and biologically driven data dimensionality reduction. Toxicol Lett. 2014;230(2):234‑43.

29. Walker DI, Uppal K, Zhang L, Vermeulen R, Smith M, Hu W, et al. High-resolution metabolomics of occupational exposure to trichloroethylene. Int J Epidemiol. 2016;45(5):1517‑27.

30. Kaluarachchi MR, Boulange CL, Garcia-Perez I, Lindon JC, Minet EF. Multiplatform serum metabolic phenotyping combined with pathway mapping to identify biochemical differences in smokers. Bioanalysis. 2016;8(19):2023‑43.

31. Fall T, Salihovic S, Brandmaier S, Nowak C, Ganna A, Gustafsson S, et al. Non-targeted metabolomics combined with genetic analyses identifies bile acid synthesis and phospholipid metabolism as being associated with incident type 2 diabetes. Diabetologia. 2016;59(10):2114‑24.

32. Park WH, Kang S, Lee HK, Salihovic S, Bavel B van, Lind PM, et al. Relationships between serum-induced AhR bioactivity or mitochondrial inhibition and circulating polychlorinated biphenyls (PCBs). Scientific Reports. 2017;7:9383.

33. Bonvallot N, Canlet C, Blas-Y-Estrada F, Gautier R, Tremblay-Franco M, Chevolleau S, et al. Metabolome disruption of pregnant rats and their offspring resulting from repeated exposure to a low dose of a pesticide mixture representative of environmental contamination in Brittany. Under review.

34. Cabaton NJ, Canlet C, Wadia PR, Tremblay-Franco M, Gautier R, Molina J, et al. effects of low doses of Bisphenol A on the metabolome of perinatally exposed CD-1 mice. Environ Health Perspect. 2013. 121(5):586-93.

35. Tremblay-Franco M, Cabaton NJ, Canlet C, Gautier R, Schaeberle CM, Jourdan F, et al. Dynamic metabolic disruption in rats perinatally exposed to low doses of Bisphenol A. PLoS ONE. 2015;10(10):e0141698.

36. David A, Abdul-Sada A, Lange A, Tyler CR, Hill EM. A new approach for plasma (xeno)metabolomics based on solid-phase extraction and nanoflow liquid chromatography-nanoelectrospray ionisation mass spectrometry. J Chromato A. 2014;72‑85.

37. David A, lange A, Abdul-Sada A, Tyler CR, Hill EM. Disruption of the prostaglandin metabolome and characterization of the pharmaceutical exposome in fish exposed to wastewater treatment works ellfuent as revealed by nanoflow-nanospray mass spectrometry-based metabolomics. Environ. Sci. Technol. 2017;51(1):616-24.

38. Chetwynd AJ, David A, Hill EM, Abdul-Sada A. Evaluation of analytical performance and reliability of direct nanoLC-nanoESI-high resolution mass spectrometry for profiling the (xeno)metabolome. J Mass Spectrom. 2014;49(10):1063-9.

39. Southam AD, Lange A, Al-Salhi R, Hill EM, Tyler CR, Viant MR. Distinguishing between the metabolome and xenobiotic exposome in environmental field samples analysed by direct-infusion mass spectrometry based metabolomics and lipidomics. Metabolomics. 2014;1050‑8.

40. Crockford DJ, Maher AD, Ahmadi KR. 1H NMR and UPLC-MS(E) statistical heterospectroscopy: characterization of drug metabolites (xenometabolome) in epidemiological studies. Anal Chem. 2008;6835‑44.

41. Jamin E, Bonvallot N., Tremblay-Franco M, Cravedi JP, Chevrier C, Cordier S, et al. Untargeted profiling of pesticide metabolites by LC-HRMS: an exposomics tool for human exposure evaluation. Anal Bioanal Chem. 2014;406:1149‑61.

42. Darde T, Lecluze E, Becker E, Potdevin V, Collin O, Jégou B, et al. ChemPSy: Identification, classification and prioritization of novel endocrine disruptors by integrating massive toxicogenomics datasets. Ten years of "Programme National de Recherche - Environnement Santé Travail, ANSES; Paris, November 2016.

43. Darde T, Gaurdiault P, Beranger R, Lancien C, Caillarec-Joly A, Sallou O, et al. TOXsIgN: a cross-species repository for toxicogenomics signatures. *In revision*.

44. National Academies of Sciences, Engineering, and Medicine. Using 21st century science to improve risk-related evaluations. 2017. Washington, DC: The National Academies Press. https://doi.org/10.17226/24635.

Table 1: Studies using metabolomics to reveal biological pathways associated with environmental exposures in human

Refs.	Population, number of individuals, hypothesis of exposure	Measurement of exposure	Samples, metabolomics analyses
(8)	Volunteers. n=178. Proximity of foundry, contaminated environment with metals	Urinary Cd & NAG (+ 8-oxodG in samples < p15 & > p85 Cd). 2 groups: < p15 and > p85 of urinary Cd	1st morning spot urine, ^{1}H NMR, PLS
(9)	Volunteers, pregnant women (cohort participants). n=83. Proximity to agricultural activities	SIG: 3 groups according to the surface of land dedicated to agricultural activities in the town of residence	1st morning spot urine, 1st trimester, ^{1}H NMR, PLS-DA
(10)	Volunteers adult females ≥ 40y, rural areas. n=94. Diet exposure via contaminated soils	Urinary Cd & NAG + Pb, Cu. 2 groups: urinary Cd > or < 5 µg/L (according to WHO)	1st morning spot urine, LC-QTOF-MS, GC-MS, OPLS-DA
(11)	Volunteers, general population. adult males. n=1027 (cohort participants)	Urinary As (2 inorganic + 3 organic forms). 2 groups: < p20 and > p80 of urinary As	1st morning spot urine, HPLC-QTOF-MS, PLS-DA
(12)	Elderly (352) & children (214). n=566. PAHs polluted rural area < 2 km downwind of a coking plant.	9 urinary PAHs metabolites. 2 groups dichotomized according to localization: exposed (359) vs non exposed (197)	1st morning spot urine, UHPLC-TOF-MS, OPLS-DA
(13)	Volunteers, adult males. n=24. Proximity to a waste incinerator (evaluation of a set of biomarkers in an independent human cohort exposed to dioxins)	SIG: 2 groups. The first corresponds to males living or working near the waste incinerator (24). Healthy male volunteers constitute a control group (24)	1st morning spot urine, UPLC-QTOF-MS, OPLS-DA
(14)	Volunteers, 70y males. n=965 (cohort participants)	Plasmatic DDE & HCB	Blood, fasted individuals, UPLC-TOF-MS, multivariate linear regression
(15)	Volunteers, adult females, rural areas with Cd contaminated soils. n=21.	Urinary Cd & NAG + Pb, Cu. 3 groups (high, low and control): urinary Cd > 15, 5-15 or < 5 µg/L	1st morning spot urine, GC-MS, OPLS-DA
(16)	Volunteers, < 40 km of petrochemical complex. n=160. Potentially exposed to V & PAHs	Urinary V, 1-OHP (+ oxidative stress markers). 2 groups: highly exposed: <10 km + > p75 1-OHP & V (80) vs lower exposed : > 10km (80)	Blood, ^{1}H NMR, OPLS-DA
(17)	Volunteers, adult males from general population. n=364 (cohort participants)	Urinary metabolites of DEHP & DBP. 3 groups: terciles according to Σmetabolites	1st morning spot urine, HPLC-QTOF-MS, PLS-DA
(18)	Healthy volunteers, general population. n=34	Serum POPs (DDE, HCB, β-HCH, PCB28, 138, 153). 2 groups according to individual POP concentrations	Serum collected fasted (3 samples/ ind), ULPC-QTOF-MS, OPLS-DA

Table 1: Studies using metabolomics to reveal biological pathways associated with environmental exposures in human (continued)

Refs.	Population, number of individuals, hypothesis of exposure	Measurement of exposure	Samples, metabolomics analyses
(19)	Volunteers, general population close to oil refineries / coal-fired power plants. n=252. Potentially exposed to PAHs and metals	SIG and urinary 1-OHP & V, Ni, Cu, As, Sr, Cd, Hg, Tl (+ oxidative stress markers). 2 groups: highly exposed close to the complex (111) and lower exposed (141) further away	1st morning spot urine, GC-TOF-MS, PLS-DA
(20)	Volunteers, pregnant women (cohort participants). n=67	Serum PCBs in women and cord-blood. 2 groups: 1st and last quartile	Blood collected at 32w pregnancy and in cord-blood at delivery, HILIC-MS/MS, PCA, random forest
(21)	Volunteers, female and male children 7-9 year old. n=18 (birth cohort participants)	Urinary total BPA (deglucuronidation). 2 groups: lower and higher quartile (9 vs 9 with >501 µg/g creat and < 4 µg/g creat). Separated in low and high BMI in each group	1st morning spot urine, UPLC-QTOF-MS, PLS-DA
(22)	Volunteers, pregnant women (cohort participants). n=50	As (inorganic and total) in maternal urine prior to the time of delivery and in cord blood	Cord-blood at delivery, ^{1}H NMR, multivariate linear regressions, pFDR
(23)	Volunteers, pregnant women. n=246	Total urinary As. 3 groups according to terciles	1st morning spot urine, 1st trimester, UPLC-QTOF-MS, PLS-DA
(24)	Workers in a copper foundry. n=391. Exposed to lead, Cd, As	Not included in the statistical analyses (As, Pb, Cd at work shift). 2 groups: exposed (359) vs non-occupationally exposed (32)	Blood collected the morning before work, ^{1}H NMR, PLS-DA
(25)	Male welders and office workers. N=51. Exposed to welding fumes.	Personal sampling of fine particles. Cr, Ni and Mn detection. Inflammatory marker measurements. 2 groups: exposed (35) vs non exposed – office workers (16)	1st morning spot urine, ^{1}H NMR, PCA and pFDR
(26)	Boilermakers recruited at an apprentice welding school. n=11. Exposed to welding fumes	Personal integrated measurement of PM2.5. Individuals are their own controls with samples collected before and after the work	Blood collected before and after work, GC and LC-MS, PCA and LMM (linear mixed-effect model)
(27)	Workers from chlorophenoxy herbicide production plants. n=144. Exposed to TCDD following an accident	Plasmatic 2,3,7,8-TCDD. 2 groups: exposed (81) vs non exposed (63)	Blood collected during home visits, UHPLC-QTOF-MS, PLS
(28)	Workers from herbicide production plant intoxicated by dioxins & V. Yushchenko. n=23. Exposed to TCDD following an accident	Plasmatic 2,3,7,8-TCDD. 2 groups: exposed (11) vs non exposed (11) and positive control (V. Yushchenko)	24h-Urine, UHPLC-QTOF-MS, OPLS-DA
(29)	Workers from cleaning process plants. n=175. Exposed to TCE	Air personal measurement of TCE + other VOCs. 2 groups according to the TCE median (80 exposed vs 95 non exposed)	Blood, LC-MS, linear regressions, pFDR

Table 2: Biomarkers that have been changed in blood or urine when populations are exposed to environmental contaminants

Exposure (ref.)	Sample used	Metabolites that are modified between exposed and non-exposed groups: number (n) and name	Suggested altered pathway/ mechanism
As (tot) (23)	Urine	n=9. LysoPC(14:0), glutathione, leukotriene E4 metabolites, cystathionine ketimin, dihydropyridine, thiocysteine, p-cresol glucuronide, vanillactic acid	Oxidative stress
As (tot) (11)	Urine	n=5. testosterone, guanine, hippurate, acetyl-N-formyl-5-methoxykynurenamine, serine	Endocrine disruption and oxidative stress
iAs (22)	Cord blood	n=17. Aceto-acetate, acetone, betaine, ethanol, glutamate, glycerol, glycine, isoleucine, lactate, mannose, methionine, O-acetylcholine, pyruvate, serine, taurine, tyrosine, 3-hydroxybutyrate	Impaired vitamin metabolism, TCA cycle, and amino-acid metabolism → oxidative stress
Metals, Cd (close to foundry) (8)	Urine	n=6. Citrate, 3-hydroxyisovalerate, 4-deoxyerythronic acid, dimethylglycine, creatinine, creatine	Impaired mitochondrial metabolism & one-carbon metabolism. Oxidative stress
Metals, Pb, Cd, As (copper foundry) (24)	Blood	n=7. LDL, VLDL, unsaturated lipids, 1-methylhistidine, phenylalanine, tyrosine, glutamate	Impaired lipid metabolism & amino-acid metabolism
Cd (contaminated areas) (10)	Urine	n=27. Glutamine, cysteine, tyrosine, N-methyl-L-histidine, L-histidinol, taurine, phenylacetylglutamine, hippurate, pyroglutamic acid, galactose, myoinositol, xanthine, urea, deoxyadenosine monophosphate, creatine, creatinine, 17-α-hydroxyprogesterone, tetrahydrocortisone, estrone & corticosterone	Impaired amino acid metabolism, galactose metabolism, purine metabolism, creatine pathway, steroid hormone biosynthesis impairment. N-methyl-L-histidine appears as a more sensitive biomarker of renal function than U-NAG
Cd (contaminated areas) (15)	Urine	n=27; 8 of which have a dose-response relationship: glucose, 3-α-mannobiose, cellobiose, phosphate, glycine, threonine, azelaic acid, butanoic acid	Impaired carbohydrate and glucose metabolism, amino-acid metabolism, bone metabolism, TCA cycle, mitochondrial disorder. Modification of human microbiome. Increase of all metabolites suggests impaired tubular reabsorption
PAHs, V (close to petro-chemistry) (16)	Blood	n=18. VLDL, LDL, lipids, isoleucine, valine, alanine, lysine, glutamine, tyrosine, histidine, phenylalanine, pyruvate, lactate, α- /β-glucose, n-acetylglycoprotein	Impaired amino-acid metabolism, carbohydrate metabolism by elevating PPAR and insulin signaling. Oxidative/ nitrosative stress
PAHs, metals (close to petro-chemistry) (19)	Urine	n=45 in children, n=42 in elderly. Alanine, aspartate, glutamate, phenylalanine, tryptophan metabolism in children. Glycine, serine, threonine, alanine, aspartate, glutamate metabolism, & aminoacyl-tRNA biosynthesis in elderly	Impaired amino-acid metabolism associated with oxidative stress markers in urine. Oxidative/ nitrative DNA damage, lipid peroxidation. Age-dependent response
PAHs (close to coking plant) (12)	Urine	n=18 (elderly non-smokers). 3-methylhistidine, pyroglutamic acid, uric acid, 10 carnitines, 2-isopropylmalic acid, decenedioylglucuronide, azelaic acid, decenedioic acid, hydroxytetradecanedioic acid	Impaired amino-acid metabolism, purine metabolism, lipid metabolism and glucuronic acid metabolism. Dodecadienyl-carnitine as a reliable biomarker of PAH exposure. Oxidative stress hypothesis → lipid peroxidation, mitochondrial dysfunction, up-regulation of UGTs & depletion of antiox.

Table 2: Biomarkers that have been changed in blood or urine when populations are exposed to environmental contaminants (continued)

Exposure (ref.)	Sample used	Metabolites that are modified between exposed and non-exposed groups: number (n) and name	Suggested altered pathway/ mechanism
Welding fumes, metals, fine PM ± smoking (25)	Urine	n=10. Creatine, taurine, trimethylamine-N-oxide/ betaine, acetone, creatinine, glycine, gluconate, hippurate, S-sulfocystein, serine.	Impairment of carbohydrate metabolism, amino-acid metabolism. Oxidative stress.
Welding fumes PM2.5 (26)	Blood	n=3. Eicosapentaenoic acid, docosapentaenoic acid n3, n6.	Impairment of unsaturated fatty acids metabolism.
2,3,7,8-TCDD (herbicide production plant) (28)	Urine	n~11 in exposed workers. Etiocholanolone conjugates, androsterone glucuronide and features associated to bile acids: glycocholic acid, its glucuronide metabolite, glycodeoxycholic acid, chenodeoxycholic acid sulfate or ursodeoxycholicacid sulfate, glucuro-and sulfoconjugates of glycochenodeoxycholic, glucuroconjugated forms of hydroxyandrosterone or hydroxyetiocholanolone, hydroxyDHEA or hydroxytestosterone, andestrone, glycoursodeoxycholic acid glucuronide and sulfate. n=24 in VY samples (+ drugs metabolites associated with VY treatment). Some steroids such as DHEA sulfate, androsterone sulfate and androsterone glucuronide	Altered cholesterol metabolism and bile acid biosynthesis in exposed workers. Hypothesis of cholesterol homeostasis dysregulation following TCDD exposure mediated by AhR. Results for VY sample consistent with altered steroid metabolism and liver damage, compatible with an increased expression of cytochrome P450s, persistent hepatotoxicity, bile acid homeostasis dysregulation and oxidative stress.
Dioxins (close to waste incinerator) (13)	Urine	Targeted analysis based on the 24 putative conjugated steroids and bile acids obtained from (10) (VY samples). n=9 unambiguously identified biomarkers. Dehydroepiandrosterone 3β-sulfate, androsterone 3α-glucuronide, androsterone 3α-sulfate, pregnanediol 3α-glucuronide, 11-ketoetiocholanolone 3α-glucuronide, glucuronide conjugates of 11β-hydroxyandrosterone, glycochenodeoxycholic acid, glycocholic acid and glycoursodeoxycholic acid sulfate.	Impairment of steroid metabolism and bile acid biosynthesis. A set of biomarkers valuable in characterizing various dioxin exposure situations.
2,3,7,8-TCDD (herbicide prod. plant) (27)	Blood	No obvious metabolic perturbations after multiple testing correction.	TCDD exposure at levels present in this study does not lead to significant perturbations of the serum metabolome.
POPs (DDE, HCB, β-HCH, PCB28-138-153) (18)	Blood	n=40 but only 10 unambiguously identified, including sphingolipids, glycerophosphocholines, glycerophosphoethanolamines	Lipid metabolism impairment associated to DDE, HCB and PCBs revealing a similar mode of action.

Table 2: Biomarkers that have been changed in blood or urine when populations are exposed to environmental contaminants (continued)

Exposure (ref.)	Sample used	Metabolites that are modified between exposed and non-exposed groups: number (n) and name	Suggested altered pathway/ mechanism
PCBs (20)	Blood	n=14 in maternal samples. Phosphorylcholine, hypoxanthine, cytosine, putrescine, carbamoyl phosphate, N6-acetyl-L-lysine, glutathione, uracil, norepinephrine, citraconic acid, xanthosine, kynurenic acid, serine, N-acetyl-glucosamine-1-phosphate. n=10 in cord samples. p-hydroxybenzoate, dihydroorotate, purine, ethanolamine, guanidoacetic acid, hydroxyproline, sedoheptulose 1,7-bisphosphate, betaine, tyrosine, glucosamine.	Biomarkers related to glutathione and amino acid metabolism in maternal serum and the amino acid metabolism, ubiquinone and other terpenoid-quinone biosynthesis in cord serum. These pathways are linked with lipid synthesis, mitochondrial electron transport, fetal survival and growth → disruption of energy metabolism.
TCE (cleaning process) (29)	Blood	n=15 (confirmed metabolites) + TCE metabolites and unknown chlorinated compounds. 7α-hydroxycholest-4-en-3-one, checnodeoxycholic acid, creatine, cysteine, glutamine, homocysteine, indolelactic acid, methylthioadenosine, palmitocarnitine, phenylacetic acid, taurine, tryptophan, tyrosine, uric acid, α-linolenic acid.	Disruption in purine catabolism and decreases in sulphur amino acid and bile acid biosynthesis pathways. Some metabolites changes are consistent with known toxic effects of TCE (immunosuppression, hepatotoxicity and nephrotoxicity, neurotoxicity).
Pesticides (close to agricultural areas) (9)	Urine	n=5. Glycine, threonine, lactate, glycerophosphocholine, citrate.	Impairment of amino-acids metabolism, oxidation/reduction pathways and mitochondrial function.
DEHP & DBP (17)	Urine	n=16. Acetylneuraminic acid, carnitine C8:1, carnitine C18:0, carnitine C16:2, cysteine, phenylglycine, phenylpyruvic acid, glutamylphenylalanine, diacetylspermine, alanine, taurine, tryptophan, ornithine, methylglutaconic acid, hydroxyl-PEG2, keto-PGE2.	Increased oxidative stress and fatty acid oxidation. Decreased prostaglandin metabolism. Disruption of urea cycle, tryptophan and phenylalanine metabolism.
BPA (21)	Urine	n=35. Cholesterol, 4-methylpentanal, 22β-hydroxycholesterol, progesterone, pregnanediol, corticosterone, cortisol, cortolone, dehydroandrosterone, androstenedione, testosterone-glucuronide, estrone 3-sulfate, *N*-acetylmuramic acid, *N*-acetylmuramic acid 6-phosphate, D-glucosamine$^{(-)}$, D-glucosamine-6-phosphate, CMP-*N*-acetylneuraminic acid$^{(-)}$, CMP-*N*-glycoloylneuraminate$^{(+)}$, fructose 6-phosphate, D-phenylalanine, phenylacetylglycine, phenylpropanoate, malonyl-CoA$^{(-)}$, crotonoyl-CoA$^{(-)}$, anthranilic acid, *N*-acetylserotonin, 4-hydroxyphenylacetylglutamine, 3-methoxytyramine$^{(-)}$, formyl pyruvate, L-glutamyl-5-phosphate, N^2-acetyl-L-ornithine, L-carnitine, N^6-acetyl-N^6-hydroxy-L-lysine, aerobactin, 5-acetamidopentanoate.	Disruption of steroidogenesis pathways in females, but inconclusive effects in males. Alteration of pathways related to amino acid and nucleotide biosynthesis, phenylalanine metabolism, tryptophan metabolism, tyrosine metabolism, lysine degradation, pyruvate metabolism and arginine biosynthesis. Alteration in glucose homeostasis were also observed. Male children were less affected than females by these metabolic variations.