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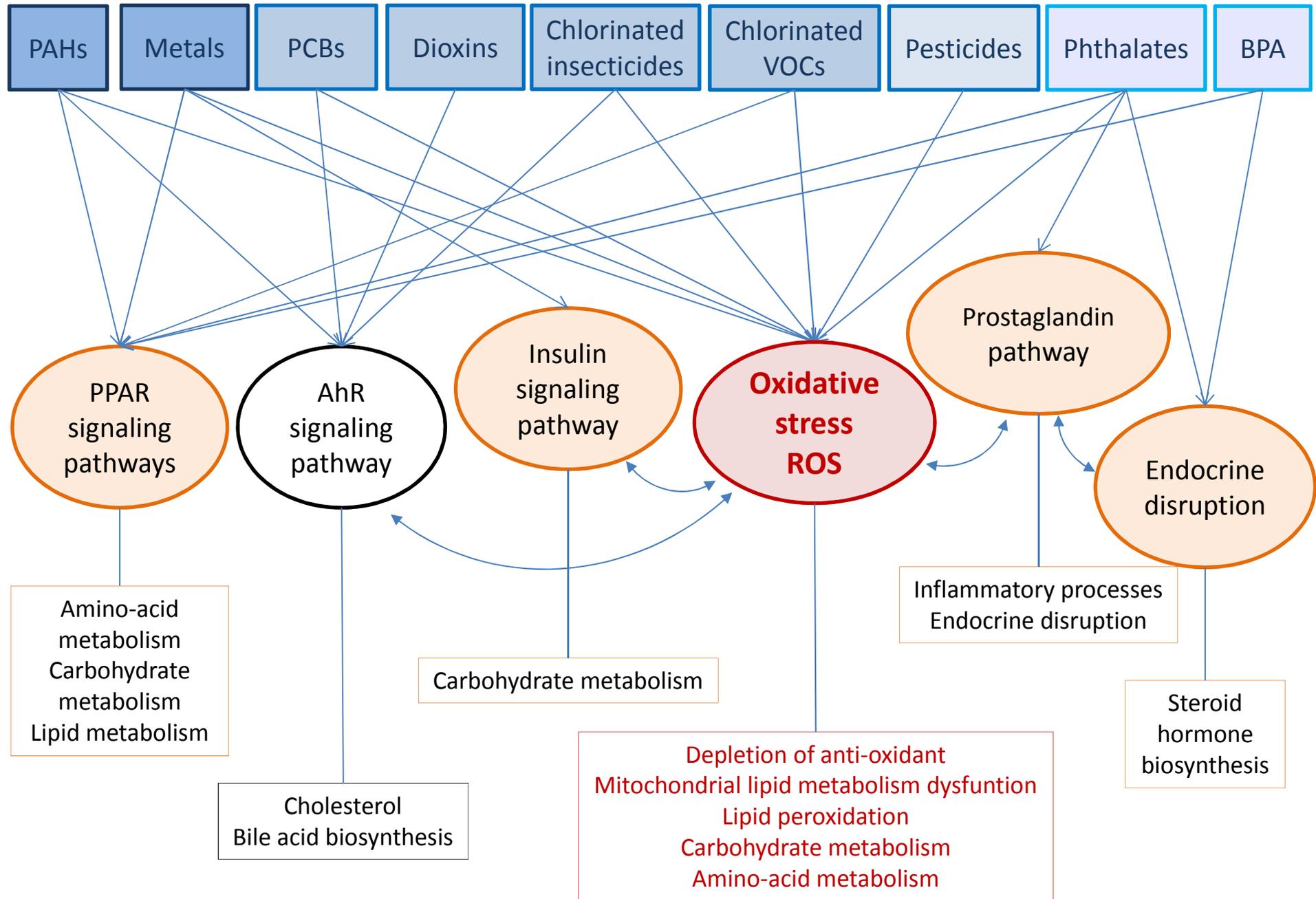
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1 **Metabolomics as a powerful tool to decipher the biological effects**
2 **of environmental contaminants in humans**

3 Authors: Nathalie Bonvallet^{1,2*}, Arthur David^{1,2}, Frédéric Chalmel^{1,3}, Cécile Chevrier^{1,3},
4 Sylvaine Cordier^{1,3}, Jean-Pierre Cravedi⁴, Daniel Zalko⁴.

5

6 ¹ INSERM UMR1085 IRSET, Rennes, France

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

8 ³ University of Rennes I, Rennes, France

9 ⁴ Toxalim, Université de Toulouse, INRA, INP-ENVT, INP-EI-Purpan, Université de
10 Toulouse Paul Sabatier, Toulouse, France

11

12 * Corresponding author: Nathalie Bonvallet

13 E-mail: nathalie.bonvallet@ehesp.fr

14 EHESP School of Public Health, Avenue du Professeur Léon Bernard, CS74312, 35043

15 Rennes Cedex, France. Tel: +332 99 02 26 22. Fax: +332 99 02 26 75.

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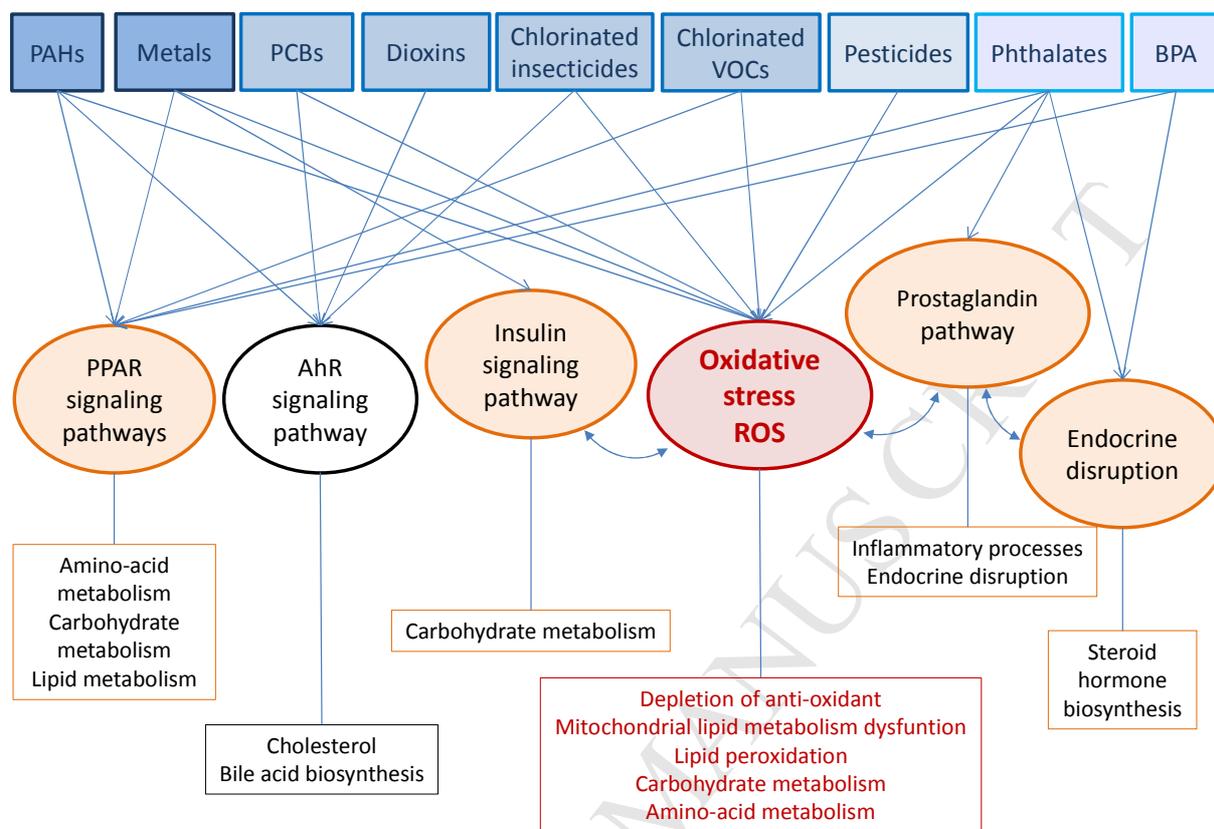
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18 metabolomics, environmental contaminants, biomarkers, human exposure

19

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24

25

26 Abstract

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

44

45 **Introduction**

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

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

78 **Description of covered populations and metabolomics approaches** 79 **used**

80 The search identified 22 publications that applied metabolomics to biological fluids of human
81 populations environmentally exposed to contaminants (Table 1). Sixteen publications (8–23)
82 studied the general population, including – for 7 of them – specifically vulnerable individuals:
83 pregnant women, children or elder persons (9,12,14,20–23). The other studies concerned
84 occupational exposure (24–29). Most studies were related to heavy metals: n=10, chlorinated
85 persistent organic compounds (POPs): n=6 and polycyclic aromatic hydrocarbons (PAHs):
86 n=3, followed by particulate matter (fine particles and PM_{2.5}), pesticides, phthalates,
87 bisphenol A (BPA) and trichloroethylene (TCE). For the general population, the proximity of
88 a polluted area was studied in half of the cases (foundries, coking plants, waste incinerators,
89 petrochemical complexes, agricultural areas, contaminated soils). For workers, all studies
90 suggested an association between specific professional activities and occupational exposure
91 (foundry and metals, herbicide production and dioxins, cleaning processes factories and TCE,
92 boiler makers and welding fumes + PM). Except for pesticide exposure, which was estimated
93 from geographic information systems (GIS) (data on agricultural activities matched with

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

101 **Metabolic features related to environmental exposures**

102 A summary of the reported metabolic changes and suggested pathways is presented in Table
103 2.

104 **Metals and Polycyclic aromatic hydrocarbons (PAHs)**

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

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

131 **Persistent organic pollutants (POPs)**

132 Metabolomics studies that have investigated POPs exposure focused on dioxins and PCBs
133 (13,18,20,27,28), and to a lesser extent, organochlorine pesticides (DDE, HCB and HCH)
134 (14,18). Most of them showed dysregulations in cholesterol, steroid metabolism and bile acid
135 biosynthesis which are consistent with AhR mediation and liver injury. Conjugated steroids
136 and bile acids were considered as valuable biomarkers characterizing the exposure to several
137 dioxins with various exposure scenarios (poisoning, acute/high occupational exposure, and
138 chronic/low doses exposure in general population near a waste incinerator (13,28)). These
139 biomarkers were also associated with prevalent type 2 diabetes in four Swedish cohorts (31),
140 supporting associations between POPs and metabolic diseases. Surprisingly, (27) did not
141 identify any significant metabolic feature after multiple testing correction. Impairment of lipid

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

148 **Chlorinated volatile organic compounds**

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

159 **Pesticides**

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

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

170 **Plasticizers**

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

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

190 **Discussion**

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

195 **Metabolomics for identifying mechanisms of chemical toxicity**

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

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

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

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

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

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

255

256 **The importance of studying mixtures: some perspectives?**

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

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

270 **Conclusion**

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

282 **References**

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

- 285 2. Fiehn O. Metabolomics - the link between genotypes and phenotypes. *Plant Mol Biol.*
286 2002;48:151-71.
- 287 3. Diaz SO, Pinto J, Graça G, Duarte IF, Barros AS, Galhano E, et al. Metabolic
288 biomarkers of prenatal disorders: an exploratory NMR metabonomics study of 2nd trimester
289 maternal urine and blood plasma. *J Proteome Res.* 2011;10(8):3732-42.
- 290 4. Kenny LC, Broadhurst DI, Dunn W, Brown M, North RA, McCowan L, et al. Robust
291 early pregnancy prediction of later preeclampsia using metabolomic biomarkers.
292 *Hypertension.* 2010;56(4):741-9.
- 293 5. Mamas M, Dunn W, Neyses L, Goodacre R. The role of metabolites and
294 metabolomics in clinically applicable biomarkers of disease. *Arch Toxicol.* 2011;85(1):5-17.
- 295 6. Kim K. Urine metabolomics analysis for kidney cancer detection and biomarker
296 discovery. *Mol Cell Proteomics.* 2009;8:558-70.
- 297 7. Bonvallet N, Tremblay-Franco M, Chevrier C, Canlet C, Debrauwer L, Cravedi JP, et
298 al. Potential input from metabolomics for exploring and understanding the links between
299 environment and health. *J Toxicol Environ Health, Part B-Crit Rev.* 2014;17:21-44.
- 300 8. Ellis J, Athersuch T, Thomas L, Teichert F, Perez-Trujillo M, Svendsen C, et al.
301 Metabolic profiling detects early effects of environmental and lifestyle exposure to cadmium
302 in a human population. *BMC Med.* 2012;10(1):61.
- 303 9. Bonvallet N, Tremblay-Franco M, Chevrier C, Canlet C, Warembourg C, Cravedi JP,
304 et al. Metabolomics tools for describing complex pesticide exposure in pregnant women in
305 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.

- 329 18. Carrizo D, Chevallier OP, Woodside JV, Brennan SF, Cantwell MM, Cuskelly G, et
330 al. Untargeted metabolomic analysis of human serum samples associated with exposure levels
331 of Persistent organic pollutants indicate important perturbations in sphingolipids and
332 glycerophospholipids levels. *Chemosphere*. 2017;168:731-8.
- 333 19. Chen C-HS, Yuan T-H, Shie R-H, Wu K-Y, Chan C-C. Linking sources to early
334 effects by profiling urine metabolome of residents living near oil refineries and coal-fired
335 power plants. *Environ Int*. 2017;102:87-96.
- 336 20. Eguchi A, Sakurai K, Watanabe M, Mori C. Exploration of potential biomarkers and
337 related biological pathways for PCB exposure in maternal and cord serum: a pilot birth cohort
338 study in Chiba, Japan. *Environ Int*. 2017;102:157-64.
- 339 21. Khan A, Park H, Lee HA, Park B, Gwak HS, Lee H-R, et al. Elevated metabolites of
340 steroidogenesis and amino acid metabolism in preadolescent female children with high
341 urinary bisphenol A levels: a high-resolution metabolomics study. *Toxicol Sci*. 2017. DOI
342 10.1093.
- 343 22. Laine JE, Bailey KA, Olshan AF, Smeester L, Drobna Z, Styblo M, et al. Neonatal
344 metabolomic profiles related to prenatal arsenic exposure. *Environ Sci Technol*.
345 2017;51(1):625-33.
- 346 23. Li H, Wang M, Liang Q, Jin S, Sun X, Jiang Y, et al. Urinary metabolomics revealed
347 arsenic exposure related to metabolic alterations in general Chinese pregnant women. *J*
348 *Chromatogr A*. 2017;1479:145-52.
- 349 24. Dudka I, Kossowska B, Senhadri H, Latajka R, Hajek J, Andrzejak R, et al.
350 Metabonomic analysis of serum of workers occupationally exposed to arsenic, cadmium and
351 lead for biomarker research: A preliminary study. *Environ Int*. 2014;68:71-81.

- 352 25. Kuo CH, Wang KC, Tian TF, Tsai MH, Chiung YM, Hsieh CM, et al. Metabolomic
353 characterization of laborers exposed to welding fumes. *Chem Res Toxicol.*
354 2012;25(3):676-86.
- 355 26. Wei Y, Wang Z, Chang C, Fan T, Su L, Chen F, et al. Global metabolomic profiling
356 reveals an association of metal fume exposure and plasma unsaturated fatty acids. *PLoS ONE.*
357 2013;8(10):e77413.
- 358 27. Hosnijeh FS, Pechlivanis A, Keun HC, Portengen L, Bueno-de-Mesquita HB,
359 Heederik D, et al. Serum metabolomic perturbations among workers exposed to 2,3,7,8-
360 tetrachlorodibenzo-p-dioxin (TCDD). *Environ Mol Mutagen.* 2013;54(7):558-65.
- 361 28. Jeanneret F, Boccard J, Badoud F, Sorg O, Tonoli D, Pelclova D, et al. Human urinary
362 biomarkers of dioxin exposure: analysis by metabolomics and biologically driven data
363 dimensionality reduction. *Toxicol Lett.* 2014;230(2):234-43.
- 364 29. Walker DI, Uppal K, Zhang L, Vermeulen R, Smith M, Hu W, et al. High-resolution
365 metabolomics of occupational exposure to trichloroethylene. *Int J Epidemiol.*
366 2016;45(5):1517-27.
- 367 30. Kaluarachchi MR, Boulange CL, Garcia-Perez I, Lindon JC, Minet EF. Multiplatform
368 serum metabolic phenotyping combined with pathway mapping to identify biochemical
369 differences in smokers. *Bioanalysis.* 2016;8(19):2023-43.
- 370 31. Fall T, Salihovic S, Brandmaier S, Nowak C, Ganna A, Gustafsson S, et al. Non-
371 targeted metabolomics combined with genetic analyses identifies bile acid synthesis and
372 phospholipid metabolism as being associated with incident type 2 diabetes. *Diabetologia.*
373 2016;59(10):2114-24.

- 374 32. Park WH, Kang S, Lee HK, Salihovic S, Bavel B van, Lind PM, et al. Relationships
375 between serum-induced AhR bioactivity or mitochondrial inhibition and circulating
376 polychlorinated biphenyls (PCBs). *Scientific Reports*. 2017;7:9383.
- 377 33. Bonvallet N, Canlet C, Blas-Y-Estrada F, Gautier R, Tremblay-Franco M, Chevolleau
378 S, et al. Metabolome disruption of pregnant rats and their offspring resulting from repeated
379 exposure to a low dose of a pesticide mixture representative of environmental contamination
380 in Brittany. Under review.
- 381 34. Cabaton NJ, Canlet C, Wadia PR, Tremblay-Franco M, Gautier R, Molina J, et al.
382 effects of low doses of Bisphenol A on the metabolome of perinatally exposed CD-1 mice.
383 *Environ Health Perspect*. 2013. 121(5):586-93.
- 384 35. Tremblay-Franco M, Cabaton NJ, Canlet C, Gautier R, Schaeberle CM, Jourdan F, et
385 al. Dynamic metabolic disruption in rats perinatally exposed to low doses of Bisphenol A.
386 *PLoS ONE*. 2015;10(10):e0141698.
- 387 36. David A, Abdul-Sada A, Lange A, Tyler CR, Hill EM. A new approach for plasma
388 (xeno)metabolomics based on solid-phase extraction and nanoflow liquid chromatography-
389 nanoelectrospray ionisation mass spectrometry. *J Chromato A*. 2014;72-85.
- 390 37. David A, lange A, Abdul-Sada A, Tyler CR, Hill EM. Disruption of the prostaglandin
391 metabolome and characterization of the pharmaceutical exposome in fish exposed to
392 wastewater treatment works effluent as revealed by nanoflow-nanospray mass spectrometry-
393 based metabolomics. *Environ. Sci. Technol*. 2017;51(1):616-24.
- 394 38. Chetwynd AJ, David A, Hill EM, Abdul-Sada A. Evaluation of analytical performance
395 and reliability of direct nanoLC-nanoESI-high resolution mass spectrometry for profiling the
396 (xeno)metabolome. *J Mass Spectrom*. 2014;49(10):1063-9.

- 397 39. Southam AD, Lange A, Al-Salhi R, Hill EM, Tyler CR, Viant MR. Distinguishing
398 between the metabolome and xenobiotic exposome in environmental field samples analysed
399 by direct-infusion mass spectrometry based metabolomics and lipidomics. *Metabolomics*.
400 2014;1050-8.
- 401 40. Crockford DJ, Maher AD, Ahmadi KR. ¹H NMR and UPLC-MS(E) statistical
402 heterospectroscopy: characterization of drug metabolites (xenometabolome) in
403 epidemiological studies. *Anal Chem*. 2008;6835-44.
- 404 41. Jamin E, Bonvallet N., Tremblay-Franco M, Cravedi JP, Chevrier C, Cordier S, et al.
405 Untargeted profiling of pesticide metabolites by LC-HRMS: an exposomics tool for human
406 exposure evaluation. *Anal Bioanal Chem*. 2014;406:1149-61.
- 407 42. Darde T, Lecluze E, Becker E, Potdevin V, Collin O, Jégou B, et al. ChemPSy:
408 Identification, classification and prioritization of novel endocrine disruptors by integrating
409 massive toxicogenomics datasets. Ten years of "Programme National de Recherche -
410 Environnement Santé Travail, ANSES; Paris, November 2016.
- 411 43. Darde T, Gaurdiault P, Beranger R, Lancien C, Caillarec-Joly A, Sallou O, et al.
412 TOXsIgN: a cross-species repository for toxicogenomics signatures. *In revision*.
- 413 44. National Academies of Sciences, Engineering, and Medicine. Using 21st century
414 science to improve risk-related evaluations. 2017. Washington, DC: The National Academies
415 Press. <https://doi.org/10.17226/24635>.

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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	1 st 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	1 st morning spot urine, 1 st 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)	1 st 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	1 st 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)	1 st 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)	1 st 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	1 st 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	1 st 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	1 st 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	1 st 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	1 st morning spot urine, 1 st 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)	1 st 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, vanillic 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/ nitrate 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 \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 PM_{2.5} (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 ursodeoxycholic acid sulfate, glucuro- and sulfoconjugates of glycochenodeoxycholic, glucuroconjugated forms of hydroxyandrosterone or hydroxyetiocholanolone, hydroxyDHEA or hydroxytestosterone, andestrone, glyoursodeoxycholic 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 3 β -sulfate, androsterone 3 α -glucuronide, androsterone 3 α -sulfate, pregnanediol 3 α -glucuronide, 11-ketoetiocholanolone 3 α -glucuronide, glucuronide conjugates of 11 β -hydroxyandrosterone, glycochenodeoxycholic acid, glycocholic acid and glyoursodeoxycholic 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, N ⁶ -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. 7 α -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, 22 β -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-glycolylneuraminic acid ⁽⁻⁾ , fructose 6-phosphate, D-phenylalanine, phenylacetylglutamine, 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.