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1 **Title** Chronic oral exposure to pesticides and their consequences on metabolic regulation: role
2 of the microbiota

3

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19

20 **Abstract**

21 Pesticides have long been used in agriculture and household treatments. Pesticide residues can
22 be found in biological samples for both the agriculture workers through direct exposure but also
23 to the general population by indirect exposure. There is also evidence of pesticide
24 contamination in utero and trans-generational impacts.

25 Whilst acute exposure to pesticides has long been associated with endocrine perturbations,
26 chronic exposure with low doses also increases the prevalence of metabolic disorders such as
27 obesity or type 2 diabetes. Dysmetabolism is a low-grade inflammation disorder and as such
28 the microbiota plays a role in its etiology. It is therefore important to fully understand the role
29 of microbiota on the genesis of subsequent health effects. The digestive tract and mostly
30 microbiota are the first organs of contact after oral exposure. The objective of this review is
31 thus to better understand mechanisms that link pesticide exposure, dysmetabolism and
32 microbiota.

33 One of the key outcomes on the microbiota is the reduced Bacteroidetes and increased
34 Firmicutes phyla, reflecting both pesticide exposure and risk factors of dysmetabolism. Other
35 bacterial genders and metabolic activities are also involved. As for most pathologies impacting
36 microbiota (including inflammatory disorders), the role of prebiotics can be suggested as a
37 prevention strategy and some preliminary evidence reinforce this axis.

38 **Keywords**

39 Microbiota, pesticides, metabolic syndrome, prebiotic, dysbiosis, low dose

40

41 **Introduction**

42 There is a wide range of environmental pollutants from agriculture. In 2015, pesticide purchases
43 increased to reach 68 thousand tons, making France the third largest user of pesticides in the
44 world and the largest user in Europe. As an example, the region of Picardy is known for its
45 excessive consumption of pesticides of about 3,900 tonnes annually used in the agricultural
46 sector [1]. Pesticides are therefore present in our environment and can be absorbed by exposure
47 to aerosols (skin, respiratory tract) or ingested with food via our digestive tract, thus interacting
48 with the intestinal microbiota.

49 Microbiota acts as an interface between food and digestive cells and thus plays a role in the
50 maturation and digestive function of the intestine. In addition, the intestinal microbiota allows
51 the assimilation of nutrients, helps digest fibres, participates in the synthesis of certain vitamins
52 and regulates the absorption of fatty acids, calcium and magnesium [2–4]. It also stimulates
53 immune defences, and acts as a protective barrier against pathogenic microbes and toxins [5,
54 6]. The composition and diversity of the microbiota change in successive stages. At birth, the
55 newborn passes from the in-utero compartment (often considered sterile) into an environment
56 rich in microorganisms. By the age of 3, the composition and diversity of the microbiota are
57 more stable stabilized and subsequently remain relatively stable throughout life, although some
58 changes have been observed in the elderly [7]. The microbial environment can nevertheless be
59 disturbed from time to time by factors such as antibiotic treatments, immune system failure,
60 infections, changes in dietary habits, and other changes to the environment. This disturbance,
61 also called dysbiosis, is often characterized by a decrease in the numbers of beneficial
62 microorganisms and a simultaneous increase in the number of potentially pathogenic
63 microorganisms [8, 9]. Like any ecosystem, the intestinal microbiota is usually able to return
64 to its initial state a few weeks after a disturbance; this phenomenon is referred to as resilience

65 [10]. In contrast, persistent instability or imbalance (i.e. intestinal dysbiosis) has often been
66 linked to pathologies such as inflammatory bowel disease [11] [12], obesity, and diabetes [8,
67 13].

68 The aim of this review is therefore to better understand the role of the microbiota as a link
69 between pesticide exposure and metabolic perturbations. We will first present an update on the
70 relationship between pesticides and metabolic dysfunction, with a focus on diabetes and
71 obesity. The second stage of this review will focus on available data on pesticides and
72 microbiota, with a focus on those parameters associated with metabolic regulation.

73 **1- Exposure to pesticides and dysmetabolism**

74 Thousands of various chemicals are used as pesticides. This term refers to any substance used
75 to control harmful organisms. It encompasses herbicides, fungicides, insecticides and
76 antimicrobials. The agricultural use is by far the most important consumption of pesticides, but
77 they are also used in industry and homes. The most frequently used chemical families are
78 organochlorines (OC), organophosphates (OP), carbamates, triazines and pyrethroids [14].
79 Although their main role is protection of the crops, pesticides have side effects and they
80 constitute a significant and persistent threat to the environment and to the health of living beings
81 through their residues which are detected in food, water and ground [15].

82 Air, water, soil, food ... pesticides are omnipresent in our daily life. They thus become diffuse
83 and permanent pollution. This chronic exposure to various pesticides, even at low doses, raises
84 the question of the cumulative effect and the cocktail effect. By encountering other molecules,
85 they can combine; their effects become more pronounced and be much more dangerous, just as
86 they can aggravate some existing health. [16, 17]

87 Pesticides are persistent organic pollutants with low degradation power: they can easily impact
88 the human digestive system through daily food. Even at low doses of exposure they cause
89 undesirable effects. They are considered carcinogenic, mutagenic or reprotoxic following acute
90 exposure [18]. They are also part of the large family of endocrine disruptors, Behind these
91 terms, a multitude of pathologies such as Parkinson's disease, various cancers, malformations,
92 infertility ...). [19, 20].

93 Of particular interest is the impact of a pesticide on the digestive tract, as it is the first organ in
94 contact with ingested food contaminants. As an example, exposure to the organophosphate
95 chlorpyrifos (CPF) early in foetal development disrupts the environment, permeability and

96 functions of the intestine [21], associated with disruption of metabolic regulation, including
97 prediabetics parameters [16]. Understanding the potential impact on human health of exposure
98 to pesticides is important from a public health perspective, especially in light of a potential link
99 to obesity and diabetes.

100 **1.1- Epidemiological evidence for dysmetabolism associated with exposure to pesticides**

101 When studying the impact of pesticides on human health, there have been a number of
102 demonstration of the link between exposure to phytosanitary substances and obesity, as
103 examples with the organochlorine dichloro-diphenyl-trichloroethane (DDT) [22] or the
104 organophosphate glyphosate [23]. Obesity is characterized by the expansion of the mass of
105 adipose tissue, which is often accompanied by metabolic dysfunctions. The results of a meta-
106 analysis, including 8 prospective epidemiological studies in the United States, Mexico and
107 various European countries, demonstrated a significant positive association between exposure
108 to the pesticide DDT and its metabolite dichlorodiphenyldichloroethylene (DDE) and adiposity
109 [24]. The relationship was evaluated in 6,770 children and young adults aged 6 to 19. The
110 relationship was strong overall, but inconclusive when adjusted for lipid intake. According to
111 data from the 2003-2004 and 2005-2006 National Health and Nutrition Survey (NHANES),
112 residues of 2,5-dichlorophenol but not 2,4-dichlorophenol in urine were associated with an
113 increase in obesity in adults and children [25].

114 Epidemiological evidence of transgenerational impact has been described in the literature with
115 increased risks of childhood obesity or in adulthood after maternal exposure to DDT [26, 27].
116 The link was not found for prenatal exposure to organophosphorus pesticides and childhood
117 adiposity, although there was a moderate increased risk for slow OP metabolizers [28].
118 Polymorphism in metabolizing enzymes was again shown to influence risks of childhood
119 obesity after prenatal exposure to pesticides [29]. These data support the extension of the
120 developmental origins of health and disease (DOHAD) hypothesis to environmental exposures
121 in early life.

122 In addition, most of the literature on pesticide exposure and chronic metabolic disorders
123 concerns the increased incidence of type 2 diabetes. Environmental chemicals can increase the
124 risk of diabetes due to effects on glucose metabolism, mitochondrial dysfunction, and endocrine
125 disruption mechanisms. The application of pesticides presents a significant risk, it has been
126 shown that exposure to pesticides can lead to abnormal glucose metabolism, increasing the risk
127 of diabetes in agricultural populations. Long-term exposure from handling certain pesticides,

128 especially OC and OP insecticides, may be associated with an increased risk of diabetes [30].
129 A study that included 33,457 approved applicators enrolled in the agricultural health study
130 showed the effects of using the OC insecticides aldrin, chlordane and heptachlor for more than
131 100 days had an increased risk of diabetes of 51%, 63% and 94%, respectively [31]. A recent
132 study suggests that the onset of diabetes in Thai farmers (866 participating cases with diabetes
133 mellitus and 1021 healthy controls) was associated with exposure to pesticides [32]. Of the 35
134 individual pesticides studied, those with a statistically significant increased risk of diabetes
135 were endosulfan, mevinphos, carbaryl, and benlate. This finding is consistent with previous
136 epidemiological studies. Work in 187 farmers occupationally exposed to anti-cholinesterase
137 OPs demonstrated that these users are susceptible to diabetes [33]. Fasting blood sugar,
138 cholesterol, and glucose tolerance were significantly elevated. Amongst OP, monocrotophos
139 (MCP), CPF and methyl parathion showed significant correlation and regression with HbA1c
140 values in pesticide users in India [34]. Another study included 98 non-diabetic farmers who
141 handle agricultural insecticides during their fieldwork, the period of exposure to agricultural
142 pesticides was 15 to 20 years to reveal that there was a positive correlation between the
143 concentration blood malathion, waist circumference and insulin resistance. It was also observed
144 that the increase in the average values of the waist circumference and the body mass index was
145 accompanied by a significant increase in the average values of the blood concentration of
146 malathion. These results suggest that chronic exposure to malathion in non-diabetic farmers
147 may induce insulin resistance [35]. A large prospective cohort study Agricultural Health Study
148 was launched in the USA in North Carolina and in Iowa. 26,235 people were enrolled in the
149 study, including 19,776 registered pesticide applicators and 6,459 spouses of registered
150 agricultural applicators. Its aims were to see how agriculture, lifestyle and genetic factors
151 influence the health of populations [36]. Data from 13,637 farm wives who reported having
152 personally mixed or applied pesticides at enrolment provided comprehensive data on the
153 diagnosis of diabetes. Five pesticides were positively associated with diabetes, including three
154 organophosphates, fonofos, phorate and parathion [37]. On the other hand, a study composed
155 of 150 pesticide sprayers and 150 non-agricultural workers aged 40 to 60 in Thailand aimed to
156 assess the association between exposure to organophosphates and insulin resistance. Overall,
157 no association between OP exposure and insulin resistance in pesticide sprayers and the general
158 population [38].

159 It is important therefore to understand if the dysmetabolic risks are shared equally with the
160 general population, not directly exposed to high concentrations of the pesticides. In Japan, a

161 study was carried out on 1374 participants, who were not directly exposed to 10 polychlorinated
162 dibenzo-p-dioxins (PCDD), 7 polychlorinated dibenzofurans (PCDF) and 12 dioxin-like
163 polychlorinated biphenyls (DL-PCB) (Uemura Hirokazu et al. 2009; Uemura et al. 2008).
164 Blood and lipid concentrations of residues of PCDD, PCDF and DL-PCB were significantly
165 associated with metabolic syndrome. High blood pressure, high triglycerides, and glucose
166 intolerance were most closely associated with these pollutants, suggesting that the body burden
167 levels of dioxins, particularly those of DL-PCB, are associated with the metabolic syndrome.
168 PCB and PCDF are important and persistent organic pollutants (POP) in humans.
169 Polychlorinated biphenyls (PCB) were also associated with increased incidence of diabetes in
170 the Anniston cohort in the United States [39] and this was significant in women and population
171 under 55 years of age. This increased incidence of diabetes in women with the highest PCB
172 residues was also observed in the Yucheng "petroleum disease" cohort in Taiwan. Yucheng
173 women who had previously been exposed to PCB and PCDF, suffered from an increased
174 incidence of diabetes [40]. In an exploratory study set in Korea, low-dose background exposure
175 to OC pesticides was strongly associated with type 2 diabetics, although the absolute
176 concentrations of OC pesticides were not higher than in control non-diabetic population [41].
177 The association between diabetes and serum levels of PCB, DDE, hexachlorobenzene (HCB)
178 and mirex has been studied in a 352 adult Native American (Mohawk) population, indicating
179 once again that exposure to OC compounds increases the risk of developing diabetes [42].

180 Finally, researchers examined the association between pesticide use during pregnancy and
181 gestational diabetes mellitus (GDM) in 11,273 wives of licensed pesticide applicators [43]. The
182 results suggest that activities involving exposure to agricultural pesticides specifically four
183 herbicides (2,4,5-Trichlorophenoxyacetic acid; fenoprop; atrazine; or butylate) and three
184 insecticides (diazinon, phorate, or carbofuran) during the first trimester of pregnancy can
185 increase the risk of GDM. The MIREC (Maternal-Infant Research on Environmental
186 Chemicals) study included 1,274 women who had had a single birth and did not have pre-
187 existing diabetes to determine associations between pesticides, perfluoroalkyl substances
188 (PFAS) and PCB measured in early pregnancy and impaired glucose tolerance and GDM [44].
189 In this study the evidence was only consistent for positive associations between the chemicals
190 that examined and GDM or impaired glucose tolerance (IGT) during pregnancy.

191

192 **1.2- Mechanisms of dysmetabolism associated with pesticides**

193 As presented in the previous section, growing evidence links widespread exposure to pesticides
194 and global epidemics of type 2 diabetes and obesity. But do we have further indications from
195 preclinical studies on the mechanisms involved? We will be concentrating on selected
196 compounds, namely members of the OP pesticides, for this section. Their chemical structures
197 are shown in figure 1.

198 **Monocrotophos, a phosphate**

199 Monocrotophos (0.9 and 1.8 mg/kg b.w.) treatment of adult Wistar rats over 180 days caused a
200 dose and time-dependent increase in blood glucose response and insulin resistance through
201 pancreatic beta-cells dysfunction. This was associated with a non-significant increase on body
202 weight but a significant and dose-dependent increase in the weight of the white fat pads and an
203 increase in the diameter of the pancreatic islets, all of which led to hyperglycemia,
204 hyperinsulinemia and dyslipidemia [45].

205 **Chlorpyrifos, a thiophosphate**

206 Wistar rats to a dose of 3.5 mg / kg b.w. CPF from gestation to adulthood has effects on the
207 body weight and organophosphates such as CPF may increase the risk of diabetes mellitus [30].
208 Animals showed increased glycemia associated with decreased fasting insulin without affecting
209 lipid profiles at 60 days of age. Elderly male apoE3-TR and C57BL/6N mice were orally
210 exposed to CPF at 2 mg / kg b.w. for 8 consecutive weeks. The results of the study show that
211 long-term exposure to CPF increased significantly fasting glucose and cholesterol in plasma as
212 well as insulin resistance markers. CPF also induced higher body weight gain and food intake,
213 possibly through hormonal regulation of leptin and ghrelin [46].

214 HepG2 hepatocarcinoma cells were treated with different concentrations of CPF (10 to 2500
215 μM) or cadmium (Cd) (5 to 100 μM) for 24 h. The CPF-Cd complex induced an accumulation
216 of lipids (triglycerides and cholesterol) in the hepatocytes, whereas the CPF or cadmium
217 administered individually did not have such an effect [47]. To determine whether CPF exposure
218 increased intracellular lipid accumulation, McA-RH7777 hepatoma cells were incubated at
219 doses of 1.25 to 80 μM at CPF for 48 h, which confirmed the hypothesis of significant increase
220 in intracellular accumulation of neutral lipids associated with increased de novo lipogenesis and
221 decreased fatty acid-induced triglyceride secretion with unchanged fatty acid oxidation [48].

222 **Malathion, a dithiophosphate**

223 Male Sprague-Dawley rats fed malathion at doses of 100, 500 and 1500 ppm for 4 weeks
224 induced neurotoxic depression of cholinesterase activity together with induction of oxidative
225 stress as measured by lipid peroxidation markers [49]. At the lower dose of 100, 200 and 400
226 ppm for 4 weeks plasma glucose and hepatic glycogenolysis and gluconeogenesis enzymes
227 were increased, although the effect was not dose-dependent [50]. Similarly, when malathion
228 was fed to Wistar rats at the dose of 100 mg/kg b.w. for 32 days, it increased fasting glucose
229 levels and muscle and liver glycogen rate whilst reducing hepatic protein and lipid rate [51].
230 Exposure of rats to malathion may thus induce diabetes associated with stimulation of hepatic
231 gluconeogenesis and glycogenolysis in favour of the release of glucose into the blood.

232 In vitro treatment of primary culture of pancreatic islet cells with malathion concentrations
233 ranging from 25 to 625 $\mu\text{g} / \text{mL}$ led to increased insulin content at 5 h, independent of the dose
234 [52]. This was however associated with time-dependent accumulation of glucagon in the islet
235 cells but reduction of insulin secretion with both low and high glucose stimulation.

236 **Acephate, a phosphoramidate**

237 Wistar rat were exposed orally to 2.5 mg / kg b.w. acephate during pregnancy (day 7 of
238 gestation) and lactation (day 21 of lactation), causing increased body weight gain and food
239 intake in the dams, mostly during the lactation phase [53]. Intraperitoneal glucose tolerance
240 tests increased glycemic peak both during pregnancy and lactation, suggesting signs of
241 gestational diabetes mellitus. Interestingly, offsprings were born with a significant weight
242 deficiency compared to control animals but this was reversed (although not significantly) at 90
243 days of age, associated with increased food intake after weaning. Similarly, whilst fasting
244 glucose was not affected at weaning time (21 days of age), all glycemic and lipidic markers
245 were affected at 90 days. This indicates that alterations in maternal metabolism of glucose could
246 program the offspring to be susceptible to type 2 diabetes as adults, even if exposure to the
247 pesticide is only through perinatal maternal interactions.

248 **Glyphosate, a glycine-derivative OP**

249 3T3-L1 fibroblasts are an excellent model for studying adipocyte differentiation [54].
250 glyphosate treatment during exponential growing phase strongly reduced cell proliferation and
251 induced apoptosis in a dose and time-dependent manner. Interestingly, when freed from the
252 pesticide treatment, and after a lag time of 24 hours, the cells started proliferating again,
253 indicating the reversibility of the pesticide effect. Glyphosate, finally, prevented 3T3-L1 cells
254 to differentiate into adipocytes but this could again be reversed if pesticide treatment was

255 interrupted for the final step of differentiation. Same authors later observed that fibroblast to
256 adipocyte differentiation was prevented by glyphosate through inhibition of PPAR γ , a
257 transcription factor involved in adipogenesis [55]. They further demonstrated an increased lipid
258 peroxidation and oxidative stress enzymes such as superoxide dismutase (SOD) which is
259 indicative of its ability to modify cell physiology.

260 Overall and despite some chemical specificities, the impact of OP on weight gain and glucose
261 metabolism seems to find its origins in perturbation at molecular levels through insulin
262 secretion from pancreatic cells, lipogenesis in hepatic cells or oxidative stress in adipocytes.
263 The role of PPAR γ may be of special interest as this transcription factor is involved in a large
264 number of physiological functions, especially when considering energy regulation. The role of
265 pesticides on the hormonal regulation of appetite and food intake is also a target of interest.

266

267 **2- Growing interest in the role of the microbiota**

268 Obesity now affects almost the entire planet, and the increase in the prevalence of obesity over
269 the past 25 years is such that it cannot be due to genetic factors alone. Unfortunately, medical
270 treatments fail in the long term because they do not adapt to recent changes in lifestyles [56].
271 Thus, new and alternative strategies are urgently needed for the prevention and long-term
272 treatment of obesity.

273 The gut microbiota has been recognized as an important pathophysiological factor in the
274 development and maintenance of obesity [56]. The human intestinal microbiota is a very
275 complex entity, composed mainly of more than 10^{14} bacteria and archaea as well as viruses,
276 fungi and protozoa. Bacteria belonging to five phyla dominate the microbiota, i.e. *Firmicutes*,
277 *Bacteroidetes*, *Actinobacteria*, *Proteobacteria* and *Verrucomicrobia* [57]. Normally, the gut
278 microbiota has a particular distribution along the intestine as its density increases from the
279 proximal intestine to the distal intestine (bacteria are rare in the stomach, duodenum and
280 jejunum, while their concentration in the ileum goes up to 10^7 cells per gram of luminal content
281 and in the colon goes up to 10^{12} cells per gram of luminal content). The intestinal microbiota is
282 a dynamic entity. It is changed by diet, lifestyle, antibiotics, and genetic makeup [57].

283

284 **2.1 Microbiota in the dysmetabolic response**

285 The important role of the gut microbiota in maintaining human health has required a better
286 understanding of the temporal dynamics of gut microbial communities as well as the host and
287 environmental factors behind these dynamics. Genetics, birth pattern, infant feeding patterns,
288 use of antibiotics, sanitary living conditions, and long-term eating habits all help shape the
289 makeup of the gut microbiota [58]. Significant weight changes have been associated with
290 microbial instability. Long-term weight loss has been shown to alter the ratio of *Bacteroidetes*
291 and *Firmicutes*, the two major bacterial phyla residing in the human gastrointestinal tract [58].

292 Obesity has a microbial component, which could have potential therapeutic implications [59].
293 A quantitative and qualitative imbalance of the intestinal microbiota (dysbiosis) is probably the
294 basis of many disorders, including obesity [60]. Analysis of 154 individuals yielded 9,920
295 nearly complete bacterial 16S rRNA sequences and 1,937,461 partial sequences, plus 2.14
296 gigabases of their microbiomes. The results reveal that the human gut microbiota is shared
297 among family members, but each person's gut microbial community varies in the specific
298 bacterial lineages present. Obesity is associated with variations in the phylum in the microbiota,
299 with reduced bacterial diversity [61].

300 The relative proportion of *Bacteroidetes* is decreased whilst *Firmicutes* population is increased
301 in obese people compared to lean people, and this proportion increases with weight loss [59,
302 60, 62]. The other phyla also play a role in obesity. Clarke et al reported that the gut microbiota
303 in obese people contained a lower proportion of *Verrucomicrobia* [63]. This was confirmed by
304 a study concluding that obesity is probably linked to a higher proportion of *Actinobacteria* and
305 *Firmicutes*, with a decrease in *Bacteroidetes*, *Verrucomicrobia* and *Faecalibacterium*
306 *prausnitzii* [64]. To confirm the intestinal alterations observed and test whether the
307 *Lactobacillus* or *Bifidobacterium* species found in the human intestine are associated with
308 obesity or lean status, the stools of 68 obese and 47 controls are analyzed targeting *Firmicutes*,
309 *Bacteroidetes*, *Methanobrevibacter smithii*, *Lactococcus lactis*, *Bifidobacterium animalis* and
310 seven species of *Lactobacillus*. Reduced levels of *M. smithii* were confirmed to be associated
311 with obesity, and higher levels of *B. animalis*, *L. paracasei* or *L. plantarum* were associated
312 with normal weight while higher levels of *L. reuteri* were associated with obesity (Million et
313 al. 2012). A reduced number of *Bifidobacterium* and *Bacteroides* and an increased number of
314 *Staphylococcus*, *Enterobacteriaceae* and *Escherichia coli* have been detected in overweight
315 women compared to pregnant women of normal weight. The numbers of *E. coli* were higher in
316 women with excessive weight gain than in women with normal weight gain during pregnancy,
317 while *Bifidobacterium* and *Akkermansia muciniphila* showed the opposite trend [67]. However,

318 these results have been contradicted by other studies. Duncan et al monitored major groups of
319 fecal bacteria using fluorescent in situ hybridization (FISH) in obese and non-obese subjects
320 under weight maintenance conditions, and in obese male volunteers undergoing weight loss for
321 4 weeks [68]. They did not indentify relationship between BMI or absolute weight loss and the
322 relative populations of major bacterial groups, including *Bacteroidetes*. Another study showed
323 no difference between obese and non-obese individuals in the proportion of *Bacteroidetes*
324 measured in fecal samples, and no significant change in the percentage of *Bacteroidetes* in the
325 feces of obese dieters [69]. *Bacteroides* and *Staphylococci* were significantly higher in
326 overweight women than in women of normal weight. Maternal weight and BMI before
327 pregnancy correlated with higher concentrations of *Bacteroides*, *Clostridium*, and
328 *Staphylococcus* [70]. *Bacteroidetes* increased during the weight loss period following gastric
329 bypass surgery [62]. The relative proportion of *Bacteroidetes* to *Firmicutes* increases with
330 weight loss [59]. More specifically, weight loss programs in adolescents led to an increase in
331 the number of *Bacteroides* and *Lactobacillus* group and a decrease in the *Clostridium*
332 *coccoides*, *Bifidobacterium longum* and *Bifidobacterium adolescentis* group [71]. It has also
333 been associated with a decrease in the amounts of *Bifidobacterium* and *Ruminococcus* [72, 73].
334 *Akkermansia muciniphila* is a recently identified mucin-degrading bacterium living in the
335 mucus layer and accounts for 3-5% of community bacteria. *A. muciniphila* is associated with a
336 healthier metabolic state and better clinical outcomes after caloric restriction in overweight or
337 obese adults. Further results indicate that an increased abundance of *Akkermansia muciniphila*
338 in obese or overweight subjects exposed to a low-calorie diet is associated with healthier
339 metabolic condition, including improved lipid and carbohydrate metabolism and decreased
340 body fat from the waistline [74]. Several lesser-known bacteria, namely *Desulfovibrionaceae*,
341 have been positively associated with obesity or type 2 diabetes. This indicates the importance
342 of certain genera or species which exist in small proportions in the pathogenesis of obesity [74].
343 Recent studies have shown changes in the communities that are part of the gut microbiota of
344 individuals who achieve weight loss with an increase in certain species of the genus *Bacteroides*
345 (*Bacteroides fragilis*), *Lactobacillus* and *Prevotella* in parallel with a reduction in species of
346 the genus *Bifidobacterium* (*B. longum*), *Clostridium* (*C. coccoides*, *C. histolyticum*),
347 *Eubacterium* (*E. rectale*) and *Akkermansia* [66].

348 Finally, Type 2 diabetes in humans is also associated with changes in the composition of the gut
349 microbiota. The proportions of *Firmicutes* branch and *Clostridia* class were significantly
350 reduced in the diabetic group compared to the control group. In addition, the ratios of

351 *Bacteroidetes* to *Firmicutes* as well as the ratios of the *Bacteroides-Prevotella* group to *C.*
352 *coccoides-E. rectale* group positively and significantly correlated with plasma glucose
353 concentration but not with BMI [75]. This seems in opposition to the modulations linked to
354 obesity.

355

356 **2.2 Exposure to pesticides and microbiota**

357 As mentioned earlier, there is a wide variety of pesticide families. Due to their chemical
358 structures, they can play different roles in the microbiota. Little is known about the
359 epidemiological evidence for the modulation of the microbiota by pesticides, but from the first
360 part of this review it is likely that we will find commonalities between pesticide-induced
361 dysbiosis and dysbiosis associated with obesity. Some evidence can be drawn from *in vivo* and
362 *in vitro* experiments [76].

363 Organophosphates (OPs) have been widely used since the 1950s, resulting in biologically
364 relevant and toxic consequences on host health [34].

365 Chlorpyrifos is a typical OP insecticide commonly used to treat fruits, vegetable crops and
366 vineyards, metabolized to a more toxic molecule, 3,5,6-trichloro-2-pyridinol, by
367 biotransformation and which can lead to deterioration of host metabolism, increased bacterial
368 translocation and changes in the composition of the microbiota [77–79]. Perinatal exposure to
369 CPF (5mg / kg b.w.) reduced the body weight and length of the pups and inhibited their
370 intestinal development [77]. In addition, CPF (1 to 5 mg / kg b.w.) induced higher levels of
371 *Bacteroides*, *Enterococcus* and *Clostridium*, but lower levels of *Lactobacillus* spp and
372 *Bifidobacterium* spp in rat intestines [30, 77]. In mice orally exposed to CPF (1 mg / kg b.w.)
373 from postnatal day 10 (PND) to PND 15, *Akkermansia muciphila* exhibited greater abundance
374 in the APOE4 genotype, but was reduced by exposure to CPF [80]. The effect of chronic
375 exposure to CPF at a dose of 1 or 5 mg was studied in the Simulated Human Intestinal Microbial
376 Ecosystem Model (SHIME®) inoculated with healthy human faeces for up to 60 days.
377 Exposure to CPF induces community microbial dysbiosis associated with proliferation of
378 *Enterobacteriaceae*, *Bacteroides* spp and decreased levels of *Lactobacillus* spp and
379 *Bifidobacterium* spp [16, 77].

380 Glyphosate is the active component of Roundup (Monsanto), the most widely used herbicide
381 in the world. The growth of *Enterococcus faecalis* bacteria isolated from cattle and horse faeces

382 has been shown to be inhibited by low concentrations of glyphosate (450 mg / mL) for 5 days
383 [81].

384 Sensitivity to glyphosate depends on the bacterial strain. The effect of glyphosate on the poultry
385 microbiota (0.075 to 5 mg / mL) for 5 days has been demonstrated by the high resistance of
386 pathogenic bacteria, including *Salmonella enteritidis*, *Salmonella gallinarum*, *Salmonella*
387 *typhimurium*, *Clostridium perfringens* and *Clostridium botulinum*, and the increased
388 susceptibility of most beneficial bacteria such as *Enterococcus faecalis*, *Enterococcus faecalis*,
389 *Enterococcus faecalis badius*, *Bifidobacterium adolescentis* and *Lactobacillus* spp [82].

390 Other pesticides were studied and the main results are summarized in table 1 [34, 83] for clinical
391 studies and table 2 for preclinical evidence. Dysbiosis affecting the distribution of *Bacteroidetes*
392 to *Firmicutes* is also observed for DDT[84], cabendazim [85, 86], diazinon [87], or 2,3,7,8-
393 Tetrachlorodibenzofuran[88]. The decrease in *Verrucomicrobia* has also been observed with
394 DDT [84]. Other pesticides are presented as well [21, 34, 89–91]. This therefore confirms that
395 the dysmetabolism induced by the pesticides is at least partially linked to the dysbiosis during
396 the transit of the pesticide in the digestive tract. Some pesticides are metabolized and activated
397 by microbial activity, as appears to be the case for PCB or DDT. Regardless of the consequences
398 on dysmetabolism, there is therefore also a role of the microbiota in the biotransformation and
399 bioactivation of pesticides.

400

401 **2.3 Other consequences of dysbiosis and implications for dysmetabolism**

402 The CPF-induced dysbiosis of the microbiome altered the mucous barrier, increased bacterial
403 translocation in the spleen and liver, and stimulated the innate immune system [92, 93].

404 The weak mucosal barrier is one of the elements suggested by Cani et al linking dysbiosis and
405 obesity to low grade inflammation [94]. Obesity is associated with chronic low-grade systemic
406 inflammation that is due to an innate immune response to lipopolysaccharide (LPS)
407 translocation. It is an intrinsic constituent of the Gram-negative bacterial cell wall. It is
408 considered an endotoxin and is found in low concentrations in the blood of healthy people [95].

409 The composition of the gut microbiota has been shown to differ in lean and obese humans and
410 animals and change rapidly in response to dietary factors. A diet rich in fat changes the
411 composition of the intestinal microbiota leading to increased production and intestinal
412 permeability of LPS, leading to its high plasma concentration and the development of

413 "metabolic endotoxemia" [96]. Cani et al found that normal endotoxemia increased or
414 decreased during feeding or fasting, on a 4 weeks high fat diet chronically increased plasma
415 LPS concentration two to three times the threshold that defines metabolic endotoxemia [97].

416 Dysbiosis and subsequent LPS translocation have been shown to reduce the expression of host
417 genes that code for tight gut junction proteins like ZO-1 and occludin - necessary for the
418 standard character of gastrointestinal permeability [98]. Such an alteration in gastrointestinal
419 epithelial integrity induced by the microbiota could lead to intestinal translocation of the whole
420 bacterium. It was observed that in mice on a high fat diet, bacterial absorption is higher than in
421 mice on a standard diet [97, 98]. In a study on CPF (5 mg / kg b.w.) and intestinal permeability
422 during the pre and postnatal periods, epithelial homeostasis in the rat was disrupted, and organ
423 development and maturation was affected as well. There were significant changes in intestinal
424 tight junctions (TJ) gene expression, with downregulation of ZO-1 and occludin and
425 upregulation of claudins 1 and 4, and translocation bacterial to the spleen [93, 99].

426 LPS has been shown to induce low grade inflammation leading to the development of obesity.
427 In a comparative study, it was shown that when low doses of LPS were given to mice for 4
428 weeks, animals developed similar obesity at 4 weeks than a high fat diet [98]. Interestingly, a
429 high fat diet is not only directly responsible for obesity, but it also indirectly worsens it by
430 increasing the absorption of LPS endotoxins by integrating it into chylomicrons [98]. As a high
431 fat diet increases the formation of chylomicron, more chylomicron is available for LPS to be
432 integrated and therefore more absorption of this endotoxin compared to the low fat diet. As
433 obese people have more *Firmicutes* (Gram positive) and less *Bacteroidetes* (Gram negative)
434 than lean individuals, the origin of increased LPS may be questioned. Recent observations show
435 that the microbiota of obese people is rich in *Prevotellaceae* (a subgroup of *Bacteroidetes*),
436 which is a good source of LPS [98]. In obese people, dysbiosis appears to be related to increases
437 in the phylum *Firmicutes*, the genus *Clostridium*, and the species *Eubacterium rectale*,
438 *Clostridium coccoides*, *Lactobacillus reuteri*, *Akkermansia muciniphila*, *Clostridium*
439 *histolyticum* and *Staphylococcus aureus* [100].

440 Another consequence of dysbiosis is the altered production of short chain fatty acids (SCFA).
441 The total amount of SCFA was higher in obese than lean groups [101]. While propionate is
442 thought to be a beneficial SCFA, the authors also reported a modulation of the *Firmicutes* to
443 *Bacteroidetes* ratio in favour of *Bacteroidetes*, putting these results into question. The literature
444 and the results of the various studies are not so clear because SCFAs are produced by
445 fermentation of dietary fibers and are often associated with the prevention of several

446 pathologies linked to inflammation or oxidative stress [102]. Increased SCFA production
447 however may be linked to bacteria from the *Firmicutes* phylum with the ability to degrade
448 certain non-digestible polysaccharides into monosaccharides and SCFA which are taken up by
449 host, thus leading to increased energy uptake from molecules that would otherwise be excreted
450 in the stool [61, 103, 104]. It has been suggested that more efficient energy harvesting in obese
451 people is associated with hydrogen transfer between taxa.

452

453 **3- Food prevention through regulation of the microbiota**

454 Prebiotics are defined as "non-digestible food ingredients which selectively stimulate the
455 growth and activity of bacterial species already implanted in the colon, and thus improve the
456 health of the host" [105]. While any nutrient that enters the large intestine can have prebiotic
457 effects, the majority of known prebiotics are carbohydrates. Specifically, oligosaccharides like
458 inulin-type fructans and galactooligosaccharides (GOS) are well supported for their prebiotic
459 effects on microbial composition, mainly due to the increased proportions of *Bifidobacterium*
460 and *Lactobacillus* [106]. Thus, supplementation with prebiotics could provide substrates for the
461 fermentation of the gut microbiota and improve the composition of the microbiota, which will
462 ultimately be beneficial for the health of the host. Examples of substances with prebiotic
463 properties are fructooligosaccharides, gluco-oligosaccharides, isomaltooligosaccharides,
464 maltooligosaccharides, lactulose, soy raffinose oligosaccharides, stachyose, xylo-
465 oligosaccharides and inulin resistant starch. Recently, research has been carried out to confirm
466 the prebiotic properties of new substances such as resistant dextrins derived from potato starch.
467 These formulations have a bifidogenic effect and stimulate the growth of the intestinal
468 microbiota, thus limiting the growth of *Clostridium* strains [107, 108].

469 Data from metagenomic studies comparing the gut microbiota of healthy and diseased
470 individuals or exposed to a substance (e.g., MetaHit and HMP projects) have allowed
471 characterization of different bacterial groups or repressed species under specific pathological
472 conditions [107, 109]. Growth studies can then be performed under specific conditions to
473 identify substrates which can selectively stimulate the growth or activity of these bacteria, and
474 thus have the potential to correct the dysbiosis associated with the disease when administered
475 under form of prebiotics. Knowledge of specific bacterial species present in less or in
476 abundance in certain pathological states offers the opportunity to use prebiotics in a targeted
477 manner in order to offer them as a preventive strategy [107].

478 Few prevention studies have been performed to validate the potential mechanisms of action and
479 the feasibility of the prevention or curative action of prebiotics and what would be the required
480 dose in pesticide-induced dysmetabolism. However, some preliminary data can be used to
481 enhance the protective effect of dietary fiber following CPF treatment. Co-treatment with inulin
482 has been shown to prevent dysbiosis as well as early markers of dysmetabolism in rats
483 chronically exposed to CPF [30].

484 However there are numerous reports of prebiotics being used in weight management and
485 diabetes. Daily consumption of yacon syrup, providing 0.14 g / kg b.w. fructooligosaccharides
486 (FOS) resulted in a significant decrease in body weight, waist circumference and body mass
487 index as well as a decrease in insulin resistance [110]. It also increased the frequency of bowel
488 movements and the feeling of fullness while a dose of 0.29 g / kg b.w. FOS resulted in adverse
489 events including digestive perturbations. Weight reduction was observed also with 21 g
490 oligofructose for 12 weeks together with decreased glycemia and hormonal regulation of food
491 intake [111]. The concentration of GLP-1 and the concentrations of proglucagon mRNA were
492 significantly higher in fructan-fed rats compared to controls. After 8 h of food deprivation, the
493 active ghrelin in plasma remained significantly lower treatment rats than in control rats [112].
494 As these have been linked in earlier sections with pesticide-associated dysmetabolism [46] there
495 is a strong potential for functional impact of a fibre intervention.

496 As previously shown, the gut microbiota regulates biological systems related to obesity, such
497 as nutrient intake, fat accumulation, and energy storage. A diet rich in fat induces an
498 accumulation of large adipocytes, promotes differentiation factors activated by the gamma
499 receptor activated by peroxisome proliferators (PPAR γ) and leads to a considerable increase in
500 the expression of G proteins coupled to the 43 receptor (GPR43) in the subcutaneous fatty tissue
501 [113]. Again, pesticides have been reported in previous sections to regulate PPAR γ expression
502 [55]. Therefore targeting PPAR γ may improve both dietary and pesticide-associated
503 perturbations of the adipose tissue. Prebiotic supplementation of obese animals also decreased
504 body weight gain, fat cell size, adiposity, and insulin resistance [94]. Inulin-type fructans (ITF)
505 reduced PPAR γ activation, and GPR43 expression together with modulation of the microbiota
506 in favour of *Bifidobacteria* and recovery of both *Bacteroidetes* and *Firmicutes* in mice [114].
507 In addition, oligofructose improved glucose tolerance, increased plasma glucagon-like peptide-
508 1 levels, and reduced body fat development, oxidative stress and low grade inflammation in
509 genetically obese mice [115].

510 Collectively, these results suggest that the gut microbiota modulates biological systems that
511 regulate nutrient availability, energy storage, body fat development, and inflammation in the
512 host, each associated with the obese phenotype. Significantly, the number of bifidobacteria is
513 inversely correlated with body fat, glucose intolerance and LPS level [112, 116, 117].

514 **Conclusion**

515 While the amount of evidence linking pesticide exposure to dysmetabolism is growing steadily,
516 the relation to dysbiosis is often missing in terms of epidemiological evidence. An increasing
517 amount of preclinical data is slowly becoming available, linking the three topics through
518 mechanistic evidence. While there is still a long way to go to fully understand the complex
519 relationships between pesticide exposure and dysmetabolism, the intermediate role of the
520 microbiota allows already for some prevention hypotheses to be tested.

521 Pesticides represent a large panel of chemical structures and as such the conclusions cannot be
522 extrapolated from one family to the next but there are some common grounds including
523 dysregulation of the Bacteroidetes to Firmicutes ratio in the process leading to dysregulation of
524 energy metabolism and pathologies like diabetes or obesity. More research needs to be done to
525 fully understand the impact of the various pesticides at physiological doses in chronic exposure,
526 maybe even as cocktail exposure to be more representative of the actual environment present
527 for consumers. Only when we have a full understanding of the impact of chronic low dose
528 exposure to pesticides can we be sure what prevention strategies are more likely to be effective.

529 One of the objectives of this review is to show the link between pesticide and dysmetabolism
530 via microbiota modulation with a strong impact both for direct populations but also indirect
531 exposure for offspring (transgenerational effect). The paradigm that relied on the notion of a
532 dose is called into question because: "it is not the dose that makes the poison" but rather the
533 consequence of chronic exposure to xenobiotics at low doses. Their work emphasizes that the
534 effect is even more marked for sensitive populations, the importance of the vulnerability
535 window for in utero exposure. Through this review, we wish to highlight the contribution of the
536 microbiota in the xenobiotic response, the activation of pesticides (bioactivation /
537 metabolization) but also the modulation of bacterial populations for a health impact on the host.
538 Intestinal dysbiosis is associated with pesticides in a manner similar to that proposed as
539 representative of the metabolic syndrome. In fact, the mechanisms of action via bacterial
540 translocation, intestinal permeability and bacterial dysmetabolism lead to low-grade

541 inflammation and the appearance of endogenous disturbances of energy homeostasis and
542 increase the risk of developing chronic inflammatory pathologies.

543 Finally, this review aims to educate the reader on the consequences of exposure to pesticides
544 on microbial homeostasis and the increasing incidence of obesity and T2D in the world
545 population. This is why the role of prebiotics in prevention has an essential role in the
546 management of pesticide exposure.

547

548 **Perspectives**

549 Many of the works presented focus on one pesticide molecule at a time, but few are multi-
550 exposure, and the cocktail effect is still little studied (whereas in reality we are exposed to
551 multiple pesticide residues). A recent report seems to indicate that exposure to a cocktail of 6
552 pesticides at or below tolerated daily intake concentration during the perinatal period did not
553 induce significant dysmetabolism but indirect signs of dysbiosis were found in the pups [118],
554 indicating an increased risk factor for later development of metabolic diseases. It would be
555 desirable to be vigilant with regard to children's nutrition and the presence of pesticide residues
556 (even at very low doses) which potentiate the nutritional imbalances in our 21st century diet. It
557 is an alert to help reduce these pesticides worldwide. In terms of prevention, we have focused
558 on prebiotics, it would be interesting to explore other preventive strategies to also potentiate the
559 positive (beneficial) effects; probiotics, synbiotics, fecal transplantation ... because the
560 microbiota is at the heart of the preventive strategy "taking care of your microbiota means
561 taking care of your health".

562

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894 Table 1: Pesticides and their microbiota impact in relation to dysmetabolism; clinical
 895 evidence

	Pesticide	cohort	Link to dysmetabolism	Link to dysbiosis	References
Organochlorine pesticides	8 OC pesticides	(Korea n=83)	Link to BMI and waist circumference	Increased Methanobacteriales	Lee, 2011 [34]
Organophosphate insecticides	MCP	(India n=3080)	Increased prevalence of diabetes in exposed populations	(subgroup n=120) OP metabolizing activity (esterase) Increased acetate levels	Velmurugan, 2017 [83]

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897 *BMI: body mass index; OP: organophosphate, OC: Organochlorine; MCP: Monocrotophos.*

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Table 2: Pesticides and their microbiota impact in relation to dysmetabolism; animal evidence

	Pesticide	dose and duration of exposure	Link to dysmetabolism	Link to dysbiosis	References
Organophosphates	CPF	1-5 mg/kg, GD1-D21 Rats	No impact or Increased body weight in adults Low body mass and short body length at birth	Higher numbers of <i>Enterococcus</i> spp, <i>Clostridium</i> spp, <i>Staphylococcus</i> spp and <i>Bacteroides</i> spp, <i>Clostridium</i> spp <i>Lactobacillus</i> spp and <i>Bifidobacterium</i> spp significantly lower	Joly-Condet 2015 [21]
		0.3-3 mg/kg, 9 wks Rats	Changes in Plasma Glucose Levels and Lipid Profile Significant difference in body weight	Increase in potentially pathogenic flora and decrease in beneficial flora	Fang, 2018 [90]
		1 mg/kg, 30 days. Mice	Abnormal permeability	<i>Firmicutes</i> decreased while <i>Bacteroidetes</i> increased <i>Lactobacillaceae</i> decreased while <i>Bacteroidaceae</i> increased	Zhao, 2016 [89]
	Diazinon	4 mg/L, 13 weeks Mice	Impaired energy metabolism Male animals more responsive Abnormal translocation Reduced body weight gain	Altered microbiota composition <i>Lachnospiraceae</i> , <i>Ruminococcaceae</i> , <i>Clostridiaceae</i> , <i>Erysipelotrichaceae</i>	Gao, 2017 [87]
	MCP	10x TMID, 180 days Mice	Increased glycemia, glucose intolerance	OP metabolizing activity (esterase) Increased acetate levels	Velmurugan 2017 [34]
Organochlorines	TCDF	24 µg/kg, 5 days	Triggers inflammation and alters hepatic lipogenesis, gluconeogenesis, and glycogenolysis	Reduction in the <i>Firmicutes/ Bacteroidetes</i> ratio with enrichment of <i>Flavobacteriia</i> and <i>Butyrivibrio</i> spp., and depletion of <i>Clostridia</i> and <i>Oscillobacter</i>	Zhang, 2015 [88]
	DDT	2 mg/kg, 21 days Rats	Increased weight gain Increased fasting glucose and insulin Perturbed lipid metabolism	Reduced <i>Bacteroidetes</i> and <i>Proteobacteria</i> Increased <i>Firmicutes</i>	Zhan, 2019 [84]
	PCP	0-100 µg/L, 28 days	Reduced body weight	Increased <i>Bacteroidetes</i> to <i>Firmicutes</i> ratio Increase in <i>Bacteroides</i> and decrease in <i>Chryseobacterium</i> , <i>Microbacterium</i> , <i>Arthrobacter</i> and <i>Legionella</i>	Kan, 2015 [91]
benzimidazoles	CBZ	100-500 mg/kg, 28 days	Hepatic lipid accumulation Increased TG, cholesterol, HDL and LDL	Increased <i>Firmicutes</i> , <i>Proteobacteria</i> and <i>Actinobacteria</i> Decreased <i>Bacteroidetes</i>	Jin, 2015 [83]
		0.2-5 mg/kg, 14 weeks	significant increase in body fat, hepatic and epididymal fat increased serum TG and glucose levels	decrease in the number of <i>Bacteroidetes</i> and <i>Verrucomicrobia</i> and increase in the number of <i>Actinobacteria</i>	Jin, 2018 [83]

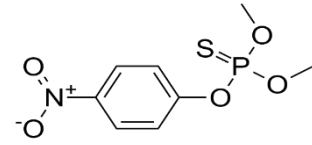
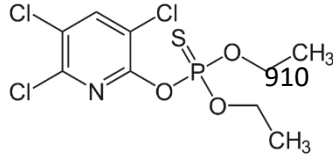
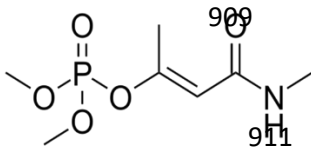
901 *CBZ: Carbendazim; CPF: Chlorpyrifos; TCDF: Tetrachlorodibenzofuran ; MCP:*
902 *Monocrotophos ; TG: triglyceride (TG); LDL: Low Density Lipoprotein; HDL :High Density*
903 *Lipoprotein, GD: gestation days; TMDI: theoretical maximum daily intake. DDT:*
904 *Dichlorodiphenyltrichloroethane. PCP: Pentachlorophenol.*

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907 Figure 1 : Chemical structure of typical organophosphate pesticides

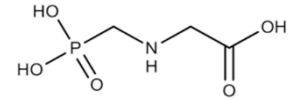
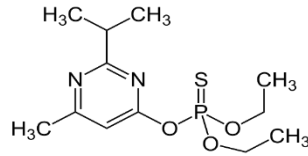
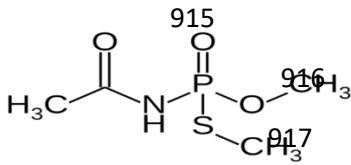
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920 From left to right and top to bottom : monocrotophos, chlorpyrifos ethyl, methyl parathion,

921 acephate, diazinon and glyphosate

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