



HAL
open science

Effects of Chlorpyrifos on gut dysbiosis and barriers integrity in women with a focus on pregnancy and prebiotic intervention: insights from advanced in vitro human models

Maria Abou Diwan, Avénie Huet, Juliette Poiriez, Claire-Joly Condette, Stéphane Delanaud, Emmanuel Sevin, Aurélie Corona, Larbi Rhazi, Flore Depeint, Halima Ouadid-Ahidouch, et al.

► To cite this version:

Maria Abou Diwan, Avénie Huet, Juliette Poiriez, Claire-Joly Condette, Stéphane Delanaud, et al.. Effects of Chlorpyrifos on gut dysbiosis and barriers integrity in women with a focus on pregnancy and prebiotic intervention: insights from advanced in vitro human models. *Environmental Pollution*, 2024, pp.125533. 10.1016/j.envpol.2024.125533 . hal-04837526

HAL Id: hal-04837526

<https://u-picardie.hal.science/hal-04837526v1>

Submitted on 3 Feb 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Effects of Chlorpyrifos on gut dysbiosis and barriers integrity in women with a focus on pregnancy and prebiotic intervention: Insights from advanced in vitro human models[☆]

Maria Abou Diwan^{a,b}, Avénie Huet^c, Juliette Poiriez^b, Claire Joly Condette^a, Stéphane Delanaud^a, Emmanuel Sevin^b, Aurélie Corona^a, Larbi Rhazi^d, Flore Depeint^e, Halima Ouadid-Ahidouch^c, Fabien Gosselet^b, Véronique Bach^a, Pietra Candela^{b,1}, Hafida Khorsi-Cauet^{a,*,1}

^a PERITOX—Périnatalité et Risques Toxiques—UMR_I 01 UPJV/INERIS, Centre Universitaire de Recherche en Santé, CURS-UPJV, Université Picardie Jules Verne, CEDEX 1, 80054, Amiens, France

^b Laboratoire de la Barrière Hémato-Encéphalique (LBHE), UR 2465, Université d'Artois, 62300, Lens, France

^c Laboratoire de physiologie cellulaire et moléculaire (LPCM), UR UPJV 4667, Université Picardie Jules Verne, UPJV, 80039, Amiens, France

^d UP 2018.C103 Transformations & Agro-Ressources, Institut Polytechnique UniLaSalle, Beauvais, France

^e Institut Polytechnique UniLaSalle, Université d'Artois IDEALISS, URL 7519, 60000, Beauvais, France

ARTICLE INFO

Keywords:

Gut-microbiota-BBB axis

Chlorpyrifos

Pregnant women

Human in vitro models

Pesticide-induced gut dysbiosis

Bacterial translocation

ABSTRACT

Chlorpyrifos (CPF), a commonly used organophosphate pesticide, poses potential risks to human health, particularly affecting the gut microbiota (GM), intestinal barrier (IB), and blood-brain barrier (BBB). CPF-induced gut dysbiosis compromises the integrity of both the IB and the BBB, leading to increased intestinal permeability, inflammation, and bacterial translocation, all of which may impact neurological health. Although CPF's effects on the GM are documented, limited research explores how these impacts differ in women, particularly during pregnancy. To address this gap, this study investigates CPF's effects using three advanced human in vitro models: the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) to mimic the gut environment of women of child-bearing age and pregnant women, a Caco-2 model for the IB, and a BBB model to assess CPF's effects and the protective role of the prebiotic inulin.

Microbiological analyses of SHIME® supernatants, including bacterial culture and quantification of short-chain fatty acids (SCFAs) and CPF metabolites, were conducted to assess gut composition and pesticide degradation. We also examined the effects of CPF-induced dysbiosis on IB and BBB permeability to FITC-Dextran, focusing on bacterial translocation after 4 h of exposure to CPF-treated SHIME® supernatants.

Our results revealed significant intestinal imbalance, marked by an increase in potentially pathogenic bacteria in the GM of both non-pregnant and pregnant women exposed to CPF. This dysbiosis led to a significant shift in SCFAs ratio and increased IB permeability and bacterial translocation across the IB, but not the BBB. Notably, inulin supplementation restored GM balance and prevented bacterial translocation, highlighting its potential as a preventive measure against CPF-induced dysbiosis.

This study enhances our understanding of the health risks associated with CPF exposure in women, with implications for maternal and fetal health, and underscores the importance of considering physiological states such as pregnancy in toxicological research.

[☆] This paper has been recommended for acceptance by Wen Chen.

* Corresponding author.

E-mail address: hafida.khorsi@u-picardie.fr (H. Khorsi-Cauet).

¹ These authors contributed equally to this work.

<https://doi.org/10.1016/j.envpol.2024.125533>

Received 29 October 2024; Received in revised form 5 December 2024; Accepted 12 December 2024

Available online 13 December 2024

0269-7491/© 2025 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The link between the gut microbiota (GM) and the brain, often referred to as the gut-brain axis, describes the bidirectional communication between the GM, which consists of the microorganisms living in the gut—mainly bacteria—and the “barriers” that separate it from the rest of the body (Morais et al., 2021). These barriers include the intestinal barrier (IB), which limits the absorption of food contaminants, and the blood-brain barrier (BBB), which restricts the entry of harmful molecules and cells into the brain (Abou Diwan et al., 2023). This axis of communication was long overlooked in medical and scientific research, but in recent decades, extensive studies have uncovered its critical role in both mental and physical health.

Animal studies indicate that the disruption of the GM due to pesticide exposure can impact human health by altering IB function and having long-term effects on the BBB (Joly Condette et al., 2016; Galea, 2021; Balaguer-Trias et al., 2022). The pervasive use of pesticides in modern agriculture has raised significant concerns about their impact on the key players of gut-brain axis. Among these, Chlorpyrifos (CPF), an organophosphate insecticide, has been widely used due to its effectiveness in pest control. CPF is a neurotoxic pesticide that inhibits acetylcholinesterase (AChE) which leads to an accumulation of acetylcholine, causing continuous nerve signal transmission. CPF has also been linked to endocrine disruption as this pesticide can interfere with the endocrine system by mimicking or blocking thyroid or reproductive hormones (Balaguer-Trias et al., 2022; Lahimer et al., 2023). However, its potential adverse effects on human health, particularly on GM, IB, and BBB, have not been extensively studied and have become a growing area of research. For instance, recent studies have shown that chronic CPF exposure can lead to alterations in the GM, a condition known as dysbiosis that can cause harmful microbial metabolites to enter the bloodstream (bacterial translocation) across the IB (Zhao et al., 2016; Joly Condette et al., 2016; Linares et al., 2021). CPF exposure has been associated with an overgrowth of potentially harmful bacteria as well as a reduction in beneficial bacteria, mainly producing short-chain fatty acids (SCFAs), which play a crucial role in maintaining gut health and barrier function (Dalile et al., 2019). These changes in microbial composition and function can disrupt the homeostasis of the gut ecosystem, contributing to adverse health effects (Reygner et al., 2016a; Djekkoun et al., 2022a; Joly Condette et al., 2022).

Since the GM plays a crucial role in maintaining the integrity of IB and the BBB, dysbiosis can result in the impairment of both barriers (Réquilé et al., 2018; Doroszkiewicz et al., 2021; Di Vincenzo et al., 2023). These effects are thought to occur through mechanisms such as increased intestinal permeability, chronic inflammation, and bacterial translocation that influence neurological functions (Sampson et al., 2016; Nagpal and Yadav, 2017; Rutsch et al., 2020). However, further research is needed to fully understand these complex interactions and to develop strategies for mitigating the potential health risks.

In addition, the effects of CPF on the GM are well-documented but there is a lack of research on how these effects vary in women, particularly during pregnancy. Hormonal and immunological changes during pregnancy can influence the composition of the GM (Edwards et al., 2017; Fuhler, 2020). Consequently, pesticide-induced dysbiosis may differ between non-pregnant and pregnant women. As a result, the effects of dysbiosis on the IB and the BBB may vary depending on pregnancy status, potentially leading to distinct health impacts for both the mother and the fetus (Developmental Origins of Health and Disease (DOHaD) “windows of vulnerability”) (Wright, 2017; Balaguer-Trias et al., 2022). In contrast, supplementation with the prebiotic inulin has been suggested as a potential strategy to mitigate pesticide effects, although little is known about its efficacy in women and pregnant individuals (Reygner et al., 2016b; Djekkoun et al., 2022a; Sheng et al., 2023; Wang et al., 2023). Inulin is a soluble dietary fiber found in over 36 000 plant species as a reserve polysaccharide including Jerusalem artichoke, chicory root, onion, garlic, barley, and dahlia. It is widely

recognized as a prebiotic with significant effects on GM regulation by promoting the growth of beneficial bacteria such as *Bifidobacteria* and *Lactobacilli* (Wang et al., 2023). Inulin is not digested by the human body but is fermented by these beneficial bacteria, resulting in the production of SCFAs that help restore GM balance and exert anti-inflammatory effects. Previous studies showed that CPF, either alone or in combination with a high fat diet induces intestinal dysbiosis leading to a disruption in the delicate balance of the GM (Reygner et al., 2016b; Réquilé et al., 2018; Djekkoun et al., 2022a, b; Abou Diwan et al., 2024).

Although inulin supplementation has shown promise in mitigating pesticide-induced dysbiosis, there is a critical lack of understanding regarding its efficacy during pregnancy. Moreover, pesticide-induced dysbiosis during pregnancy could have significant consequences, particularly for the IB and the BBB, which play pivotal roles in maintaining maternal and fetal health.

Therefore, to address this research gap, it is important to study pesticide-induced dysbiosis during pregnancy and its effects on these critical body barriers. In particular, our study aims to evaluate the impact of CPF on the GM, the IB, and the BBB and was designed to analyze the potential beneficial effects of inulin during CPF exposure. We combined three original *in vitro* human models: (1) an artificial gut simulator SHIME® (Simulator of the Human Intestinal Microbial Ecosystem) to mimic the human gut environment and assess the GM (Van de Wiele et al., 2015; Joly Condette et al., 2022), (2) a Caco-2 cell model to examine the IB (Sevin et al., 2013), and (3) an endothelial cell and pericyte co-culture model to investigate the BBB (Versele et al., 2022). By analyzing fecal samples from women of child-bearing age and pregnant women, we aim to elucidate how CPF affects the GM differently during pregnancy and to explore its consequences on IB and BBB permeability, as well as bacterial translocation across these barriers.

2. Materials and methods

2.1. Chemicals

Chlorpyrifos ((O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate) with 98.5 % purity, Sigma-Aldrich), being a lipophilic molecule, was dissolved in rapeseed oil prior to administration of 1 mg in the first *in vitro* reactor (stomach) containing 200 mL of sterile feed medium. Exposure to CPF started after 15 days of stabilization of the intestinal flora of the *in vitro* SHIME model.

2.2. *In vitro* models

2.2.1. SHIME® model

Definition of the model The Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) (Van de Wiele et al., 2015; Joly Condette et al., 2022) model is an *in vitro* representation of the digestive tract, spanning from the stomach to the descending colon (R1 to R6). It comprises six distinct double-wall reactors connected by peristaltic pumps and is maintained at a constant temperature of 37 °C with daily pH and redox voltage monitoring.

Feces collection The initiation of the SHIME® requires an inoculum of human feces. In this study, we used fecal inoculum from 2 groups of women volunteers: of reproductive age and pregnant. Our inclusion criteria selected five healthy adults for each group: with no symptoms of gastrointestinal diseases and were not treated with antibiotics during the six months prior to feces collection. Additionally, the selected donors had not received any hormonal treatments for hormonal dysregulation or disease in the six months prior to feces collection, although some donors were using oral contraception. Regarding menstrual cycle control, we did not select non-pregnant donors based on a specific phase of their menstrual cycle, but those who were in their menstrual phase informed us. Consequently, all phases of the menstrual cycle are represented among the donors. The collection of women feces from the Amiens University Hospital, with the help of Dr. Arthur Foulon, and

their inoculation in the SHIME® were approved by the Ethical committee for non-interventional research (CERNI) of Picardy Jules Verne University (Evaluation number 2023-07-5/2023–27).

SHIME® experimental process A 15-day stabilization period of the microbiota (pH and redox potential) is required before beginning exposure. Supernatants from the *in vitro* digestion were collected on the 15th day of stabilization, representing the control. Following this stabilization period, daily exposure to 1 mg/day of CPF began. Sample collections were done 15 days and 30 days post-exposure to study respectively short-term and long-term effects of CPF alone and the combination of CPF and inulin (in the case of co-exposure to CPF and inulin, 10 g of inulin extract was added to the stomach directly after CPF solution addition (Joly Condette et al., 2022)). The supernatants were sampled three times in the final week of each period to allow statistical analysis.

The selection of the CPF dose (1 mg/day) was based on prior studies that identified this concentration as appropriate for evaluating the effects of CPF on GM and epithelial cell permeability.

In previous studies, we investigated both the NOAEL dose (1 mg/kg/day) and a toxic dose (5 mg/kg/day) in rat models, focusing on CPF's inhibitory effects on acetylcholine (Joly Condette et al., 2016; Darwiche et al., 2018; Abou Diwan et al., 2024). Furthermore, we have published findings on the effects of a 1 mg/day CPF dose on human GM using the SHIME® model, which included a mixed population of men and women (Joly Condette et al., 2022). This dose corresponds to CPF residues commonly detected in daily meals, as calculated using ANSES data (Joly et al., 2013). For this study, the 1 mg/day CPF dose was selected to reflect realistic human oral exposure levels, approximating 0.016 mg/kg/day for a 60-kg individual. This value considers both dietary intake and environmental exposure, ensuring that the experimental conditions are relevant to real-world exposure scenarios.

2.2.2. IB model

Caco-2 cell culture protocol has been previously detailed in our colleagues studies (Sevin et al., 2013, 2019). Caco-2 cells ($0.4 \times 10^{+5}$ cells), at passage 026, were seeded in 25 cm² plastic flasks and maintained with complete medium, which was refreshed every two days. The medium consisted of Dulbecco's Modified Eagle's Medium (DMEM) high glucose (4500 mg/L) with L-glutamine (584 mg/L), supplemented with 10% Fetal Bovine Serum (FCS, Life Technologies, Waltham, MA, USA), 1% non-essential amino acids (excluding L-glutamine), and 1% penicillin-streptomycin solution. After 3 days of incubation, when the cells reached 80–90% confluence, they were trypsinized and reseeded at a density of 5×10^5 cells in 75 cm² flasks with complete medium. When the Caco-2 cells reached a high density (approximately 0.5×10^5 cells/cm²), after 5–6 days, they were passage into 6-well plate on 0.4 µm or 1 µm polycarbonate or polyethylene terephthalate membrane inserts respectively. Cells were seeded at densities of either 40 000 cells/cm² and cultured for 21–25 days in complete medium, with media changes every two days.

2.2.3. Human *in vitro* BBB model

The BBB model consisted of a co-culture of human brain pericytes (hBPs) in the lower compartment and human brain-like endothelial cells (hBLEC) on the Transwell insert. hBPs were obtained from the team of Pr. Takashi Kanda (Department of Neurology and Clinical Neuroscience Clinical Neuroscience of Yamaguchi University in Japan) (Shimizu et al., 2011). They were isolated in accordance with the Declaration of Helsinki and the protocol was approved by the ethics committee of the Medical Faculty (IRB#: H18-033-6) of the University of Yamaguchi. These cells were characterized for pericyte markers as described in previous published work (Menaceur et al., 2023). After thawing, hBPs (passages 15–25) were seeded in gelatin-coated Petri dishes and cultured in DMEM high glucose, FCS, L-glutamine, and antibiotics. Human hematopoietic stem cells were isolated from umbilical cord blood, with informed consent and approval from the French Ministry of

Higher Education and Research, (CODECOH Number DC2011-1321) (Cecchelli et al., 2014; Heymans et al., 2020). These cells were sorted, cultured, and differentiated into ECs in EGM-2 medium with VEGF (PeproTech Inc, Cranbury, NJ, USA). For the BBB model, hBPs were seeded in gelatin-coated dishes, and CD34⁺ ECs were seeded on inserts (1 µm, SARSTEDT, Nümbrecht, Germany) covered with Matrigel™ (Corning, NY, USA). After 6 days of co-culture of ECs with hBPs in a 12 well-plate (Costar, 3512, Corning, NY, USA), the BBB model is ready for treatment to SHIME supernatants.

2.3. Microbiological and cellular analysis

2.3.1. SHIME® bacterial composition analysis

After a 15-day stabilization period of the SHIME® microbial flora, control samples were collected prior to exposure to CPF (or CPF + inulin) to identify the bacterial population before exposure in 4 reactors: ileum, proximal colon, transverse colon and descending colon. Samples were collected again after 15 days of exposure and on the 30th day of exposure. To identify bacterial genera, various culture media were utilized, and inoculation was directly performed after sampling, using the bead spreading method in anaerobic (48h–4 days) and aerobic conditions (24–48h) at 37 °C. Bacterial colonies that were isolated on the Petri dishes were counted using an automated system (Scan® 500, Interscience) and the results were expressed as log CFU (Colony Forming Unit)/mL of the sample volume. The use of log of CFU is based on the extremely large raw values obtained (high powers of 10).

2.3.2. SHIME® bacterial fermentation metabolites assay

SCFAs (acetate, butyrate and propionate) of R3 to R6 samples were analyzed by gas chromatography as previously described (Lecerf et al., 2012; Réquillé et al., 2018) with modifications. 1 mL of sample were centrifuged for 10 min at 10 000 g at 4 °C and filtered through regenerated cellulose syringe filter 0.45 µm. Then, 0.9 mL of supernatant was mixed with 100 µL of internal standard (methylbutyric acid). SCFAs were quantified using a gas chromatograph (FOCUS GC System, ThermoFisher Scientific) equipped with an autosampler (AS-3000, ThermoFisher Scientific) and a flame ionization detector (FID). Samples (1 µL) were injected in split mode (1/10) at 270 °C. Chromatographic separation was performed through a ZB-FFAP column (30 m × 0.25 mm × 0.25 µm, Phenomenex) with dihydrogen as the carrier gas at a 1 mL/min gas flow. The oven temperature program was defined as follows: isotherm at 100 °C for 2 min, temperature gradient from 100 °C to 145 °C at 10 °C/min, isotherm at 145 °C for 0.2 min, temperature gradient from 145 °C to 220 °C at 45 °C/min and final isotherm at 220 °C for 1 min. The FID detection was operated at 280 °C with dihydrogen and air flows at, respectively, 30 mL/min and 300 mL/min.

2.3.3. Bacterial translocation across IB and BBB models

Bacterial translocation that refers to the passage of bacteria or bacterial metabolites from the intestinal lumen to sterile organs was measured in our study to assess the effects of CPF and the potential protective role of inulin supplementation.

The reproducibility of our bacterial translocation experiments has been previously validated and published *in vitro* and *in vivo* studies focusing on the IB (Réquillé et al., 2018; Djekkoun et al., 2022a; Abou Diwan et al., 2024). For this study, we extended this validated technique to analyze bacterial translocation across both the IB and BBB.

Specifically, we assessed the translocation of viable and cultivable bacteria. To check whether potential gut dysbiosis led to bacterial translocation across the IB, we applied 1/5 diluted SHIME® supernatants (distal colon alone R6 and a pool of the three colon segments) to the apical side of the IB model as previously described (Réquillé et al., 2018). Additionally, separate BBB model inserts were exposed to aliquots collected from Caco-2 basolateral compartment after SHIME® exposure to mimic gut microbiota-BBB “like” axis.

Each SHIME® condition and control were conducted in triplicate

(three membrane inserts per condition for each model) to ensure accuracy and reproducibility of permeability (measured during bacterial translocation experiment). However, for the bacterial culture aimed at assessing bacterial translocation of bacteria that crossed the apical towards the basolateral compartment of barriers, we pooled the triplicates before bacterial culture since our aim for this experiment is to emphasize the biological implications of our bacterial translocation results rather than focusing solely on statistical metrics.

Apical and basolateral aliquots inoculated on bacterial culture media were incubated at 37 °C for 24–48 h. If bacterial translocation occurs, colonies are detected on Petri dishes where aliquots of basolateral compartment were inoculated. The resulting colonies were enumerated using an automated system (Scan® 500, Inter-science, St Nom la Breteche, France) and expressed as log CFU. The percentage of translocation was calculated by dividing the colony count in basolateral compartment by the colony count in apical compartment and multiplying by 100 (Abou Diwan et al., 2024).

2.3.4. Permeability measurement during bacterial translocation experiment

Fluorescent dye passage across the barrier, from the apical (luminal) compartment, simulating the intestinal lumen, to the basolateral compartment, which simulates the blood content, in the presence of SHIME® supernatants was used as an indicator of permeability. To confirm the integrity of the barrier in the absence of SHIME® exposure, negative control conditions, where barriers were not exposed to SHIME® supernatants, were tested for dye passage.

Barriers' permeability to the non-permeant fluorescent molecule Fluorescein 4 KDa Isothiocyanate-Dextran (FD4) was measured in the IB and BBB models after 4 h of exposure to 1/5 diluted SHIME® supernatants at 37 °C during the bacterial translocation experiment. FD4 is at a concentration of 40 µM in Ringer HEPES solution that were used to dilute SHIME supernatants. Amount of FD4 in apical and basolateral compartments of cell culture was determined using fluorescence spectrophotometry at 495–525 nm (Biotech, Synergy H1, Colmar, France).

The permeability coefficient (Pe in cm/min) of FD4 was calculated in Ringer HEPES buffer using the previously established equation (Dehouck et al., 1990; Culot et al., 2008; Sevin et al., 2013, 2019).

2.4. CPF assay in SHIME® reactors

CPF parent molecule and its metabolites assay in SHIME reactors (R1 to R6) was performed using liquid chromatography (LC) with a coupled mass spectrometer (MS) and a flight time model 6545 (MS-QTOF) (Agilent, France) by the International Medical Institute of Toxicology and Pharmacology (I-MITOX) as previously described (Brousse et al., 2019).

2.5. Statistical analysis

The bacterial composition data (log CFU/mL) were expressed as mean ± standard error of mean (SEM). The SCFAs data were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using GraphPad Prism 8.0.1 statistical software (GraphPad Software, Inc., San Diego, CA, USA). One-way and two-way analyses of variance (ANOVA) were employed, with $p < 0.05$ being the statistical significance threshold for all analyses. The statistical analysis of permeability data expressed as mean ± standard deviation (SD) was conducted using the non-parametric test Kruskal-Wallis' test.

3. Results

3.1. Gut microbiota bacterial composition

3.1.1. CPF exposure of women of childbearing age GM

After the microbiological analysis of isolated colonies on culture media, the results indicate whether short-term and/or long-term

exposure to CPF impacts the GM of women of reproductive age. We chose to use bar charts to represent our findings, to facilitate comparison and more effectively highlight significant results. The x-axis represents the different reactors with a time scale (with bars assigned to each day: CPF 0 (control period), CPF 15 for short-term pesticide exposure and CPF 30 for long-term exposure for the different reactors (R3 → R6). The y-axis represents the Log CFU, as the raw values obtained are at very high powers of 10.

In this section, we focus on the microbial composition of SHIME® of women of childbearing age. Starting with the analysis of total aerobic flora, we observed a bacterial count of approximately 8 Log₁₀ CFU/mL in the different reactors during control period. However, no significant variation in the bacterial count was observed after exposure to CPF compared to control (Fig. 1 A). Similarly, total anaerobic flora didn't show significant bacterial count changes (Fig. 1 B). Subsequently, our analysis of the microbial composition at the phylum level revealed alterations in the concentrations of specific gut bacteria, after CPF exposure. We observed a significant increase of 35 % of *Clostridium* spp colony count in proximal ($p < 0.01$, vs CPF0) and transverse colon ($p < 0.001$, vs CPF0 and $p < 0.01$ vs CPF15) (Fig. 1 C). In the distal colon we detected a decrease of *Clostridium* spp after 15 days ($p < 0.01$, vs CPF0) that was reversed to a significant increase of approximately 40 % after 30 days ($p < 0.001$, vs CPF15). Additionally, our results showed a significant increase of *Enterococcus* spp post-30 days exposure in proximal colon ($p < 0.05$, vs CPF0 and vs CPF15) (Fig. 1 D). In contrast, the abundance of potentially beneficial bacteria, such as *Lactobacillus* spp., significantly decreased after 15 days pesticide exposure the transverse ($p < 0.001$, vs CPF0) and distal colon after 15 ($p < 0.0001$, vs CPF0) and 30 days ($p < 0.001$, vs CPF0) (Fig. 1 F). Meanwhile, *Bifidobacterium* spp. exhibited a slight and progressive, yet non-significant, decline in the ileum over the 30-day exposure period (Fig. 1 E). In the proximal colon, *Bifidobacterium* spp. decreased after 15 days but did not show a significant change after 30 days.

These results indicate that CPF exposure significantly modulated GM composition of women of reproductive age: CPF reduced the number of beneficial *Lactobacillus* and increased the number of potentially pathogenic phyla: *Clostridium* spp and *Enterococcus* spp.

3.1.2. CPF exposure of pregnant women GM

In a second stage, we focused on the microbial composition of SHIME® of pregnant women. Total aerobic flora results showed a significant increase after 15 days of CPF exposure in both proximal and distal colon ($p < 0.01$, vs CPF0) while no significant change in total anaerobic flora was detected (Fig. 2 A and B). Our phylum-level analysis revealed modifications in the concentrations of specific gut bacteria following CPF exposure. As in SHIME® of women of childbearing age, we found increased *Clostridium* spp count in both proximal and distal colon at the 30th day of exposure ($p < 0.05$, vs CPF0) (Fig. 2 C). Parallely, *Enterococcus* spp counts were more significantly increased post-30 days in all colon segments ($p < 0.001$ in PC; $p < 0.01$ in TC; $p < 0.0001$ in DC, vs CPF0) (Fig. 2 D). In contrast, *Bifidobacterium* spp., significantly decreased after 30 days pesticide exposure in the transverse colon ($p < 0.05$, vs CPF0). *Lactobacillus* spp exhibited a significant decline in the ileum over the 30-day exposure period ($p < 0.05$, vs CPF0 and vs CPF15) and in distal colon ($p < 0.05$, vs CPF0) (Fig. 2 F).

Overall, CPF exposure significantly altered the GM in both pregnant and non-pregnant women, with notable increases in potentially pathogenic *Clostridium* spp. and *Enterococcus* spp., and a reduction in beneficial *Lactobacillus* spp.; however, the changes were more pronounced in pregnant women, indicating a heightened sensitivity during pregnancy.

3.1.3. CPF and inulin exposure of women GM

In a third stage, we focused on the effect of the combination of CPF and the prebiotic inulin on the microbial composition of SHIME® of women. No significant variation in the bacterial count total aerobic and

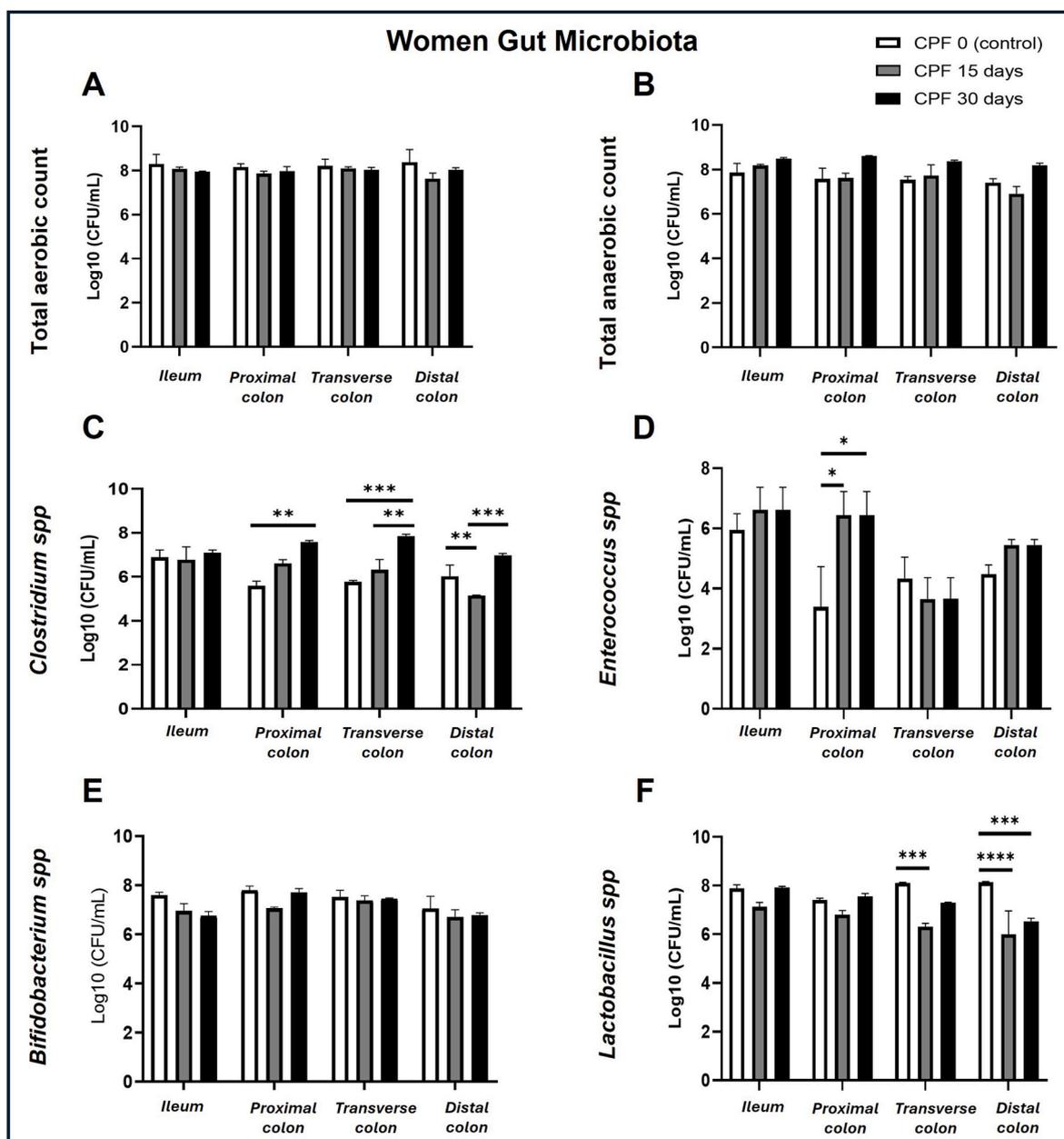


Fig. 1. Effect of 15 and 30 days (CPF15 and CPF30) of CPF exposure (1 mg/day) on bacterial count (log CFU/mL) of total aerobic and anaerobic flora, *Clostridium* spp., *Enterobacteria*, *Lactobacillus* and *Bifidobacterium* in the ileum and colon reactors of the SHIME® of childbearing age female volunteers. Data is illustrated as mean \pm SEM (n = 6–9) using analysis of variance (ANOVA) and Tukey's test. Significance * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. CPF0: Control; CPF15: 15 days of CPF; CPF30: 30 days of CPF.

anaerobic flora was observed after exposure to CPF and inulin compared to control (Fig. 3 A et B). With a closer look at bacterial phyla, we found a stable evolution of *Clostridium* (Fig. 3 C) and *Enterobacteria* (Fig. 3 D) and *Enterococcus* (data not shown). *Bifidobacterium* count slightly increased in transverse colon after 15 days of CPF and inulin co-exposure, but this variation was not statistically significant. In contrast, *Lactobacillus* bacteria showed a significant increase in proximal colon after 15 days ($p < 0.05$, vs CPF0), that was maintained until the 30th day of co-exposure. Similarly, in transverse colon, *Lactobacillus* increased progressively until the 30th day of co-exposure ($p < 0.05$, vs CPF0).

In contrast to the significant alterations observed with CPF exposure alone, the combined exposure to CPF and inulin resulted in a remarkable stability across the majority of bacterial phyla. This suggests that inulin may have a protective effect, preventing the disruptive impact of CPF on

the GM composition.

3.2. Gut microbiota SCFAs analysis

In parallel with GM composition analysis, accurate quantification of SCFAs is essential as they are key indicators of gut microbial and metabolic activity. In the SHIME® model representing the GM of non-pregnant women, a significant increase of total SCFAs (mol/L) was observed in the proximal colon ($p < 0.001$, vs control) (Fig. 4 B) after 30 days of CPF exposure but no impact on total SCFAs was detected in ileum (Fig. 4 A), transverse (Fig. 4 C) and distal colon (Fig. 4 D). However, a significant decrease in propionate ratio (Fig. 4 a.3, b.3, c.3, d.3) was seen after 30 days of CPF exposure in the proximal ($p < 0.001$, vs control; $p < 0.0001$, vs CPF15), transverse ($p < 0.05$ vs control), and distal colon ($p < 0.05$ vs control), accompanied by an increase in butyrate in the

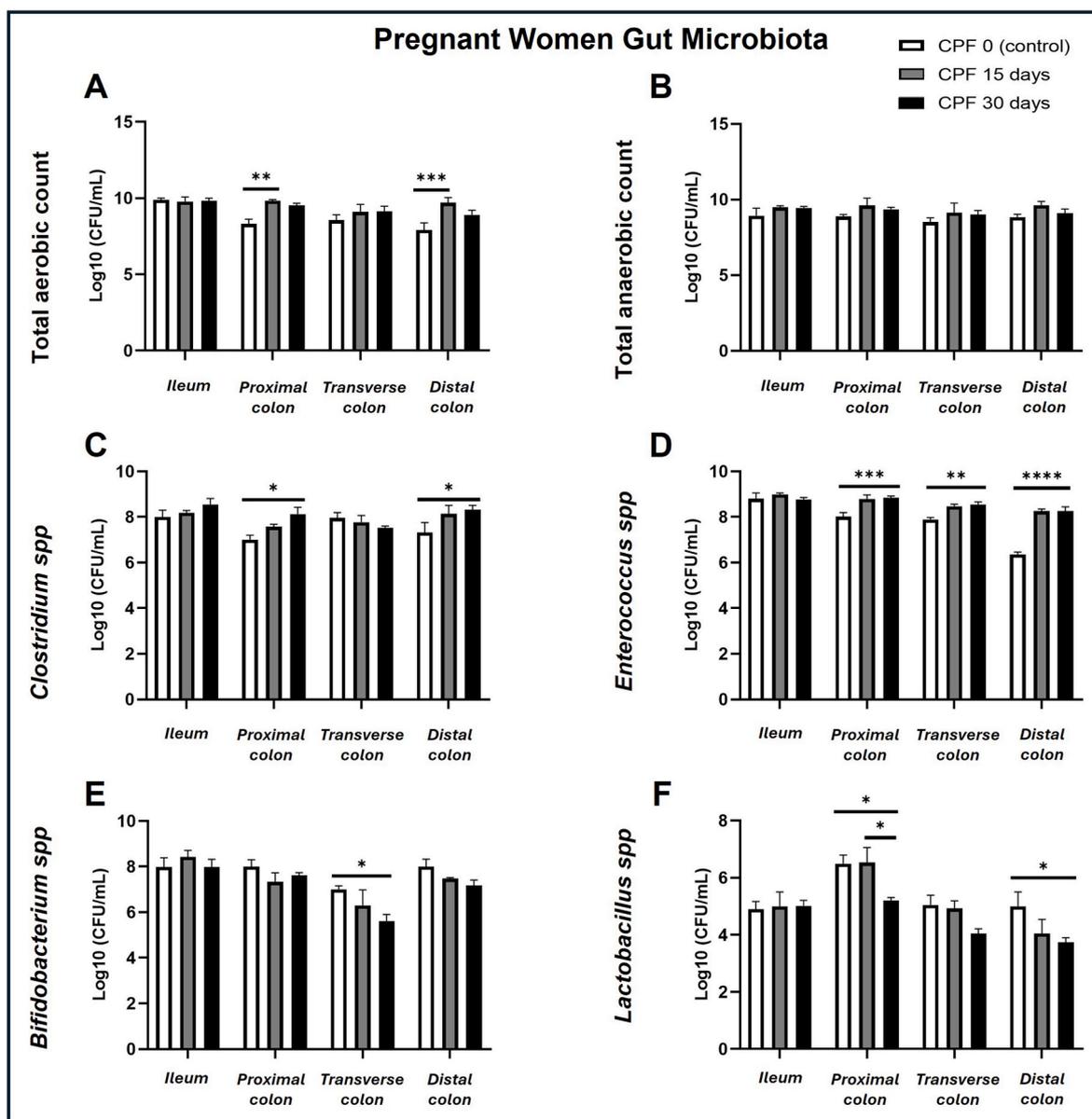


Fig. 2. Effect of 15 and 30 days (CPF15 and CPF30) of CPF exposure (1 mg/day) on bacterial count (log CFU/mL) of total aerobic and anaerobic flora, *Clostridium* spp., *Enterococcus*, *Lactobacillus* and *Bifidobacterium* in the ileum and colon reactors of the SHIME® of pregnant female volunteers. Data is illustrated as mean \pm SEM (n = 6–9) using analysis of variance (ANOVA) and Tukey's test. Significance * p < 0.05; **p < 0.01, ***p < 0.001, ****p < 0.0001. CPF0: Control; CPF15: 15 days of CPF; CPF30: 30 days of CPF.

proximal colon (p < 0.0001, vs CPF15 and vs CPF30), but a decrease in butyrate in the distal colon after 30 days (p < 0.05 vs control). In the distal colon, there was a tendency for the acetate ratio to increase from 65 to 80 (Fig. 4 d.1), although this was not significant due to the progressive increase between CPF0 (control), CPF15days, and CPF30days, to the expense of butyrate and propionate significant decrease.

In the SHIME model representing the GM of pregnant women, total SCFAs increased in the proximal colon after 30 days of CPF exposure (p < 0.01, vs control). Additionally, a significant increase in total SCFAs was detected in the transverse and distal colon after 15 and 30 days of CPF treatment. Regarding SCFAs ratios, a 20% decrease in acetate (from 80 to 60) was observed after 15 days of CPF treatment in the distal colon (p < 0.05 vs control), accompanied by a 20% increase in propionate (p < 0.001, CPF15 vs control and CPF30 vs control). This increase in propionate was also significant in the ileum, proximal colon, and transverse colon after 15 days, and remained significant in the transverse and distal colon after 30 days. A 10% increase in butyrate (Fig. 4

a.2, b.2, c.2, d.2) was noted in the ileum (5% after 15 days of CPF and 5% after 30 days), while a 10% decrease in butyrate was observed in the proximal colon.

In the case of GM supplemented with inulin, changes in total SCFAs were only detected in distal colon: significant increase after 15 days of exposure to CPF + preb (p < 0.001, vs control) that was restored to control levels after 30 days. However, an approximate 10% increase in butyrate was observed in the ileum after 15 and 30 days of exposure (p < 0.001, vs control), but a decrease in butyrate was noted in the proximal colon after 15 days of, and also in the transverse colon after 15 and 30 days to the favor of an increase in propionate after 30 days in the transverse and distal colon.

3.3. CPF assay in SHIME® reactors

The assay of CPF and its metabolites was conducted to assess their biotransformation by GM across SHIME® reactors, spanning from the

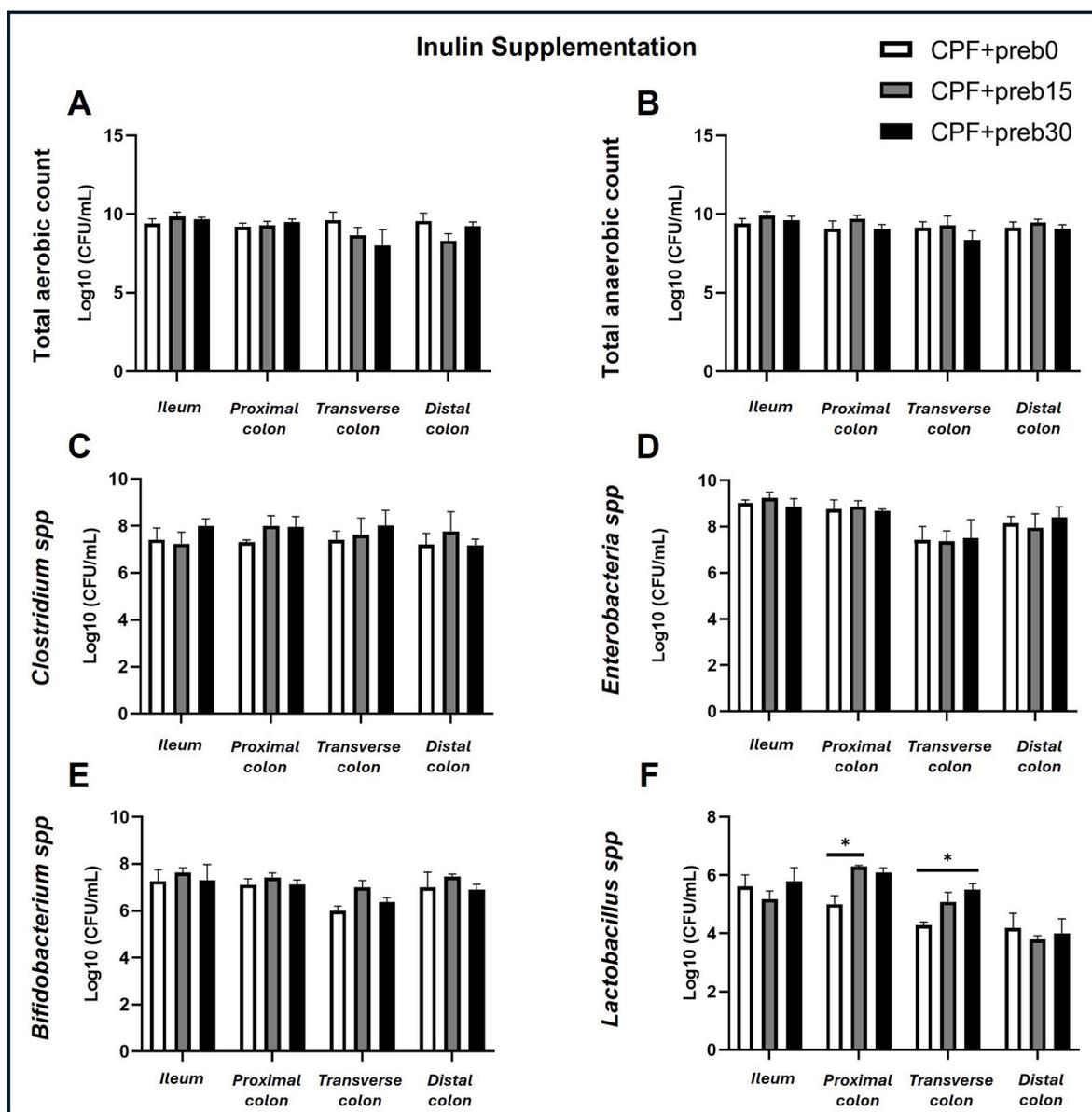


Fig. 3. Effect of 15 and 30 days (CPF + preb15 and CPF + preb30) of CPF exposure (1 mg/day) and the prebiotic inulin (10 mg/day) on bacterial count (log CFU/mL) of total aerobes and anaerobes, *Clostridium* spp., *Enterobacteria*, *Lactobacillus* and *Bifidobacterium* in different colon reactors of the SHIME®. Values are expressed as mean \pm SEM using the nonparametric Kruskal-Wallis test. Significance * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$. CPF + preb0: Control; CPF + preb15: 15 days of CPF and inulin; CPF + preb30: 30 days of CPF and inulin.

stomach to the distal colon. It was crucial to ascertain the presence of CPF in donors' fecal samples prior to the control period and exposure. Our findings indicated an absence of CPF and its metabolites in the reactors before exposure in both SHIME® experiments: CPF non-pregnant women (Fig. 5A1 and A2), CPF pregnant women (Fig. 5B1 and B2) and CPF + prebiotic (Fig. 5C1 and C2). This implies that the fecal inoculum was devoid of CPF and its metabolites, ensuring that the observed effects on the GM were attributable solely to the CPF introduced into the stomach reactor (R1). Following the collection of all control sample replicates, CPF administration started, as evidenced by the increased CPF concentrations in reactor samples between the control and 30-day exposure period. The highest CPF concentration was observed in the stomach at day 10 of exposure in the SHIME CPF experiments and at day 15 in the SHIME CPF + prebiotic. Post this peak, CPF concentrations began to decline progressively across all reactors. During this decline, minor increases were noted, corresponding to the daily addition of CPF. Notably, in the SHIME supplemented with the prebiotic, the decline in

CPF concentration was more rapid, with lower detections in the colon reactors compared to SHIME CPF. Furthermore, the final CPF concentration was lower in the SHIME CPF + prebiotic than in SHIME CPF. Specific metabolites of CPF (Chlorpyrifos-oxon and 3,5,6-trichloro-2-pyridinol (TCP)) were not detected across reactors.

3.4. Effect of CPF-treated women GM on bacterial translocation across IB and BBB models and permeability measurement

To assess the impact of GM exposure to CPF on IB integrity, we measured the permeability to FD4 (Pe FD4) in Caco-2 cells after a 4-h exposure to both untreated SHIME® colon supernatants (control group) and CPF-exposed SHIME® colon supernatants.

The graph of intestinal permeability in the IB model reveals significant variations across different treatment groups (Fig. 6B, left graph). Under negative control conditions, where barriers were not exposed to SHIME® supernatants (ctrl), negligible dye passage was observed, with

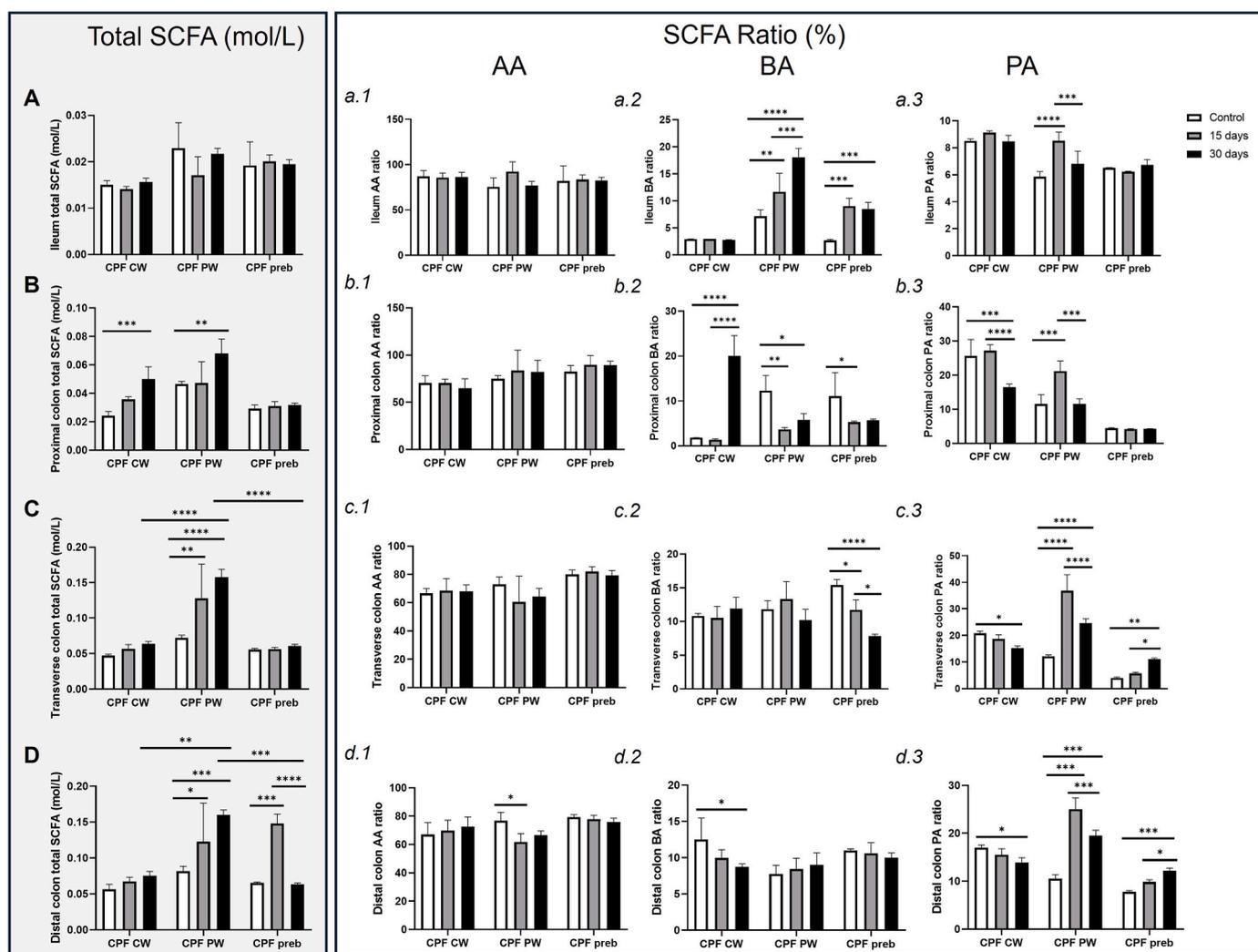


Fig. 4. Effect of 15 and 30 days of CPF in non-pregnant women (CPF CW) and pregnant women (CPF PW) and to CPF preb on the Total SCFAs levels (A. Ileum B. Proximal colon C. Transverse colon and D. Distal colon) and ratio of acetate (AA column: a.1, a.2, a.3 and a.4 in the respective reactors), butyrate (BA column: b.1, b.2, b.3 and b.4) and propionate (PA column: c.1, c.2, c.3 and c.4). Values are expressed as mean \pm SD using two-way ANOVA test. Significance * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Pe values remaining below 0.2×10^{-5} cm/s. This confirms the integrity of the barrier in the absence of SHIME® exposure, indicating a stable and intact IB. After exposure to CPF (15 days)-treated SHIME® supernatants, no significant variation of Pe FD4 was detected. However, upon exposure to CPF (30 days)-treated SHIME® supernatants there is a marked increase in permeability, suggesting that CPF disrupts the IB function. Specifically, the permeability value rises from a baseline of approximately 0.13×10^{-5} cm/min in the control group to around 0.8×10^{-5} cm/min in the Caco-2 cells. In contrast, the cells exposed to CPF + inulin-treated SHIME® supernatants had permeability values similar to the control, suggesting that inulin maintains the integrity of the IB.

Immunofluorescence analysis of the tight junction protein ZO-1 confirmed these permeability results. In the control group, ZO-1 (green) was consistently localized along the Caco-2 cell membrane (Fig. 6 Ca), indicating intact tight junctions. After exposure to CPF-treated SHIME® supernatants, ZO-1 staining became discontinuous and faded, consistent with the increased permeability and disruption of tight junctions. In contrast, ZO-1 staining remained intact in the CPF + inulin group, further supporting the protective role of inulin against CPF-induced tight junction disruption.

To assess the potential indirect effects of CPF on the BBB, endothelial cells were exposed to aliquots collected from the basolateral

compartment of Caco-2 cells after 4 h of SHIME® exposure, where CPF may have been metabolized. Despite the observed disruption in the IB, no significant changes in FD4 permeability were detected in the BBB endothelial cells after exposure to these basolateral aliquots (Fig. 6 B, right graph), indicating that gut-derived factors did not compromise BBB integrity under these conditions.

In parallel, we assessed the possibility that the increased Pe FD4 in Caco-2 cells could lead to bacterial translocation from the apical to the basolateral compartments and into the endothelial cells of the BBB.

We first assessed bacterial translocation across Caco-2 cells exposed to CPF-treated SHIME® supernatants (from the distal colon or a mix of all colon segments). As shown in Fig. 6 D, significant differences in bacterial translocation were observed among treatment groups, indicating disruption of IB function. In the control group, the percentage of bacterial translocation remained low, confirming that exposure to non-treated SHIME® supernatants did not compromise IB integrity. However, exposure to CPF (30 days)-treated SHIME® supernatants led to a marked increase in translocation, with percentages rising from 0% in the control group to 30% in the CPF15 group and 87% in the CPF30 group (distal colon). Similarly, in Caco-2 cells exposed to colon mix SHIME® supernatants, translocation percentages increased from 0% in the control to 30% in CPF15 and 92% in CPF30. In contrast, inulin maintained

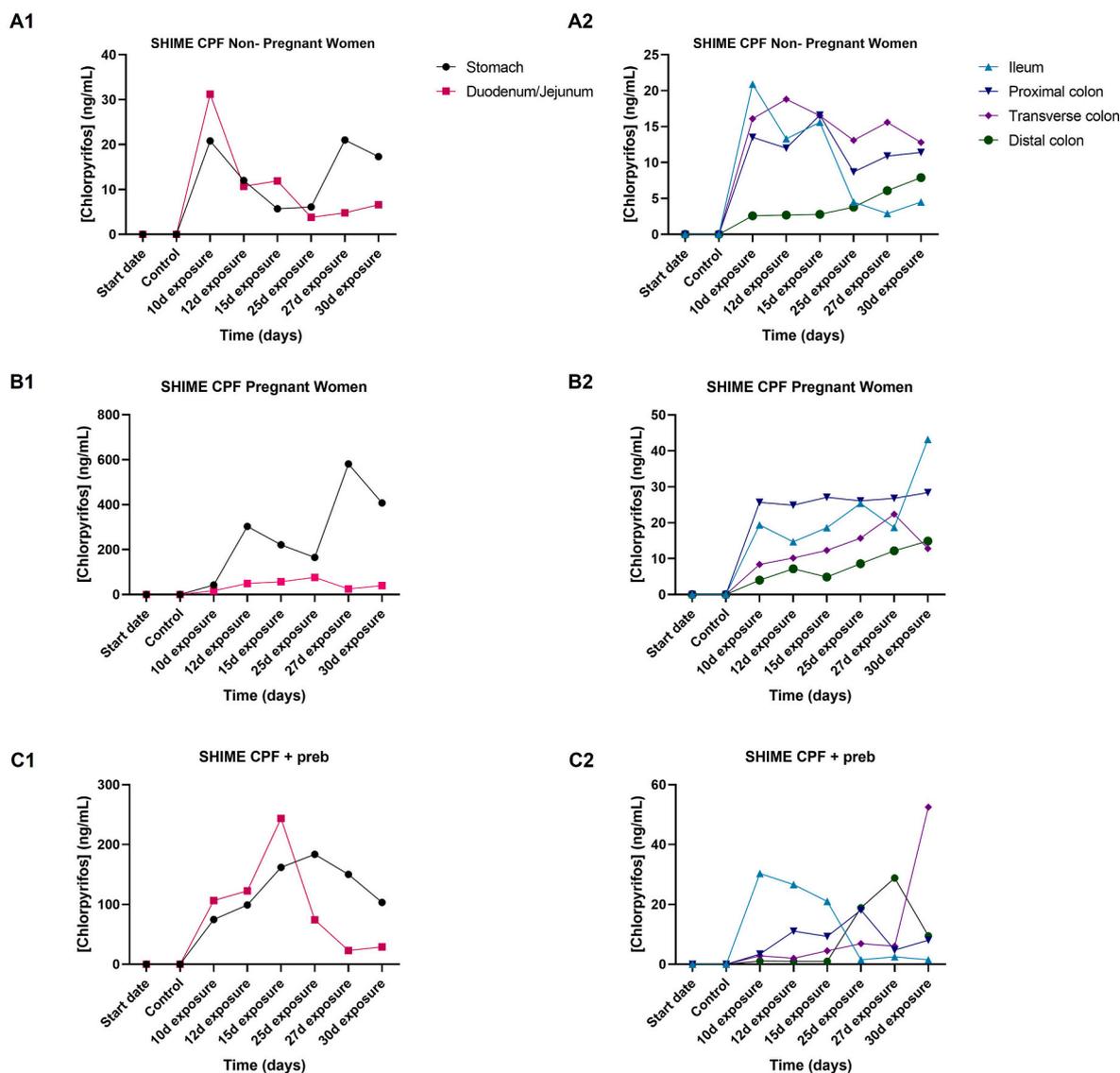


Fig. 5. Variation of CPF concentration in SHIME® reactors +from the start date (fecal inoculum before stabilization period) to 30 day of exposure to the CPF in non-pregnant women (A1 and A2), CPF in pregnant women (B1 and B2), and CPF + preb (C1 and C2).

gut barrier stability, with translocation percentages comparable to the control, ranging from 0% to 13% (distal colon) and 3%–11% (colon mix). Next, we examined the hypothetical possibility that the increase in Pe FD4 in Caco-2 cells could affect bacterial translocation to the endothelial cells of the BBB. Importantly, no bacterial translocation was detected across the BBB, confirming the stability of BBB permeability after 4 h of exposure to Caco-2 basolateral samples.

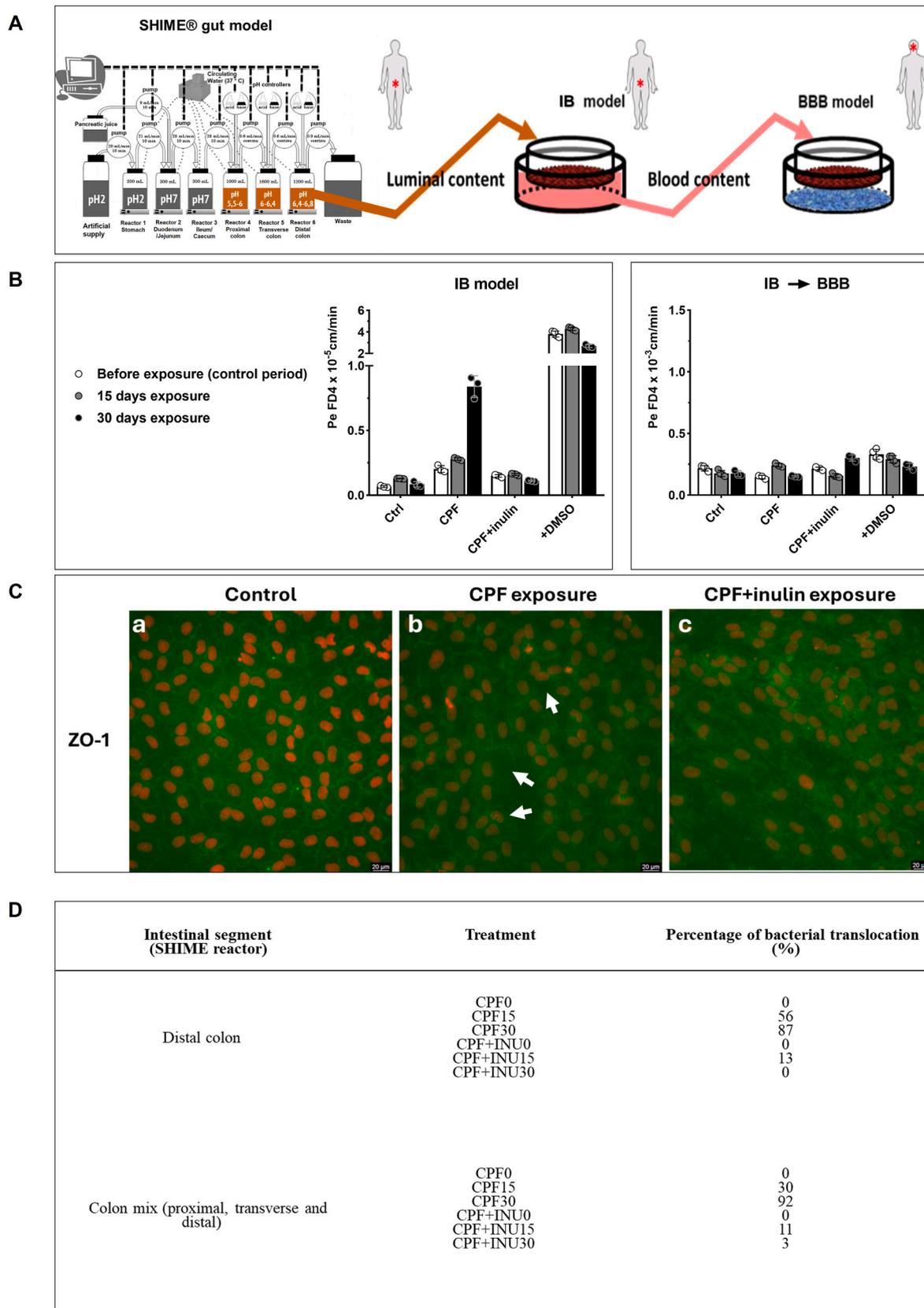
4. Discussion

4.1. Impact of CPF on gut microbiota-BBB axis and prevention by inulin

The GM-BBB axis represents a complex and dynamic communication network that links the gut, the IB, and the brain. The GM plays a crucial role in maintaining intestinal homeostasis by regulating the permeability of the IB and modulating immune responses by producing metabolites (e.g. SCFAs). In turn, the IB serves as the first line of defense and acts as a crucial checkpoint against harmful pathogens and toxins, preventing bacterial translocation and ensuring that only essential nutrients and metabolites are absorbed into the bloodstream (Abou Diwan et al., 2023). The BBB is a selective barrier that shields the brain from

harmful substances circulating in the blood and works in parallel with the IB to maintain central nervous system (CNS) homeostasis (Abbott et al., 2010). Communication between the gut and the brain occurs through various pathways, including microbial metabolites, immune signaling, and systemic circulation. Dysregulation in this axis—whether through pesticide exposure, altered GM composition, increased intestinal permeability, or BBB disruption—can initiate a cascade of effects contributing to neuroinflammation and neurodegenerative diseases (Matsuzaki et al., 2023). While some studies have explored these dynamics, there is limited research specifically focusing on pregnant women. Our study specifically aims to evaluate the impact of CPF on the GM, IB, and BBB, and to analyze the potential protective effects of inulin during CPF exposure.

The primary objective of this study was to assess the consequences of pesticide-induced gut dysbiosis in the SHIME® model (Van de Wiele et al., 2015; Joly Condetto et al., 2022) on IB and BBB permeability, utilizing an IB model of Caco-2 cells (Sevin et al., 2013) and a BBB co-culture model of human brain-like endothelial cells and human pericytes (Versele et al., 2022). Additionally, our research explores whether gut dysbiosis induced by CPF exposure can lead to bacterial translocation across the IB and BBB. Since bacterial translocation is a critical



(caption on next page)

Fig. 6. Effect of 15 and 30 days (CPF15 and CPF30) of CPF exposure (1 mg/day) \pm inulin (10 mg/day) of GM on IB and BBB models. (A) Schematic representation of the combination of the three human in vitro models. The SHIME® model is represented by six reactors (stomach to distal colon), maintained at 37 °C, controlled pH, and connected by peristaltic pumps. Artificial supply is connected to the stomach while pancreatic juice is connected to duodenum/jejunum reactor. (B) Permeability to non-permeant FD4 in both barriers IB and BBB models. Ctrl: permeability of negative control in the absence of SHIME; CPF: in the presence of SHIME® exposed to CPF (grey for the experiment after 15 days of exposure and black for experiment after 30 days of exposure to CPF); CPF + inulin: in the presence of SHIME® exposed to CPF + inulin (grey for the experiment after 15 days of exposure and black for experiment after 30 days of exposure to CPF + inulin); DMSO: positive control of permeability in the absence of SHIME®. (C) Immunostaining of Zonula Occludens 1 (ZO-1) (green color) of Caco-2 cells. Nuclei appear in red. Scale bar = 20 μ m. White arrows indicate discontinuous staining. (D) Bacterial translocation percentage of SHIME® total flora (of distal colon and colon mix of the three colon segments) across the IB (Caco-2 cells) at CPF0 (SHIME® samples collected before CPF exposure), CPF15 (SHIME® samples collected 15 days after CPF exposure) and CPF30 (SHIME® samples collected 30 days after CPF exposure). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

factor in the development of systemic inflammation and has been linked to various diseases, understanding this process is essential for evaluating the broader implications of pesticide exposure on human health.

In our previous studies, we utilized a rat model to investigate the effects of chronic CPF exposure on the maternal GM and the subsequent transgenerational impact on offspring, both in utero and postnatally (Djekkoun et al., 2022a, b; Abou Diwan et al., 2024). Recognizing the value of combining in vivo and in vitro approaches for a comprehensive understanding of pesticide toxicity, our current research employed in vitro models that more closely simulate human physiology. The novelty of our study lies in the integration of three highly standardized in vitro models, effectively mimicking the GM-BBB axis.

Gut microbiota composition Our study revealed that CPF exposure significantly disrupts GM composition, leading to an increase in pathogenic bacteria, particularly *Clostridium* spp. and *Enterococcus* spp., while decreasing beneficial bacteria like *Lactobacillus* spp. Notably, co-exposure to the prebiotic inulin mitigated some of these adverse effects, restoring *Lactobacillus* populations and stabilizing pathogenic taxa. This indicates that inulin may play a protective role in maintaining GM balance and intestinal integrity, positioning it as a potential therapeutic intervention against pesticide-induced gut alterations.

Overall, CPF exposure resulted in intestinal dysbiosis in both pregnant and non-pregnant women, with a more pronounced increase in *Clostridium* spp. and *Enterococcus* spp. observed in pregnant women, suggesting heightened sensitivity during this critical period. While our study aimed to demonstrate the robustness and statistical confidence of our results through p-value that was less than 0.0001 in some conditions, we acknowledge that bacterial numbers can exhibit substantial variability. Therefore, statistical significance does not always equate to biological relevance. In light of this, our aim was to emphasize the biological relevance and significance of our findings on gut dysbiosis, rather than focusing solely on statistical metrics.

Our findings in pregnant women GM underscore the need for further research into the implications of pesticide exposure on gut health, particularly in vulnerable populations.

Barrier's permeability Our findings indicate that an increase in pathogenic bacteria due to CPF exposure can exacerbate IB inflammation and permeability, leading to bacterial translocation. Permeability assays with Caco-2 cells demonstrated that SHIME® supernatants from CPF-treated conditions compromised gut barrier integrity, evidenced by increased FD4 permeability. This suggests that CPF, upon metabolism by gut bacteria, weakens the IB.

Immunofluorescence analysis of ZO-1 further supported these permeability findings. Importantly, while CPF compromised the IB, it did not lead to bacterial translocation across endothelial cells, indicating the BBB's resilience under these conditions. The BBB is a highly selective and tightly regulated barrier (Abbott et al., 2010), reinforced by more complex tight junctions which may explain its increased resistance to CPF effects under our experimental conditions (Obermeier et al., 2013; Saunders et al., 2015). This observation reinforces the notion that the BBB remains unaffected by CPF exposure, highlighting its robust protective mechanisms. These results emphasize the role of the GM-BBB axis and the critical importance of maintaining IB integrity to prevent systemic exposure to harmful bacteria. Moreover, the absence of

bacterial translocation (0 bacterial colonies detected) across the BBB further validates the reliability of our technique.

Notably, co-treatment with the prebiotic inulin reduced permeability and bacterial translocation, highlighting its protective effects against CPF-induced disruption of the gut barrier. Overall, these findings underscore inulin's therapeutic potential in preserving gut health in the presence of harmful substances like CPF.

SCFAs ratio alterations SCFAs are crucial metabolites produced by gut bacteria and play a vital role in maintaining gut health and barrier function. Acetate is the most abundant SCFA (acetate 60: butyrate 20: propionate 20) and serves as a precursor for butyrate production (Dalile et al., 2019; Zhang et al., 2023). In pregnant women, CPF exposure led to a decrease in butyrate, essential for colonocyte integrity and anti-inflammatory and neuroprotective functions, favoring propionate production. This shift is related to the decrease of butyrate-producing bacteria (such as *Faecalibacterium prausnitzii*) and could compromise gut barrier function and increase inflammation (Li et al., 2024). Conversely, the decrease of propionate to the favor of butyrate in CPF treated non-pregnant women GM in proximal colon is potentially negative for systemic metabolism, as propionate has been linked to cholesterol reduction and anti-inflammatory effects. The decrease of propionate, as seen after CPF exposure in transverse colon of non-pregnant women, and the decrease of both (butyrate and propionate) in distal colon, compromising the gut barrier and metabolism, can explain the increased bacterial translocation we observed across the IB. These findings align with our bacterial culture results, which revealed a rise in the potentially pathogenic bacteria (e.g. pathogenic *Clostridium* and *Enterococcus*) to the expense of beneficial bacteria (*Bifidobacterium*, *Lactobacillus* and beneficial *Clostridium* of cluster IV and XIV) (Guo et al., 2020).

In contrast, the increased butyrate ratio in ileum by inulin supplementation was not accompanied with propionate ratio decrease. In distal colon, inulin increased propionate ratio but butyrate didn't decrease which explains the beneficial effects of inulin we observed in bacterial culture (increase of *Bifidobacterium* and *Lactobacillus*) and the reduced risk of bacterial translocation (Djekkoun et al., 2022a; Qin et al., 2023; Abou Diwan et al., 2024). In addition, the time-dependent variations of butyrate and propionate confirm the link with a stable saccharolytic activity of the GM due to the prebiotic. Together, these data highlight the detrimental impact of CPF on GM composition, SCFAs production, and barrier integrity, emphasizing the potential benefits of interventions such as inulin supplementation.

CPF semi-quantitative assay While previous studies focused on CPF degradation by soil bacteria and insect's intestine, our study reported for the first time CPF concentrations in all intestinal segments of the human in vitro model. In this study, we successfully quantified CPF concentrations from day 0 (pre-exposure) to day 30 of exposure, representing a significant advancement in research on organophosphate metabolism alone and in the presence of prebiotics.

Our CPF assay results demonstrated an initial spike in CPF levels in the stomach, after CPF exposure started, due to direct ingestion, influenced by the acidic environment. As CPF moved to the duodenum, levels fluctuated, showing a secondary peak due to further digestion and absorption aided by bile and pancreatic enzymes. In the transverse colon,

CPF levels increased more slowly, reflecting the slower transit time and ongoing absorption and metabolism, with GM potentially altering CPF levels.

Our results showed that in the SHIME experiment supplemented with the prebiotic inulin, the decline in CPF concentration was more rapid, and lower CPF levels were detected in the colon reactors compared to the SHIME CPF experiment without inulin. Additionally, the final CPF concentration was lower in the SHIME CPF + prebiotic experiment than in the SHIME CPF. Inulin, as a prebiotic, promotes the growth of beneficial bacteria, which may be more efficient at breaking down CPF. Therefore, inulin may enhance the biotransformation and clearance of CPF by the GM. This enhanced microbial activity could lead to a more rapid decline in CPF concentration.

Another possible explanation is that inulin may bind to CPF, reducing its bioavailability. The binding capacity of fibers to pesticide residues has been studied previously (Ta et al., 1999), suggesting that they could limit CPF absorption in the gut by binding to it, thereby reducing its concentration in the colon reactors.

Extending the time points of assessment provides a comprehensive understanding of CPF metabolism, revealing long-term effects and aiding in the development of mitigation strategies.

Furthermore, specific metabolites of CPF, such as CPF-oxon and TCP, were not detected across reactors. The absence of CPF-oxon and TCP does not necessarily indicate that the microbial community in our model did not metabolize CPF. The observed decrease in CPF concentration from the stomach to the distal colon suggests that CPF was indeed metabolized by the GM. If CPF had been randomly degraded, we would expect to see uniform degradation across all intestinal segments. Instead, the progressive reduction in CPF concentration from the stomach to the colon supports the conclusion that the GM actively metabolized CPF as it passed through the different intestinal segments.

Moreover, this absence indicates that either these metabolites were not produced in detectable amounts by the semi-quantitative technique HPLC – MS- QTOF (which requires a more efficient quantitative method) or were rapidly further metabolized by the GM. Studies have indeed indicated that CPF-oxon may be degraded in the acidic environment of the stomach and/or undergo extensive first-pass metabolism within the gut (Eaton et al., 2008).

Overall, our results highlight the potential role of prebiotics like inulin in modulating the biotransformation and clearance of environmental toxins such as CPF in the gut, by increasing bacterial species capable of degrading CPF (Harishankar et al., 2013). Investigating the specific bacterial strains involved in CPF biotransformation could provide deeper insights into the mechanisms at play. This could have significant implications for dietary interventions aimed at reducing the toxic burden of such compounds in human and could be particularly beneficial in populations with high exposure to CPF.

Results consistency with existing literature Our findings align with previous in vitro studies that have demonstrated the detrimental effects of CPF on GM and barrier integrity (Réquillé et al., 2018; Guibourdenche et al., 2021; Joly Condet et al., 2022). For instance, a study reported similar gut dysbiosis by analyzing GM bacterial phyla (Zhao et al., 2016). The studies mentioned above have shown that CPF disrupts tight junction proteins, leading to increased intestinal permeability in Caco-2 cells after direct exposure to CPF or exposure to CPF-treated gut samples. This mirrors our results, where CPF exposure resulted in compromised gut barrier function.

Moreover, studies focusing on the role of the GM in regulating IB function have shown that dysbiosis can compromise barrier integrity (Di Vincenzo et al., 2023). These alterations in intestinal permeability are associated with several factors, including dietary components, microbial dysbiosis, and environmental toxins. This increased permeability is linked to systemic inflammation and metabolic disorders (Takiishi et al., 2017).

Numerous studies have documented significant alterations in GM composition due to environmental exposures, particularly pesticides

and other chemicals. For instance, Diazinon showed an impact on composition of bacterial populations (*Lachnospiraceae*, *Ruminococcaceae*, and *Clostridiaceae*) (Gao et al., 2017; Meng et al., 2020). Glyphosate, a widely used herbicide, has also been shown to alter GM balance by decreasing the relative abundance of *Firmicutes* and *Lactobacillus* and increasing *Fusobacteria* and proinflammatory cytokines IL-1 β , IL-6, and TNF- α (Tang et al., 2020). Additionally, a study by Mao and colleagues demonstrated that exposure to glyphosate, resulted in a marked decrease in the diversity of gut microbial communities in rodent models (Mao et al., 2018). This reduction in diversity was accompanied by an increase in pathogenic bacteria, highlighting the potential for environmental toxins to disrupt microbial balance. The impact of glyphosate on GM suggests that this herbicide is unlikely to remain unchanged as it passes through the digestive system. In fact, its chemical structure, specifically its phosphonate group, makes it highly reactive in the acidic environment of the stomach and the enzymatic conditions of the intestines. This leads to its metabolization contributing to the observed disruptions in microbial diversity (Lesseur et al., 2022).

Furthermore, studies found that exposure to pesticides can disrupt integrity in intestinal epithelial cells, resulting in heightened permeability and increased risk for inflammatory diseases. As an example, the fungicide Imazalil decreased *Firmicutes* population inducing gut dysbiosis and disrupted IB (Jin et al., 2016, 2021).

The protective effects of inulin observed in our study are also supported by previous research, which demonstrated that inulin supplementation can restore gut balance and IB function and reduce neuroinflammation (Hoffman et al., 2019; Uerlings et al., 2020). Moreover, a recent study demonstrated beneficial inulin effects on BBB permeability (Wang et al., 2023). These comparisons highlight the consistency of our findings with existing literature and underscore the potential of inulin as a therapeutic intervention. Overall, these findings underscore the sensitivity of the GM to environmental influences and the potential consequences of such disruptions on health. Understanding these dynamics is essential for developing targeted interventions to preserve gut health, particularly in vulnerable populations, including pregnant women.

While our findings align with many previous studies of CPF impacts on GM, the specific interaction between the GM, the IB, and the BBB remains an emerging field of research. Unlike many other studies that focused solely on the IB or GM, our study provides a novel exploration of the GM-BBB axis, emphasizing the importance of investigating pesticide effects across multiple biological barriers in vulnerable population (pregnant women). This comparison further highlights the necessity of combining in vitro models with in vivo studies to provide a more comprehensive understanding of the long-term effects of environmental contaminants like CPF on human health.

4.2. Strength and novelty of the in vitro approach to study the consequences of the GM dysbiosis on the human IB and BBB

In vivo and in vitro models are both essential tools in studying pesticide toxicity, each offering unique advantages. In vivo models, which involve studying pesticides in living organisms, provide a holistic understanding of how these chemicals interact with biological systems. However, these models are often expensive, time-consuming, and raise ethical concerns related to animal testing. In contrast, in vitro models offer a controlled environment where experimental conditions such as pH, temperature, and nutrient availability can be precisely manipulated, leading to higher reproducibility of results, which is crucial for validating scientific findings. These models are cost-effective, allow high-throughput screening and reduce the need for animal experimentation, addressing ethical issues.

Our mechanistic approach, using three standardized human in vitro models that mimic the GM-BBB axis, enables a detailed exploration of microbial interactions and enhances in vitro studies by incorporating key processes often missing in standard experiments. However, it is

important to note that this model greatly simplifies the complexity of the GM-BBB axis, as it does not account for the full range of these physiological interactions that occur in a living organism.

4.3. Limits and perspectives

Future studies should prioritize investigating the underlying mechanisms behind CPF's selective effects on the IB compared to the BBB. Understanding how inulin provides its protective benefits—particularly optimal dosages and its efficacy when used in conjunction with other interventions—will be essential. Additionally, long-term studies are necessary to evaluate the chronic impacts of CPF exposure on both the IB and BBB, potentially extending exposure durations to 24 h or several days to capture sustained effects. Furthermore, to better represent actual population exposures, future models could be designed to examine the combined effects of multiple pesticides, building on previous research that has explored the interactions between CPF and glyphosate in relation to brain health (Benbrook et al., 2021; Zhang et al., 2023). Such comprehensive investigations could significantly enhance our understanding of pesticide toxicity and its implications for human health.

To hypothetically improve our mechanistic approach and better mimic the gut-brain axis, we could explore the integration of more complex models.

5. Conclusion

In summary, our study provides insights into the impact of CPF on GM, IB, and BBB. By using *in vitro* models and fecal samples from women of child-bearing age and pregnant women, we elucidated the differential responses of the GM to CPF during pregnancy and its subsequent effects on the IB and BBB permeability, as well as bacterial translocation. Our findings revealed the detrimental effects of CPF on GM composition and IB integrity, while highlighting the protective role of inulin.

Importantly, while the dose of 1 mg/day CPF used in this study (approximately 0.016 mg/kg/day for a 60-kg individual) might be higher than typical environmental exposure levels for the general population, it aligns with potential exposure scenarios for individuals in high-risk settings, such as occupational environments or areas with elevated pesticide use. This dose provides valuable insight into the upper-limit risks associated with CPF exposure, especially for vulnerable populations like pregnant women. By addressing these exposure scenarios, our findings emphasize the importance of understanding the health implications of both typical and heightened CPF exposure levels in real-world contexts. Future studies should further investigate the long-term protective effects of prebiotics like inulin in mitigating pesticide-induced dysbiosis and barrier dysfunction.

CRediT authorship contribution statement

Maria Abou Diwan: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Avénie Huet:** Methodology, Formal analysis. **Juliette Poiriez:** Methodology. **Claire Joly Condette:** Writing – original draft, Methodology, Conceptualization. **Stéphane Delanaud:** Software, Methodology. **Emmanuel Sevin:** Writing – review & editing, Software, Methodology, Formal analysis, Data curation. **Aurélié Corona:** Methodology. **Larbi Rhazi:** Methodology. **Flore Depeint:** Writing – review & editing, Validation, Methodology, Data curation. **Halima Ouadid-Ahidou:** Visualization, Project administration, Funding acquisition. **Fabien Gosselet:** Writing – review & editing, Funding acquisition. **Véronique Bach:** Visualization, Resources, Funding acquisition. **Pietra Candela:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Hafida Khorsi-Cauet:** Writing – review &

editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Ethical approval

The study protocol was approved by the Ethical committee for non-interventional research (CERNI) of Picardy Jules Verne University (Evaluation number 2023-07-5/2023–27).

The ethics committee of the Medical Faculty at the University of Yamaguchi Graduate School (IRB#: H18-033-6) approved the study protocol for isolating pericytes from human tissue, ensuring compliance with the Declaration of Helsinki as revised in Somerset West in 1996. Additionally, the French Ministry of Higher Education and Research (CODECOH Number DC2011-1321) approved the protocol for isolating endothelial cells, and all related experiments were conducted following this approved protocol.

Consent to participate

Participation in this study was entirely voluntary, and participants could withdraw at any time without any penalty. Participants were given a detailed explanation of the study's purpose, procedures, potential risks, and benefits. Confidentiality of the participants' data was maintained throughout the study, since the protocol ensures that the processed data is anonymous. It does not allow for the identification of individuals, even indirectly, in any way; therefore, it is not considered personal data, and there is no correspondence table linking each person's identity to a random number referring to a series of individual data.

Consent to publish

Not applicable.

Funding

This research received funding from the French State and the Hauts-de-France Region as part of CPER MOSOPS 2021–2027 project. Maria Abou Diwan received a doctoral fellowship from the 'Conseil régional du Nord-Pas-de-Calais' (50%), Picardy University (25%), and the Artois University (25%).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Maria ABOU DIWAN reports financial support was provided by Regional Directorate of Public Finance of the Hauts de France. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors extend their gratitude to the gynecologist Dr. Arthur Foulon at the University Hospital of Amiens for identifying suitable participants and providing fecal samples. Our appreciation goes to all women volunteers who donated fecal samples for research. We also thank members of the research management team of Picardy Jules Verne University for their assistance with the ethics approval application for this study. We thank I-MITOX laboratory, especially Dr. Nouredine Sadeg, Dr. Florent Brousse and Dr. Gilles Malonga for pesticides metabolites analysis. We finally thank Pr. Fumitaka Shimizu and Pr. Takashi Kanda from the Department of Neurology and Clinical Neuroscience of Yamaguchi University in Japan for the human brain pericytes.

Data availability

Data will be made available on request.

References

- Abbott, N.J., Patabendige, A.A.K., Dolman, D.E.M., et al., 2010. Structure and function of the blood-brain barrier. *Neurobiol. Dis.* 37, 13–25.
- Abou Diwan, M., Djekkoun, N., Marie-Christine, B., et al., 2024. Maternal exposure to pesticides induces perturbations in the gut microbiota and blood – brain barrier of dams and the progeny, prevented by a prebiotic. *Environ. Sci. Pollut. Res.* <https://doi.org/10.1007/s11356-024-34969-1>.
- Abou Diwan, M., Lahimer, M., Bach, V., et al., 2023. Impact of Pesticide Residues on the Gut-Microbiota – Blood – Brain Barrier Axis : A Narrative Review.
- Balaguer-Trias, J., Deepika, D., Schuhmacher, M., Kumar, V., 2022. Impact of contaminants on microbiota: linking the gut–brain Axis with neurotoxicity. *Int. J. Environ. Res. Publ. Health* 19. <https://doi.org/10.3390/ijerph19031368>.
- Brousse, F., Malonga, G., Yang, G., Sadeg, N., 2019. Assessment of exposure to organic and inorganic pollutants in children's hair. *Int J Public Heal Res* 7, 18–22.
- Cecchelli, R., Aday, S., Sevin, E., et al., 2014. A stable and reproducible human blood-brain barrier model derived from hematopoietic stem cells. *PLoS One* 9. <https://doi.org/10.1371/journal.pone.0099733>.
- Culot, M., Lundquist, S., Vanuxeem, D., et al., 2008. An in vitro blood-brain barrier model for high throughput (HTS) toxicological screening. *Toxicol. Vitro* 22, 799–811. <https://doi.org/10.1016/j.tiv.2007.12.016>.
- Dalile, B., Van Oudenhove, L., Vervliet, B., Verbeke, K., 2019. The role of short-chain fatty acids in microbiota–gut–brain communication. *Nat. Rev. Gastroenterol. Hepatol.* 16, 461–478. <https://doi.org/10.1038/s41575-019-0157-3>.
- Darwiche, W., Gay-Quéheillard, J., Delanaud, S., et al., 2018. Impact of Chronic Exposure to the Pesticide Chlorpyrifos on Respiratory Parameters and Sleep Apnea in Juvenile and Adult Rats, pp. 1–12.
- Dehouck, M.-P., Méresse, S., Delorme, P., et al., 1990. An easier, reproducible, and mass-production method to study the blood–brain barrier in vitro. *J. Neurochem.* 54, 1798–1801. <https://doi.org/10.1111/J.1471-4159.1990.TB01236.X>.
- Di Vincenzo, F., Del Gaudio, A., Petito, V., et al., 2023. Gut microbiota, intestinal permeability, and systemic inflammation: a narrative review. *Intern Emerg Med.* <https://doi.org/10.1007/s11739-023-03374-w>.
- Djekkoun, N., Depeint, F., Guibourdenche, M., et al., 2022a. Chronic perigestational exposure to chlorpyrifos induces perturbations in gut bacteria and glucose and lipid markers in female rats and their offspring. *Toxics* 10, 138.
- Djekkoun, N., Depeint, F., Guibourdenche, M., et al., 2022b. Perigestational exposure of a combination of a high - fat diet and pesticide impacts the metabolic and microbiotic status of dams and pups : a preventive strategy based on prebiotics. *Eur. J. Nutr.* <https://doi.org/10.1007/s00394-022-03063-y>.
- Doroszkiewicz, J., Groblewska, M., Mroczko, B., 2021. The role of gut microbiota and gut–brain interplay in selected diseases of the central nervous system. *Int. J. Mol. Sci.* 22. <https://doi.org/10.3390/ijms221810028>.
- Eaton, D.L., Daroff, R.B., Autrup, H., et al., 2008. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. *Crit. Rev. Toxicol.* 38, 1–125. <https://doi.org/10.1080/10408440802272158>.
- Edwards, S.M., Cunningham, S.A., Dunlop, A.L., et al., 2017. The maternal gut microbiome during pregnancy. *MCN. Am J Matern Nurs* 42, 310–317. <https://doi.org/10.1097/NMC.0000000000000372>.
- Fuhler, G.M., 2020. The immune system and microbiome in pregnancy. *Best Pract. Res. Clin. Gastroenterol.* 44–45, 101671. <https://doi.org/10.1016/j.BPG.2020.101671>.
- Galea, I., 2021. The blood – brain barrier in systemic infection and in inflammation. <https://doi.org/10.1038/s41423-021-00757-x>.
- Gao, B., Bian, X., Chi, L., et al., 2017. Organophosphate Diazinon altered quorum sensing, cell motility, stress response, and carbohydrate metabolism of gut microbiome. *Toxicol. Sci.* 157, 354–364. <https://doi.org/10.1093/toxsci/kfx053>.
- Guibourdenche, M., Haug, J., Chevalier, N., et al., 2021. Food contaminants effects on an in vitro model of human intestinal epithelium. *Toxics* 9, 135. <https://doi.org/10.3390/TOXICS9060135>, 2021, Vol 9, Page 135.
- Guo, P., Zhang, K., Ma, X., He, P., 2020. Clostridium species as probiotics: potentials and challenges. *J. Anim. Sci. Biotechnol.* 11, 1–10. <https://doi.org/10.1186/S40104-019-0402-1/FIGURES/2>.
- Harishankar, M.K., Sasikala, C., Ramya, M., 2013. Efficiency of the intestinal bacteria in the degradation of the toxic pesticide, chlorpyrifos. *3 Biotech* 3, 137–142. <https://doi.org/10.1007/s13205-012-0078-0>.
- Heymans, M., Figueiredo, R., Dehouck, L., et al., 2020. Contribution of brain pericytes in blood-brain barrier formation and maintenance: a transcriptomic study of cocultured human endothelial cells derived from hematopoietic stem cells. *Fluids Barriers CNS* 17, 1–28. <https://doi.org/10.1186/S12987-020-00208-1/FIGURES/7>.
- Hoffman, J.D., Yanckello, L.M., Chlipala, G., et al., 2019. Dietary inulin alters the gut microbiome, enhances systemic metabolism and reduces neuroinflammation in an APOE4 mouse model. <https://doi.org/10.1371/journal.pone.0221828>.
- Jin, C., Yuan, X., Wang, C., et al., 2021. Maternal exposure to imazalil disrupts intestinal barrier and bile acids enterohepatic circulation tightly related IL-22 expression in F0, F1 and F2 generations of mice. *J. Hazard Mater.* 403, 123668. <https://doi.org/10.1016/J.JHAZMAT.2020.123668>.
- Jin, C., Zeng, Z., Fu, Z., Jin, Y., 2016. Oral imazalil exposure induces gut microbiota dysbiosis and colonic inflammation in mice. *Chemosphere* 160, 349–358. <https://doi.org/10.1016/j.chemosphere.2016.06.105>.
- Joly Condette, C., Djekkoun, N., Reygnier, J., et al., 2022. Effect of daily co-exposure to inulin and chlorpyrifos on selected microbiota endpoints in the SHIME® model. *Environ. Pollut.* 302, 118961. <https://doi.org/10.1016/J.ENVPOL.2022.118961>.
- Joly Condette, C., Elion Dzon, B., Hamdad, F., et al., 2016. Use of molecular typing to investigate bacterial translocation from the intestinal tract of chlorpyrifos-exposed rats. *Gut Pathog.* 8, 1–12. <https://doi.org/10.1186/s13099-016-0129-x>.
- Joly, C., Gay-Quéheillard, J., Léké, A., Chardon, K., Delanaud, S., Bach, V., Khorsi-Cauet, H., 2013. Impact of chronic exposure to low doses of chlorpyrifos on the intestinal microbiota in the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) and in the rat. *Environ Sci Pollut Res Int* 20 (5), 2726–2734. <https://doi.org/10.1007/s11356-012-1283-4>. Epub 2012 Nov 8. PMID: 23135753.
- Lahimer, M., Djekkoun, N., Tricotteaux-Zarqaoui, S., et al., 2023. Impact of perinatal coexposure to chlorpyrifos and a high-fat diet on kisspeptin and GnRH presence and reproductive organs. *Toxics* 11, 789. <https://doi.org/10.3390/TOXICS11090789>, 2023, Vol 11, Page 789.
- Lecerf, J.M., Dépeint, F., Clerc, E., et al., 2012. Xylo-oligosaccharide (XOS) in combination with inulin modulates both the intestinal environment and immune status in healthy subjects, while XOS alone only shows prebiotic properties. *Br. J. Nutr.* 108, 1847–1858. <https://doi.org/10.1017/S0007114511007252>.
- Lesueur, C., Pathak, K.V., Pirrotte, P., et al., 2022. Urinary glyphosate concentration in pregnant women in relation to length of gestation. *Environ. Res.* 203, 111811. <https://doi.org/10.1016/j.envres.2021.111811>.
- Li, Y., Liu, A., Chen, K., et al., 2024. Sodium butyrate alleviates lead-induced neuroinflammation and improves cognitive and memory impairment through the ACS2/H3K9ac/BDNF pathway. *Environ. Int.* 184, 108479. <https://doi.org/10.1016/J.ENVINT.2024.108479>.
- Linares, R., Francés, R., Gutiérrez, A., Juanola, O., 2021. Bacterial translocation as inflammatory driver in crohn's disease. *Front. Cell Dev. Biol.* 9, 703310. <https://doi.org/10.3389/FCCELL.2021.703310/BIBTEX>.
- Mao, Q., Manservigi, F., Panzocchi, S., et al., 2018. The Ramazzini Institute 13-week pilot study on glyphosate and Roundup administered at human-equivalent dose to Sprague Dawley rats: effects on the microbiome. *Environ Heal.* <https://doi.org/10.1186/s12940-018-0394-x>.
- Matsuzaki, R., Gunnigle, E., Geissen, V., et al., 2023. Pesticide exposure and the microbiota-gut-brain axis. *ISME J.* 17, 1153–1166. <https://doi.org/10.1038/s41396-023-01450-9>.
- Menaceur, C., Hachani, J., Dib, S., et al., 2023. Highlighting in vitro the role of brain-like endothelial cells on the maturation and metabolism of brain pericytes by SWATH proteomics. *Cells* 12, 1010. <https://doi.org/10.3390/CELLS12071010/S1>.
- Meng, Z., Liu, L., Yan, S., et al., 2020. Gut microbiota: a key factor in the host health effects induced by pesticide exposure. *J. Agric. Food Chem.* <https://doi.org/10.1021/acs.jafc.0c04678>.
- Morais, L.H., Schreiber, H.L., Mazmanian, S.K., 2021. The gut microbiota–brain axis in behaviour and brain disorders. *Nat. Rev. Microbiol.* 19, 241–255. <https://doi.org/10.1038/s41579-020-00460-0>.
- Nagpal, R., Yadav, H., 2017. Bacterial translocation from the gut to the distant organs: an overview. *Ann. Nutr. Metab.* 71, 11–16. <https://doi.org/10.1159/000479918>.
- Obermeier, B., Daneman, R., Ransohoff, R.M., 2013. Development, maintenance and disruption of the blood-brain barrier. *Nat. Med.* 19, 1584–1596. <https://doi.org/10.1038/NM.3407>.
- Qin, Y.Q., Wang, L.Y., Yang, X.Y., et al., 2023. Inulin: properties and health benefits. *Food Funct.* 14, 2948–2968. <https://doi.org/10.1039/D2FO01096H>.
- Réquilé, M., González Alvarez, D.O., Delanaud, S., et al., 2018. Use of a combination of in vitro models to investigate the impact of chlorpyrifos and inulin on the intestinal microbiota and the permeability of the intestinal mucosa. *Environ. Sci. Pollut. Res.* 25, 22529–22540. <https://doi.org/10.1007/s11356-018-2332-4>.
- Reygnier, J., Condette, C.J., Bruneau, A., et al., 2016a. Changes in composition and function of human intestinal microbiota exposed to chlorpyrifos in oil as assessed by the SHIME® model. *Int. J. Environ. Res. Publ. Health* 13. <https://doi.org/10.3390/ijerph13111088>.
- Reygnier, J., Lichtenberger, L., Elmhiri, G., et al., 2016b. Inulin supplementation lowered the metabolic defects of prolonged exposure to chlorpyrifos from gestation to young adult stage in offspring rats. *PLoS One* 11. <https://doi.org/10.1371/journal.pone.0164614>.
- Rutsch, A., Kantsjö, J.B., Ronchi, F., 2020. The gut-brain Axis : how microbiota and host inflammasome influence brain physiology and pathology. *Front. Immunol.* 11, 1–24. <https://doi.org/10.3389/fimmu.2020.604179>.
- Sampson, T.R., Debelius, J.W., Thron, T., et al., 2016. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell* 167, 1469–1480.e12. <https://doi.org/10.1016/J.CELL.2016.11.018>.
- Saunders, N.R., Dziegielewska, K.M., Möllgård, K., Habgood, M.D., 2015. Markers for blood-brain barrier integrity: how appropriate is Evans blue in the twenty-first century and what are the alternatives? *Front. Neurosci.* 9. <https://doi.org/10.3389/FNINS.2015.00385>.
- Sevin, E., Dehouck, L., Fabulas-da Costa, A., et al., 2013. Accelerated Caco-2 cell permeability model for drug discovery. *J. Pharmacol. Toxicol. Methods* 68, 334–339. <https://doi.org/10.1016/j.vascn.2013.07.004>.
- Sevin, E., Dehouck, L., Versele, R., et al., 2019. A miniaturized pump out method for characterizing molecule interaction with ABC transporters. *Int. J. Mol. Sci.* 20, 5529. <https://doi.org/10.3390/IJMS20225529>, 2019, Vol 20, Page 5529.
- Sheng, W., Ji, G., Zhang, L., 2023. Immunomodulatory effects of inulin and its intestinal metabolites. *Front. Immunol.* 14, 1224092. <https://doi.org/10.3389/FIMMU.2023.1224092/BIBTEX>.
- Shimizu, F., Sano, Y., aki, Abe M., et al., 2011. Peripheral nerve pericytes modify the blood–nerve barrier function and tight junctional molecules through the secretion of

- various soluble factors. *J. Cell. Physiol.* 226, 255–266. <https://doi.org/10.1002/JCP.22337>.
- Ta, C.A., Zee, J.A., Desrosiers, T., et al., 1999. Binding capacity of various fibre to pesticide residues under simulated gastrointestinal conditions. *Food Chem. Toxicol.* 37, 1147–1151. [https://doi.org/10.1016/S0278-6915\(99\)00110-6](https://doi.org/10.1016/S0278-6915(99)00110-6).
- Takiishi, T., Ideli, C., Fenero, M., et al., 2017. Intestinal barrier and gut microbiota : shaping our immune responses throughout life. *Tissue Barriers* 5, 1–12. <https://doi.org/10.1080/21688370.2017.1373208>.
- Tang, Q., Tang, J., Ren, X., Li, C., 2020. Glyphosate exposure induces inflammatory responses in the small intestine and alters gut microbial composition in rats. *Environ. Pollut.* 261, 114129. <https://doi.org/10.1016/j.envpol.2020.114129>.
- Uerlings, J., Schroyen, M., Willems, E., et al., 2020. Differential effects of inulin or its fermentation metabolites on gut barrier and immune function of porcine intestinal epithelial cells. *J. Funct. Foods* 67, 103855. <https://doi.org/10.1016/j.jff.2020.103855>.
- Van de Wiele, T., Van den Abbeele, P., Ossieur, W., et al., 2015. The simulator of the human intestinal microbial ecosystem (SHIME®). *Impact Food Bioact Heal Vitr Ex Vivo Model* 305–317. https://doi.org/10.1007/978-3-319-16104-4_27.
- Versele, R., Sevin, E., Gosselet, F., et al., 2022. TNF- α and IL-1 β Modulate Blood-Brain Barrier Permeability and Decrease Amyloid- β Peptide Efflux in a Human Blood-Brain Barrier Model.
- Wang, L., Wang, Z., Lan, Y., et al., 2023. Inulin attenuates blood-brain barrier permeability and alleviates behavioral disorders by modulating the TLR4/MyD88/NF- κ B pathway in mice with chronic stress. *J. Agric. Food Chem.* 71, 13325–13337. https://doi.org/10.1021/ACS.JAFC.3C03568/ASSET/IMAGES/LARGE/JF3C03568_0007.JPEG.
- Wright, R.O., 2017. Environment , susceptibility windows , development , and child health. *Curr. Opin. Pediatr.* 211–217. <https://doi.org/10.1097/MOP.0000000000000465>.
- Zhang, D., Jian, Y.P., Zhang, Y.N., et al., 2023. Short-chain fatty acids in diseases. *Cell Commun. Signal.* 211 (21), 1–20. <https://doi.org/10.1186/S12964-023-01219-9>, 2023.
- Zhao, Y., Zhang, Y., Wang, G., et al., 2016. Effects of chlorpyrifos on the gut microbiome and urine metabolome in mouse (*Mus musculus*). *Chemosphere* 153, 287–293. <https://doi.org/10.1016/j.chemosphere.2016.03.055>.