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Maternal exposure to pesticides induces perturbations in the gut microbiota and blood–brain barrier of dams and the progeny, prevented by a prebiotic

Maria Abou Diwan^{1,2} · Narimane Djekkoun¹ · Marie-Christine Boucau² · Aurélie Corona¹ · Lucie Dehouck² · Maurice Biendo¹ · Fabien Gosselet² · Véronique Bach¹ · Pietra Candela² · Hafida Khorsi-Cauet¹

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Abstract

Exposure to pesticide residues during the first 1000 days of life can disrupt body homeostasis and contribute to chronic metabolic diseases. Perinatal chlorpyrifos (CPF) exposure alters gut microbiota (GM) balance, potentially affecting offspring's health. Given the GM influence on brain function, the primary aim is to determine if pesticide-induced dysbiosis (microbial imbalance) affects indirectly other organs, such as the blood–brain barrier (BBB). The secondary objective is to evaluate the prebiotics protective effects, particularly inulin in promoting microbial balance (symbiosis), in both mothers and offspring. A total of 15 or more female rats were divided in 4 groups: control, oral CPF-exposed (1 mg/kg/day), exposed to inulin (10 g/L), and co-exposed to CPF and inulin from pre-gestation until weaning of pups. Samples from intestines, spleen, liver, and brain microvessels underwent microbiological and biomolecular analyses. Bacterial culture assessed GM composition of living bacteria and their translocation to non-intestinal organs. RT qPCR and Western blotting detected gene expression and protein levels of tight junction markers in brain microvessels. CPF exposure caused gut dysbiosis in offspring, with decreased *Lactobacillus* and *Bifidobacterium* and increased *Escherichia coli* ($p < 0.01$) leading to bacterial translocation to the spleen and liver. CPF also decreased tight junction's gene expression levels (50 to 60% decrease of CLDN3, $p < 0.05$). In contrast, inulin partially mitigated these adverse effects and restored gene expression to control levels. Our findings demonstrate a causal link between GM alterations and BBB integrity disruptions. The protective effects of inulin suggest potential therapeutic strategies to counteract pesticide-induced dysbiosis.

Keywords Gut-microbiota-BBB axis · Pesticides · Chlorpyrifos · Prebiotics · Perinatal period · Rat model · Dysbiosis · Bacterial translocation

Highlights

- First in vivo study examining Chlorpyrifos (CPF) and inulin's combined effects on the blood–brain barrier (BBB)
- Use of a rat model to mimic perinatal exposure effects on gut microbiota (GM) and BBB
- Analysis of sex-specific responses to indirect CPF-exposure in offspring
- Inulin mitigates CPF-induced gut dysbiosis and bacterial translocation to sterile organs
- CPF reduces tight junction gene expression in brain microvessels, partially restored by inulin
- Findings suggest inulin as a potential preventive strategy against pesticide-induced GM dysbiosis

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Introduction

The rising global population has increased food demand, leading to widespread pesticide use, which poses significant health concerns, particularly through chronic oral exposure (Mostafalou and Abdollahi 2016). This has led to restrictions of pesticide use and the prohibition of some substances, like dichloro-diphenyl-trichloroethane (DDT) and the shift to lower-risk alternatives: organophosphates and carbamates (Abubakar et al. 2019). Organophosphates, acetylcholinesterase (AChE) inhibitors, have been used in large quantities for many years which led to accumulation in soils and water despite having a half-life of less than 200 days (Supreeth and Raju 2017). Chlorpyrifos (CPF), a widely used organophosphate insecticide, is known to exert its toxic effects primarily by inhibiting AChE, a pivotal enzyme in nerve impulse

transmission (Darwiche et al. 2018; El Khayat El Sabbouri et al. 2020). The irreversible inhibition of AChE is due to metabolic activation of CPF to CPF-oxon. This leads to the accumulation of acetylcholine (ACh) at synapses and neuromuscular junctions, resulting in overstimulation of the nervous system, causing neurotoxicity, oxidative stress, and inflammation through the PI3K/AKT/JNK pathway (Zhang et al. 2023).

The nervous system is not the only target of CPF toxicity. Recent studies have shown that CPF exposure may disrupt body homeostasis by affecting the gut microbiota (GM) and the blood–brain barrier (BBB), although the exact mechanisms remain unclear (Li and Ehrich 2013; Joly Condetta et al. 2015; Balaguer-Trias et al. 2022; Abou Diwan et al. 2023). Bidirectional communication exists between GM and BBB and is part of the endocrine network in the gut-microbiota-brain axis. However, the causal relationship between CPF exposure, changes in GM, and alteration of the BBB has not been elucidated.

GM is a major gut component and a critical regulator of food contaminants and body homeostasis. This complex community of microorganisms, primarily bacteria of two major phyla *Bacillota* and *Bacteroidota*, is implicated in many physiological functions including absorption, metabolism, the endocrine system, and immunity (Valdes Ana et al. 2018; Matsuzaki et al. 2023).

In pregnant dams, CPF exposure has been associated with an imbalance in the intestinal flora known as intestinal dysbiosis characterized by a decrease in the potentially beneficial flora and an increase in the potentially pathogenic flora (Jin et al. 2016; Djekkoun et al. 2022a, b). This imbalance can be countered by inulin, a prebiotic that promotes the growth of beneficial bacteria like *Bifidobacterium* and *Lactobacillus* and prevents intestinal dysbiosis and bacterial translocation (the passage of bacteria across the intestinal barrier (IB) (Reygnier et al. 2016; Réquillé et al. 2018). In fact, prebiotics are emerging as one of the most frequently used substances to maintain a healthy microbiome or restore its balance when bacterial equilibrium is disrupted (Uerlings et al. 2020). As a soluble dietary fiber found in many plant species such as chicory and artichoke, inulin is fermented by the beneficial bacteria (*Bifidobacterium* and *Lactobacillus*) of the GM to beneficial metabolites including short-chain fatty acids (SCFAs) (Wang et al. 2023). It is now a well-known prebiotic with an outstanding beneficial regulation of intestinal microbiota and is well-tolerated up to 10 g/day in healthy young adults (Bonnema et al. 2010; Holscher et al. 2014). However, the consequences of intestinal microbiota modulations by dietary fibers on BBB and the alleviation of pesticide effects by prebiotics have not been well documented.

In numerous investigations, the GM has been scrutinized as an autonomous entity, largely neglecting the implications of pesticide-triggered intestinal dysbiosis on the BBB.

Given the interconnectedness of the gut-brain axis, investigating inulin's effects on the GM, and BBB is crucial. CPF-induced gut dysbiosis may compromise the BBB, a vital barrier that protects the brain, potentially leading to neurological disorders. The integrity of the BBB relies on tight junctions, and their disruption could permit harmful substances to enter the brain, causing central nervous system (CNS) damage. The long-term effects of such disruptions, especially in offspring, are not well understood (Li and Ehrich 2013; Lee et al. 2018; Réquillé et al. 2018; Cresto et al. 2023).

While most research has focused on the effects of CPF alone, our study uniquely explores the combined impact of CPF and inulin on GM and BBB during pregnancy. Notably, this is the first in vivo study to address the effect of CPF and inulin on the BBB, highlighting the novelty of our work. By examining inulin's protective role against CPF-induced dysbiosis and its influence on BBB integrity, this research makes a significant contribution to the field. Using a rat model to mimic the perinatal period in vivo, we assessed how pesticide exposure during gestation and lactation affects GM and BBB integrity in both mothers and offspring. Our analyses had a specific focus on sex-specific responses, as differences in CPF exposure effects between males and females have been documented (Shaffo et al. 2018; Guardia-Escote et al. 2018).

Materials and methods

Animals and study design

Animal housing

Wistar rats (Janvier laboratory—Saint Berthevin, France) were housed in pairs under stable environmental conditions, with an ambient temperature 23 ± 1 °C, a relative humidity of 26% and a 12-h light/dark cycle).

Daily animals' welfare monitoring and experiment protocol approval were previously detailed in our studies (Guibourdenche et al. 2021; Djekkoun et al. 2022a; Lahimer et al. 2023). The experiment protocol was approved by the local ethic committee (C2EA-96), the Regional Directorate for Health, Animal Protection, and the Environment in Amiens, France, as well as the Ministry of Research (reference number APAFIS #8207–2016121322563594 v2, approved on September 5, 2017). All animals were treated in compliance with EU Directive 2010/63.

Experimental design

Following a 1-week acclimation period, 16 mothers (7-week-old, body weight (BW) 225 ± 4.9 g) were randomly assigned to different experimental conditions ($n = 4$ /group). For the

reproduction protocol, each group was configured to include four females. This setup was designed to ensure a minimum of 6 to 10 female/male offspring for the analysis of passive impacts. Females were divided into 4 groups: C (control, standard chow (SC) diet, Serlab, Montataire, France), CPF (Chlorpyrifos), CPFI (CPF + inulin) and I (inulin). CPF females received 1 mg/kg of BW/day of CPF (O, O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate solution (purity 99.8% ± 0.1%, supplied by LGC Standards Laboratory, Molsheim, France). Dissolved in organic rapeseed oil, CPF was orally administered to the animals on a daily basis for 5 consecutive days, followed by a 2-day break, using gavage. 1 mg/kg BW represents the “no observed adverse effect level” (NOAEL) for the inhibition of cerebral cholinesterase activity in rats within the CPF groups. The remaining two groups were subjected to the mentioned diet and supplemented with prebiotics: 10 g/L inulin in water. The mean intake of inulin was 3.73 mg ± 0.04 mg/g of BW/day (Djekkoun et al. 2022a) (estimated based on the volume of water consumed daily, as dams were housed individually from pregnancy until lactation).

The 16 female dams were mated and upon pregnancy confirmation, housed individually, and exposed until the end of lactation. They were divided into 4 groups based on maternal exposure: CTRL group (standard chow), CPF

group (standard chow + CPF), inulin (standard chow + inulin), CPF-inulin (standard chow + CPF and inulin). The day of birth was marked as post-natal day 1 (PND1). Each litter averaged 10 pups, with 54% males. Offspring were monitored from birth until their early adult stage (60 days). They were not given any treatment and had unrestricted access to standard food and water. Animals were sacrificed at PND60 (young adults). Animals were fasted, the night before sacrifice and euthanized using intraperitoneal sodium pentobarbital (EXAGON 140 mg/kg–0.35 mL/kg) (Axience SAS, Pantin, France). Brain samples, intestinal segments, and other organs (liver, spleen, AND adipose tissue) were collected under sterile conditions and stored at – 80 °C (Fig. 1).

Microbiological analysis

Bacterial composition of intestinal microbiota and bacterial translocation

As described in Djekkoun et al. (2022b, a), the organs were measured in weight, placed within a sterile stomacher bag, and then blended using Ringer’s saline solution (Bio-Rad, Marne la Coquette, France). Subsequently, serial dilutions were prepared by a factor of ten. Following this, 1 mL of the homogenized intestinal segments and sterile organs was

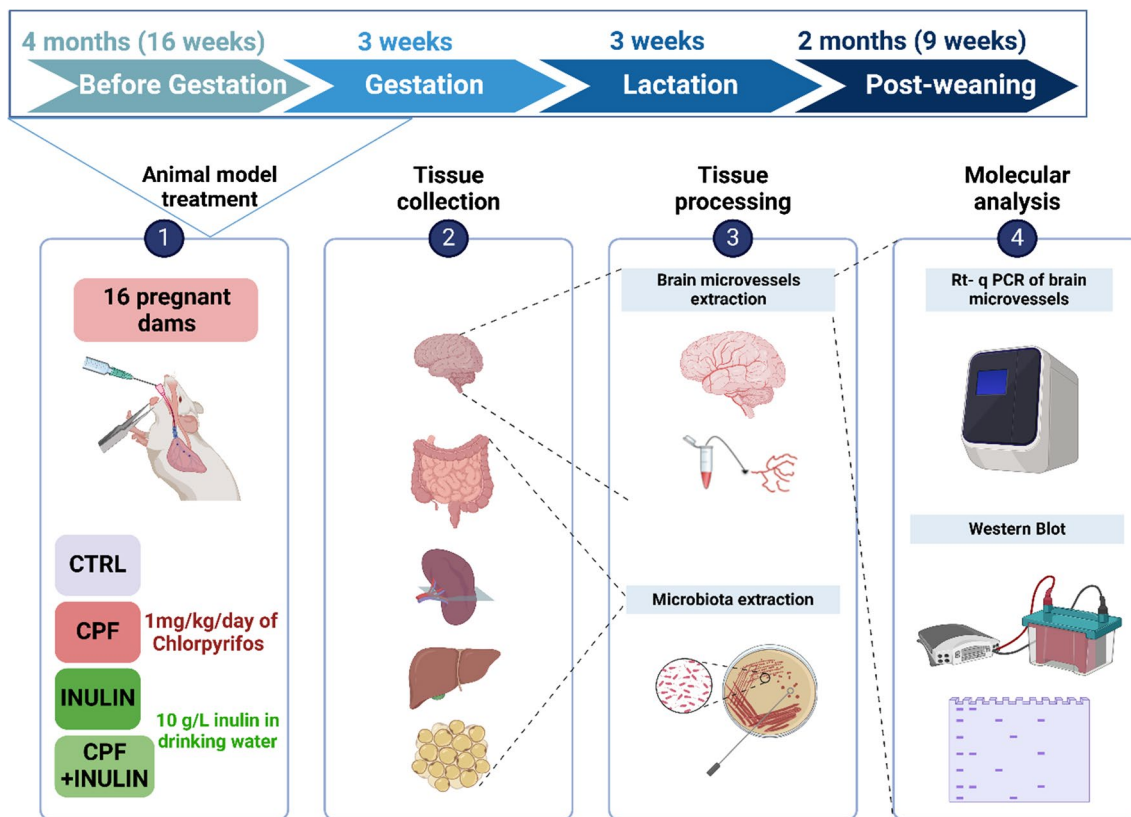


Fig. 1 Experimental study design

evenly distributed onto various selective and non-selective media to determine the presence of aerobic and anaerobic microbiota qualitatively and quantitatively (Bactron Anaerobic, Sheldon Manufacturing, Cornelius, OR, USA). The cultures were incubated at 37 °C for a period ranging between 48 h and 4 days, and bacterial identification was performed using established microbiological methods. The isolated colonies on the Petri dishes were enumerated using an automated system (Scan® 500, Inter-science, St Nom la Bretèche, France) and expressed as log (colony forming unit CFU)/g of tissue.

Bacterial fermentation metabolites

SCFAs (acetate, butyrate and propionate) were analyzed by gas chromatography in diluted gut microbiota samples and quantified by comparison to a standard curve (Djekkoun et al. 2022a).

Microvessel isolation from rats' brain (BBB)

Cortices

The protocol for isolating microvessels from rat's brain protocol was adapted from a previously detailed study of our laboratory colleagues (Coisne et al. 2005). Cortices were isolated, excluding the striatum, brain white matter, cerebellum, and optic nerves. The outer vessels and meninges were carefully removed. The tissue was homogenized in HBSS 1 × with 10 mM HEPES and 0.1% BSA. The homogenate was combined with 30% dextran in HBSS 1 ×, with 10 mM

HEPES and 0.1% BSA and centrifuged at 3000 g for 25 min (min) at 4 °C. The resulting pellet, containing microvessels, was further purified by repeated centrifugation. The vascular fraction was dissociated and filtered through a 59-µm nylon mesh to separate large vessels from capillaries. The enriched capillaries fraction was filtered through a 5-µm nylon mesh and suspended in ice-cold HBSS 1 × with 10 mM HEPES and 0.1% BSA. The filtrate was divided into equal volumes and centrifuged at 1000 g for 7 min at 4 °C. The resulting pellets were used for RNA and protein extractions, with NucleoZOL (lysis buffer of Macherey–Nagel NucleoSpin RNA extraction set for NucleoZOL) and RIPA 1 × lysis buffer (Merck Millipore, USA) added, respectively. The lysates were homogenized and frozen at – 80 °C until further extractions.

mRNA extraction and RT-PCR

Microvessel mRNA extraction was then performed using the commercially available Macherey–Nagel NucleoSpin® RNA extraction set for NucleoZOL. The RNA quantity and purity were assessed using a spectrophotometer, measuring absorbance at A260 and A280 nm. Reverse transcription (RT) was conducted in a total volume of 20 µL, comprising 250 ng of RNA, RT-MIX (BioRad iScript™ Reverse Transcription Supermix), and RNase-free water. RT and qPCR amplifications method we adopted were previously detailed (Versele et al. 2022). GAPDH and ubiquitin were used as housekeeping genes to normalize mRNA. Primers of housekeeping genes and targets are listed in Table 1 (Life Technologies, Thermo Fisher Scientific, Courtaboeuf, France).

Table 1 q-PCR primer pair nucleotide sequences (*Rattus norvegicus*), forward (F) and reverse (R) 5' to 3' primer sequence (accession numbers of cDNA from NCBI database)

Role	Gene	Sequences (5'-3')	Accession number
Housekeeping genes	GAPDH	F: AAGGTCGGTGTGAACGGATT R: CTTTGTCAACAAGAGAAGGCAGC	NM_017008.4
	Ubiquitin	F: CACCAAGAAGGTCAAACAGGAA R: TCAAACCCAAGAACAAGCACAA	NM_017314.1
Tight junction proteins	Occludin	F: TGACTACAGAGAGGAGAGCGAA R: CTGCAGATCCCTTAACTTGCT	NM_031329.2
	Claudin 3	F: CCAACACCATCATCCGGGATTT R: GTACAACCCAGTTCCCATCTCT	NM_031700.2
	Claudin 5	F: CTGGTTCGCCAACATCGTAG R: CCAGCTCGTACTTCTGAGACA	NM_031701.2
	ZO-1 (associated to TJ)	F: CTCTCAACAGAAAGCAGAAGCC R: TGCTTAACTGCAGAGGCTGA	NM_001106266.1
Adhesion molecules	ICAM-1	F: AGACCACTGTGCTTTGAGAACT R: GCTCTGGGAACGAATACACAGT	NM_012967.1
	VCAM-1	F: ACACAGCAGTCAAATGGAGTCT R: GGTGGGTTCTTTCGGAGCAA	NM_012889.2
Inflammation markers	NLRP3	F: ACCCACAGTGTAACTTGAGAA R: GTAGGTACAGGTGTGTGAGGTT	NM_001191642.1
	TLR4	F: ATTGTTCCTTTCTGCCTGAGA R: TCTGATCCATGCATTGGTAGGT	NM_019178.2

Endothelial cells, neuronal, and pericyte markers were used to verify the purity of our endothelial cells microvessel-enriched fraction.

Protein assay and western blot

Protein lysates were thawed on ice and centrifuged at 10,000 rpm at 4 °C for 10 min to eliminate cellular debris. The protein assay of supernatant was performed using Bradford method (BioRad, France), at 595 nm (spectrophotometer Synergy H1, BioTek, France).

Samples with 20 µg of protein were prepared in laemmli buffer (BioRad, Munich, Germany) with beta-mercaptoethanol (Sigma-Aldrich, Germany) and then heated at 95 °C for 5 min and electrophoresed using precast Criterion™ TGX™ gels (Bio-rad). Proteins were transferred to nitrocellulose membranes (GE Healthcare, Germany), blocked with Tris-buffered saline containing 0.1% Tween (TBST) 20 and 5% skimmed milk and incubated with primary antibodies (Table 2) overnight at 4 °C, except for β-actin (20 min at room temperature). After washing with TBST, membranes were incubated with a secondary antibody conjugated with horseradish peroxidase (Table 3). Protein bands were visualized using enhanced chemiluminescence reagent (GE Healthcare) and imaged with the Azure c600 Western blot Imaging system (Azure Biosystems, Dublin, Ireland). Band densities were quantified using AzureSpot 2.0 Gel Analysis software (Azure Biosystems, Dublin, Ireland), normalized to β-actin.

Statistical analysis

The bacterial composition data were expressed as mean ± standard deviation, unless otherwise specified. SPSS (version 25.0, IBM Corp., Armonk, NY, USA) and Stat View software (version 5.0, Abacus Concepts Inc., Berkeley, CA, USA) were used to conduct statistical analyses. Both one-way and two-way analyses of variance (ANOVA), as well as a chi-squared test, were employed (Djekkoun et al. 2022a). The statistical significance threshold was set at $p < 0.05$ for all analyses.

Although for female dams we were limited to 4 groups of 4 dams, we had 7 to 10 pups in each offspring group. The gut microbiota results we showed were those of male offspring

Table 3 Secondary antibodies used for the western blot experiment

Secondary antibodies	Dilution	Reference
Goat anti-rabbit HRP	1:8000	Dako PO44801
Goat anti-mouse HRP	1:5000	Dako PO44701

that were analyzed statistically with $n > 6$. In parallel, for the results illustrating the effects on BBB in offspring, we also had 7 to 10 brains per group and for each microvessel extraction (4 to 5 extractions/group), we needed to pool two brains coming from the same groups to: (1) have enough material for RT-q PCR and western blot analysis, (2) ensure the minimum number allowing statistical analysis, and (3) respect the number of rats allowed in the study based on ethical and animal protection.

The data of RT-qPCR analysis were presented as the average ± standard error of the mean (SEM). Statistical analysis was conducted using GraphPad Prism 8.0.1 statistical software (GraphPad Software, Inc., San Diego, CA, USA). Data from the RT-q PCR experiments were assessed employing a two-way ANOVA method when $n = 7$ to 10 (Guibourdenche et al. 2021). However, for dams' results, when having $n < 6$, we used a non-parametric test (Kruskal–Wallis test) (Deepika et al. 2022). $p < 0.05$ was set as the criterion for determining statistical significance for all analyses.

Results

Bacterial composition of intestinal microbiota

Pesticide exposure can elicit gender-specific responses (Comfort and Re 2017; Gao et al. 2017). In our previous published work, we illustrated results regarding the effect of CPF exposure on dams and female offspring bacterial composition of intestinal microbiota. Here, our attention is directed towards the microbial composition of male offspring. Starting with the analysis of total flora, no significant difference was observed between the male offspring groups (aerobic flora $p = 0.174$, anaerobic flora $p = 0.978$ CPF vs. control). Afterwards, our assessment of the microbial composition at the phylum level revealed modification of concentrations of selected gut bacteria in male offspring.

Table 2 Primary antibodies used for the western blot experiment

Primary antibodies	Size (kDa)	Dilution	Reference
Rabbit anti-Occludin	59	1:1000	ab-216327
Rabbit anti-Claudin 5	23	1:1000	GTX49370
Mouse anti-β-actin	42	1:20000	A5441B (Sigma)
Mouse anti-ICAM-1	56	1:250	MA5407 (Invitrogen)
Rabbit anti-VCAM-1 CD106	110	1:1000	PA5-80,213 (Invitrogen)

We observed a significant decrease of *Lactobacillus* spp. concentrations in male offspring treated with CPF compared to the control group ($p < 0.001$) (Fig. 2A). *Bifidobacterium* spp. results revealed a significant decrease ($p < 0.001$ vs. control group). On the contrary, we observed a significant increase, compared to the control group, in the potentially pathogenic flora within the CPF-treated males: *Escherichia coli* ($p < 0.01$), *Enterococcus* ($p < 0.001$) and *Staphylococcus* ($p < 0.001$) (Fig. 2B). Interestingly, prebiotic increased the concentration of *Lactobacillus* spp. ($p < 0.01$ vs. CPF) and *Bifidobacterium* spp. ($p < 0.001$ vs. CPF) respectively (Fig. 2A). However, we did not find a significant effect of inulin treatment on the potentially pathogenic flora compared to CPF group. These results altogether showed that CPF treatment significantly modulated both potentially beneficial and pathogenic GM populations leading to a bacterial dysbiosis in male offspring, whereas inulin beneficial effects targeted the potentially beneficial bacteria only.

In parallel to bacterial composition, we analyzed the SCFAs profile which varied based on the treatment. CPF increased the acetic acid content by 2% in the dams, though

this increase was not significant. In the offspring, the acetate ratio significantly increased by CPF exposure but inulin prevented it ($p = 0.012$; CPF = 71.3%, CPF-inulin = 53.7%). Regarding propionate and butyrate levels in both dams and offspring, there were increases but these variations were not significant in dams for either metabolite. In the offspring, CPF decreased propionic acid concentrations that were partially restored by inulin supplementation (CPF-inulin = 15.3% vs. C = 15.4% vs. CPF = 12.6%). Notably, in the offspring, the inhibition of butyrate by CPF and its recovery by inulin was significant ($p = 0.008$; CPF-inulin = 27.4% vs. CPF = 16.0%).

BT

To check if bacterial dysbiosis led to bacterial translocation (BT) of intestinal bacteria to sterile organs across the IB, we used bacterial culture methods to detect the translocation of bacteria that crossed the barrier towards spleen, liver, mesenteric adipose tissue, and gonadic adipose tissue (only data of spleen and liver are shown). To understand the factors affecting the translocation risk, we assessed the potential relationship

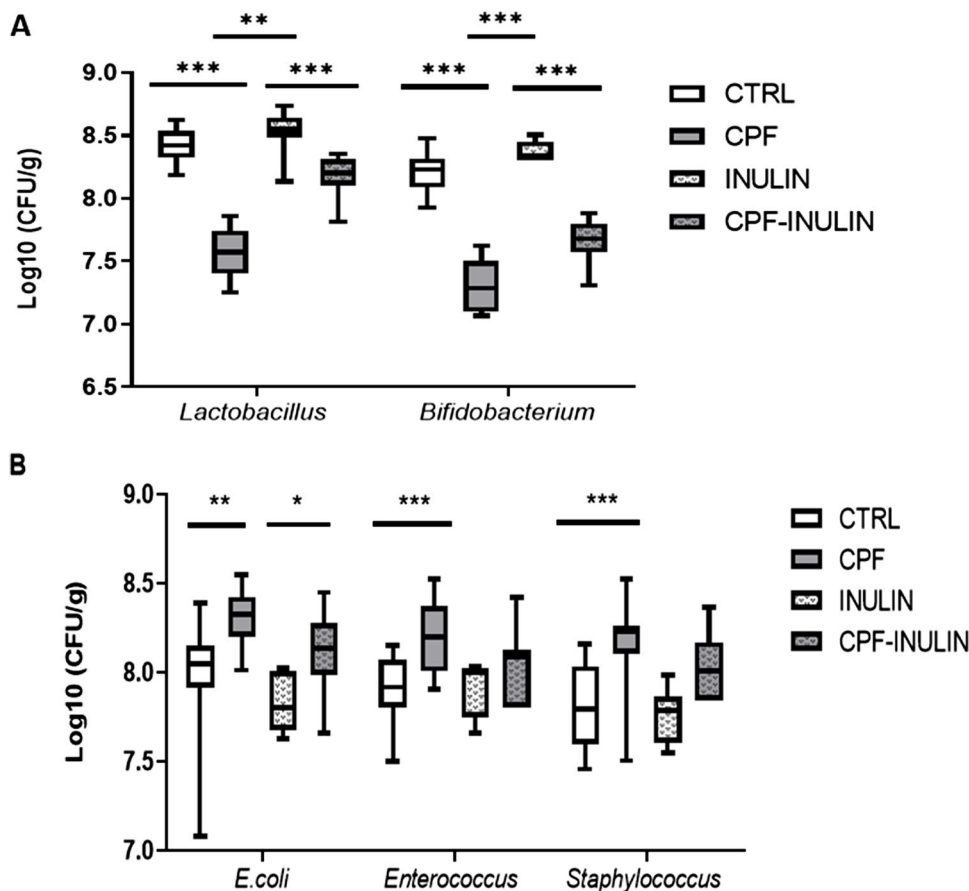


Fig. 2 Effects of chronic exposure to CPF and inulin on gut microbiota beneficial bacteria (A) and potentially pathogenic bacteria (B) in male offspring. Data is illustrated as mean \pm standard deviation

($n = 7-10$) using analysis of variance (ANOVA) and Tukey's test. Significance * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$. CTRL, control; CPF, chlorpyrifos; inulin; CPF-inulin, chlorpyrifos + inulin

between organ type, sex, treatment group, and translocation risk in the F0 (rat dams) and F1 (offspring) generations using logistic regression models performed with R® software version R-4.3.3 and Excel® software version 2010. A logistic regression model was initially used to assess the impact of organ type, gender, generation, and treatment group assignment on translocation risk. Then, the model was extended by including all variables together to explore potential confounding effects and to identify independent associations with translocation risk.

Influence of exposure on BT

In our analysis, we observed varying effects of exposure to CPF, inulin, and CPF-inulin on translocation risk with the reference group as the control category. Our results showed a large aerobic bacterial translocation to the spleen in CPF groups (25% of dams, 25% of female offspring, and 29.4% of male offspring) compared to control groups (0%) (Table 4). Overall, the CPF group had a significantly higher risk of translocation ($p < 0.001$) compared to the control group. No translocation to spleen is observed in dams and female offspring inulin groups (0%), while 14.28% of translocation is shown in male offspring inulin group compared to control (Table 4). The inulin group did not show a significant effect on translocation risk ($p = 0.6946$, inulin vs. control group). This statistical result confirms that inulin does not induce bacterial translocation contrarily to CPF pesticide. Similarly, in CPF + inulin groups, no translocation occurred except for the 20% observed in the male offspring group vs. control. In fact, the CPF-inulin group showed a less significant positive effect on translocation risk than CPF ($p = 0.0509$). Interestingly, when we compared our results to the reference category CPF, the logistic regression model showed that the CPF-inulin and inulin groups had a negative impact on translocation risk ($p < 0.001$). This suggests that being part of these groups is associated with a reduced risk of translocation compared to the CPF group. These findings

Table 4 Percentage of animals ($n = 3-10$) with bacterial translocation to sterile organs, for dams, female, and male offspring. The data are expressed as a percentage ($n = 3-10$)

Percentage of bacterial translocation (%)				
Treatment	Organ	Female dams	Female offspring	Male offspring
Control (Ctrl)	Spleen	0	0	0
CPF		25	25	29.4
Inulin		0	0	14.28
CPF + inulin		0	0	20
Control (Ctrl)	Liver	0	0	0
CPF		75	62.5	17.6
Inulin		0	0	0
CPF + inulin		25	16.6	10

show that CPF-inulin and inulin provide significant protection against translocation compared to the CPF group.

Influence of organ on BT

Compared to results in the spleen, higher levels of bacterial translocation were detected in liver of CPF groups of dams (75%) and female offspring (62.5%) but only 17.6% in the male offspring group, compared to all control and inulin groups (0%) (Table 4). Nevertheless, the logistic regression model provided no significant association between organ type and the occurrence of translocation ($p = 0.139$).

Influence of gender on BT

When analyzing results by gender, we noticed that dams and their female offspring shared similar translocation profile in the different exposure groups (the influence of gender is significant for CPF groups) in spleen, liver, and gonadic adipose tissue but not in mesenteric adipose tissue where female offspring shared similar results with male offspring. It seems that inulin reduced bacterial translocation in females more than in males. This led us to the hypothesis that CPF and inulin effects on translocation risk differ between female and male. Based on the provided logistic regression model, we did not find significant difference in translocation risk between male and female progeny ($p = 0.568$), but we did find that the interaction between sex and organ had a significant effect on translocation in individuals of the progeny ($p = 0.0160$). These findings highlight the importance of taking sex into account when assessing the effect of organs on translocation. They also indicate that gender modifies the relationship between organs and translocation, which could have significant implications for understanding the mechanisms underlying translocation.

Influence of generation on BT

Our overall descriptive comparison of results in dams to those of offspring showed differences only in the mesenteric adipose tissue where we observed similar translocation percentage in the different exposed groups between female and male offspring, but they were different from their mother translocation profile (data not shown for mesenteric adipose tissue). The logistic regression model provided no significant association between generations and the occurrence of translocation ($p = 0.8507$).

Gene expression analysis of tight junctions of microvessels (BBB)

Dams

Considering the crucial importance of maintaining the integrity of the BBB for proper brain functionality, predominantly

attributed to the tight junctional complex, we proceeded to analyze the tight junctions within microvessels following pesticide treatment. This analysis aimed to assess the integrity of the BBB in dams. In pursuit of this objective, real-time qPCR experiment was employed to assess the impact of CPF and inulin on the expression of the key tight junctions' proteins constituting the BBB. As shown in Fig. 3, our results demonstrate a significant reduction in mRNA levels following CPF exposure. Specifically, CLDN3 exhibited a 50% decrease; CLDN5 decreased by 70%, occludin by 60%, and ZO-1 by 40% ($p < 0.05$). Compared to CPF alone, inulin supplementation (CPF + inulin) tended to increase the expression of CLDN5 and OCLN but not for CLDN3 and ZO-1. These findings underscore the detrimental impact of CPF exposure on the integrity of BBB tight junctions, highlighting potential mechanisms underlying neurotoxicity associated with pesticide exposure.

Progeny

RT qPCR results

Previous studies have suggested that exposure to CPF may exert effects on offspring. These potential effects encompass microbiota abnormalities, alterations in endocrine disruptions, and reproductive issues. This study examines the impact of CPF exposure on tight junction proteins in brain microvessels of progeny rats. Following rat sacrifice, brain microvessels were extracted and the mRNA levels of tight junction proteins were analyzed. Our findings illustrated in Fig. 4(A and B) demonstrate significant alterations in female progeny rats exposed to CPF. Specifically, CPF exposure led to a substantial increase in CLDN5 mRNA levels (210%, $p < 0.001$) and OCLN mRNA levels (195%, $p < 0.05$)

compared to control groups (Fig. 4A). No significant change was observed for ZO-1. Similarly to the female progeny, our study revealed significant alterations in tight junction mRNA levels in male offspring (Fig. 4C and D). Specifically, CPF exposure led to a notable decrease in CLDN3 mRNA levels to 39% ($p < 0.05$) (Fig. 4C). Supplementation with inulin alone resulted in an 80% decrease ($p < 0.01$) in CLDN3 mRNA levels, while the combination of CPF and inulin led to a 64% decrease ($p < 0.05$). No significant variations were observed in the mRNA levels of CLDN5 and ZO-1. Furthermore, analysis of OCLN gene expression demonstrated a significant increase of 65% ($p < 0.05$) following exposure to CPF in combination with inulin compared to inulin alone. However, this increase was not significant compared to control or exposure to CPF alone. In addition, for female progeny, we explored the impact of CPF exposure on efflux pumps (glycoprotein P and breast cancer resistant protein BCRP) and pro-inflammatory markers (intercellular adhesion molecule ICAM-1, vascular cell adhesion molecule VCAM-1, Toll-like receptor TLR4, and NLR family pyrin domain containing NLRP3). Here, we detailed our results concerning pro-inflammatory markers (Fig. 4B). Our findings revealed a significant increase of 120% in ICAM-1 gene expression following CPF exposure compared to control groups. Interestingly, inulin supplementation led to a notable decrease in ICAM-1 expression to 35% compared to CPF alone ($p < 0.05$), suggesting a mitigating effect on inflammation by inulin. Additionally, we observed a significant increase in NLRP3 mRNA levels by CPF alone (241%) compared to control, which was attenuated to 170% by inulin supplementation (CPF + inulin) ($p < 0.01$). However, no significant variations were detected in VCAM-1 and TLR4 gene expression.

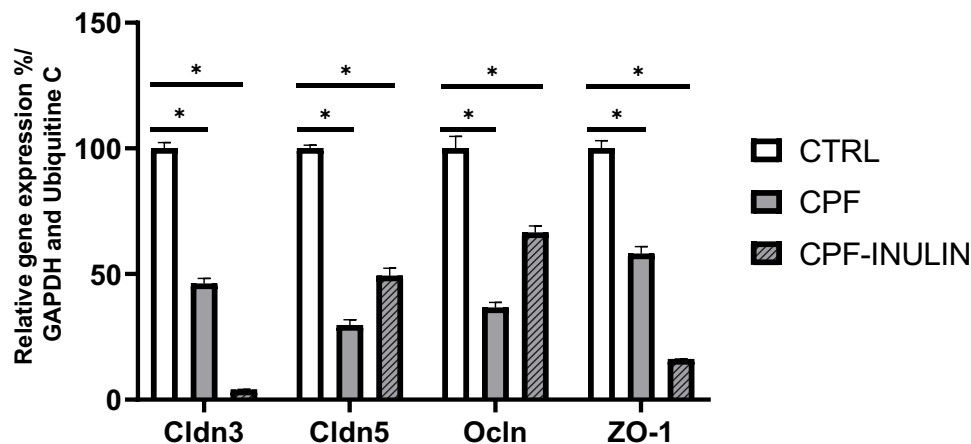


Fig. 3 Effects of chronic oral exposure to CPF and inulin on BBB tight junctions in dams. Values are expressed as mean \pm SEM using the non-parametric Kruskal–Wallis test. Significance * $p < 0.05$; **

$p < 0.01$, *** $p < 0.001$. CTRL, control; CPF, chlorpyrifos; inulin; CPF-inulin, chlorpyrifos + inulin

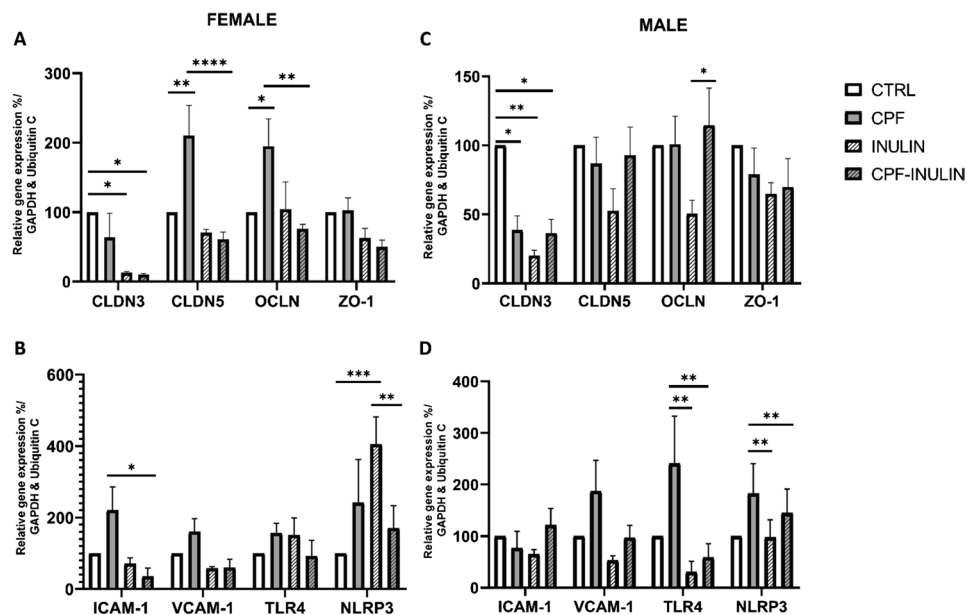


Fig. 4 Effects of chronic exposure to CPF and inulin on BBB tight junction's mRNA levels in female and male progenies. Values are expressed as mean \pm SEM ($n=7-10$) using analysis of variance

(two-way ANOVA–Tukey multiple comparisons test). Significance * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. CTRL, control; CPF, chlorpyrifos; inulin; CPF-inulin, chlorpyrifos + inulin

In parallel, our analysis of male progeny revealed significant findings regarding the expression of TLR4 and NLRP3 in response to different experimental conditions (Fig. 4D). Specifically, we observed a significant decrease in TLR4 expression in the inulin and CPF + inulin groups compared to the CPF alone exposed groups ($p < 0.01$). Additionally, our study demonstrated an increase in NLRP3 mRNA levels induced by CPF alone (182%) compared to control ($p < 0.01$). However, this increase was mitigated to 144% by inulin supplementation (CPF + inulin) ($p < 0.01$). These findings underscore the potential of inulin supplementation in modulating the expression of inflammatory markers in male progeny exposed to CPF.

Protein assay and western blot

After the analysis of the impact of CPF exposure on mRNA levels of tight junction proteins and pro-inflammatory markers in brain microvessels of rat progeny, we quantified the corresponding protein levels using western blot analysis. Our findings illustrated in Fig. 5 reveal alterations of cldn5, ocln, and ICAM-1 protein levels in both female and male progeny rats. Specifically, CPF exposure resulted in a significant increase in cldn5 protein levels (588.99%, $p < 0.05$) and ocln protein levels (206.93%, ns) compared to control groups in female rats. Conversely, inulin supplementation decreased cldn5 protein expression to levels similar to those of control group (133.36%, $p < 0.05$ CPF-inulin vs. CPF) in female offspring. Similarly, both inulin alone and inulin supplementation (CPF-inulin) restored occludin protein levels to those observed in the control group (CPF 120.62%, CPF-inulin 126.27% vs. control, non-significant).

In contrast to the observations in female offspring, we did not detect significant variations in the protein levels of cldn5 and occludin in male offspring. As a result, our statistical analysis indicated a discernible difference in the expression variation pattern of cldn5 between male and female rats ($p = 0.088 < 0.1$ (indicative) male vs. female). In addition, we explored the impact of CPF exposure on adhesion molecules (ICAM-1 and VCAM-1). In Fig. 5, we illustrate results of ICAM-1 protein levels. We found no significant variations in ICAM-1 protein levels. However, the comparison of expression variation pattern of ICAM-1 between male and female revealed a significant difference ($p < 0.05$).

These results showed that CPF altered tight junctions' protein levels especially, while inulin restored their expression to control levels in female progeny and maintained stable protein levels relatively to control in male progeny. The treatment-related variation patterns of claudin 5 and occludin protein expression closely paralleled the variation observed in mRNA levels in both male and female progeny. These findings suggest a congruence between our western blot results and those obtained from RT-qPCR analysis.

Discussion

Involved in many physiological functions, the gut microbiota stability is considered the key to the health of distant organs (Vernocchi et al. 2016; Appleton 2018; Valdes Ana et al. 2018; Morais et al. 2021). In this work, we focused on gut dysbiosis and the consequences of these disruptions on

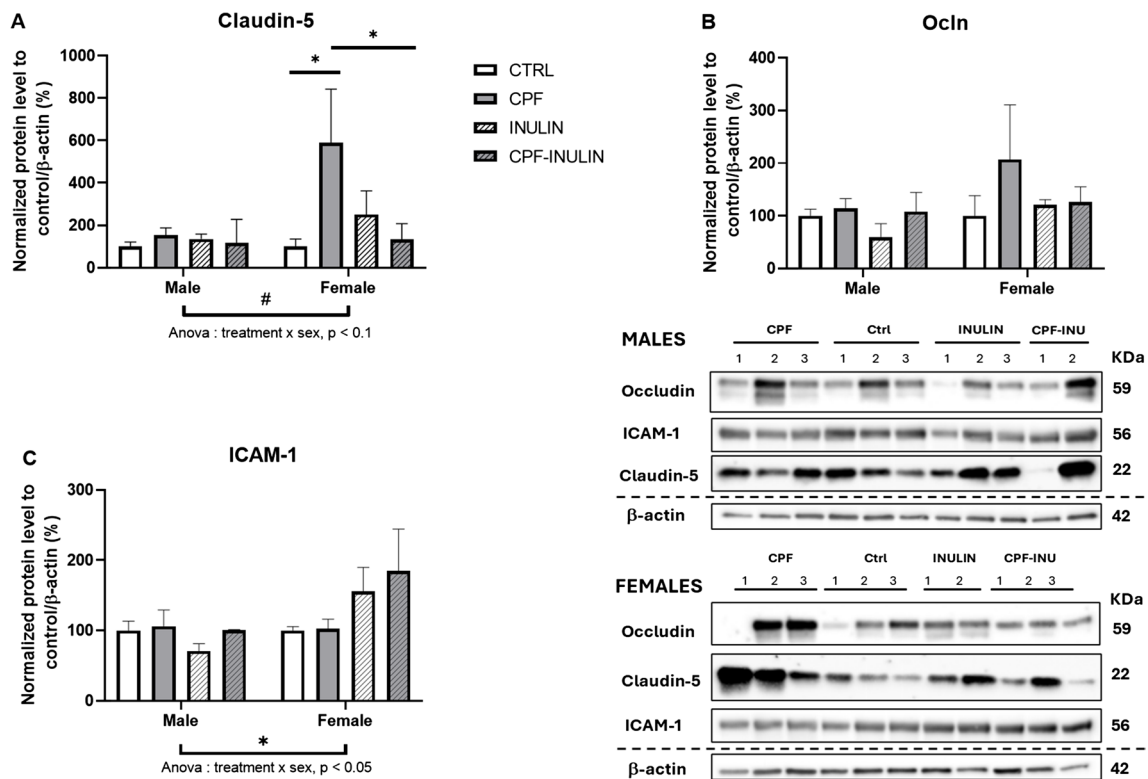


Fig. 5 Effects of chronic exposure to CPF and inulin on BBB tight junctions' protein levels in female and male progenies. Values are expressed as mean \pm SEM using analysis of variance (two-way

ANOVA–Tukey multiple comparisons test). Significance * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$. CTRL, control (100%); CPF, chlorpyrifos; inulin; CPF-inulin, chlorpyrifos + inulin

bacterial translocation to sterile organs and BBB tight junctions' expression in dams and progeny. Chronic exposure of dams only to 1 mg/kg/day of the organophosphate pesticide CPF during gestation and lactation had direct effects on GM and BBB tight junctions and adhesion molecule expression of dams and indirect effects on female and male progenies. Our animal model closely replicates the physiological events that occur during gestation. Our results revealed a significant impact on the bacterial composition of the intestinal microbiota, with marked changes in abundance and diversity of specific bacterial phyla. This highlights the complex interaction between pesticide exposure and the GM. CPF specifically disrupted gut bacteria balance, leading to intestinal dysbiosis in both dams and offspring. This dysbiosis led to bacterial translocation to sterile organs, reflecting a compromise in the IB integrity (Sarkar and Banerjee 2019; Di Vincenzo et al. 2023). Furthermore, our findings establish a causal link between alterations in the GM and disruptions in BBB integrity. The observed protective effects of inulin suggest potential therapeutic strategies for mitigating pesticide-induced dysbiosis. These findings were corroborated through multiple techniques, including bacterial isolation and identification, as well as the assessment of BBB gene and protein expression using RT-qPCR and Western blotting in brain microvessels-enriched fractions.

Impact of CPF on gut microbiota dysbiosis and bacterial translocation

In our previous published work, we demonstrated that CPF exposure induced body weight changes, disruption of lipid and glucose metabolisms in addition to dysbacteriosis in dams and progeny (Guibourdenche et al. 2021; Djekkoun et al. 2022a). Our present work confirmed these results regarding GM in male progeny where we can see a significant disruption of bacterial populations. We then compared male-related findings to our published results of female progeny and noticed that beneficial flora of male progeny showed the same significant decrease as for females, but potentially pathogenic flora had a more significant increase in male progeny compared to female exposed to CPF alone. Analysis of bacterial metabolites might explain this result. In fact, bacterial dysbiosis had an impact on the SCFAs (Joly Condette et al. 2022). As an example, we observed a significant increase of the acetic acid ratio in progeny and a significant decrease in butyric acid by CPF exposure, which was interestingly prevented by inulin. This shift in bacterial metabolites pattern is a result of the perturbation of intestinal homeostasis. As the microbial community is in continuous contact with the IB, microbial perturbations after CPF exposure have been shown to threaten the intestinal integrity

(Réquillé et al. 2018). These results confirm the decrease of beneficial bacterial flora and help us understand the subsequent mechanisms altering the IB permeability and leading to bacterial and metabolites translocation. Our results showed a large aerobic bacterial translocation to the spleen and liver in CPF groups. However, inulin prevented bacterial translocation.

Similar results in previous studies showed that CPF (0.3 and 1 mg/kg/day) exposure can disrupt the delicate balance of gut bacteria, specifically by affecting the diversity and abundance of beneficial bacteria contributing to gut dysbiosis (Joly Condetto et al. 2015; Zhao et al. 2016; Perez-Fernandez et al. 2020). Since the GM is involved in the immune and endocrine systems, the consequences of CPF-associated dysbacteriosis on intestinal permeability and inflammation have been studied by our collaborators (Yang et al. 2020; Guibourdenche et al. 2021). In fact, a “leaky” IB showing a gene expression disruption of IB tight junctions was observed in male progeny of dams exposed to 1 mg/kg/day CPF during gestation and lactation. A significant decrease of occludin gene expression was detected in different intestinal segments in addition to alterations of zonula occludens and claudin 4 expression (Condetto et al. 2014; Guibourdenche et al. 2021). A disruption of the mucus layer of the IB was also observed in the male progeny. These findings might explain the bacterial translocation that we observed in our microbiological study. No significant increased levels of pro-inflammatory cytokines and lipopolysaccharides (LPS) were observed although other published work found increased serum levels of monocyte chemoattractant protein 1 (MCP-1) and tumor necrosis factor (TNF- α) (Li et al. 2019).

Impact of CPF on brain microvessels (BBB)

Although existing literature demonstrates that pesticides can influence the microbiota and affect the BBB, our study represents the first demonstration of CPF's effects on brain microvessels following metabolism by the GM in an in vivo model.

CPF has been extensively documented to possess neurotoxic properties by altering the GM composition (Darwiche et al. 2018; El Khayat El Sabbouri et al. 2020; Cresto et al. 2023; Van Melis et al. 2023). Moreover, it has been shown that the GM plays an essential role in BBB permeability and can modulate tight junctions expression (Braniste et al. 2014). The BBB is an essential barrier in the brain and can be altered by in vitro CPF exposure. In vitro studies have shown that CPF can alter BBB integrity, with evidence of CPF and its metabolite such as CPF-oxon, crossing this barrier (Parran et al. 2005). CPF was detected on both the luminal and abluminal sides of the BBB, whereas the metabolites trichloro-2-pyridinol (TCP) and CPF-oxon were on the abluminal side only. These findings suggest that CPF can cross the BBB and that the barrier's enzymatic activity can

metabolize this pesticide (Parran et al. 2005). Additionally, Li and Ehrlich demonstrated decreased BBB electrical resistance and altered gene expression of claudin-5 and ZO-1 in vitro, indicating disruption and loss of BBB integrity (Li and Ehrlich 2013).

Our study builds on these findings by showing that CPF-induced dysbiosis affects BBB integrity through microbiota-mediated mechanisms. Previous studies added CPF directly to a BBB model, excluding the biotransformation of CPF by GM. In fact, CPF is 70% absorbed by the oral route, that is why studies on its effects on GM have been multiplied (Rathod and Garg 2017; Djekkoun et al. 2021, 2022a, b;) and have shown that *Lactobacillus fermentum* degrades CPF to TCP while *Lactobacillus lactis* and *Escherichia coli* to CPF-oxon.

We focused on microvessels as they are deemed a reliable representation of the BBB owing to their anatomical and functional resemblances to the barrier in vivo. This selection allowed us to explore the impacts of our experimental interventions on a system closely mirroring the complexities of the BBB, thus offering valuable insights into the mechanisms governing its regulation and function.

The comprehensive analysis of the RT-q PCR and western blot results revealed CPF capacity to modulate the gene expression of tight junctions both in dams and offspring, albeit with distinct effects observed between the two groups. The observed downregulation of CLDN 3, CLDN 5, OCLN, and ZO-1 mRNA levels following CPF exposure highlights the potential disruptive effects of CPF on BBB tight junctions in dams. Previous research has shown that the primary tight junction proteins intimately involved in maintaining the integrity of the BBB consist of CLDN5, CLDN3, and OCLN and a modulation of these genes can serve as an important biomarker for BBB permeability (Yuan et al. 2020). These alterations in tight junction protein expression may lead to increased permeability of the BBB, allowing for the unrestricted passage of potentially harmful substances into the CNS (Gosselet et al. 2021).

Our study aligns with previous research showing that pesticide exposure can impair BBB integrity by affecting tight junction proteins (Rempe et al. 2018). However, the effects varied between sexes: female progeny exhibited increased expression of CLDN5 and occludin, while male progeny showed decreased CLDN3 expression. These differential effects highlight the complex nature of pesticide-induced neurotoxicity and suggest a sex-dependent manner response to CPF with more pronounced effects in females.

This observation is consistent with the known role of CPF as an endocrine disruptor (Shaffo et al. 2018). The divergent effects observed in our study could be attributed to the interplay of biological factors influenced by sex, highlighting the intricate and potentially sex-specific consequences of CPF exposure on the expression of tight junction genes across different biological contexts.

Additionally, we observed a significant increase in pro-inflammatory markers ICAM-1 and NLRP3 gene expression in female progeny and TLR4 and NLRP3 in male progeny BBB, indicating CPF-induced neuroinflammation. This aligns with previous studies linking NLRP3 inflammasome activation in microglial cells by CPF exposure to neuroinflammation and its role in neurodegenerative diseases such as Parkinson and Alzheimer diseases (Weis et al. 2021). Thus, activation of neuroinflammation might explain CPF influence on BBB tight junctions.

Our exposure strategy confirmed CPF's direct effects on mothers during perinatal period and its indirect effects on the progeny. The emphasis on perinatal exposure arises from the acknowledgment that vulnerability to CPF is heightened during in utero development and early life due to the lower levels of the detoxifying enzyme paraoxonesterase in children compared to adults (Rathod and Garg 2017; Van der Zande et al. 2017). Consequently, CPF can elicit effects on neonates even at doses below those that inhibit AChE. Therefore, our study focuses on the impact of low chronic CPF exposure during the critical early days of life, referred to "windows of vulnerability" (Developmental Origins of Health and Disease DOHaD concept).

Prevention of CPF effects by inulin supplementation

Our nutritional prevention based on inulin supplementation showed potential benefits on both GM and BBB. Prebiotics are defined as ingredients that undergo selective fermentation, inducing specific modifications in the composition and/or activity of the gastrointestinal microbiota, thereby providing health benefits to the host (Gibson et al. 2017). Previous in vivo and in vitro studies highlighted the beneficial effects of inulin supplementation that prevented gut dysbiosis and reduced bacterial translocation to sterile organs (to 0% in most cases) (Djekkoun et al. 2022a). Our results confirmed the beneficial effect of inulin supplementation on bacterial composition in male progeny. In fact, inulin male-exposed groups showed higher *Lactobacillus* and *Bifidobacterium* (beneficial bacteria) counts than non-exposed to inulin groups and a significant decrease of *Escherichia coli* (potentially pathogenic bacteria) in groups exposed to inulin alone compared to CPF + inulin. Thus, our results of male progeny are consistent with our previous study on dams and their female progeny (Djekkoun et al. 2022a). We were able to confirm that inulin can restore gut "eubiosis". Our prevention strategy results corroborate with the findings of a study on zebrafish where the prebiotic countered pesticides effects (Yousefi et al. 2018). Furthermore, many in vivo studies have analyzed the bifidogenic effect of inulin on human GM. Carlett Ramirez-Farias and colleagues demonstrated that this dietary fiber increased specifically *Bifidobacterium* species: *Bifidobacterium adolescentis*, *B. longum*, and *B. Bifidum*. When analyzing fecal fermentation products, they found that the most significant

change was the increase of lactate after 16 days of 10 g/day inulin intake (Ramirez-Farias et al. 2008). On the contrary CPF increased acetate and D-lactate but decreased L-lactate (Reygner et al. 2016). In fact, while inulin increased lactate by stimulating *Lactobacillus* and *Bifidobacterium*, CPF-related decrease in the release of lactate might favor the growth of the potentially pathogenic bacteria *Enterococcus* spp. (Joly et al. 2013). In addition, a recent key study found that pesticide exposure can lead to decreased lactate concentrations in men, indicating potential disruptions in glycolysis-related metabolism and energy homeostasis (Palaniswamy et al. 2023).

The effects of the prebiotic inulin on the BBB are still an area of ongoing research and exploration. Inulin, as a prebiotic, has primarily been studied for its effects on gut health and modulation of the GM. However, emerging evidence suggests that GM can influence various aspects of brain function, including BBB integrity. This prebiotic seems to reinforce the morphometry of the IB by enhancing tight junctions' activity. This was confirmed by reduced bacterial translocation by inulin, as our results showed. Some studies have investigated the potential neuroprotective effects of inulin through its modulation of the GM and subsequent impact on systemic inflammation and neuroinflammation (Hoffman et al. 2019). Since inflammation can influence BBB permeability, there is interest in understanding whether inulin supplementation could indirectly affect BBB function.

Our RT qPCR results showed that tight junctions' expression in CPF + inulin dams seem to be closer to control levels than CPF alone-exposed dams. Furthermore, especially in female progeny, we observed a restoration of CLDN5 and OCLN mRNA levels (expression values in inulin and CPF + inulin groups are similar to those of the control group, contrary to the significant increase by CPF alone (vs. control)). This gene expression restoration was also found in the case of pro-inflammatory factors such as NLRP3 in female (CPF + inulin group) and male progeny (inulin and CPF + inulin groups), ICAM-1 in female progeny, and TLR4 in male progeny. While CPF exposure increased the expression of these molecules, inulin was able to decrease it to control levels for some, except for the case of CLDN3 where inulin did not seem to hinder CPF effects in progeny.

These first results on BBB give promising insights about the direct (dams) and indirect (progeny) beneficial effects of inulin in organs outside of GM. This indicates that GM beneficial modulation by inulin enhances brain protection by restoring BBB tight junctions, highlighting its role in gut-brain axis communication and neuroprotection (Sheng et al. 2023). The outcomes obtained from our experiments have yielded significant information regarding the impact of pesticide exposure and inulin supplementation on the BBB.

Nonetheless, we could not compare results of BBB to those in literature since our study is the first in vivo experiment to analyze the impact of CPF and inulin on BBB. Consequently,

we compared these findings to research about inulin supplementation but in a different scenario (with no pesticide exposure) of mice with chronic unpredictable mild stress (CUMS mice) that have higher BBB permeability allowing pro-inflammatory cytokines diffusion to brain. In this study, CUMS mice supplemented with inulin showed a restoration of CLDN5 and ZO-1 levels and reversed the brain inflammatory response to CUMS by inhibition of TLR4-MyD88-NF- κ B pathway to decrease TLR4 and LPS in brain (Wang et al. 2023). These results highlight the neuroprotective role of inulin fermentation metabolites (SCFAs) that can cross the BBB (Dalile et al. 2019). Another study sought to counter the CPF neurotoxicity by supplementation with another molecule: Hesperidin, a flavonoid which has been shown to exert antioxidant and anti-inflammatory effects. Supplementation with Hesperidin (50 and/or 100 mg/kg) of CPF-exposed rats (6.75 mg/kg) increased antioxidant enzymes, decreased reactive oxygen species, and reduced inflammation by inhibiting NF- κ B pathway in brain (Küçükler et al. 2024).

There remains a scarcity of *in vivo* studies, and none have specifically examined the impact on microvessels (Cresto et al. 2023). Further analysis should be done to understand how inulin fermentation products modulate BBB tight junctions. Conducting additional analyses using immunohistology to examine the levels of tight junction proteins at various locations can enhance our comprehension of how the expression of these proteins is affected by external factors. In perspective, our discoveries concerning CPF alone prompt consideration of the impact of exposure to a mixture of pesticides on our well-being, especially among vulnerable groups such as pregnant women and newborns (Zhang et al. 2022, 2023). Given our research focus on pesticide exposure during prenatal development, it would be valuable to explore the epigenetic consequences of CPF to understand the transgenerational effect observed in offspring (Chiu et al. 2021).

Conclusion

In conclusion, our study confirmed that long-term maternal exposure to deleterious environmental factors disrupts gut microbiota homeostasis, composition, and functions. Given the persistence of CPF residues in the environment and their potential link to various diseases, understanding the consequences of bacterial dysbiosis on the BBB integrity is crucial (Chin-Chan et al. 2015; Van Melis et al. 2023). We demonstrated that GM dysbiosis and subsequent bacterial translocation significantly impact the BBB, offering insights into the mechanisms that may underlie neurological disorders.

Our findings suggest that probiotic supplementation offers potential preventive measures against the adverse effects of everyday pesticide exposure. However, further research is necessary to determine the most effective probiotic treatment

approaches, including optimal dosage and duration. Additionally, raising public awareness about the health risks associated with pesticide exposure and advocating for stricter pesticide regulations are essential to minimize environmental contamination and protect human health.

Abbreviations

ACh: Acetylcholine; *AChE*: Acetylcholinesterase; *AKT*: Alpha-serine/threonine kinase; *BBB*: Blood–brain barrier; *BCRP*: Breast cancer-resistant protein; *BSA*: Bovine serum albumin; *BW*: Body weight; *CFU*: Colony forming unit; *CLDN*: Claudin; *CNS*: Central nervous system; *CPF*: Chlorpyrifos; *CPFI*: Chlorpyrifos + inulin; *CPF-oxon*: Chlorpyrifos-oxon; *CUMS*: Chronic unpredictable mild stress; *DDT*: Dichloro-diphenyl-trichloroethane; *DOHaD*: Developmental origins of health and disease; *g*: Gram; *GAPDH*: Glyceraldehyde-3-phosphate dehydrogenase; *GM*: Gut microbiota; *HBSS*: Hank's balanced salt solution; *HEPES*: 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid; *HRP*: Horse radish peroxidase; *I*: Inulin; *IB*: Intestinal barrier; *ICAM-1*: Intercellular adhesion molecule 1; *IL*: Interleukin; *JNK*: C-Jun NH₂-terminal protein kinase; *kg*: Kilogram; *L*: Liter; *LPS*: Lipopolysaccharides; μ g: Microgram; μ M: Micromolar; *mg*: Milligram; *mL*: Milliliter; *mRNA*: Messenger ribonucleic acid; *MyD88*: Myeloid differentiation primary response protein 88; *NF- κ B*: Nuclear factor-kappa B; *ng*: Nanogram; *NLRP3*: NLR family pyrin domain containing 3; *nm*: nanometer; *NOAEL*: No-observed-adverse-effect level; *OCLN*: Occludin; *PI3K*: Phosphoinositide 3-kinase; *PND*: Post natal day; *RNA*: Ribonucleic acid; *RNase*: ribonuclease; *RT-q PCR*: Reverse transcription quantitative real-time polymerase chain reaction; *rpm*: Revolutions per minute; *RIPA*: Radioimmunoprecipitation assay buffer; *SC*: Diet standard chow diet; *SCFAs*: Short-chain fatty acids; *SEM*: Standard error of the mean; *TBST*: Tris-buffered saline containing 0.1% Tween; *TCP*: 3,5,6-Trichloro-2-pyridinol; *TJ*: Tight junctions; *TLR4*: Toll-like receptor 4; *TNF- α* : Tumor necrosis factor; *VCAM-1*: Vascular cell adhesion molecule 1; *ZO*: Zonula occludens

Author contribution Conceptualization, P. C. and H. K-C; methodology, P. C., H. K-C., M-C. B., L. D., A. C. and M. B.; software, M. AD, N. D. and M-C. B.; validation, P. C., H. K-C, F. G, and V. B.; formal analysis, M. AD, N. D., M-C. B., L. D., P. C., and H. K-C; investigation, M. AD, N. D., P. C., and H. K-C; resources, F. G and V. B.; data curation, M. AD, N. D., and M-C. B.; writing—original draft preparation, M. AD, N. D., M. C. B, L. D., P. C., H. K-C, F. G, and V. B.; writing—review and editing, M. AD, N. D., M. C. B, L. D., M. B., P. C., H. K-C, F. G, and V. B.; visualization, M. AD, N. D., P. C., and H. K-C; supervision, P. C., H. K-C, F. G, and V. B.; project administration, P. C. and H. K-C; funding acquisition, P. C. and H. K-C. All the authors have read and agreed to the published version of the manuscript.

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Data availability Data is available on demand by email to the corresponding author.

Declarations

Ethics approval The study protocol was approved by the Regional Directorate for Health, Animal Protection, and the Environment (Amiens, France) and the French Ministry of Research (reference: APAFIS # 8207–2016121322563594 v2, September 5th, 2017). All the animals were treated in accordance with the European Union Directive 2010/63 and monitored daily for well-being.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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