Genomic analyses of the effects of PCB-DL and PCB-non DL short term exposures in mice

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Aims of the work

Today there is a worldwide pandemy of diabetes and obesity. The increasing prevalence of these pathologies cannot be only explained by the modification of the way of life (hypercaloric diet and sedentary lifestyle) and genetic susceptibilities. Environmental pollutant exposure seems to be also involved.

PCB are vPvB pollutants which are widespread disseminated, notably in water. Recent epidemiological studies suggested that PCB are potent endocrine disruptors and environmental exposure (notably through fish consumption) is associated to an increase of metabolic diseases such as metabolic syndrome and diabetes. However, molecular mechanisms involved in the etiology of these diseases are poorly understood.

Methods

Animals: male C57BL/6 mice were exposed at 10 or 100 µmol/kg bw for 30 days (IP at Day0 and Day15) to a PCB-DL (PCB118), or to PCB-non DL (PCB153), or to a mixture of PCB118 and 153. There were 9 mice per groups.

Blood biochemical parameters: Blood levels of glucose, cholesterol (total and HDL), triglycerides, phospholipids, and hepatic function markers (AST, ALT, ALKT) were evaluated at D30.

Genomic analyses in liver, brown adipose tissue, muscle and colon: Total RNA was extracted using Trizol Reagent®. Genomic analyses were performed at IFR125 (Marseille) using Whole Mouse Genome Microarray Kit (4x44K) from Agilent® (France). Data were analyzed using Genespring® and public data bases. Only changes greater than two fold were considered and further verified by qRT-PCR.

Key results

Effect of PCB treatments on body weight gain and liver weight

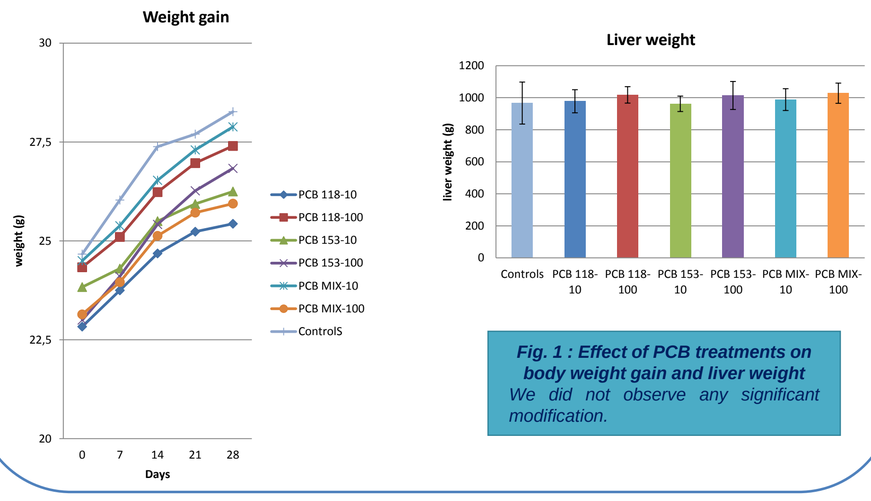

Fig. 1 : Effect of PCB treatments on body weight gain and liver weight
We did not observe any significant modification.

Effect of PCB treatments on hepatic function

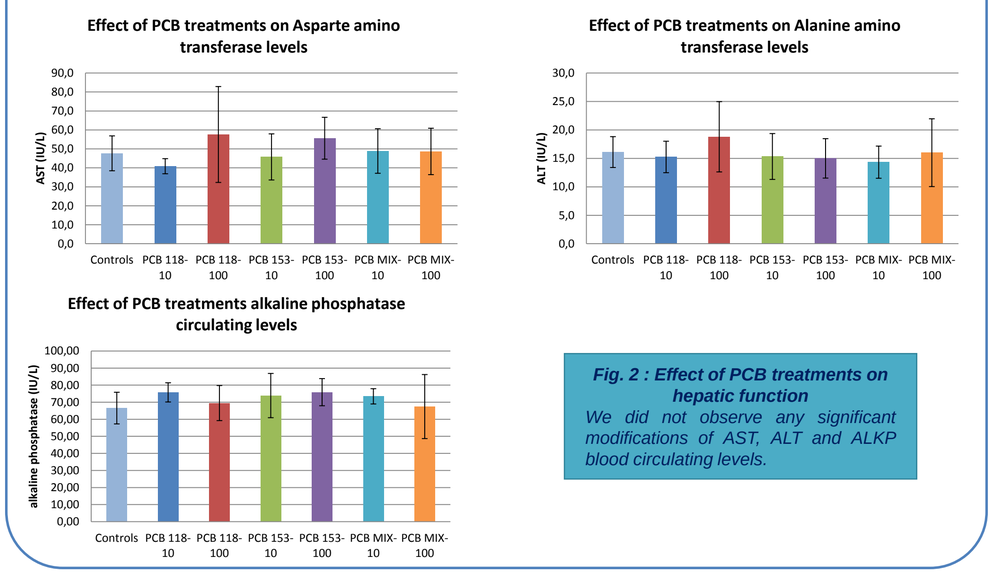

Fig. 2 : Effect of PCB treatments on hepatic function
We did not observe any significant modifications of AST, ALT and ALKP blood circulating levels.

Effect of PCB treatments on biochemical parameters

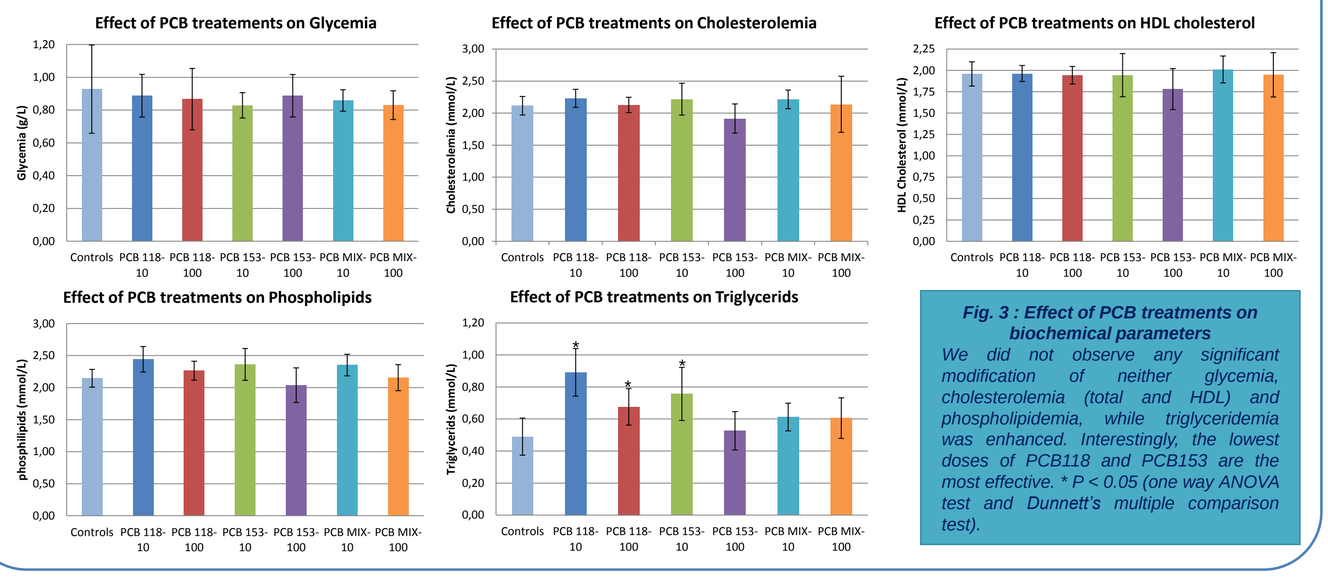

Fig. 3 : Effect of PCB treatments on biochemical parameters
*We did not observe any significant modification of neither glycemia, cholesterolemia (total and HDL) and phospholipidemia, while triglyceridemia was enhanced. Interestingly, the lowest doses of PCB118 and PCB153 are the most effective. * P < 0.05 (one way ANOVA test and Dunnett's multiple comparison test).*

Effect of PCB treatments on xenobiotic metabolizing enzymes

	LIVER						COLON					
Gene	PCB118-10	PCB118-100	PCB153-10	PCB153-100	PCB MIX-10	PCB MIX-100	PCB118-10	PCB118-100	PCB153-10	PCB153-100	PCB MIX-10	PCB MIX-100
Cyp1a1		211				59,7						2,9
Cyp1a2		4,8				3,9						
Cyp2b9		12,6	9,4	30,6	2,5	26,3						
Cyp2b10		14,0	9,5	33,9	2,6	28,7						
Cyp2c54				2,5		2,0						
Cyp2c70	*-2,7*	*-2,2*	*-2,4*	*-2,5*	*-2,9*							
Cyp11a1							2,6	2,4	2,6	3,2	2,5	2,3
Fmo3	*-2,3*		*-2,2*	*-3,4*								
Gsta1	*-2,3*	*-2,0*	*-2,3*									
Gsta2	*-2,1*		*-2,3*									
Gsto2		*-2,0*			*-2,1*							
Gpx3												
	BROWN ADIPOSE TISSUE						MUSCLE					
Gene	PCB118-10	PCB118-100	PCB153-10	PCB153-100	PCB MIX-10	PCB MIX-100	PCB118-10	PCB118-100	PCB153-10	PCB153-100	PCB MIX-10	PCB MIX-100
Cyp1a1		5,7						15,8				2,6
Cyp1a2												
Cyp2b9												
Cyp2b10												
Cyp2c54												
Cyp2c70												
Cyp11a1						2,5						
Fmo3					3,0							
Gsta1												
Gsta2										*-2,1*		
Gsto2									*-3,1*			
Gpx3	2,7		2,3		2,9							

Fig. 4 : Effect of PCB treatments on mRNA level of drug metabolizing enzyme
We observed an increase of Cyp1a1 and Cyp1a2 expression in studied tissues of PCB 118 or PCB Mix treated mice, and of Cyp2b9 and Cyp2b10 expression in the liver of PCB118, PCB 153 and PCB Mix treated mice. Cyp1a subfamily is mainly regulated by AhR and PCB118 is a potent ligand of this transcription factor, Cyp2b subfamily is mainly regulated by CAR and PCB153 is a ligand of this nuclear receptor. The induction of Cyp1a subfamily could enhance susceptibility to some environmental procarcinogens such as polycyclic aromatic hydrocarbons and arylamines.
Gst, which are involved in electrophilic compound detoxication, were repressed, notably in liver, suggesting an increased susceptibility to hepatic injury induced by xenobiotic electrophile metabolites.
Interestingly Gpx3 was induced in adipose tissue, and glutathione peroxydases play a major role in the production of reactive oxygen species which could trigger local inflammation.

Effect of PCB treatments on glucose homeostasis and insulin signaling

	LIVER						COLON					
Gene	PCB118-10	PCB118-100	PCB153-10	PCB153-100	PCB MIX-10	PCB MIX-100	PCB118-10	PCB118-100	PCB153-10	PCB153-100	PCB MIX-10	PCB MIX-100
Glucokinase	*-4,1*	*-4,3*	*-3,2*	*-3,0*								
Pygb												
Decr1												
Foxo3												
Pfkfb3												
CnR1	2,9		2,7									
	BROWN ADIPOSE TISSUE						MUSCLE					
Gene	PCB118-10	PCB118-100	PCB153-10	PCB153-100	PCB MIX-10	PCB MIX-100	PCB118-10	PCB118-100	PCB153-10	PCB153-100	PCB MIX-10	PCB MIX-100
Glucokinase							*-2,5*	*-2,2*			*-2*	*-2*
Pygb		*-2,8*	*-2,2*	*-2,4*	*-2,6*	*-4,9*						
Decr1		*-2,8*	*-2,2*	*-2,4*	*-2,6*	*-4,9*						
Foxo3		4,9					2,4			3,5	2,4	2,5
Pfkfb3							2,6	2,9	2,1	2,8		
CnR1							2,2		3,2	3,2	2,9	

Fig. 5 : Effect of PCB treatments on glucose homeostasis and insulin signaling
Our results showed that glucokinase expression was mainly decreased in liver of PCB 118 and 153 treated mice, and the inhibition of glucokinase in liver was described to be involved in diabetes pathogenesis.
In brown adipose tissue, we observed after PCB exposure, an inhibition of Pygb (glycogen phosphorylase) and Decr1 (2,4-dienoyl Co reductase). Pygb was described to be a potential target of type 2 diabetes therapy.
In muscle, we showed an increase of Foxo3 and Pfkfb3expression after PCB treatments. It was recently described that Foxo3 is involved in insulin signaling.
In liver and muscle, we observed an induction of CnR1 expression, after PCB exposure. CnR1was associated to diet-induced insulin resistance.

Effect of PCB treatments on inflammation and immune system

	LIVER						COLON					
Gene	PCB118-10	PCB118-100	PCB153-10	PCB153-100	PCB MIX-10	PCB MIX-100	PCB118-10	PCB118-100	PCB153-10	PCB153-100	PCB MIX-10	PCB MIX-100
Serpine 1												
Slc25a1												
Tank							4,1	4,0	5,2	7,9	3,7	4,9
Gata 3	3,8	4,1	2,4	3,1								
Srgn												
PU1	*-2,5*			*-2,1*			*-2,7*				*-2,2*	
Elk4												
CD28			*-2,0*	*-2,0*								
IL1β	2,3											
IL2												
IL5					*-2,5*	*-3,4*						
IL6												
Ifng	2,3					*-2,8*						
Smpd3		2,3		3,8		2,7						
	BROWN ADIPOSE TISSUE						MUSCLE					
Gene	PCB118-10	PCB118-100	PCB153-10	PCB153-100	PCB MIX-10	PCB MIX-100	PCB118-10	PCB118-100	PCB153-10	PCB153-100	PCB MIX-10	PCB MIX-100
Serpine 1					2,7	2,8						2,1
Slc25a1	*-3,7*	*-3,1*	*-2,9*	*-2,7*	*-3,8*							
Tank				3,6			11,1	7,8	11,7	7,8	12,2	6,6
Gata 3						*-18,1*						
Srgn	3,2		2,7		4,7	2,4						
PU1									*-3,9*	*-4,8*	*-3,2*	
Elk4					2,8	4,7		3,5		2,9		
CD28		*-2,0*										
IL1β										*-2,0*		
IL2							*-2,1*		*-2,2*	*-2,5*		*-3,7*
IL5		*-2,9*								*-2,5*	*-2,7*	
IL6								2,9		2,6		
Ifng							*-2,4*			*-3,5*		
Smpd3												

Fig. 7 : Effect of PCB treatments on inflammation and immune system
Pro-inflammatory cytokines and chemokines were slightly modified; we only observed with PCB118 and/or PCB153, an hepatic induction of Smpd3 which was described to be regulated by IL1β and TNFα. Moreover, we observed an increase of serpine 1 expression in brown adipose tissue after PCB Mix exposure, and serpine 1, regulated by AhR, was described to be associated with insulin resistance.

Effect of PCB treatments on lipid homeostasis

	LIVER						COLON					
Gene	PCB118-10	PCB118-100	PCB153-10	PCB153-100	PCB MIX-10	PCB MIX-100	PCB118-10	PCB118-100	PCB153-10	PCB153-100	PCB MIX-10	PCB MIX-100
Glycerolkinase												
Fasn												
Lipin 1	*-3,0*	*-2,4*	*-2,0*	*-2,1*								
Lipin 2												
PLRP1		2,9		3,9		4,5						
Pla2g1b												
Agpat2												
Lipc												
Gpd1												
Pde8b					2,9	2,8						
Mogat2	2,3	5,1		3,1	2,4	4,1						
Hsd3b4	*-3,4*			*-2,4*	*-2,2*	*-3,6*						
Rgs2												
Alox5	2,2	2,5		2,4								
	BROWN ADIPOSE TISSUE						MUSCLE					
Gene	PCB118-10	PCB118-100	PCB153-10	PCB153-100	PCB MIX-10	PCB MIX-100	PCB118-10	PCB118-100	PCB153-10	PCB153-100	PCB MIX-10	PCB MIX-100
Glycerolkinase							3,2				2,6	2,6
Fasn	*-6,5*	*-6,6*	*-5,2*	*-5,7*	*-6,8*							
Lipin 1												
Lipin 2							2,5		2,8	3,0	2,5	2,5
PLRP1												
Pla2g1b						*-4,0*	*-2,8*			*-4,1*	*-3,4*	
Agpat2	*-2,1*	*-2,0*										
Lipc							*-2,3*			*-2,8*		
Gpd1	*-2,7*	*-2,6*										
Pde8b	3,2				5,6				2,6			
Mogat2												
Hsd3b4												
Rgs2							2,6	2,5	2,5	2,1		
Alox5												

Fig. 6 : Effect of PCB treatments on lipid homeostasis
Our results showed that PCB exposure modulates the expression of various genes involved in lipid homeostasis, in liver, brown adipose tissue and muscles.
In liver, PLRP1 (pancreatic lipase related protein 1) and Mogat2 (monoacylglycerol acyltransferase-2) were induced by PCB118 and/or PCB153, while lipin1 expression was reduced.
In brown adipose tissue the expression of the fatty acid synthase was reduced by PCB118 and/or PCB153. PCB 118 treatment led to an inhibition of Gpd1 and Agpat2 expression, and Agpat2 was described to be involved in insulin sensitivity.
In muscles, we observed an induction of Lipin2 which was associated with insulin resistance. Inversely, phospholipase A2 (Pla2g1b) expression was reduced.

Conclusion

Taken together, our data demonstrated that PCB exposure do not modify specific metabolic pathways but can modulate the expression of various genes involved in insulin sensitivity, or in glucose or lipid homeostasis. Interestingly, the lowest studied doses were generally the most active, notably in adipose tissue. This is in agreement with previous reports on various endocrine disruptors.

Moreover, we observed an increase of cytochromes P450 expression associated to a reduction of GST expression, suggesting the PCB could increase susceptibility to environmental procancerogen bioactivation.