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Modulation of hepatic xenobiotic metabolizing enzymes following chronic low-dose exposure to PFOA in mice under chronic stress

Rémi Dages, Elodie N Person, Sandrine Bruel, Hervé Robert, Muriel Mercier-Bonin, Daniel Zalko, Nicolas J Cabaton

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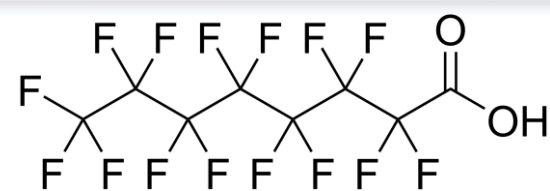
following chronic low-dose exposure to PFOA in mice under chronic stress

Rémi Dages, Elodie N. Person, Sandrine Bruel, Hervé Robert, Muriel Mercier-Bonin, Daniel Zalko and Nicolas J. Cabaton

INRAE Toxalim, Université de Toulouse, INP-ENVT, INP-EI-Purpan, Université de Toulouse 3 Paul Sabatier, Toulouse, France

Contact: nicolas.cabaton@inrae.fr

Introduction



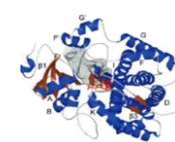
PFOA = Perfluorooctanoic acid

C₈H₁₅O₂, CAS N°335-67-1, MW 414.07

- **Perfluoroalkylated substances (PFAS)** are used in a wide range of industrial applications: coatings for cookware, food contact papers, waterproofed clothing, fire-fighting fluids, etc.^[1]
- **PFOA** raises major public health concerns. Consumers are still exposed to this extremely **persistent and bioaccumulative substance**^[2]
- **Main source of human exposure**: dietary intake, with seafood and freshwater fishes being the highest contributors^[2]
- Epidemiological surveys and results from *in vivo* studies in rodents suggest that PFAS can induce **hepatotoxicity** and exert **metabolic effects**, including effects on **lipid metabolism**^[1,2,3]

Objectives & Strategy

Metabolism is the primary defense against xenobiotics, and **Xenobiotic Metabolizing Enzymes (XMEs)** play an essential role in the protection of organisms



XMEs are involved in the **biotransformation of xenobiotics** and in a number of **endogenous anabolic** and **catabolic processes**. XMEs are expressed at the highest level in the **liver**.



Investigate the **effects of chronic low doses of PFOA on mice hepatic phase I and II functional XME activities under chronic stress**



Better **characterize the mechanisms underlying PFOA effects**



Determine **specific enzyme isoform modulation** following incubations with hepatic subcellular fractions and probe substrates combined with different analytical strategies, and under chronic stress

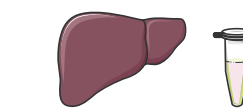
Experimental design

Animal experiment

Adult C57BL/6
♂ Mice



Treatment: 90 days, via drinking water
PFOA: 15 µg/kg b.w./day
PFOA + Corticosterone (35 mg/L the last 10 d, to mimic stress)
Control 1: no PFOA, no corticosterone
Control 2: no PFOA, corticosterone (35 mg/L the last 10 d)
n = 10 per group



Liver collection
Subcellular fraction preparation
Protein content assessment

In vitro incubations

Hepatic subcellular fractions
Specific probe substrates
Cofactors

Phase I cytochrome P450 (CYP450):
CYP2C37 = diclofenac
CYP4A10 = [¹⁴C]-lauric acid
CYP3A11 and 2A4 = [¹⁴C]-testosterone
Phase II glutathione S-transferases (GST):
1-chloro-2,4-dinitrobenzene (CDNB)



Optimal enzymatic conditions

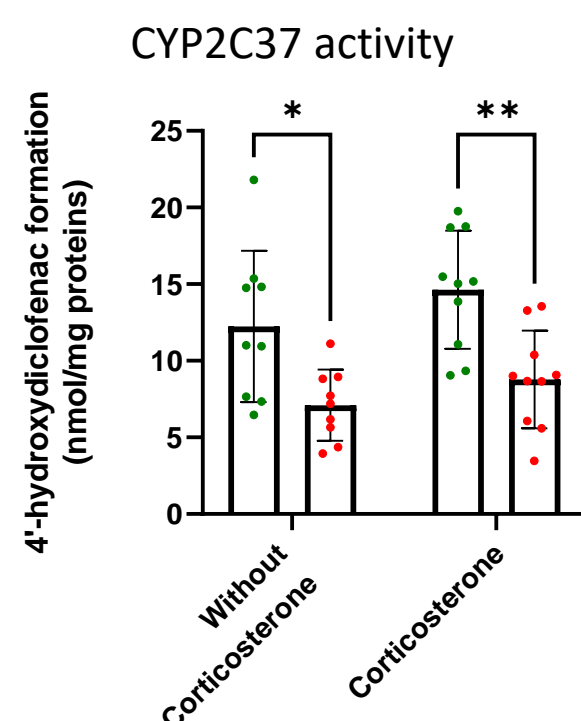
Metabolite detection and quantification



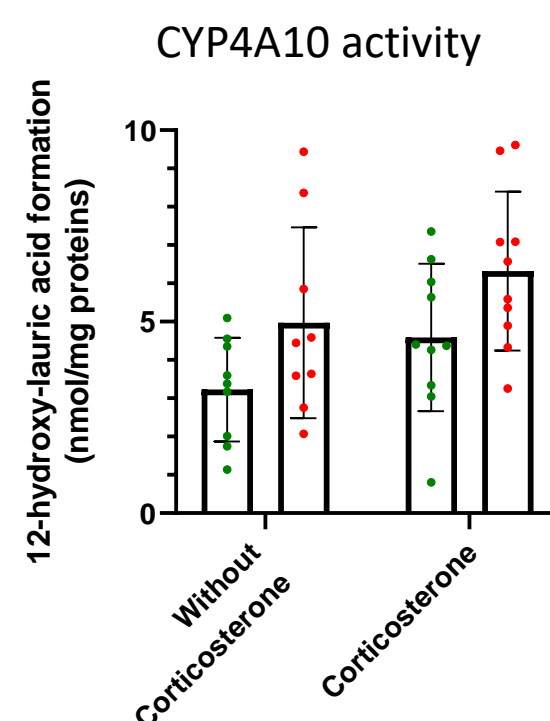
CYP2C37 = monitoring of 4'-hydroxydiclofenac formation by HPLC-UV (280 nm)
CYP4A10 = monitoring of [¹⁴C]-12-hydroylauric acid formation by Radio-HPLC
CYP3A11 and CYP2A4 = monitoring of [¹⁴C]-6β-hydroxytestosterone and [¹⁴C]-6α-hydroxytestosterone formation by Radio-HPLC, respectively
GST = monitoring CDNB conjugation by spectrophotometry

Results

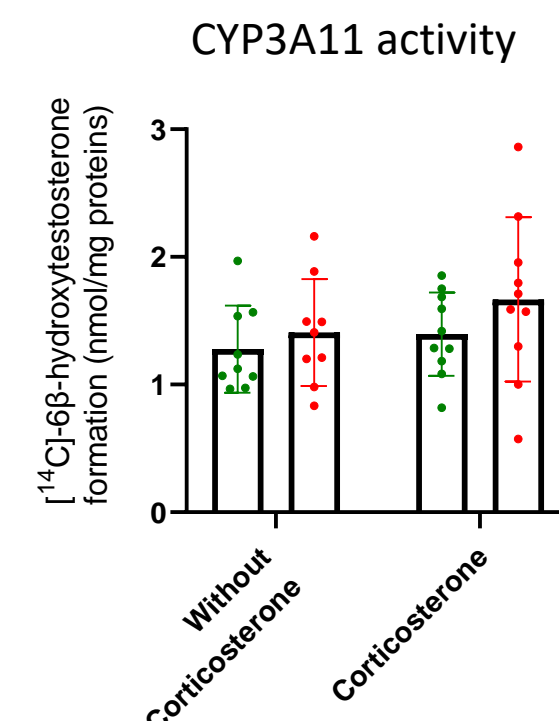
Microsomal CYP450 functional activities



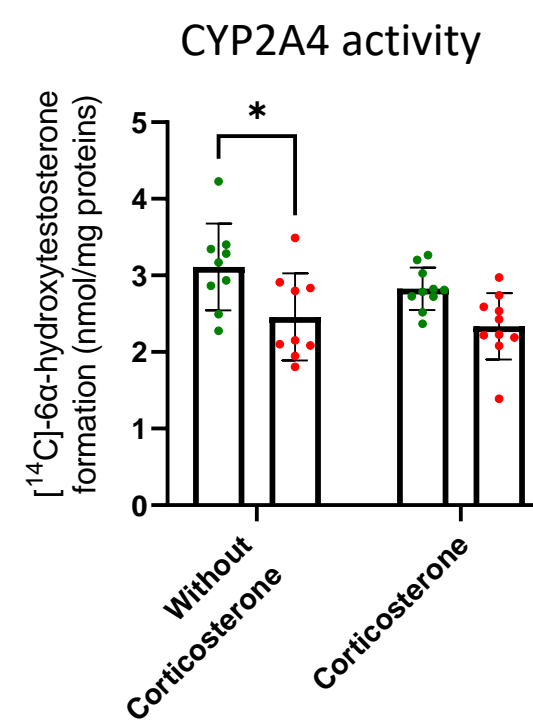
PFOA significantly decreases the activity of CYP2C37 (p<0.0001)



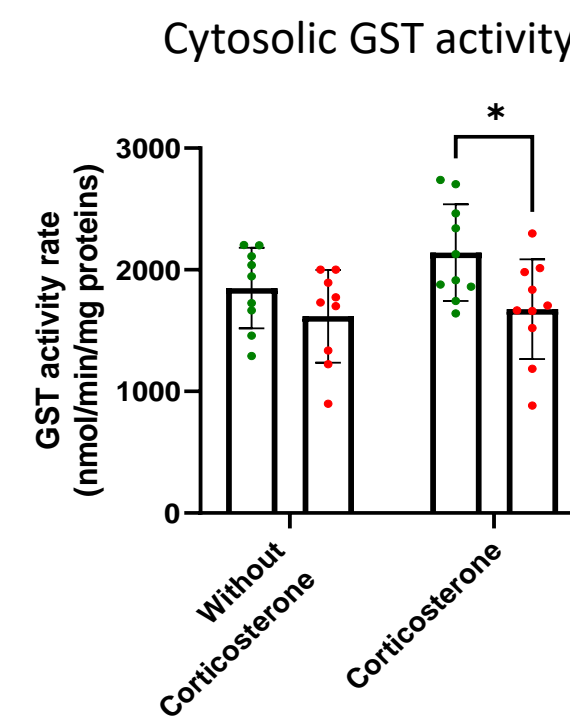
PFOA and Corticosterone significantly increase the activity of CYP4A10 (p=0.0114; p=0.0447)



PFOA does not affect the activity of CYP3A11



PFOA significantly decreases the activity of CYP2A4 (p=0.0007)



PFOA significantly decreases the cytosolic activity of GST (p=0.0083), not the microsomal activity

Cytosolic GST functional activities

Conclusions

We explored the impact of chronic low dose of PFOA on mice hepatic phase I and II functional XME activities under stress conditions.

Our study has demonstrated a significant impact of *in vivo* exposure to PFOA on hepatic phase I XME activities. **CYP2C37 activity**, an isoform involved in **liver fatty acid and eicosanoids metabolism** (endogenous substrates playing important roles in endocrine systems) upon CAR receptor regulation, was **inhibited by 15 µg PFOA/kg b.w./day**.

PFOA exposure **increases CYP4A10 activity, an isoform involved in fatty acid (ω-oxidation of medium-chain fatty acids) and eicosanoid metabolism**.

Although PFOA exposure had no significant effect on **CYP3A11 activity** (involved in the bile acid metabolism upon PXR and CAR receptor regulation), PFOA significantly decreases the activity of **CYP2A4** (involved in steroid and arachidonic acid metabolism).

Phase II cytosolic **GST activities** (involved in the steroid and prostaglandin metabolism) were significantly decreased by PFOA exposure.

These results demonstrate that low doses of exposure to PFOA *in vivo* modulates phase I and phase II XME's activities. Stress conditions can affect enzyme activities such as CYP4A10.

References

- [1] Sunderland EM. et al., 2019, J Expo Sci Environ Epidemiol. 29(2):131-147.
- [2] Knutsen HK. et al., 2018, EFSA J 16.
- [3] Li K. et al., 2017, Environ Int. Feb;99:43-54.

All the experimental procedure was carried out in accordance with the accepted standards of animal care and validated by the local ethical committee for animal experimentation N°086 (project number: APAFIS#21813) and by the French Ministry of Higher Education, Research and Innovation (agreement: C3155513)

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