GENETIC AND ENVIRONMENTAL TOXICOLOGY ASSOCIATION OF NORTHERN CALIFORNIA

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SPRING SYMPOSIUM

IMMUNOTOXICITY OF PFAS

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Conflict of Interest Statement

The author declares no conflict of interest.

I have a grant supported by Ministero dell'Istruzione, dell'Università e della Ricerca (PRIN2017, Project number 2017MLC3NF), in which PFOS is used.

Outline of the presentation

- Introduction to PFAS: uses and environmental contamination
- Health risk consideration
- PFAS and immunotoxicity
- Mechanism(s) underlying PFAS-induced immunotoxicity
- Summary and conclusions

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PERFLUORINATED COMPOUNDS

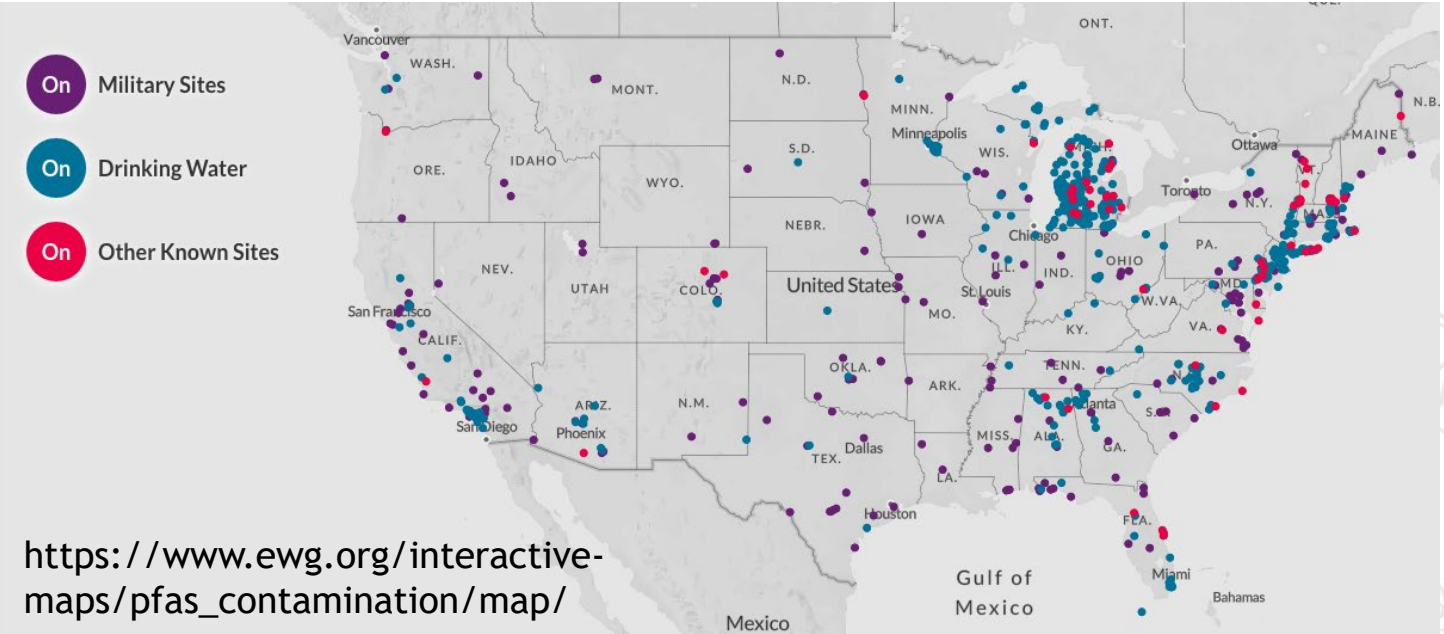
- Per- and polyfluoroalkyl substances or perfluorinated alkylated substances (PFAS), such as perfluorooctane sulfonate (PFOS) and perfluorooctane acid (PFOA), have been used extensively in commercial/industrial applications for the last 70 years.

- They possess a strong carbon-fluorine bond, which leads to their environmental persistence. Due to their widespread use, environmental persistence, they are an important class of environmental contaminants and are of major toxicological concern (Essumang et al., 2017).

- Fire-retarding foams
- Lubricants

- Food packaging
- Non-stick coatings
- Water and dirt proof

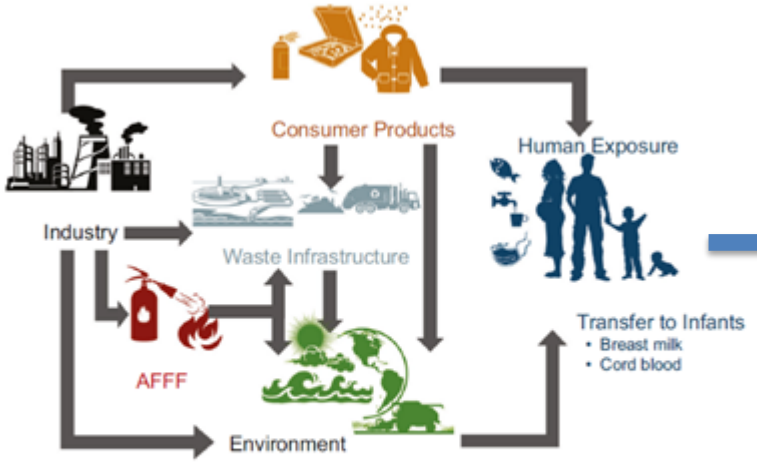
SOME FIGURES ON THE WATER CONTAMINATION



https://www.ewg.org/interactive-maps/pfas_contamination/map/

As of January 2021, 2,337 locations in 49 states are known to have PFAS contamination, including drinking water systems serving an estimated 19 million people.
(Environmental Working Group and Social Science Environmental Health Research Institute at Northeastern University)

SOME FIGURES ON THE HUMAN CONTAMINATION



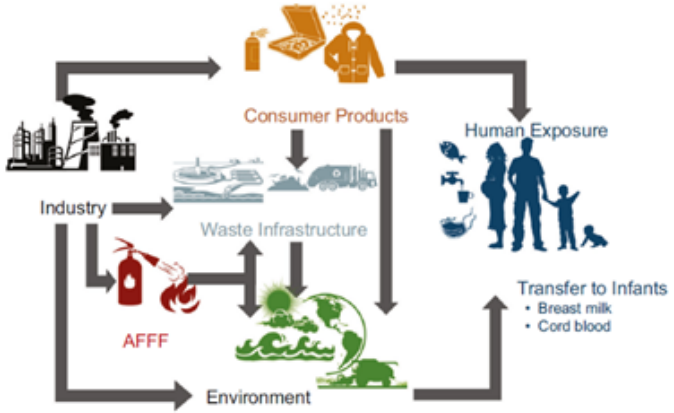
Potential major exposure pathways of PFAS to humans.
Figure from Sunderland et. al. (2019)

Food (meat and fish) and drinking water are considered the major sources of human exposure

Contamination of food with PFOS and PFOA is thought to occur mainly through two different processes (i) from bioaccumulation in aquatic and terrestrial food chains and (ii) as a result of transfer of PFAS from contact materials used in food processing and packaging.

SOME FIGURES ON THE HUMAN CONTAMINATION

Globally, biomonitoring studies have revealed that PFAS content of human serum are at ng/mL levels (Kang et al., 2018).



Potential major exposure pathways of PFAS to humans.
Figure from Sunderland et. al. (2019)

Median blood concentrations in the EU:

- **PFOS**: adult 7.7 ng/ml; children 3.2 ng/ml;
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EFSA Journal 16:5194, 2018

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ADVERSE EFFECTS

- The most commonly reported effects in experimental animals are those on the liver (increased weight, hypertrophy, increased fat content) and on the levels of thyroid hormones, cholesterol, triglycerides, and liver transaminases in serum. In addition, some PFAS were shown to cause liver tumors.

- In addition, and more importantly, effects on the immune system, as well as on the development of the mammary gland, were observed in several studies often at lower levels than those causing effects on the liver and thyroid hormones.

- **There is evidence from both epidemiology and laboratory studies that PFCs are immunotoxic, affecting both cell-mediated and humoral immunity [DeWitt et al., 2019].**

HEALTH RISK CONSIDERATION: EFSA OPINION 2020

- Based on available studies in animals and humans, effects on the immune system were considered the most critical for the risk assessment. The **decrease in antibody response at vaccination** in children was identified as the critical effect.

- After benchmark modelling of serum levels of the sum of four PFASs: PFOA, PFNA, PFHxS and PFOS, and estimating the corresponding daily intakes, the CONTAM Panel at EFSA established a new tolerable weekly intake (TWI) of 4.4 ng/kg body weight (bw) per week.

- **This TWI also protects against other potential adverse effects observed in humans. Based on the estimated exposure, but also reported serum levels, the CONTAM Panel concluded that parts of the European population exceed this TWI, which is of concern.**

EFSA Journal 2020;18(9):6223

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PFAS IMMUNOTOXICITY: animal data

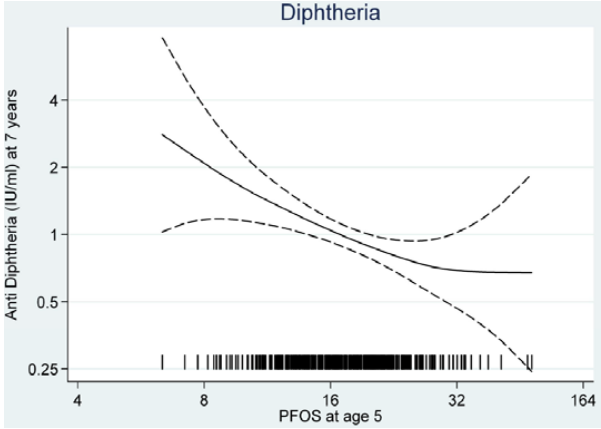
- Several studies indicated that PFOA/PFOS suppressed antibody production, caused thymus and spleen atrophy, altered cytokine production and T-cell populations [Yang et al., 2000, 2001, 2002; Dong et al., 2009; Peden-Adams et al., 2008].
- As a consequence of immunosuppression, it has been indeed demonstrated that PFOS exposure in mice resulted a significant increase in emaciation and mortality in response to influenza A virus [Guruge et al., 2009].
- The most sensitive parameter affected by PFOS is TDAR:
  - NOAEL for immunotoxicity 1.66 mg/kg for PFOS, 1 mg/kg for PFOA

PFAS AND IMMUNOTOXICITY: human data

- Consistent suppression in at least one measure of the anti-vaccine antibody response across multiple studies with evidence from developmental, childhood, and adult exposures were reported:
  - Lower antibody levels to rubella in children at age 3 with higher maternal blood levels of PFOA (prospective study in Norway, not significant for measles, Hib or tetanus)
  - Lower antibody levels to diphtheria and tetanus in children with higher maternal or child blood levels of PFOA (prospective study in Faroe Islands, antibody response and exposure measured at age 5 and 7, not always significant)
  - Lower antibody levels to mumps and rubella in children age 12-19 with higher blood levels of PFOA (cross-sectional study in United States, not significant for measles)
  - Reduced antibody response to influenza vaccination (A/H3N2) in adult residents of Ohio Valley (OH and WV) with elevated PFOA in drinking water (not significant for type B, A/H1N1 and in some analyses)
  - Reduced antibody response to diphtheria vaccination in adult hospital workers with higher blood levels of PFOA in small study in Denmark (not significant for tetanus)
  - Lower antibody levels and increased morbidity to measles in children (Guinea-Bissau, West Africa)

PFAS AND IMMUNOTOXICITY: human data

- Some studies report a correlation between PFAS levels in the body and lower resistance to disease and increased risk of infections [Dalsager et al., 2016; Granum et al., 2013].
- A relationship between higher PFAS levels and increased risk of asthma [Averina et al. 2019] as well as increased in adolescent food allergies [Buseret al. 2016] have been also reported in some studies.



Grandjean et al. JAMA 307, 391-97, 2012

PFAS IMMUNOTOXICITY: overall considerations

- The body of evidence concerning the ability of PFAS to modulate the immune system has grown in the last decade, and several studies have reported serum concentrations in rodents at immune effect levels within the range of human and wildlife exposures (DeWitt et al., 2019).

- Human epidemiological studies suggest that exposure to PFAS adversely affect serum antibody response following vaccination in children and adults (strong evidence).

- There are some suggestions that prenatal exposure to PFOS and PFOA may lead to increase propensity to infection.

- Insufficient support for causal association with allergy, autoimmunity or cancer.

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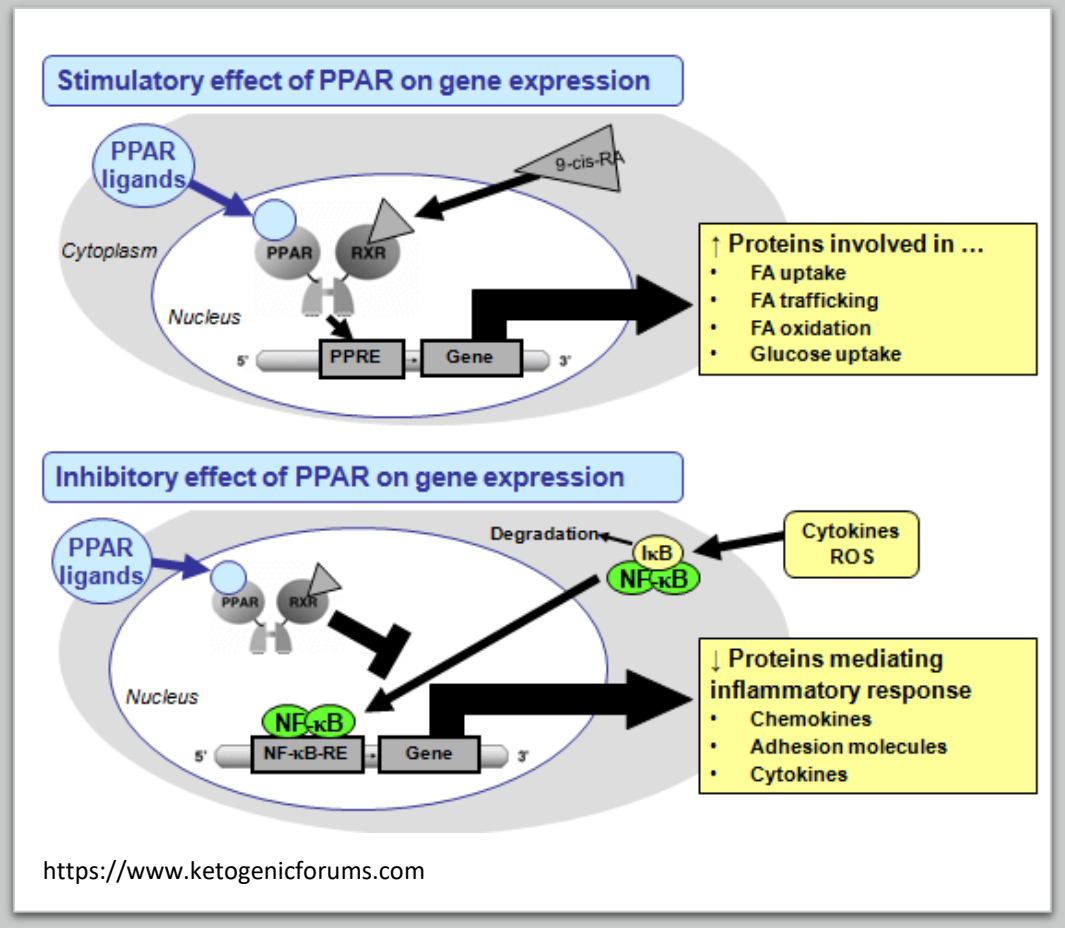
PFAS AND MECHANISMS OF ACTION

- Many PFAS are ligands of the nuclear receptor peroxisome proliferator-activated receptors (PPAR), with different kinetics, patterns and potency [EFSA, 2020].
- These receptors regulate lipid homeostasis, inflammation, adipogenesis, reproduction, wound healing, and carcinogenesis [Chinetti et al., 2000].
- Animal studies in liver indicate that approximately 85% of the genes altered by PFOA were dependent on PPARα.



https://www.ketogenicforums.com

PFAS AND MECHANISMS OF ACTION

- PFAS have been shown to interact with receptors and transcription factors other than PPARα:
  - PPARγ,
  - CAR (constitutive activated/androstane receptor),
  - estrogen receptor alpha (ERα),
  - androgen receptor,
  - glucocorticoid receptor,
  - pregnane X receptor,
  - the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2),
  - NF-κB
- In addition, there is considerable evidence from animal studies that PFAS interfere with the thyroid hormone homoeostasis.

[Rosen et al., 2008; Rosen et al., 2017; Bansal et al., 2018; Chiu et al., 2018].

PFAS AND IMMUNE SYSTEM

- **We conducted in vitro studies:**

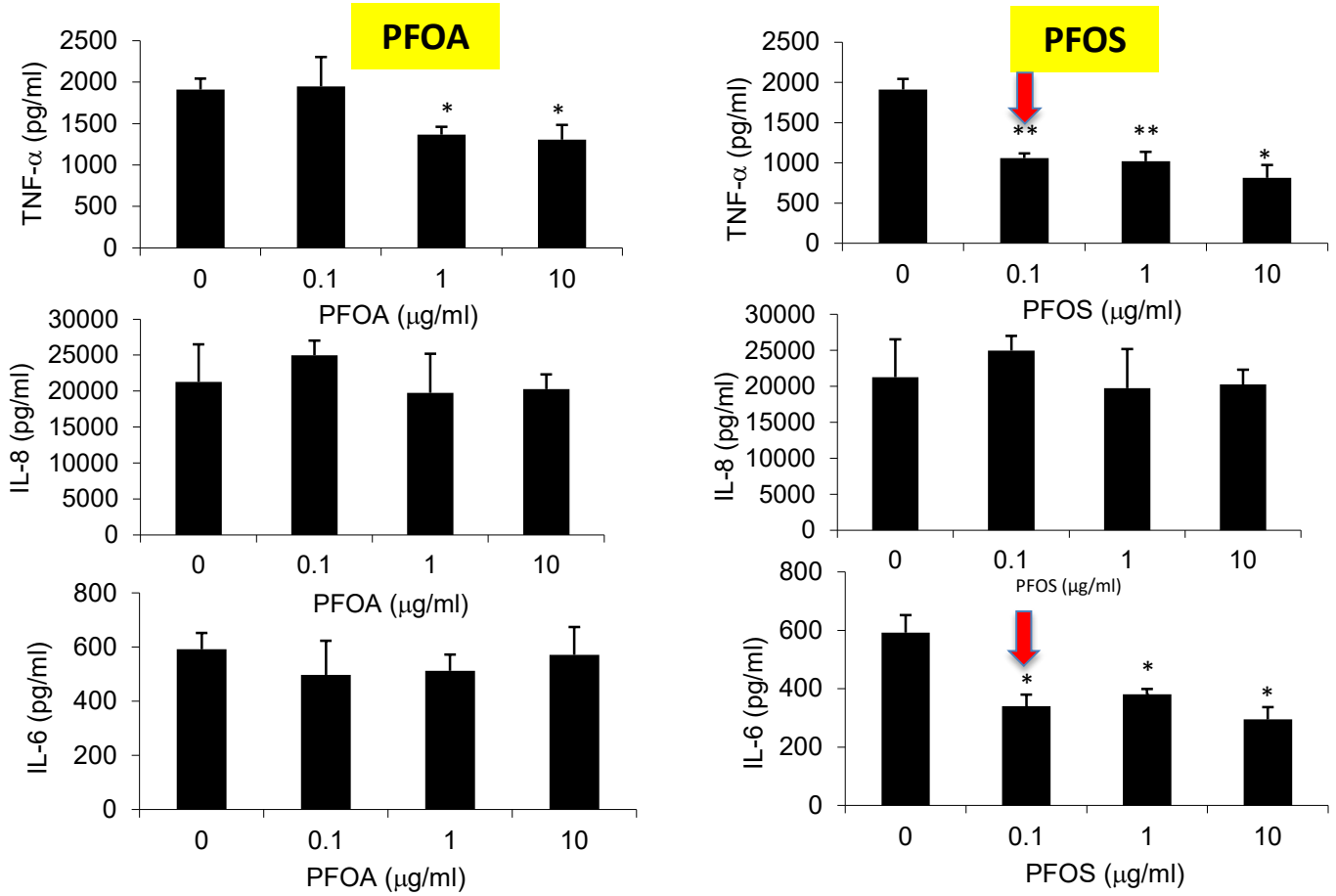
  1. **to investigate if PFAS can directly affect immune cells functionality**

  2. **to characterize the molecular mechanisms underlying such effects:**

     - **Role of NF-κB**
     - **Role of PPARα**
     - **Role of GR**

Corsini et al., 2011, 2012
Buoso et al., unpublished results

Direct Effect of PFOA and PFOS on LPS-induced cytokine release in peripheral blood leukocytes



Human general population internal dose

Median blood concentrations in the EU:

- **PFOS**: adult 7.7 ng/ml; children 3.2 ng/ml;
- **PFOA**: adult 1.9 ng/ml; children 3.3 ng/ml.
- **Occupational exposure** levels 307-458 ng/ml (Fromme et al., 2009)

Modified from Corsini et al. 2011

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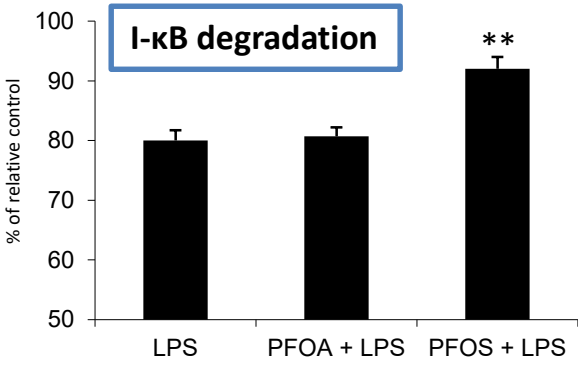
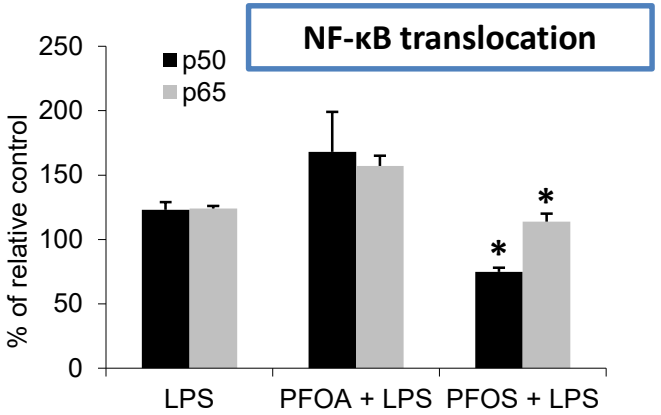
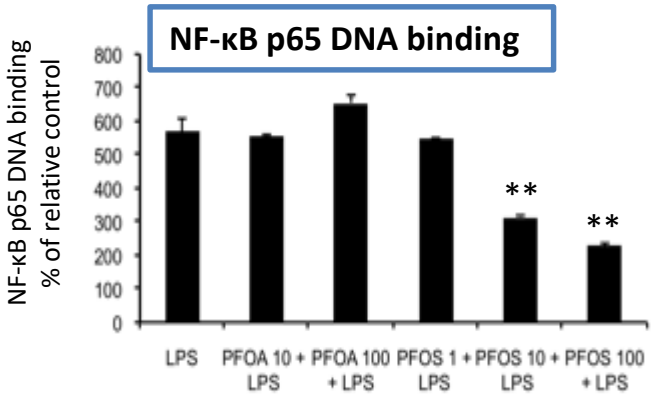
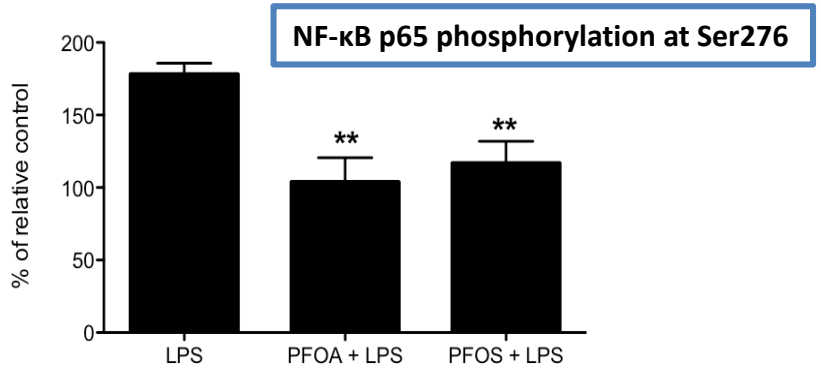
EFFECTS ON NF-κB



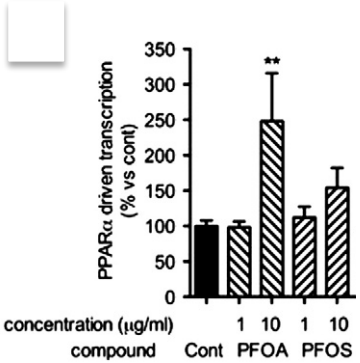


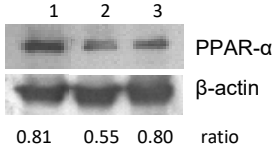


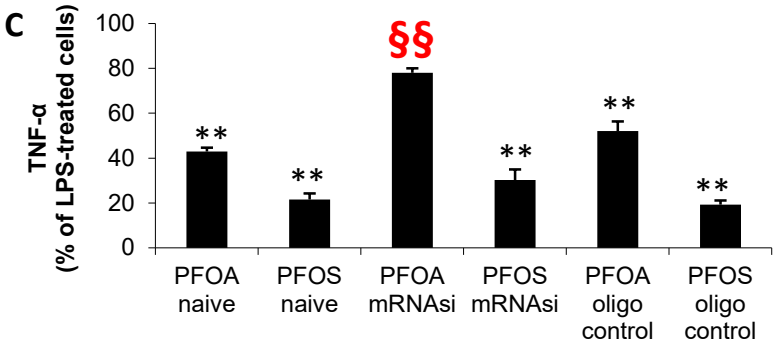


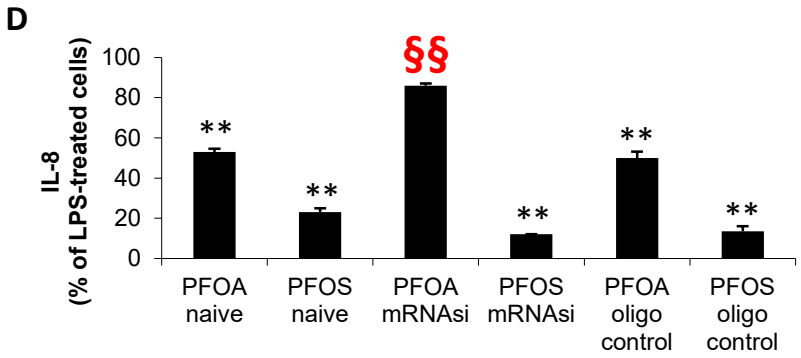
Modified from Corsini et al. 2011

Role of PPAR-α on the inhibition of PFOA and PFOS of LPS-induced cytokine release in THP-1 cells

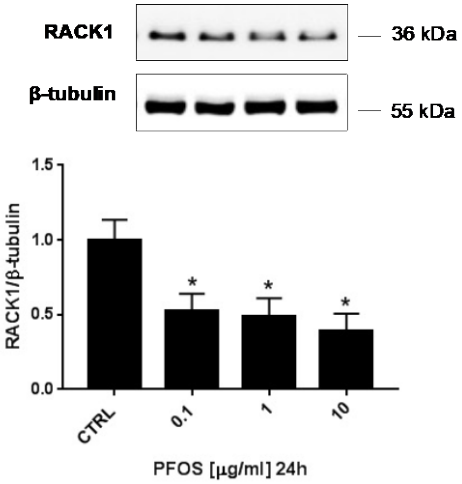


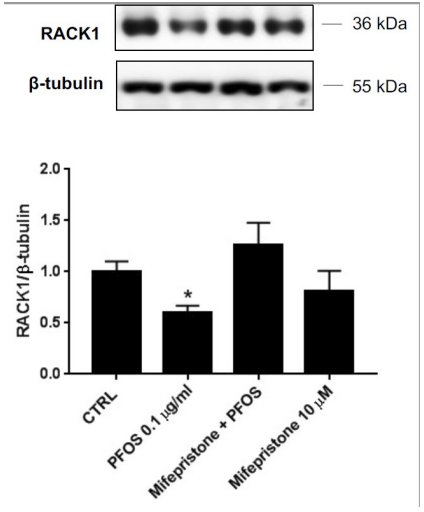


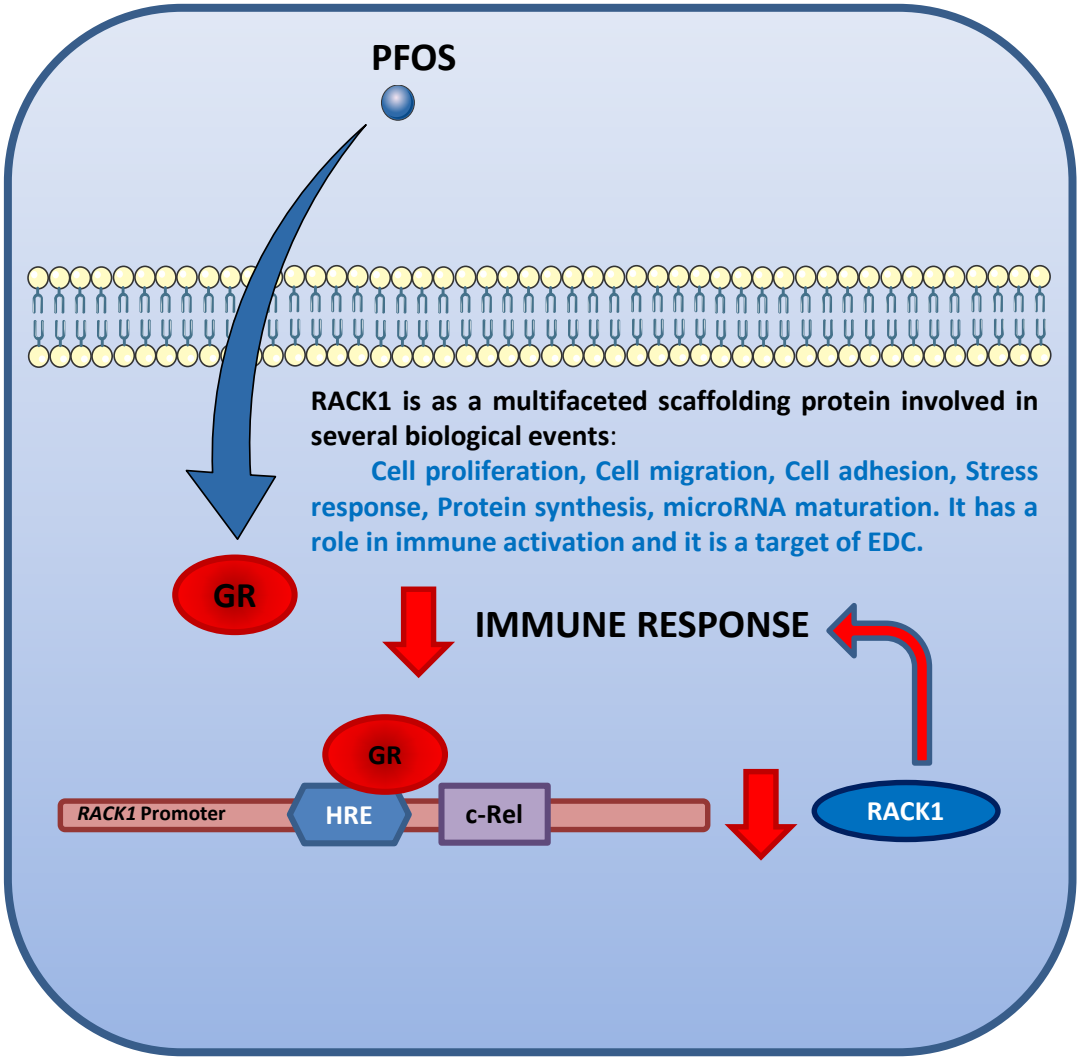




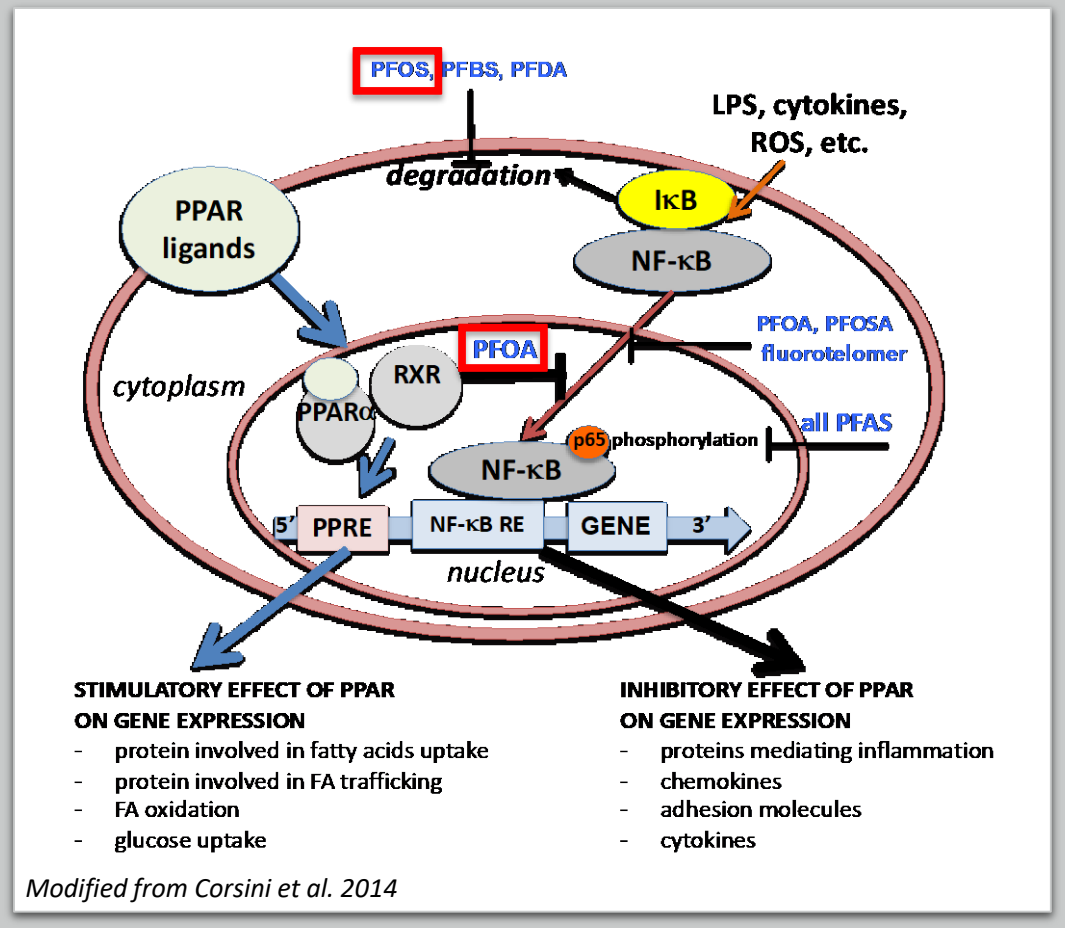
Modified from Corsini et al. 2011, 2012


RACK1
36 kDa
β-tubulin
55 kDa
RACK1/β-tubulin
1.5
1.0
0.5
0.0
CTRL
0.1
1
10
PFOS [µg/ml] 24h


RACK1
36 kDa
β-tubulin
55 kDa
RACK1/β-tubulin
2.0
1.5
1.0
0.5
0.0
CTRL
PFOS 0.1 µg/ml
Mifepristone + PFOS
Mifepristone 10 µM

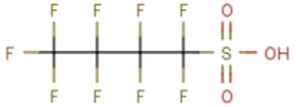

PFOS
RACK1 is as a multifaceted scaffolding protein involved in several biological events:
Cell proliferation, Cell migration, Cell adhesion, Stress response, Protein synthesis, microRNA maturation. It has a role in immune activation and it is a target of EDC.
GR
IMMUNE RESPONSE
GR
RACK1 Promoter
HRE
c-Rel
RACK1

Receptor mediated and non-receptor mediated effects of PFAS-induced immunomodulation

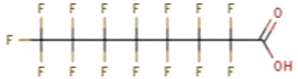


Modified from Corsini et al. 2014

In vitro characterization of the immunotoxic potential of several perfluorinated compounds



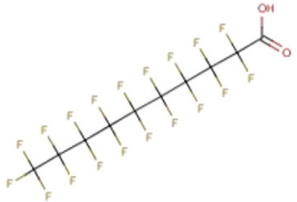
PERFLUOROBUTANE SULFONIC ACID
CAS #: 375-73-5 (PFBS)



PERFLUOROOCTANOIC ACID
CAS #: 335-67-1 (PFOA)

NH2 = **PFOSA**
CAS # 754-91-6

PERFLUOROOCTANE SULFONIC ACID
CAS #: 1763-23-1 (PFOS)



PERFLUORODECANOIC ACID
CAS #: 335-76-2 (PFDA)

1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- (8:2 Telomer)
CAS #: 678-39-7 (FLUOROTELOMER)

Compounds with similar mechanisms

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SUMMARY AND CONCLUSIONS

- In Europe, according to the EFSA opinion (2020), based on exposure, a considerable proportion of the population exceeds the established TWI for PFAS, which is of concern.
- PFAS can directly affect immune cells functionality (cytokines): PFOS > PFOA
- A different molecular mechanisms underlying such effects:
    - PFOS (PFBS, PFDA) affected LPS-induced I-κB degradation, nuclear translocation, DNA binding and p65 phosphorylation
    - PFOA (PFOSA, fluotelomer) only affect LPS-induced NF-κB p65 phosphorylation
    - Role of PPARα for PFOA
    - Role of GR in PFOS-induced RACK1 downregulation
- While concentrations of PFOA are decreasing, concentrations of other PFAS are increasing.
- In real life, we are simultaneously exposed to multiple PFASs, which interaction and joint toxicity are unclear. Further studies are needed to develop the knowledge.

THANK YOU FOR YOUR ATTENTION

QUESTIONS?