SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety

Immunotoxicology in Food and Ingredient Safety Assessment: Approaches and Case Studies

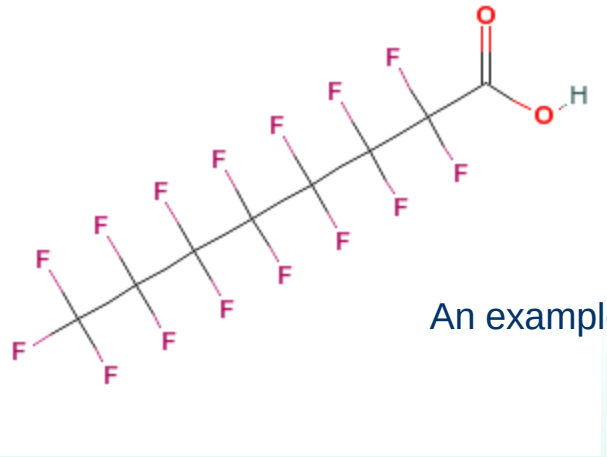
April 14, 2015

Immunomodulatory Effects of Perfluoroalkyl Substances in Rodents and Humans

Jamie DeWitt, Ph.D.
Assistant Professor
Department of Pharmacology & Toxicology
Brody School of Medicine
East Carolina University
Greenville, NC
dewittj@ecu.edu

What are “PFASs?”

Perfluoroalkyl and polyfluoroalkyl substances (PFASs): highly fluorinated aliphatic substances that contain one or more carbon atoms on which all the H substituents have been replaced by F atoms, so that they contain the perfluoroalkyl moiety CnF2n+1– (Buck et al, 2011).



An example of a PFAS (PFOA)

How are PFASs used?

- Surfactants
  - Processing aids for fluoropolymer and fluoroelastomer manufacture
  - Aqueous film-forming foams (AFFFs)

- Polymers
  - Oil, stain, grease, and water repellent coatings
  - Grease-resistant food contact paper

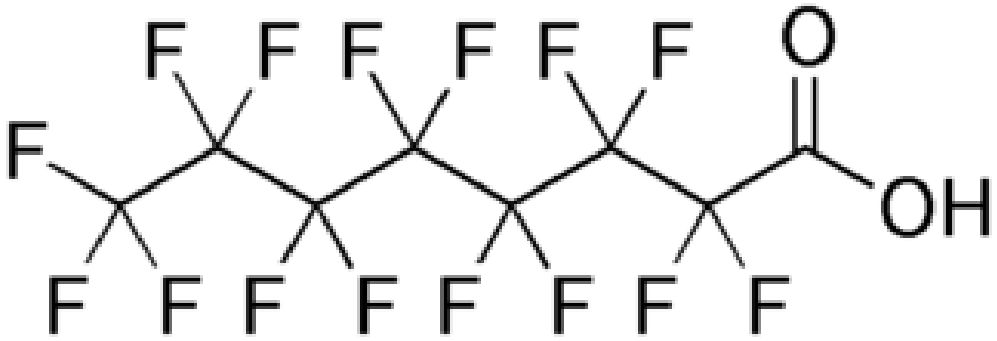
Buck et al., 2011 and US EPA, 2015

PFASs as contaminants

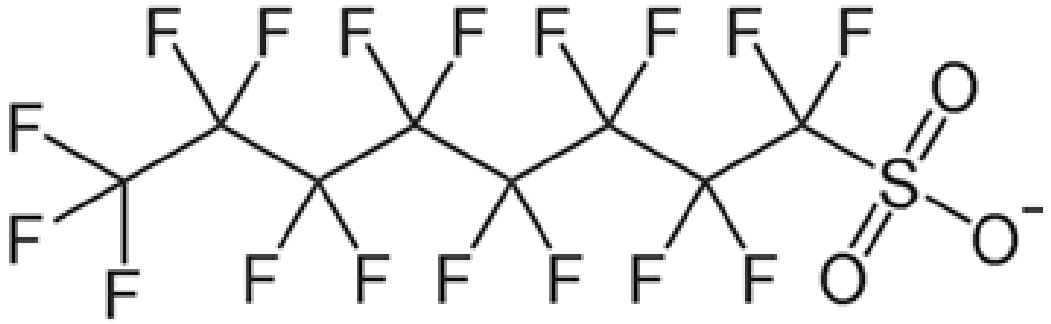
- PFASs are “emerging contaminants”
  - Agents that have “a perceived, potential, or real threat to human health or the environment by a lack of *public health standards* (emphasis added).”
  - Agents for which new sources or pathways to humans have been discovered.
  - Agents for which new detection methods or treatment technologies have been discovered.

US EPA, 2014

Two common PFASs



Perfluorooctanoic acid (PFOA) - a *perfluorinated* carboxylic acid



Perfluorooctane sulfonate (PFOS) - a *perfluorinated* sulfonic acid

PFAS in a regulatory context

- US Manufacturer of PFOS terminated production in late 1990s.

- In 2006 US EPA and eight major US companies agreed to a voluntary PFOA stewardship program:
  - A 95% reduction in facility emissions and product content levels of PFOA, precursor chemicals that can break down to PFOA, and related higher homologue chemicals.
  - A commitment to working toward eliminating these chemicals from products and emissions by 2015.

US EPA, 2015

PFASs in food

- PFASs may enter foods from
  - Soil and/or water where food is grown
  - Food packaging
  - Cookware

PFOA was most abundant PFAS in vegetable samples collected from Norway, Belgium, and Italy (perfluorinated hexanoic acid was most abundant in Czech Republic and second most abundant overall).

Highest PFOA concentration was 121 ng/kg in spinach from Italy.

Italy had highest PFOA intake estimation, followed by Belgium, Norway, and Czech Republic.

Herzke et al., 2013

PFOA and PFOS in wildlife

Up to 0.993 (PFOA) and 96.8 ng/mL (PFOS) in plasma of Loggerhead sea turtle

Up to 163 (PFOA) and 3073 (PFOS) ng/mL in plasma of Bottlenose dolphin

Up to 13 (PFOA) and 1325 (PFOS) ng/g in liver of polar bear

Not detectable (PFOA) and 450 (PFOS) ng/mL in plasma of Herring gulls

(DeWitt et al., 2012)

PFOA and PFOS in human serum, US

0.2-88 (PFOA) and 0-1656 (PFOS) ng/mL general population

17-5100 (PFOA) and 37-3490 (PFOS) ng/mL occupational

(DeWitt et al., 2012)

Highlights of immune observations

Observational
Reductions in relative spleen and thymus weights

Observational
Reductions in lymphocytes from the spleen and thymus

Functional
Reductions in antigen-specific IgM and IgG antibodies

Functional
Reductions in natural killer cell cytotoxicity

Lymphoid organ weights - PFOA

	Dose (mg/kg)	Spleen Wt.	Thymus Wt.	Reference
C57BL/6 M	30; diet	↓~40%	↓~80%	Yang et al., 2002b
BALB/c F	25 & 50; dermal	↓~30%	↓~40%*	Fairley et al., 2007
C57BL/6 F	15 & 30; water	↓~40%	↓~40%	DeWitt et al., 2008

*Reductions in thymus weight only observed at 50 mg/kg dose.

Lymphoid organ weights - PFOS

	Dose (mg/kg)	Spleen Wt.	Thymus Wt.	Reference
B6C3F1 M, F	Up to 5; gavage	No change	No change	Peden-Adams et al., 2008
C57BL/6 M	25, 50, 125; gavage	↓~40%	↓~50%	Dong et al., 2009
C57BL/6 M	20, 40; gavage	↓~30%	↓~45%	Zheng et al., 2009
C57BL/6 M	20; gavage	↓~20%	↓~25%	Zheng et al., 2011

Lymphoid organ weights

- Changes in lymphoid organ weights observed only at relatively high doses.

Did other changes accompany reductions in lymphoid organ weights?

Splenic & thymic lymphocytes - PFOA

	Dose (mg/kg)	Spleen	Thymus	Reference
C57BL/6 M	30; diet	↓ in T cells	↓ in T cells	Yang et al., 2002b
C57BL/6 F	3.75 & 7.5; water	No d-r changes in T cells; no changes in B cells	--	DeWitt et al., 2015

Splenic & thymic lymphocytes - PFOS

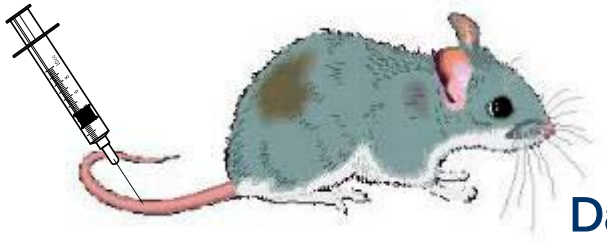
	Dose (mg/kg)	Spleen	Thymus	Reference
B6C3F1 F	Up to 5; gavage	↓ & ↑ in T cells in M	Minimal	Peden-Adams et al., 2008
C57BL/6 M	25, 50, 125; gavage	↓ In T cells	↓ In T cells	Dong et al., 2009
C57BL/6 M	20, 40; gavage	↓ In T cells	↓ In T cells	Zheng et al., 2009

Splenic & thymic lymphocytes

- Changes in splenic and thymic lymphocytes observed only at relatively high doses.

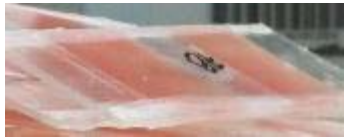
Did other changes accompany changes in lymphocyte numbers?

The TDAR

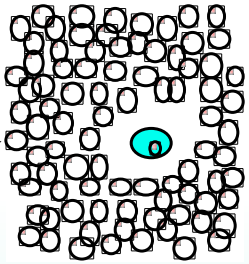


Day 4

Complement + sRBC



Incubation



Magnified

sRBC around AFC are hemolyzed = PLAQUE

Antibody Forming Cell (AFC)

Sheep RBC

The TDAR as a “gold standard”

The TDAR requires immune coordination.

- The T cell-dependent antibody response requires
  - Antigen presentation
  - T cell function
  - B cell function
  - B cell transformation
  - Cytokine production
  - Genetic/epigenetic signals

The TDAR - PFOA

Animals exposed to PFOA and immunized with hRBCs had reduced hRBC-specific IgM antibodies.

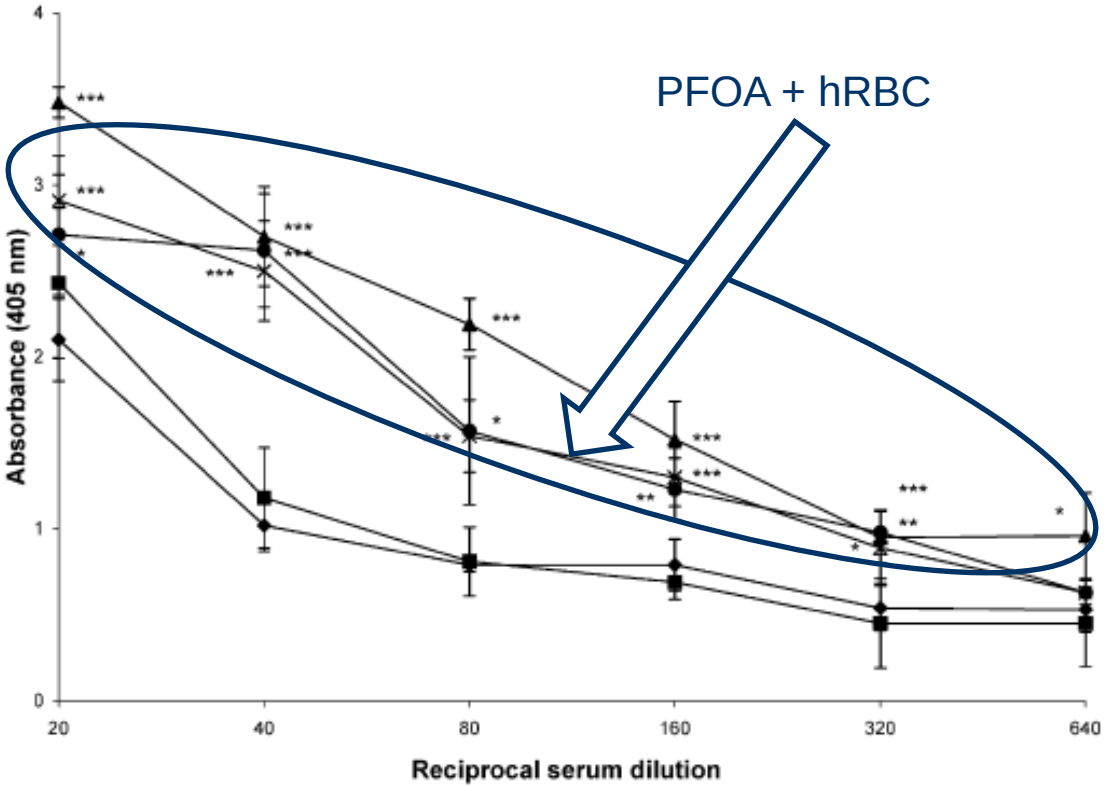
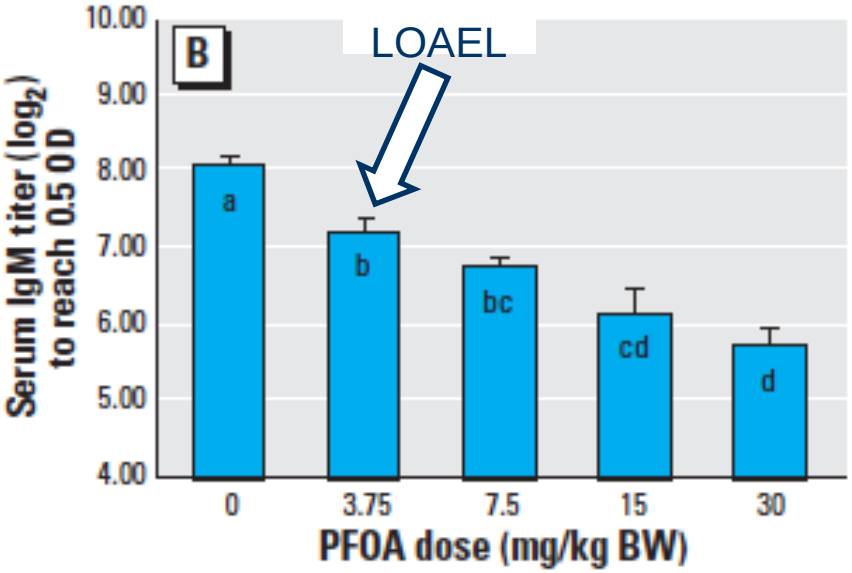
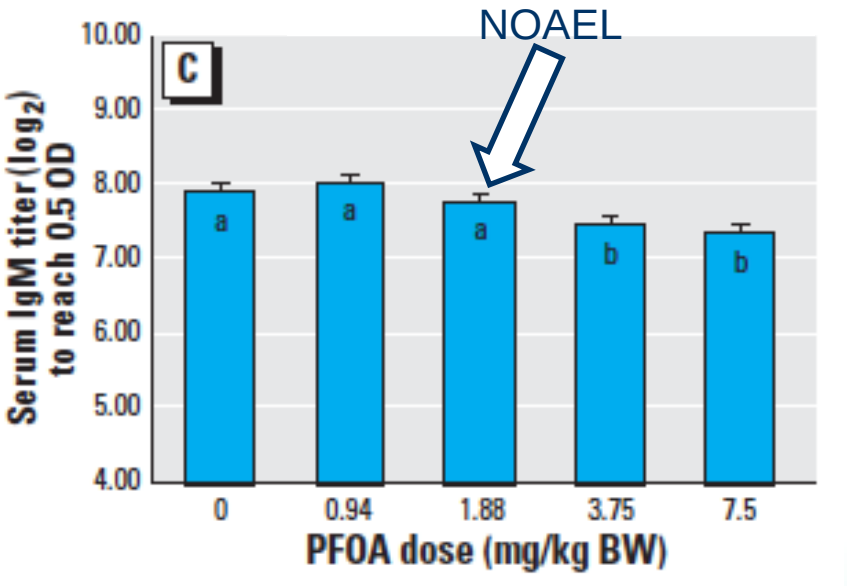


Figure modified from Yang et al., 2002a

The TDAR - PFOA



Animals exposed to PFOA and immunized with sRBCs had reduced sRBC-specific IgM antibodies.



Figures modified from DeWitt et al., 2008

The TDAR - PFOS

Animals exposed to PFOS and immunized with sRBCs had reduced sRBC-specific IgM antibodies.

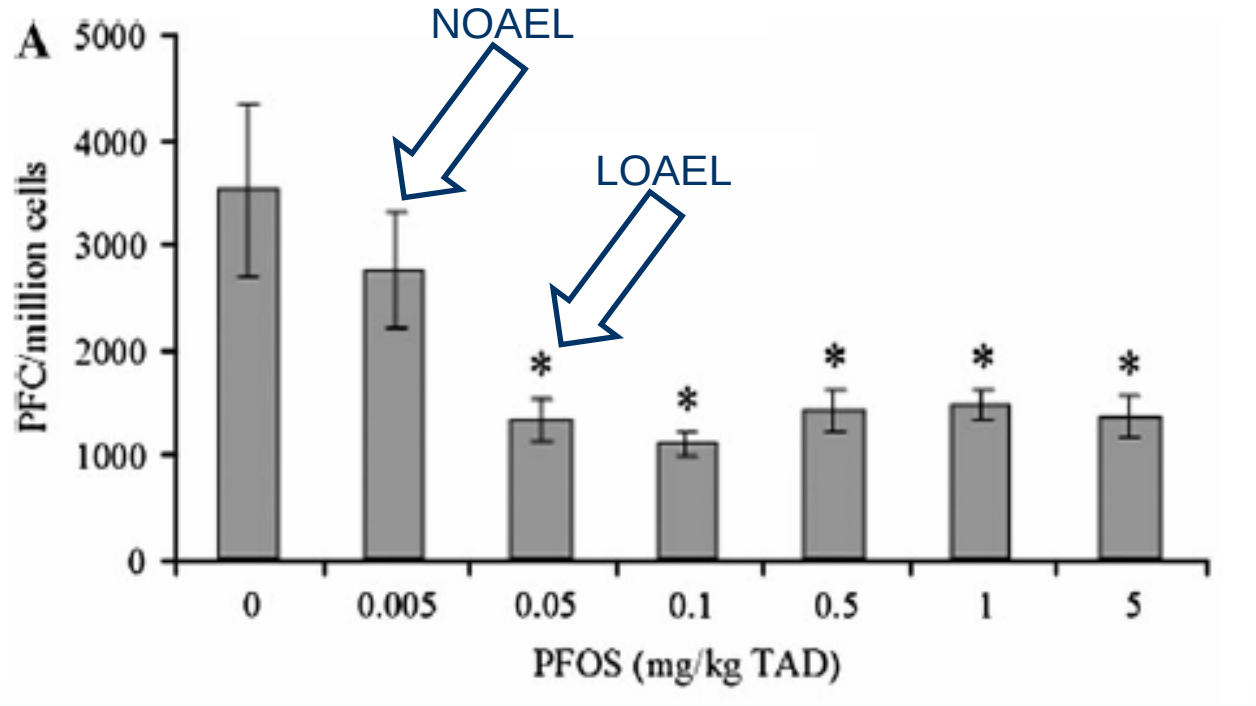


Figure modified from Peden-Adams et al., 2008

The TDAR - PFOS

Animals exposed to PFOS and immunized with sRBCs had reduced sRBC-specific IgM antibodies.

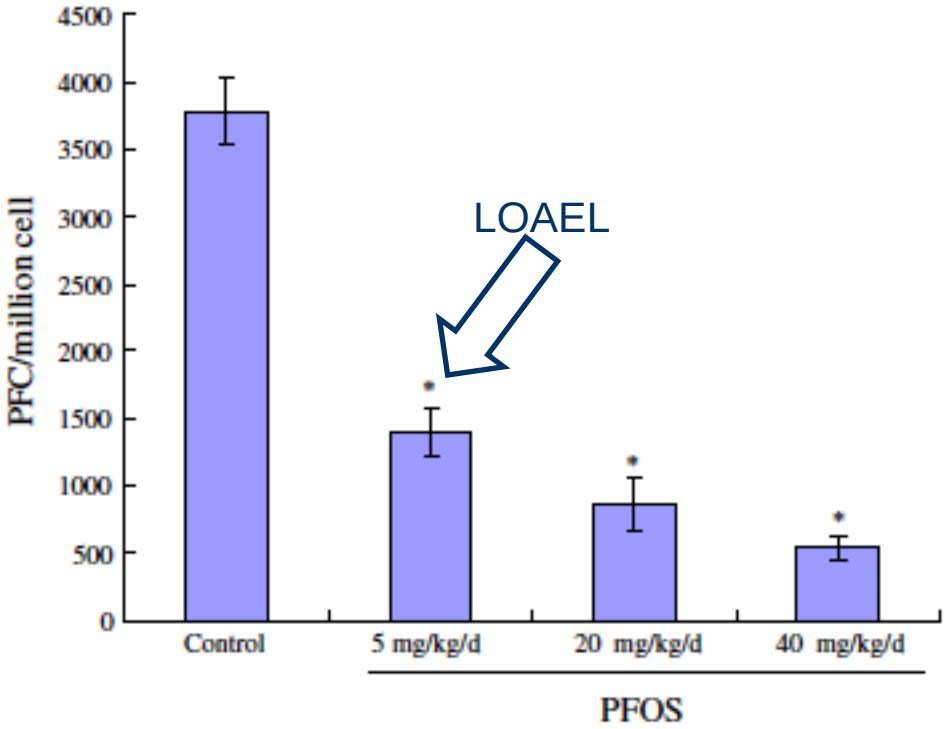


Figure modified from Zheng et al., 2009

Are PFOA & PFOS immunomodulatory?

- Suppression of the TDAR occurred in absence of impacts on lymphoid organ weights and cell subsets.
  - Both PFOA and PFOS exposure suppressed TDAR at doses below those associated with impacts on lymphoid organ weights and cell counts.
- Are data from the TDAR alone sufficient for classifying a toxicant as immunomodulatory?
  - What about impacts on other immune endpoints?

Adaptive cellular responses - PFOA

Animals exposed to PFOA, sensitized to BSA, and then challenged with BSA had responses similar to control group.

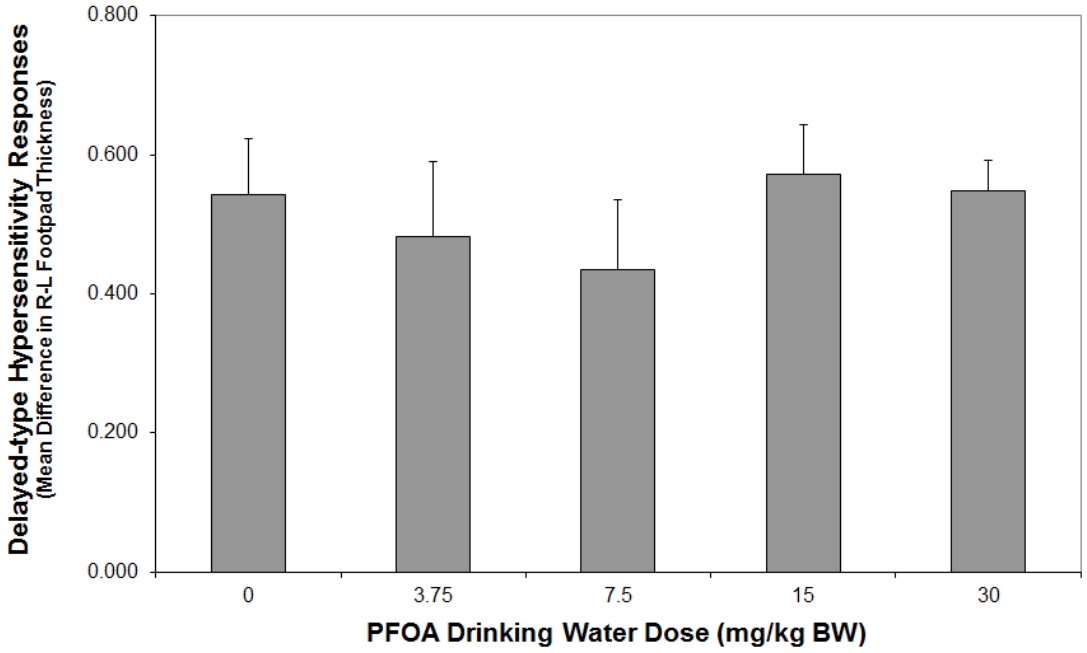


Figure generated from studies by J. DeWitt and R.W. Luebke

Innate immune cell responses - PFOS

NK cells from animals exposed to PFOS in vivo lysed a greater number of target cells compared to control group.

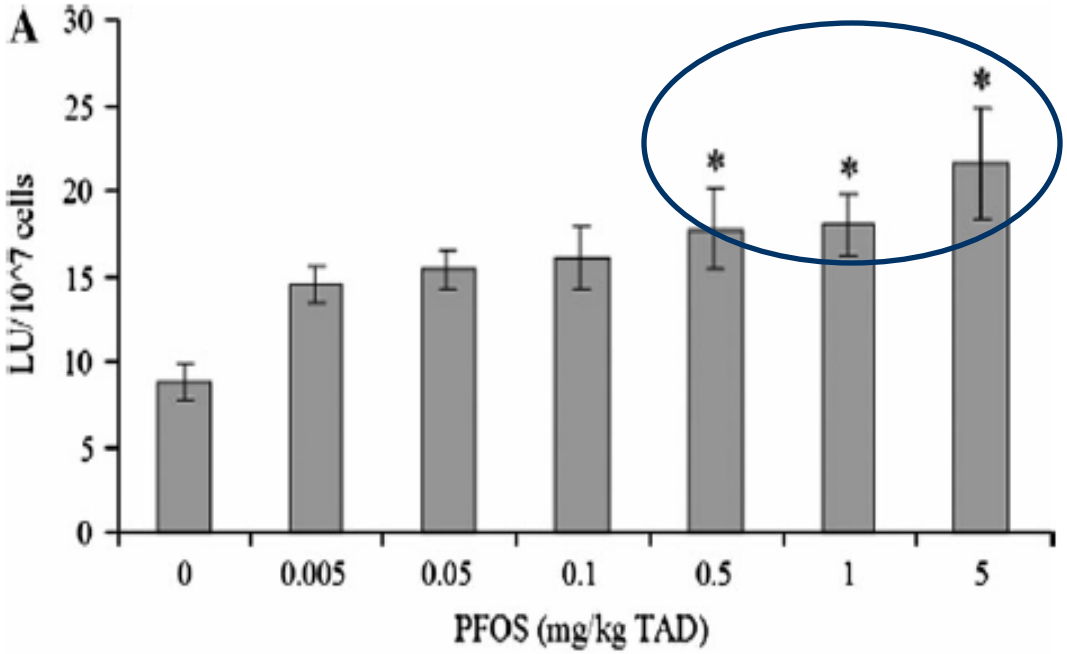


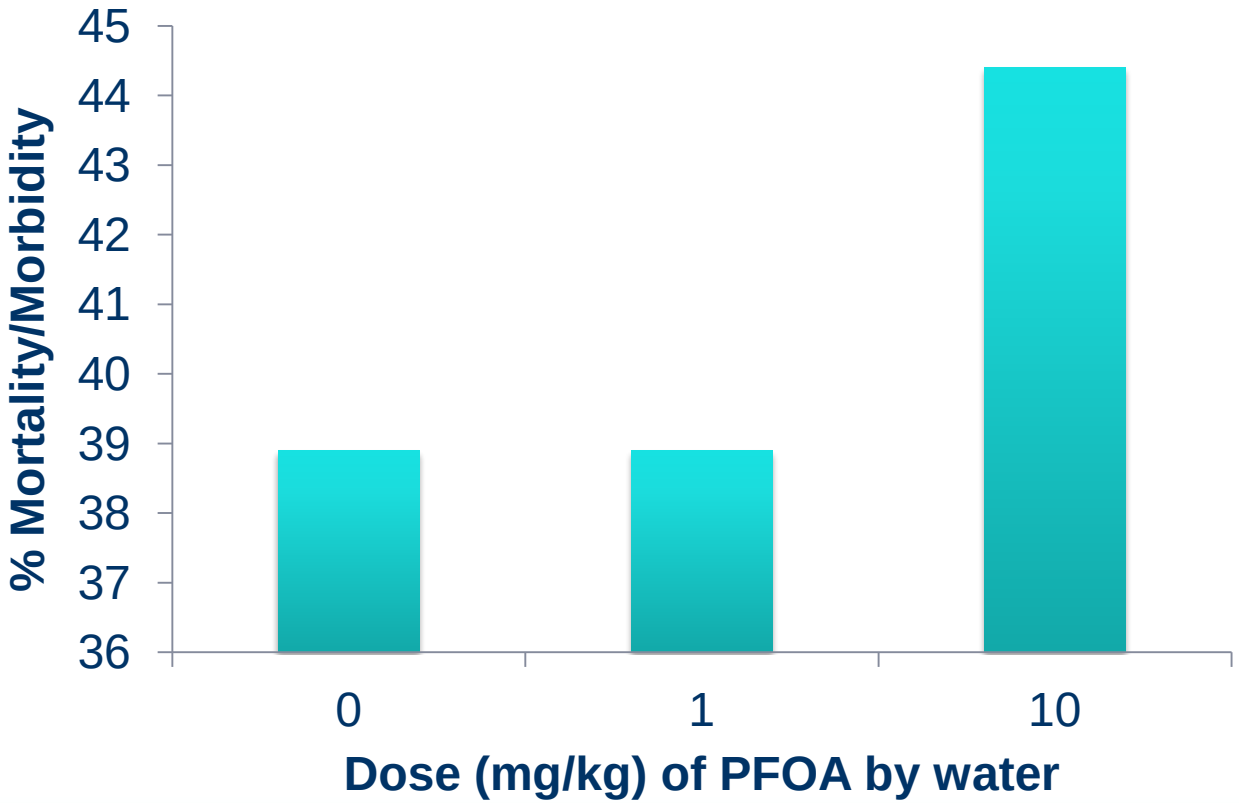
Figure modified from Peden-Adams et al., 2008

Impacts on cytokine production

- Changes in pro-inflammatory cytokines
  - IL-1b
  - TNFα
  - IL-6
- Shifts in Th1/Th2 cytokine profiles
  - Cytokine production seems to favor Th2 (PFOS only)
- *PFOA and PFOS exposure alter inflammatory pathways, but not necessarily in similar directions.*

Disease resistance - PFOA

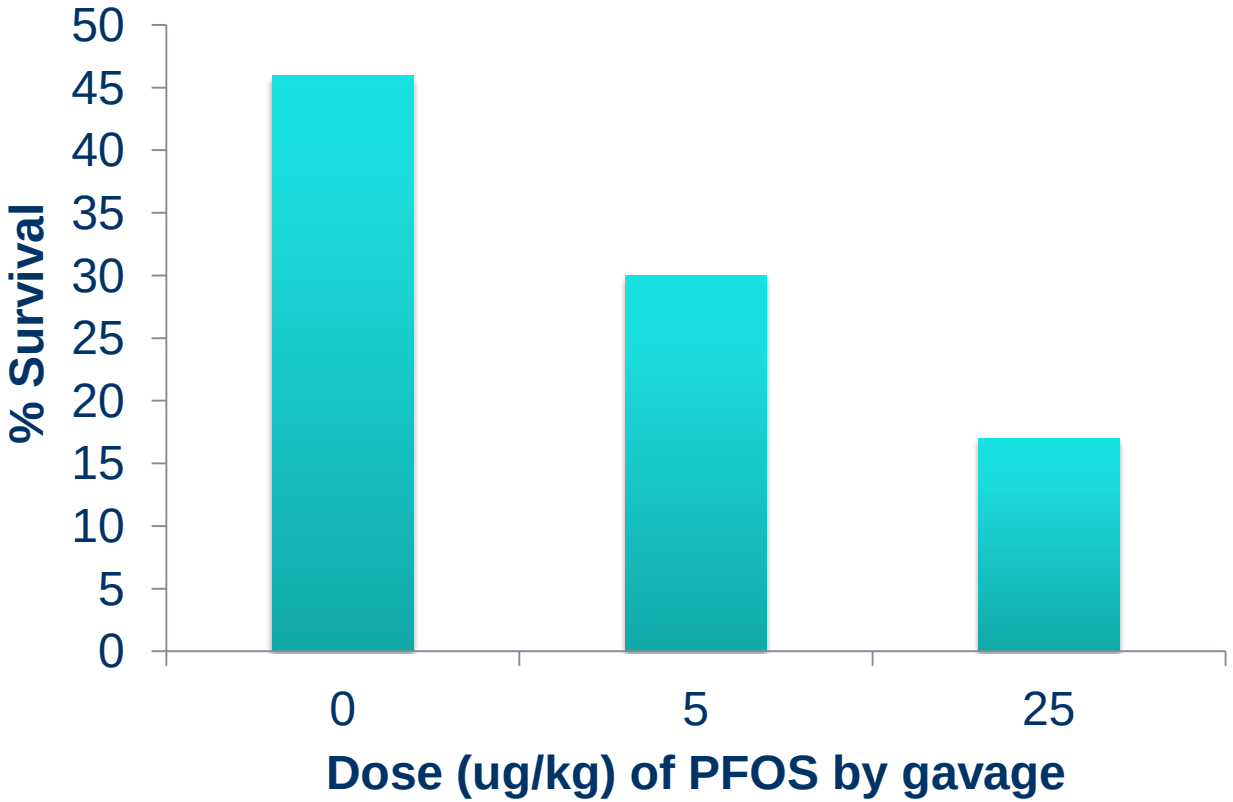
Japanese quail exposed to PFOA for eight weeks had reduced survival after infection with *E. coli* (not statistically significant).



Data from Smits & Nain, 2013

Disease resistance - PFOS

B6C3F1 mice exposed to PFOS for 21 days had reduced survival after infection with Infuenza A virus (statistically significant).

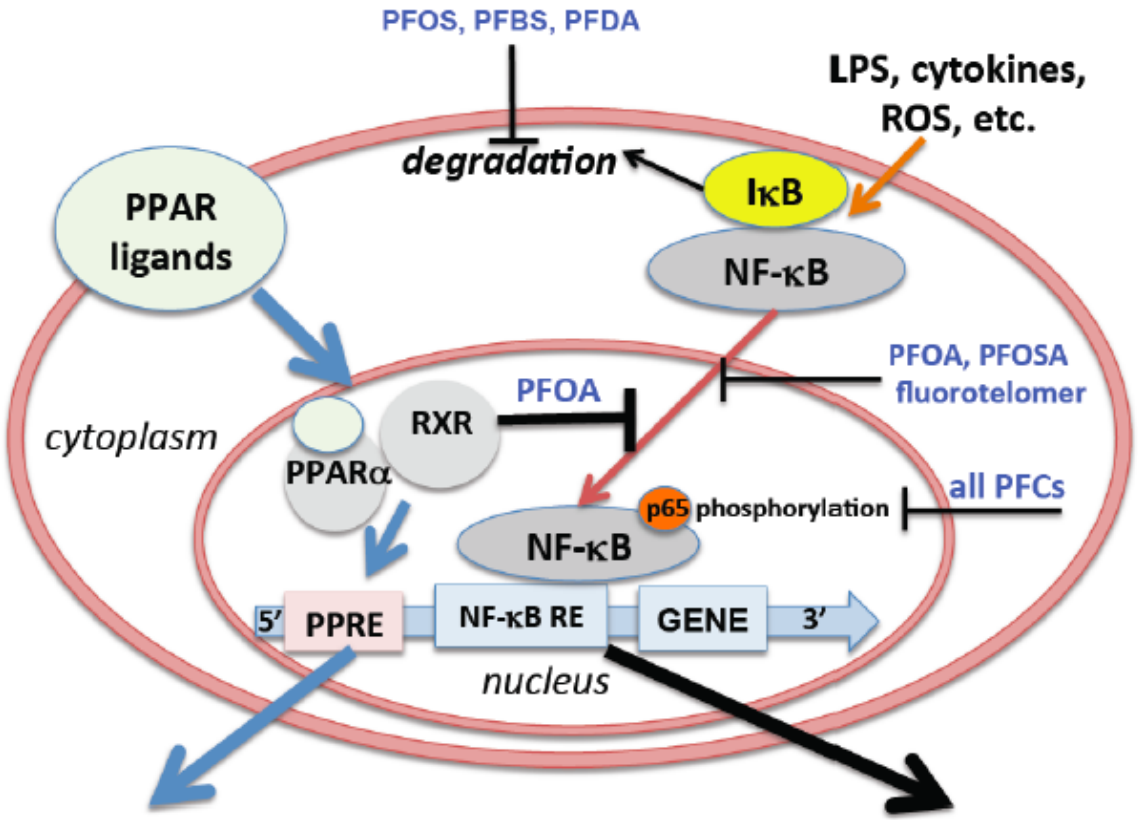


Data from Guruge et al., 2009

Summary of assays

- Suppression of the TDAR
- Alteration of cytokine production
  - Increases and decreases in inflammatory cytokines
  - Shifts in Th cytokine profiles
- Alteration of innate immune function
  - But no apparent alteration of adaptive cellular immunity
- Possible impacts in disease resistance
  - Risk of infection?

Mechanisms of immunomodulation



↑ on PPAR-related gene expression

↓ on PPAR-related gene expression

Figure modified from Corsini et al., 2014

Developmental immunotoxicity

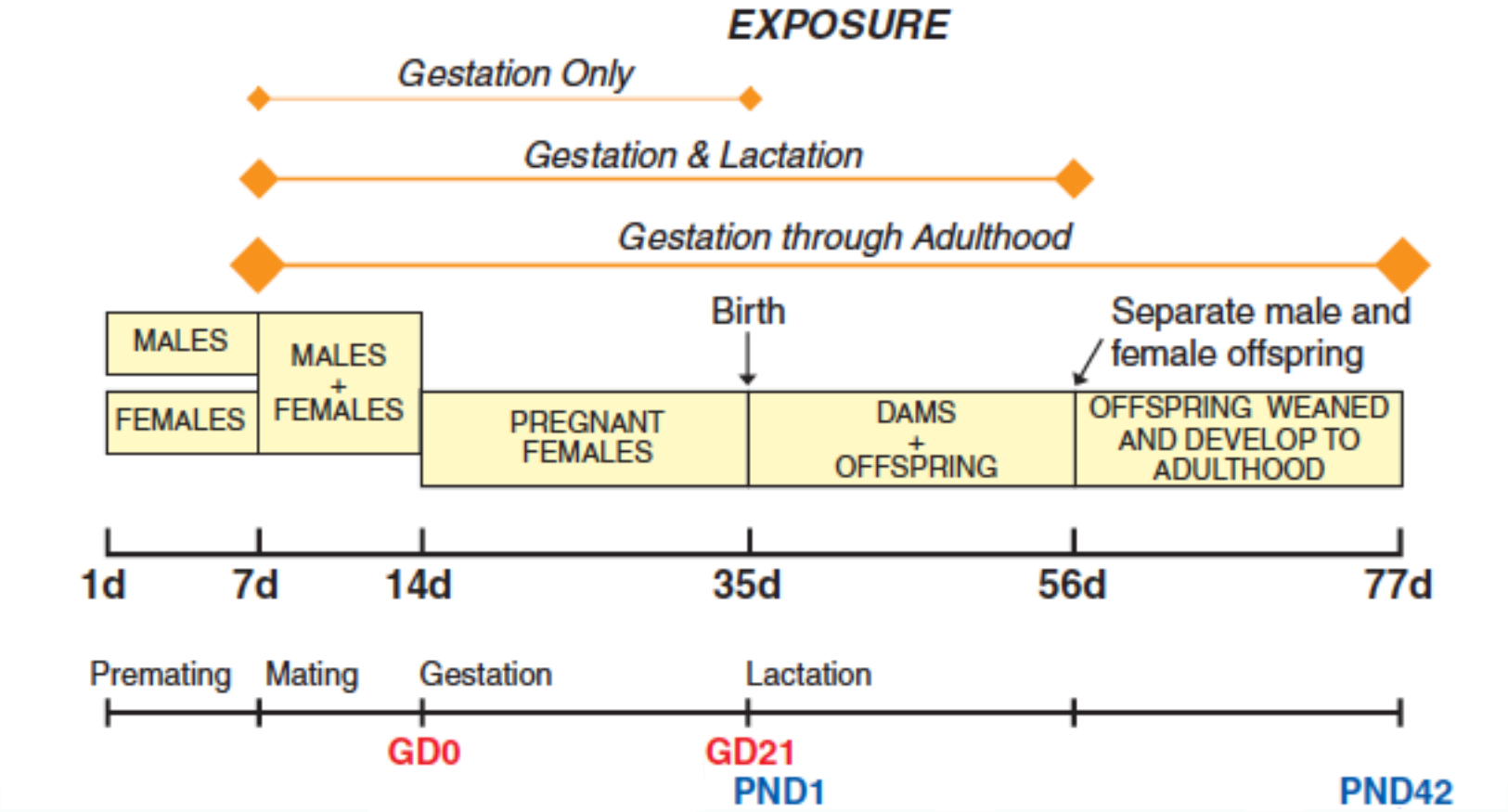
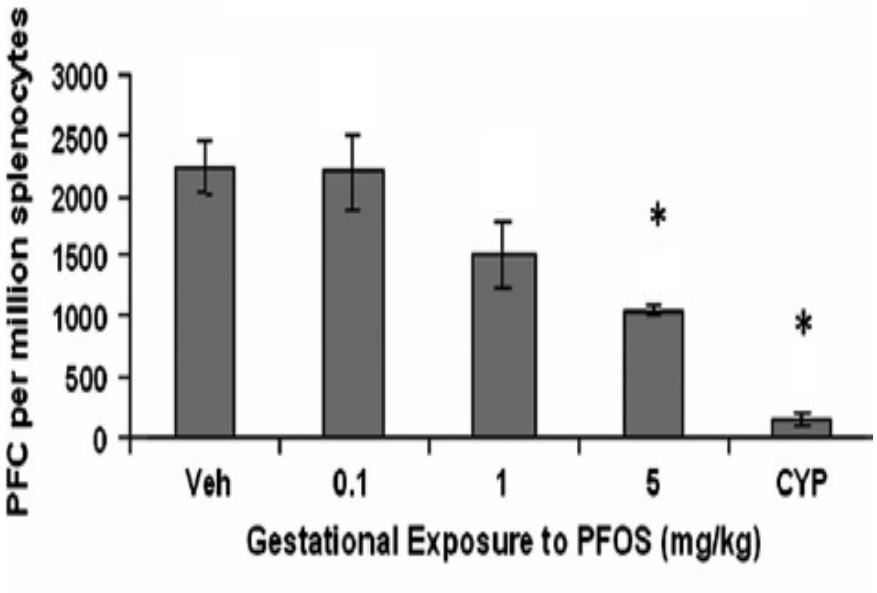
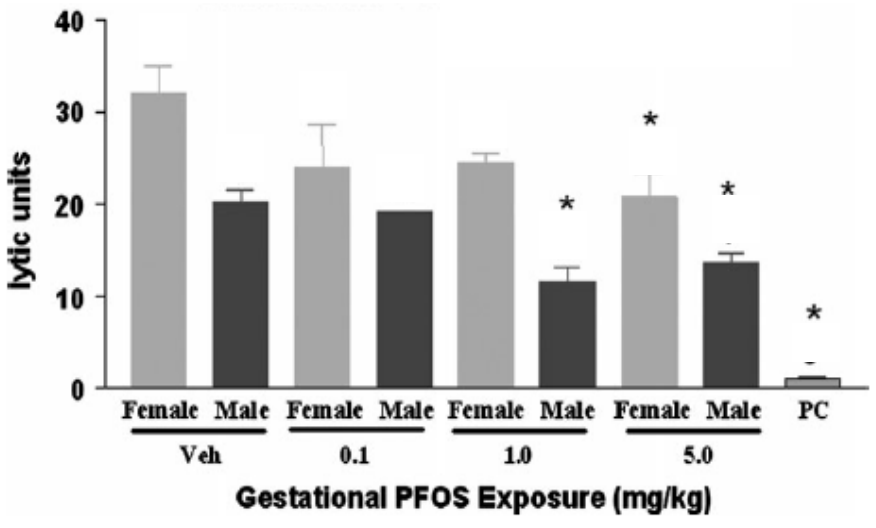


Figure modified from DeWitt et al., 2012

Developmental immunotoxicity





Figures modified from Keil et al., 2008

Evidence for functional immunomodulation in exposed humans

- Reduced vaccine responses in children
  - Prospective study of birth cohort from the National Hospital in the Faroe Islands (Grandjean et al., 2012)
    - 5-7 year old children with high serum PFASs had reduced responses to immunizations with diphtheria and tetanus; both PFOA and PFOS serum concentrations were negatively associated with antibody responses.

Evidence for functional immunomodulation in exposed humans

- Reduced vaccine responses in children
  - Prospective study of the BraMat Norwegian birth cohort (Granum et al., 2013)
    - Anti-rubella antibodies in 3 year old children were inversely associated with maternal plasma concentrations of PFASs.
    - PFOA and PFNA also were associated with number of episodes of common cold in the children.

Are PFOA & PFOS immunomodulatory?

- Both suppress the TDAR
- Both associated with changes in cytokine expression
- PFOS associated with alterations to cellular immunity
- Both associated with immune-related changes in humans

Are PFOA & PFOS immunomodulatory?

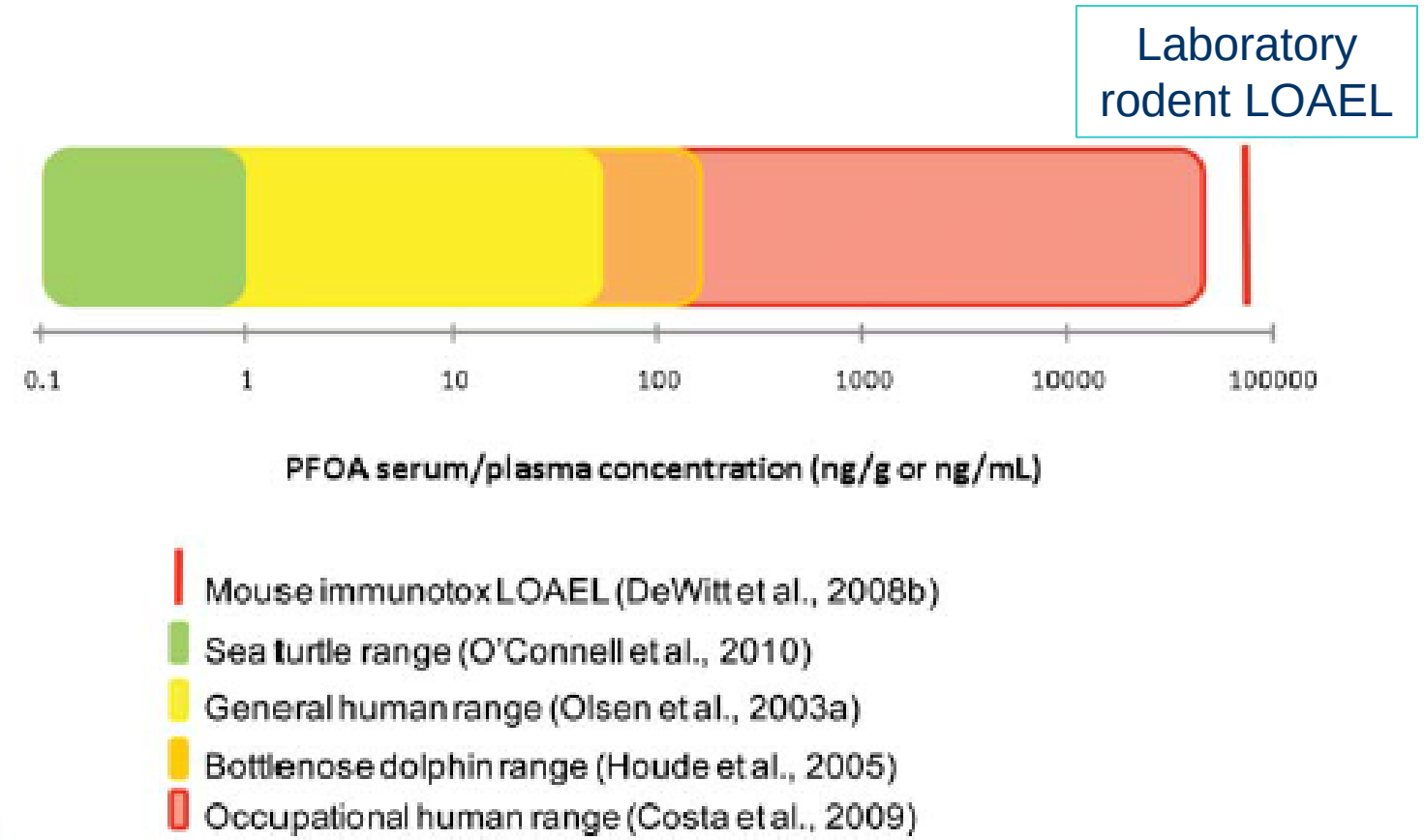


Figure modified from DeWitt et al., 2012

Are PFOA & PFOS immunomodulatory?

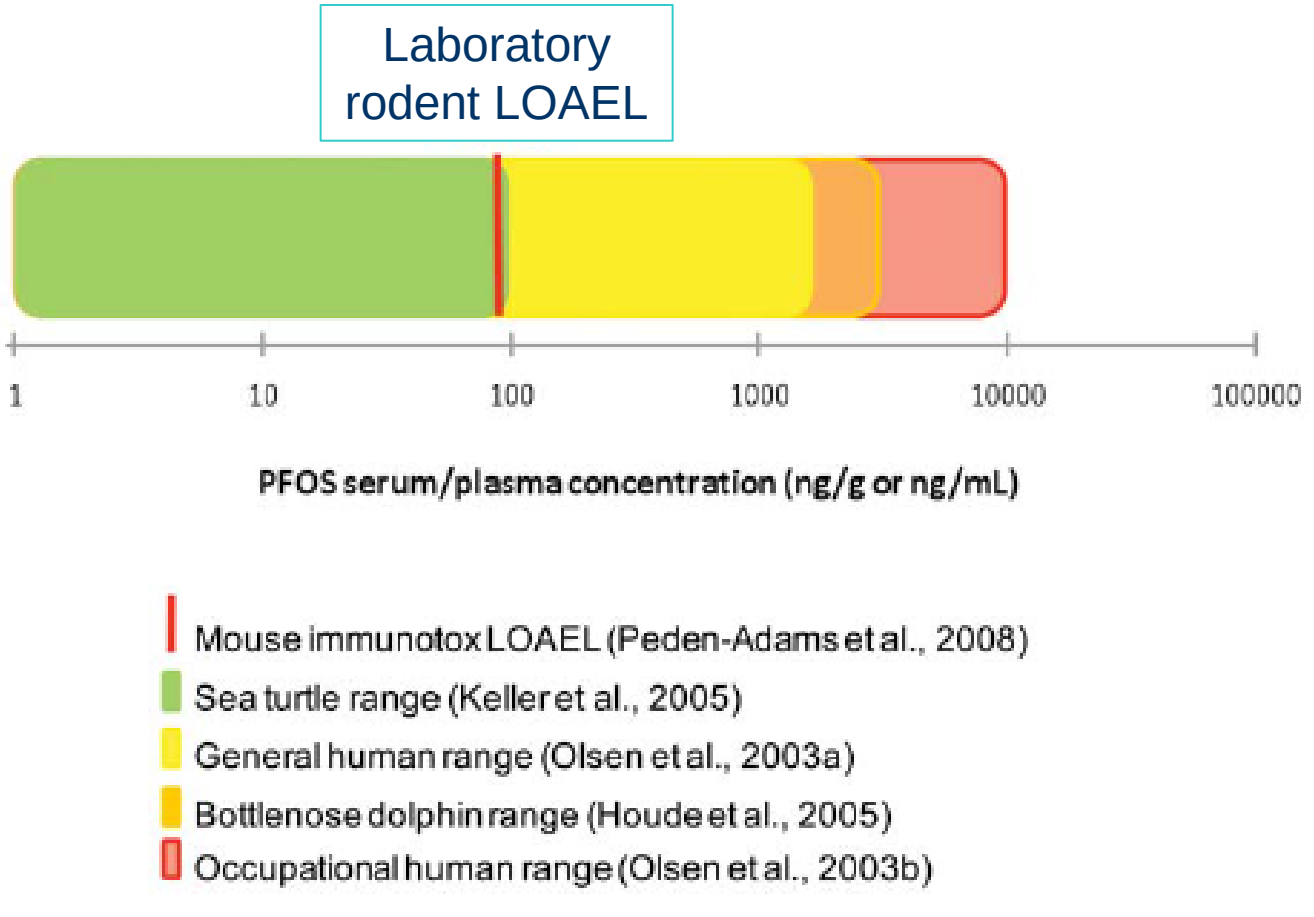


Figure modified from DeWitt et al., 2012

PFAS immunotoxicity – future needs

Unresolved questions

- Do all chain lengths act similarly with respect to the immune system?
- Do replacement compounds differ from legacy compounds?
- Do PFASs share a similar mechanism of immunomodulation?

Exposome

- What are major and minor exposure routes for the general population?
- Do children represent sensitive subpopulations?
- Do replacement compounds carry same risk of persistence as legacy compounds?

And the big one for all of you,
how does any of this impact food safety?

References

Buck et al. 2011: Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins, *Integr Environ Assess Manag*, 7: 513–541.

Corsini et al. 2014. Perfluorinated compounds: Emerging POPs with potential immunotoxicity. *Toxicol Lett*, 230:263-270.

DeWitt et al. 2008. Perfluorooctanoic acid-induced immunomodulation in adult C57BL/6J or C57BL/6N female mice. *Environ Health Perspect*, 116:644-650.

DeWitt et al. 2012. Immunotoxicity of perfluorinated compounds: Recent developments. *Toxicol Pathol*, 40:300-311.

DeWitt et al. 2012. Developmental immunotoxicity (DIT): Assays for evaluating effects of exogenous agents on development of the immune system. *Curr Protoc Toxicol*, 18:Unit 18.15.

DeWitt et al. 2015. Suppression of antigen-specific antibody responses in mice exposed to perfluorooctanoic acid: Role of PPARa and T- and B-cell targeting. *J Immunotoxicol*, 16:1-8.

Dong et al. 2009. Chronic effects of perfluorooctanesulfonate exposure on immunotoxicity in adult male C57BL/6 mice. *Arch Toxicol*. 83:805-815.

Fairley et al. 2007. Exposure to the immunosuppressant, perflurooctanoic acid, enhances the murine IgE and airway hyperreactivity response to ovalbumin. *Toxicol Sci*, 97:375-383.

Grandjean et al. 2012. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *JAMA*, 307:391-397.

Granum et al. 2013. Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. *J Immunotoxicol*, 10:373-379.

References

Guruge et al. 2009. Effect of perfluorooctane sulfonate (PFOS) on influenza A virus-induced mortality in female B6C3F1 mice. *J Toxicol Sci*, 34:687-691.

Herzke et al. 2013. Perfluorinated alkylated substances in vegetables collected in four European countries: occurrence and human exposure estimations. *Environ Sci Pollut Res Int*, 20:7930-7939.

Keil et al. 2008. Gestational exposure to perfluorooctane sulfonate suppresses immune function in B6C3F1 mice. *Toxicol Sci*, 103:77-85.

Peden-Adams et al. 2008. Suppression of humoral immunity in mice following exposure to perfluorooctane sulfonate. *Toxicol Sci*, 104:144-154.

Smits and Nain. 2013. Immunomodulation and hormonal disruption without compromised disease resistance in perfluorooctanoic acid (PFOA) exposed Japanese quail. *Environ Pollut*, 179:13-18.

US EPA. 2014. Emerging contaminants – Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). Solid Waste and Emergency Response (5106P). EPA 505-F-14-001.

US EPA. 2015. http://www.epa.gov/oppt/pfoa/index.html. Perfluorooctanoic acid and fluorinated telomers.

Yang et al. 2002a. Potent suppression of the adaptive immune response in mice upon dietery exposure to the potent peroxisome proliferator, perfluorooctanoic acid. *Int Immunopharmacol*. 2:389-397.

Yang et al. 2002b. Involvement of the peroxisome proliferator-activated receptor alpha in the immunomodulation caused by peroxisome proliferators in mice. *Biochem Pharmacol*, 63:1893-1900.

Zheng et al. 2009. Immunotoxic changes associated with a 7-day oral exposure to perflurooctanesulfonate (PFOS) in adult male C57BL/6 mice. *Arch Toxicol*, 83:679-689.

Zheng et al. 2011. Type 1 and Type 2 cytokines imbalance in adult male C57BL/6 mice following a 7-day oral exposure to perfluorooctansulfonate (PFOS). *J Immunotoxicol*. 8:30-38.