Immunotoxicological findings of PFAS:
A focus on PFOA and PFOS

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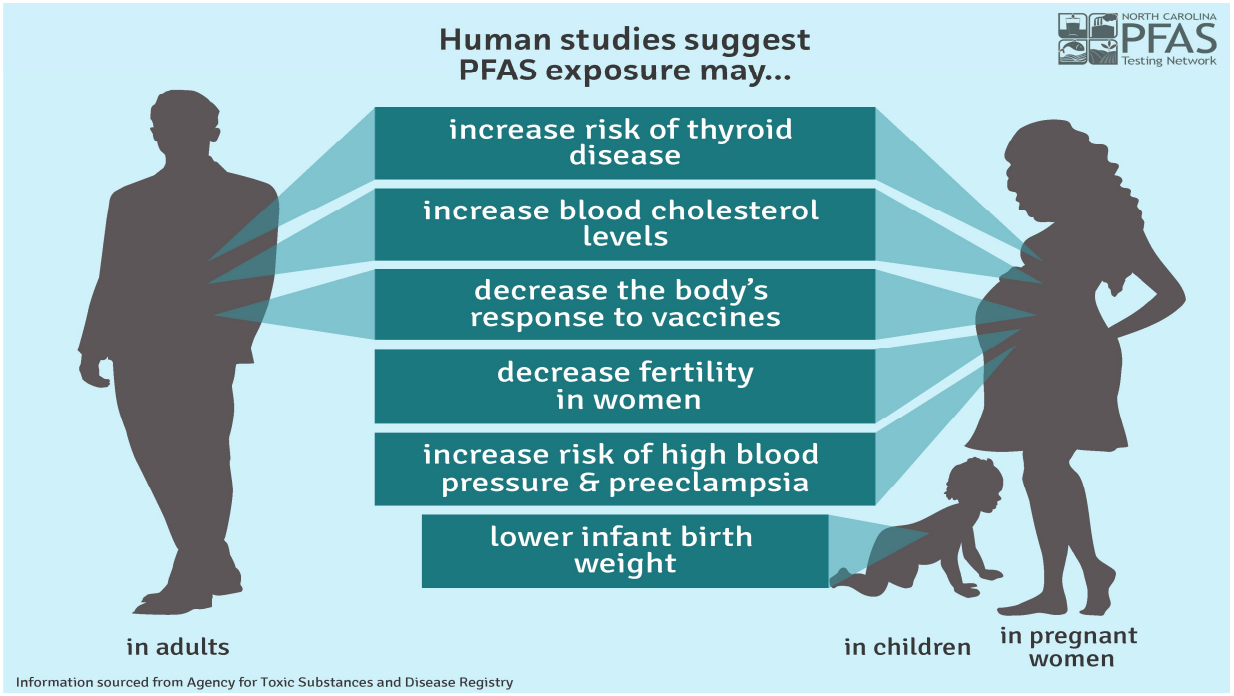
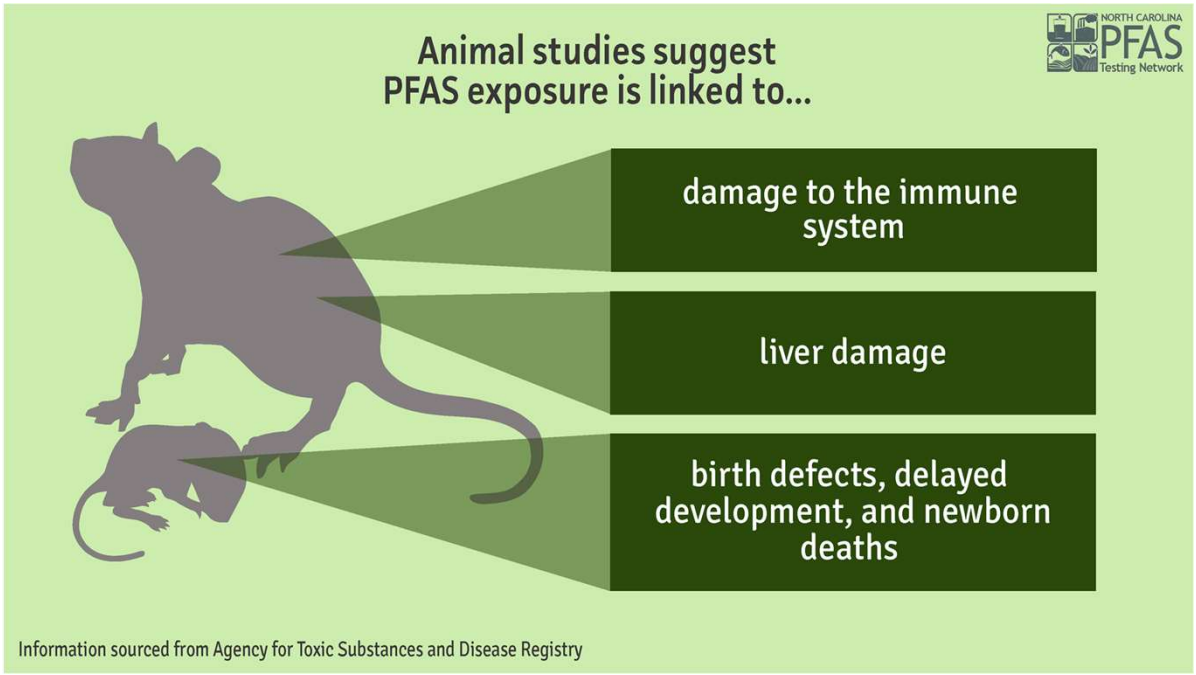
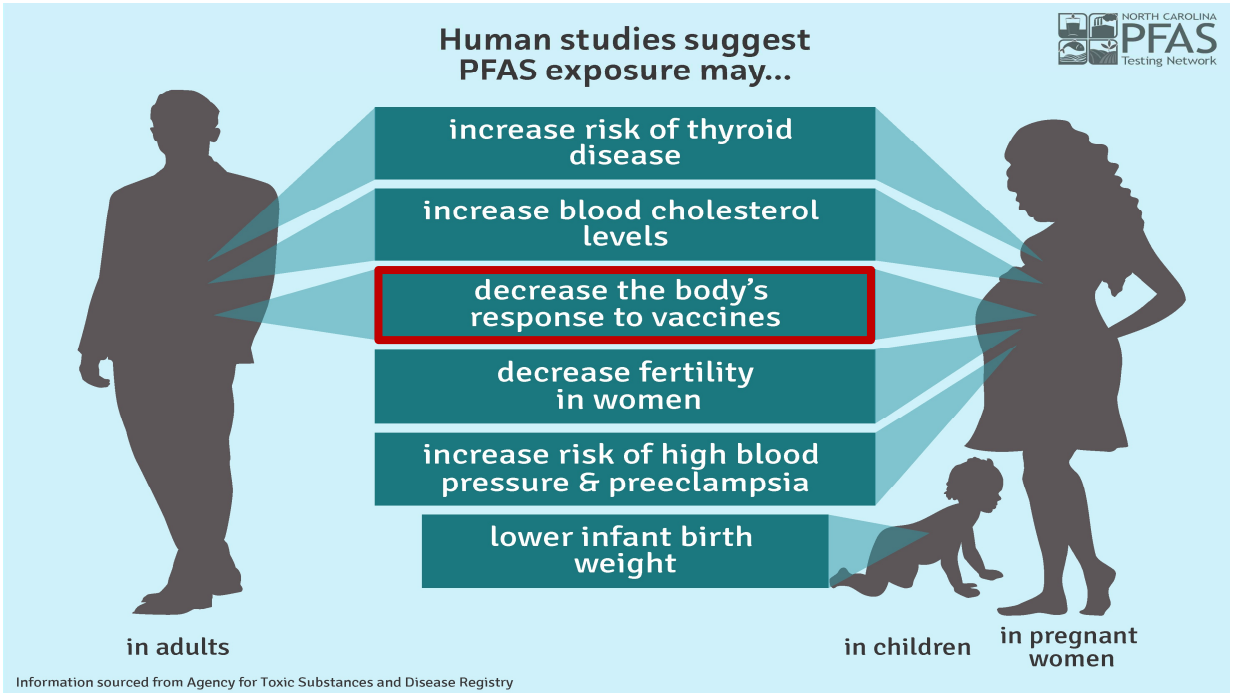
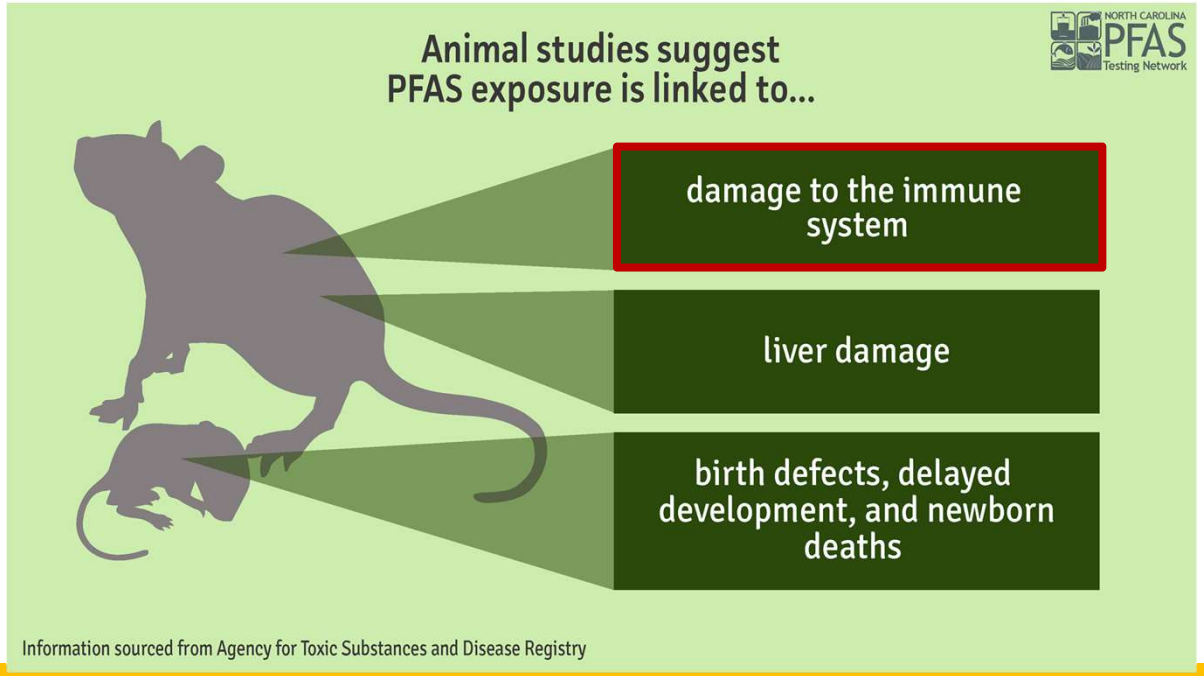


Associations between adverse health outcomes and PFAS serum concentrations in adults and children.

PFAS-exposed experimental animal models also demonstrate multiple adverse health outcomes.



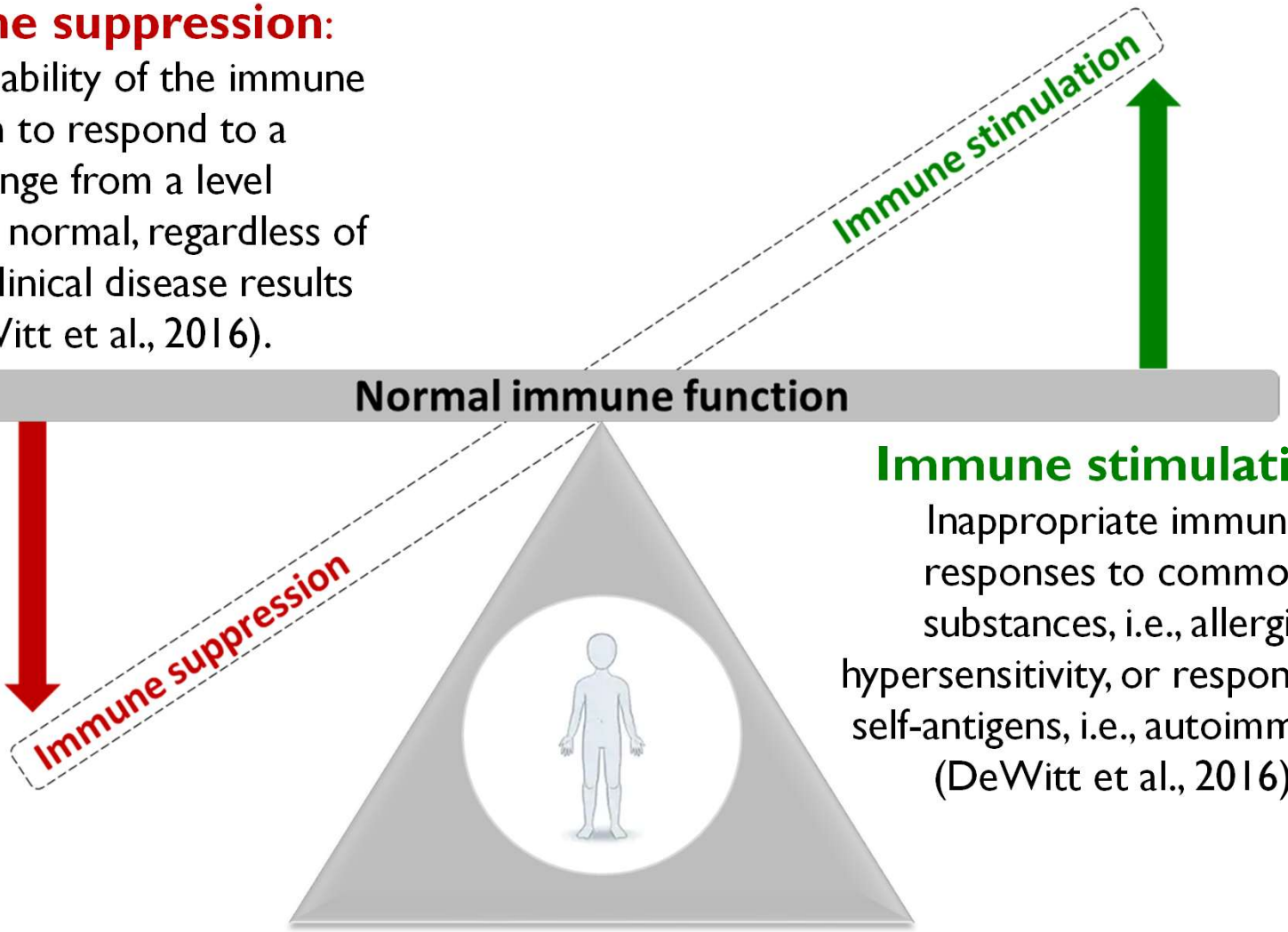




Impacts on the immune system have been documented in humans exposed to PFAS mixtures via drinking water and in animal models exposed to single PFAS.

Why should we care about immunotoxicity with respect to PFAS?

Immune suppression:
A reduced ability of the immune system to respond to a challenge from a level considered normal, regardless of whether clinical disease results (DeWitt et al., 2016).



Immune stimulation:
Inappropriate immune responses to common substances, i.e., allergic hypersensitivity, or responses to self-antigens, i.e., autoimmunity (DeWitt et al., 2016).

We can evaluate immune system responses in exposed humans, experimental animals, and cellular systems. **Primary outcomes** are those with greater predictive value for overall immunotoxicity or a health effect. **Secondary outcomes** are valuable but are more suggestive than definitive.

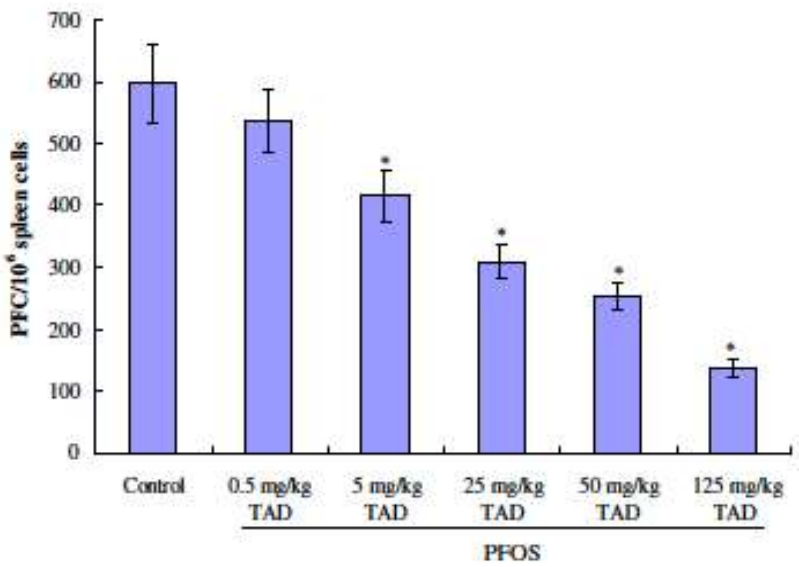
Table 5. Health Outcome Grouping and Identification of Primary and Secondary Outcomes

	Humans	Animals*	*In vitro* Assays
Primary Outcomes	Immune-related diseases and measures of immune function: ***(1) Immunosuppression*** (e.g., otitis, infections, or decreased vaccine antibody response); ***(2) Hypersensitivity-related outcomes*** (e.g., atopic dermatitis asthma, total IgE, rhinitis); ***(3) Autoimmunity*** (e.g., thyroiditis or ulcerative colitis)	Disease resistance assay or measures of immune function following *in vivo* exposure: ***(1) Immunosuppression*** disease resistance assays (e.g., host resistance to influenza) or immune function assays (e.g., antibody response [T-cell dependent IgM antibody response (TDAR)], natural killer cell [NK] activity, delayed-type hypersensitivity [DTH] response, monocyte phagocytosis); ***(2) Hypersensitivity*** (e.g., airway resistance, local lymph-node assay); ***(3) Autoimmunity*** changes in incidence or progression in animal models of autoimmune disease	***Immune function assays following <u>in vitro exposure</u>:*** ***(1) Immunosuppression*** immune function assays (e.g., natural killer cell [NK] activity, phago-cytosis or bacterial killing by monocytes, proliferation following anti-CD3 antibody stimulation of spleen cells or lymphocytes)
Secondary	***Observational immune endpoints*** (e.g., lymphocyte counts, proliferation, cytokine levels, or serum antibody levels) ***Immunostimulation**** (e.g., unintended stimulation of humoral immune function)	***Observational immune endpoints*** (e.g., lymphoid organ weight, lymphocyte counts or subpopulations, lymphocyte proliferation, cytokine production, serum antibody levels, serum or tissue autoantibody levels, or histological changes in immune organs)	***Observational immune endpoints*** (e.g., general mitogen-stimulated lymphocyte proliferation, cytokine production)

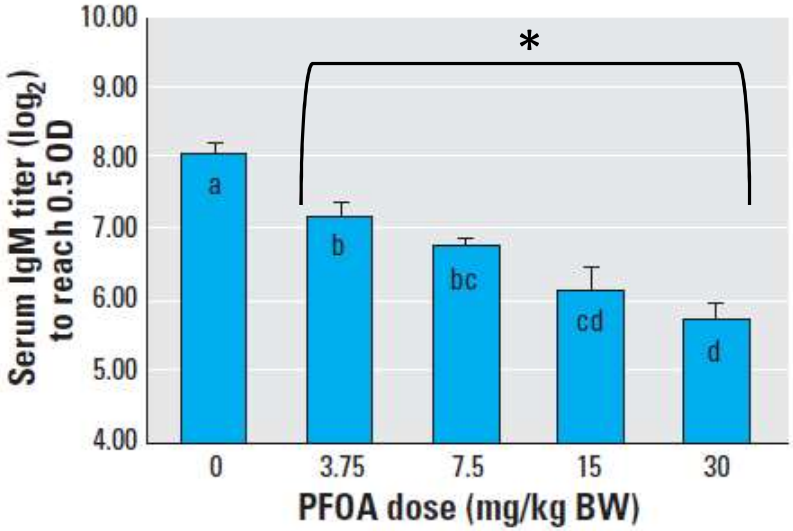
Table from: NTP, 2016.

What do we know about immunotoxicity of PFOA and PFOS?

PFOA and PFOS can induce suppression of T cell-dependent antibody responses (like a vaccine response) in rodents.



Oral PFOS exposure in male C57BL/6 mice (60d of exposure).



Oral PFOA exposure in female C57BL/6 mice (15d of exposure).

PFOS data from: Dong et al. 2009. Archives of Toxicology. PFOA data from: DeWitt et al. 2008. Environmental Health Perspectives.

PFOA or PFOS have been associated with suppression of vaccine responses in children and adults.

Table 4

Differences in Tetanus and Diphtheria Antibody Concentrations at Age 5 Years Prebooster and Age 7 Associated With a Doub Concentration of PFCs for Maternal Pregnancy Serum and Age-5 Serum in a Structural Equation Model

	Tetanus, % Change (95% CI)	*P* Value	Diphtheria, % Change (95% CI)	*P* Value	*P* Value for Same Effect[a]	Joint Change, % (95% CI)	*P* Value
Age 5 prebooster							
Maternal PFC	−20.2 (−49.2 to 25.2)	.33	−47.9 (−67.7 to −15.9)	.008	.17	−31.1 (−56.8 to 9.8)	.12
PFC at age 5 y	−20.5 (−44.4 to 13.6)	.21	−7.9 (−38.0 to 37.0)	.69	.47	−15.6 (−38.5 to 15.8)	.29
PFC at age 5 y[b]	−17.2 (−42.1 to 18.5)	.30	−1.2 (−33.6 to 46.8)	.95	.39	−11.0 (−35.2 to 22.3)	.47
Age 7							
Maternal PFC	35.1 (−25.4 to 144.6)	.32	−42.0 (−66.1 to −0.8)	.047	.007		
PFC at age 5 y	−55.2 (−73.3 to −25.0)	.002	−44.4 (−65.5 to −10.5)	.02	.42	−49.4 (−66.7 to −23.0)	.001
PFC at age 5 y[b]	−58.8 (−76.0 to −29.3)	.001	−45.5 (−66.9 to −10.3)	.02	.31	−51.8 (−68.9 to −25.1)	.001

Abbreviation: PFC, perfluorinated compound.

[a]Determined by likelihood ratio test for the same effect of PFC on the 2 types of antibodies.
[b]Adjusted for the PFC concentration in maternal pregnancy serum.

Table 3

Linear regression Coefficients of Log_{10}-Transformed influenza Antibody Titer rise and Log_{10}-Transformed influenza Antibody Titer ratio With unit increase in Log_{10} Transformed and Quartiles of PFOA and PFOS Serum Concentration ($n = 403$)

	Log PFOA (ng/ml)			Log PFOS (ng/ml)		
	Regression Coefficient	95% CI	*p* Value	Regression Coefficient	95% CI	*p* Value
a. Log_{10}-transformed PFOA/PFOS (as continuous variable)						
Influenza type B						
Log_{10}-transformed antibody titer rise ($n = 359$)[a]						
Unadjusted	−.05	(−0.16, 0.05)	.33	.09	(−0.07, 0.24)	.29
Adjusted[b]	−.02	(−0.13, 0.09)	.73	.05	(−0.11, 0.21)	.56
Log_{10}-transformed antibody titer ratio (postvaccine: prevaccine)						
Unadjusted	−.05	(−0.15, 0.05)	.30	.10	(−0.04, 0.25)	.16
Adjusted[b]	−.02	(−0.11, 0.08)	.73	.05	(−0.09, 0.18)	.52
Influenza A/H1N1						
Log_{10}-transformed antibody titer rise ($n = 322$)[a]						
Unadjusted	−.11	(−0.22, 0.01)	.07	.09	(−0.08, 0.25)	.32
Adjusted[b]	−.03	(−0.14, 0.09)	.63	.15	(−0.02, 0.32)	.08
Log_{10}-transformed antibody titer ratio (postvaccine: prevaccine)						
Unadjusted	.02	(−0.12, 0.15)	.79	.12	(−0.07, 0.32)	.22
Adjusted[b]	.07	(−0.06, 0.21)	.30	.10	(−0.11, 0.30)	.36
Influenza A/H3N2						
Log_{10}-transformed antibody titer rise($n = 372$)[a]						
Unadjusted	−.02	(−0.16, 0.13)	.81	.08	(−0.13, 0.28)	.47
Adjusted[b]	−.01	(−0.17, 0.14)	.86	.09	(−0.13, 0.32)	.42
Log_{10}-transformed antibody titer ratio (postvaccine: prevaccine)						
Unadjusted	−.15	(−0.28, −0.02)	.03	.04	(−0.15, 0.24)	.68
Adjusted[b]	−.12	(−0.25, 0.02)	.09	−.005	(−0.20, 0.19)	.96

Elevated exposure to PFOA or PFOS was associated with reduced vaccine responses in children and in adults.

Data from: Grandjean et al. 2012. JAMA; Looker et al., 2014. Toxicological Sciences.

The US National Toxicology Program determined that PFOA was presumed to be an immune hazard in humans based, in part, on a high level of evidence that PFOA suppresses the antibody response from animal studies and a moderate level of evidence from studies in humans (US NTP, 2016).

The totality of evidence from human and animal studies, not any one study, allowed the NTP to reach this conclusion.

Table 6. Evidence Profile of the Main Findings for PFOA Immunotoxicity

INITIAL CONFIDENCE for each body of evidence (# of studies)	Factors decreasing confidence "---" if no concern; "↓" if serious concern to downgrade confidence					Factors increasing confidence "---" if not present; "↑" if sufficient to upgrade confidence				FINAL CONFIDENCE RATING
	Risk of Bias	Unexplained Inconsistency	Indirectness	Imprecision	Publication Bias	Large Magnitude	Dose Response	Residual Confounding	Consistency Species/Model	
Immunotoxicity Based on Evidence for Suppression of the Antibody Response										
Human										
Initial Moderate (4 prospective studies)[a]	---	---	---	---	---	---	---	---	---	**Moderate**
Initial Low (2 cross-sectional studies)[b]	---	---	---	---	---	---	---	---	---	**Low**
Confidence Across Human Bodies of Evidence	No change for considering across study designs									**Moderate**
Animal										
Initial High (7 mammal studies)	↓	---	---	---	---	---	↑	---	---	**High**

References:

Human: Granum (2013)[a], Grandjean (2012)[a], Kielsen (2016)[b], Looker (2014)[a], Mogensen (2015)[a], Stein (2016)[b]

Animal: DeWitt (2008, 2009a, 2016), Hu (2010), Loveless (2008), Vetvicka (2013), Yang (2002a)

https://ntp.niehs.nih.gov/ntp/ohat/pfoa_pfos/pfoa_pfosmonograph_508.pdf

The US National Toxicology Program determined that PFOS was presumed to be an immune hazard in humans based, in part, on a high level of evidence that PFOS suppresses the antibody response from animal studies and a moderate level of evidence from studies in humans (US NTP, 2016).

The totality of evidence from human and animal studies, not any one study, allowed the NTP to reach this conclusion.

Table 8. Evidence Profile of the Main Findings for PFOS Immunotoxicity

INITIAL CONFIDENCE for each body of evidence (# of studies)	Factors decreasing confidence "---" if no concern; "↓" if serious concern to downgrade confidence					Factors increasing confidence "---" if not present; "↑" if sufficient to upgrade confidence				FINAL CONFIDENCE RATING
	Risk of Bias	Unexplained Inconsistency	Indirectness	Imprecision	Publication Bias	Large Magnitude	Dose Response	Residual Confounding	Consistency Species/Model	
Immunotoxicity Based on Evidence for Suppression of the Antibody Response										
Human										
Initial Moderate (4 prospective studies)[a]	---	---	---	---	---	---	---	---	---	**Moderate**
Initial Low (2 cross-sectional studies)[b]	---	---	---	---	---	---	---	---	---	**Low**
Confidence Across Human Bodies of Evidence	No change for considering across study designs									**Moderate**
Animal										
Initial High (8 mammal studies)	↓	---	---	---	---	---	↑	---	---	**High**

References:

Human: Granum (2013)[a], Grandjean (2012)[a], Kielsen (2016)[b], Looker (2014)[a], Mogensen (2015)[a], Stein (2016)[b]

Animal: Dong (2009b, 2011), Keil (2008), Lefebvre (2008), Peden-Adams (2008), Qazi (2010b), Vetvicka (2013), Zheng (2009)

https://ntp.niehs.nih.gov/ntp/ohat/pfoa_pfos/pfoa_pfosmonograph_508.pdf

PFOA and PFOS are presumed to be immune hazards to humans.

PFOA suppresses the TDAR in experimental models (high level of evidence) and humans (moderate level of evidence).

PFOS suppresses the TDAR in experimental models (high level of evidence) and humans (moderate level of evidence).

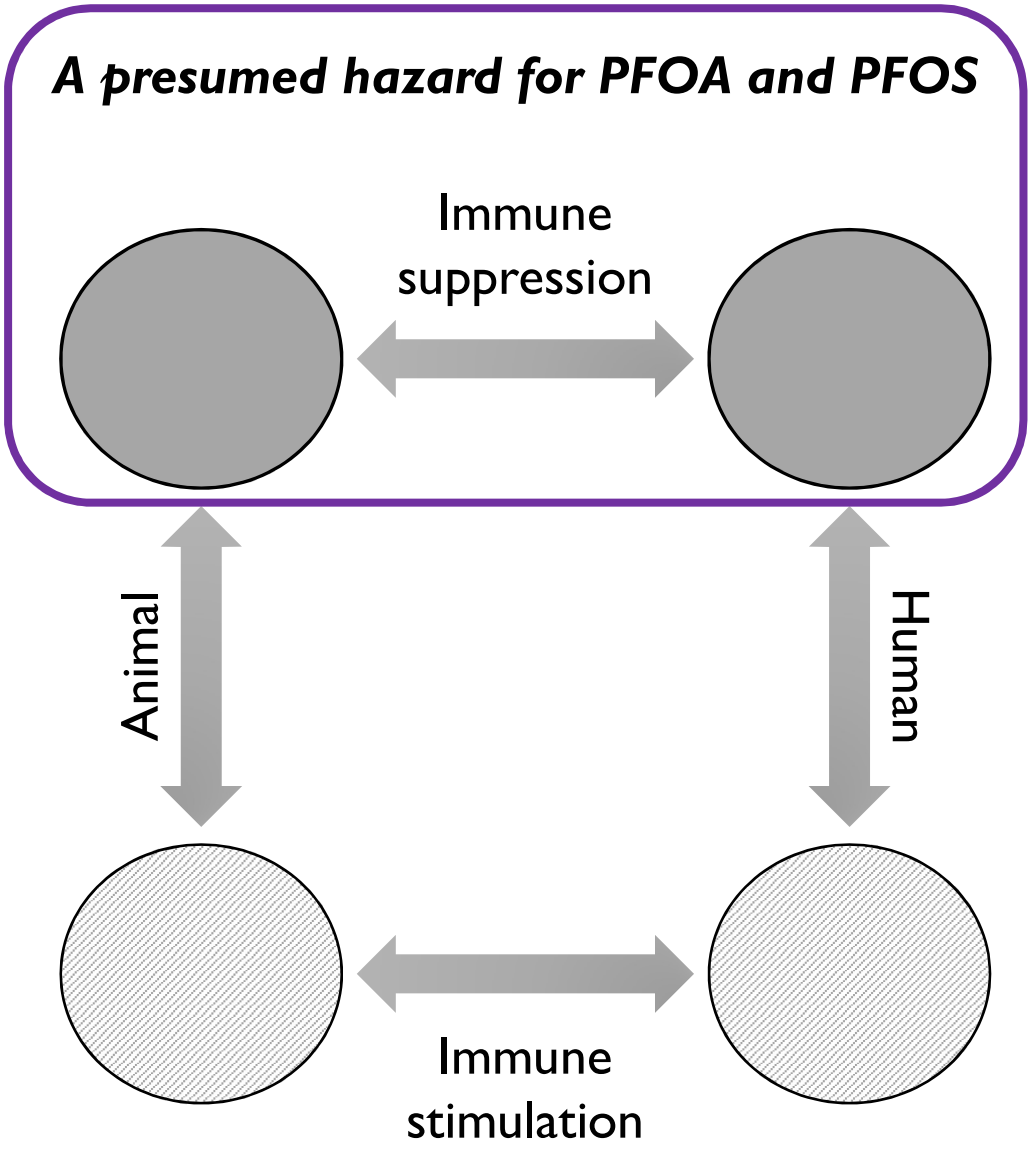
National Toxicology Program
U.S. Department of Health and Human Services

SYSTEMATIC REVIEW OF IMMUNOTOXICITY ASSOCIATED WITH EXPOSURE TO PERFLUOROOCTANOIC ACID (PFOA) OR PERFLUOROOCTANE SULFONATE (PFOS)

June 6, 2016

Other immune effects supporting this weight-of-evidence classification:

- Increased hypersensitivity-related outcomes.
- Suppression of innate immune responses (i.e., NK cell function).
- Alterations in disease resistance/infectious disease outcomes.
- Findings of autoimmunity.



Human equivalent dose (HED) for PFOA-induced immune suppression in mice calculated as 0.0053 mg/kg/day*.

Same HED for developmental toxicity (critical effect) used to calculate the reference dose for PFOA*.

The immune system also is an endpoint sensitive to PFAS.

*US EPA, 2016

Some evidence that GenX, PFHxS, PFDA, PFDeA, PFNA, PFUA, PFDoA, PFBuS, PFBS, PFHxA can affect immune endpoints in experimental models and/or exposed humans.

While much of this evidence is observational (secondary outcomes) and not functional (primary outcomes), *functional effects can occur at doses below those that affect observational endpoints.*

We had evidence of observational immune effects of PFOA and PFOS from late 70s and early 80s.

Functional immune endpoints weren't published until early 2000s.

NC has already acknowledged that evaluation of immune responses is an important step toward public health protection with respect to newly identified PFAS in the Cape Fear River.

Eight states (as of 2016) have drinking water guidelines for PFOA and PFOS that are *lower than the US EPA health advisory of 70 ng/L* (5.1-35 ng/L for PFOA and 6.5-20 ng/L for PFOS).

Agency	PFOA	PFOS	Basis of RfD
	ng/kg/day		
US EPA RfD (2016)	**20**	**20**	**----**
State RfDs (2016-2019)	**2 – 6.1** (6 states) **US EPA** (2 states)	**1.8 – 5** (7 states) **US EPA** (1 state)	These states consider more sensitive toxicity endpoints as Critical Effect and/or with Database Uncertainty Factor.
ATSDR Minimal Risk Levels (draft, 2018)	**3**	**2**	ATSDR MRLs are for intermediate exposures.
States with RfDs (PFOA and/or PFOS) below US EPA: CA, MA, MI, MN, NH, NJ, NY Endpoints: increased liver weight, developmental effects (range), decreased antibody response			

Data from: SETAC North America Focused Topic Meeting: Environmental Risk Assessment of PFAS. 2019. *Modified from presentation of Dr. Gloria Post, NJ DEP.*

Acknowledgements:

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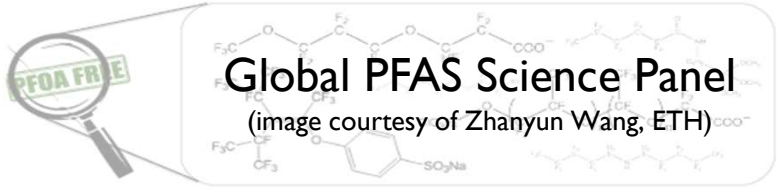
Countless colleagues and collaborators.

North Carolina Policy Collaboratory &
NC General Assembly.

NORTH CAROLINA PFAS Testing Network

ECU



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