Overview of Putative Health Effects of PFAS: *Toxicology*

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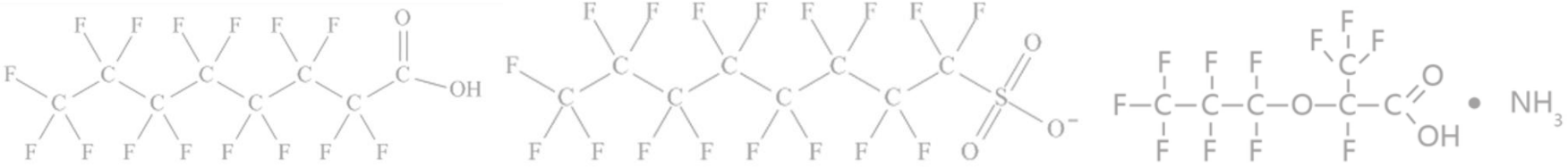

Potential conflicts of interest

I currently am funded to study toxicological effects of PFAS (sources of funding: North Carolina Policy Collaboratory & NC General Assembly, US EPA/Oregon State University (83948101), NIEHS/NC State University (1 P42 ES031009-01), NC State University Center for Human Health and the Environment, Brody Brothers Endowment)

I have spoken publicly about my understanding of PFAS toxicity, serve/have served as a plaintiff's expert witness, advocate for the need to protect the public from their exposures to PFAS, and am a proponent of the essential use concept and the class approach for PFAS management.

Why scientists who study PFAS are concerned about them

E P B M T

***E**missions*
***P**ersistence*
***B**ioaccumulation*
***M**obility*
***T**oxicity*

Multiple lines of evidence within these five categories support that ***PFAS are human and environmental health concerns***.

Some scientists have asserted that "persistence is enough" of a criterion to manage PFAS as a chemical class (Cousins et al., 2020) and others have asserted that concerns about persistence, bioaccumulation, mobility, and/or toxicity are serious enough to warrant managing PFAS as a chemical class (Kwiatkowski et al., 2020).

Why scientists who study PFAS are concerned about them

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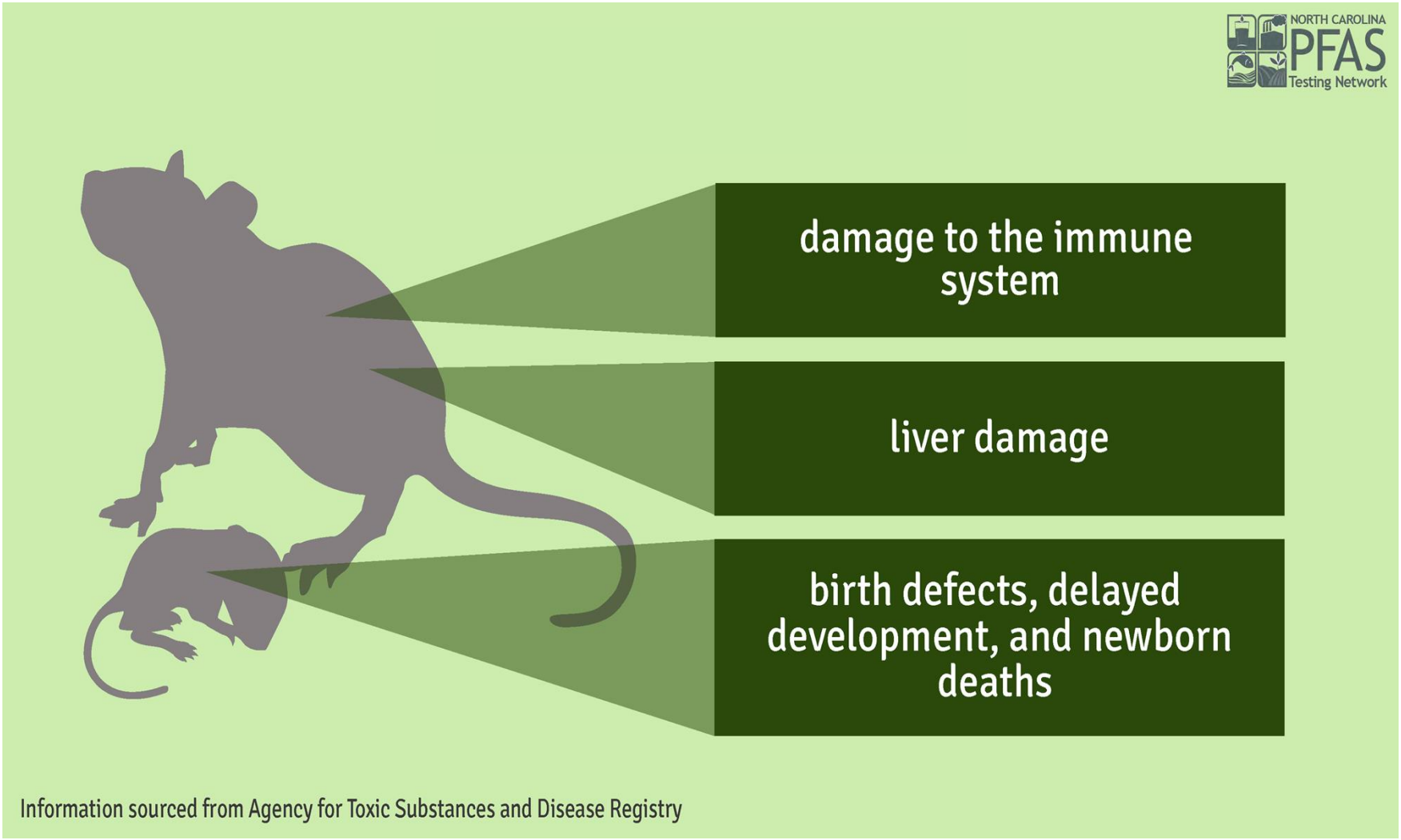
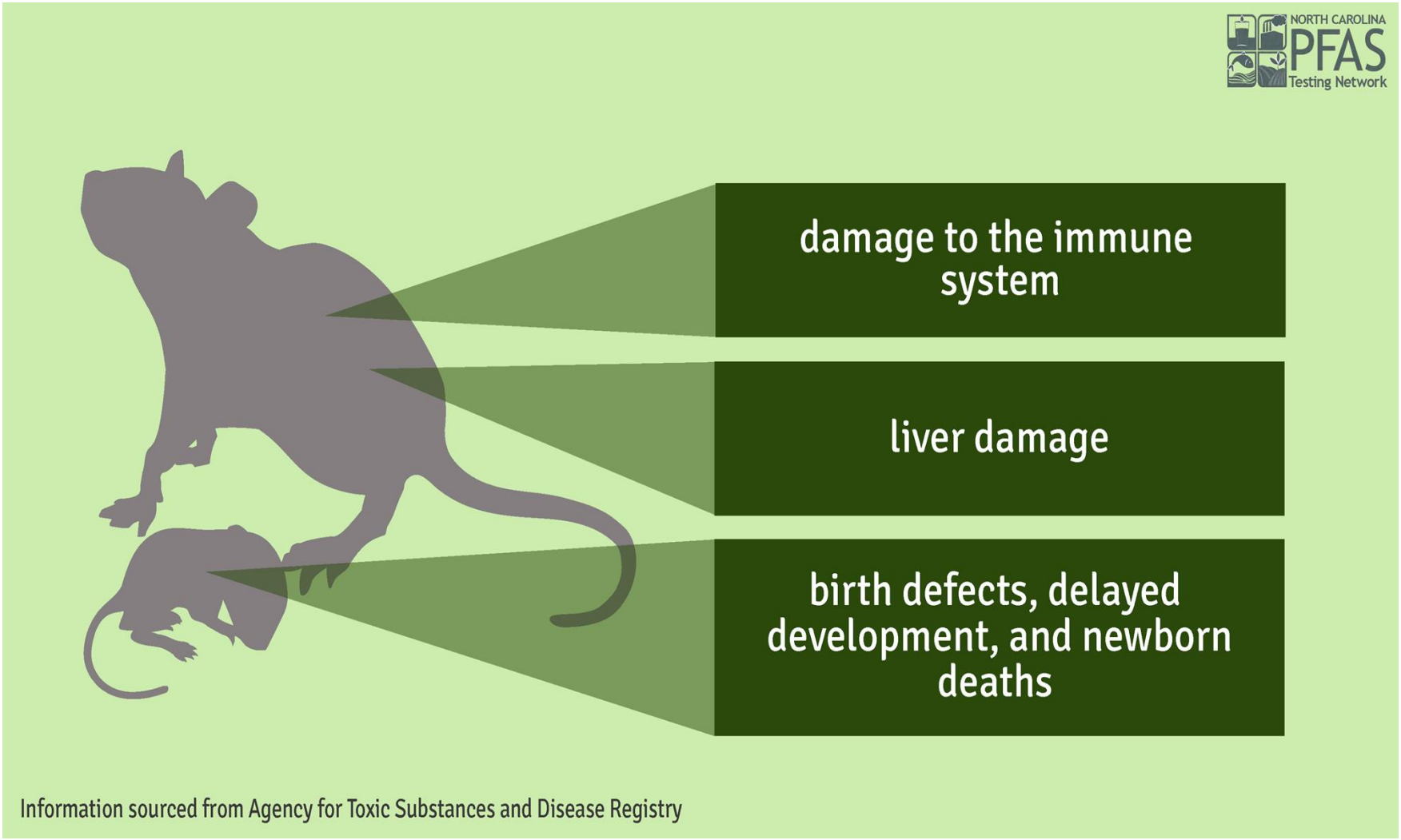
What is the toxicological evidence supporting concerns of toxicity?

Toxicological evidence



Toxicological evidence



U.S. National Toxicology Program systematic review of immunotoxicity for PFOA and PFOS

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)

PFOA and PFOS *suppress antigen-specific antibody responses* in experimental models (**high level of evidence**) and humans (moderate level of evidence).

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

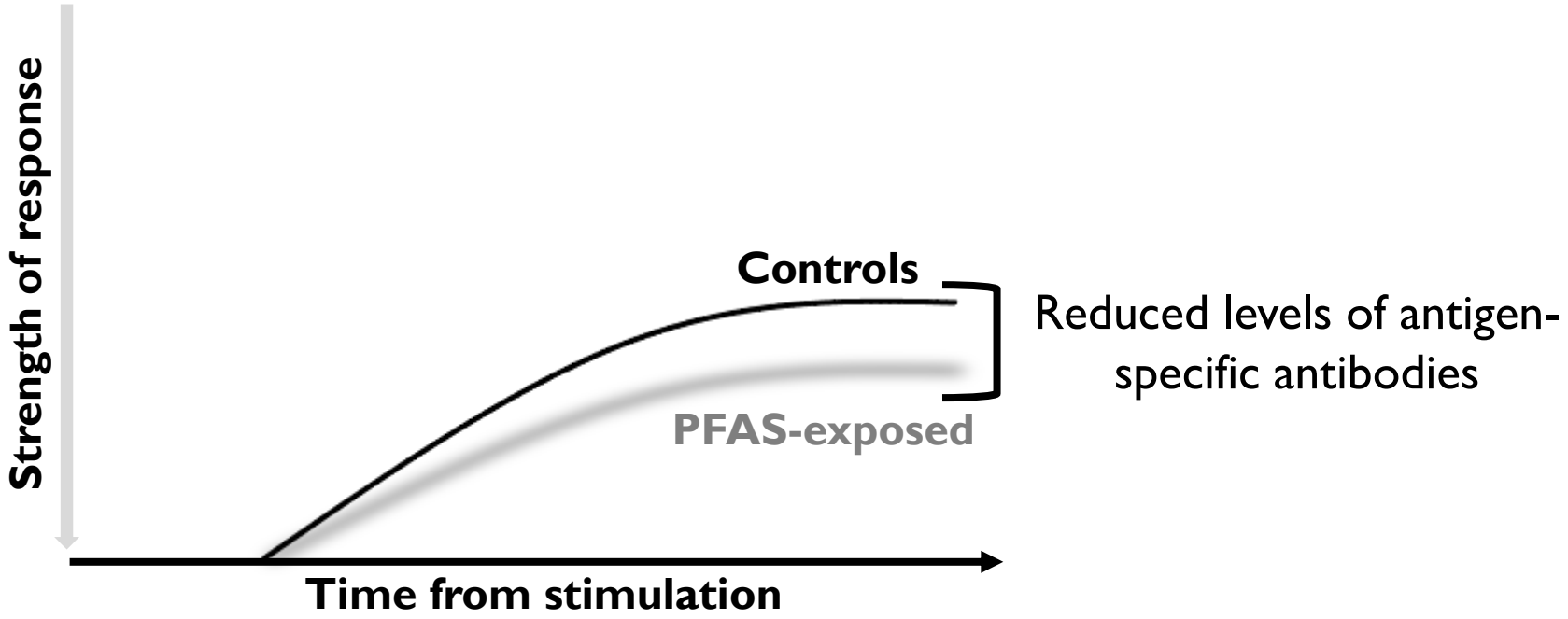
- Increased hypersensitivity-related outcomes
- Suppression of innate immune responses (i.e., NK cell function)
- Alterations in disease resistance/infectious disease outcomes
- Findings of autoimmunity

NTP conclusion: PFOA and PFOS are presumed to be immune hazards to humans.

Decreased antibody responses to antigens

Experimental models are challenged with specific antigens

Antigen-specific antibodies are measured as a marker of function

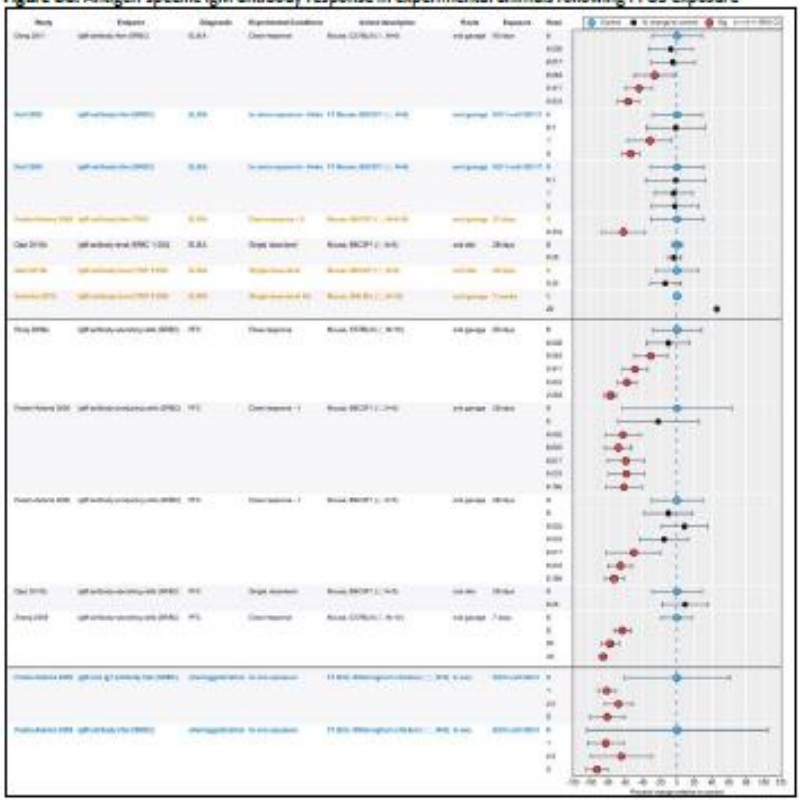


This immune functional assay in rodent models is highly translatable to humans – suppression of this response is predictive of suppression of the analogous vaccine response in humans.

Evidence of decreased antibody responses to antigens

re D6. Antigen-specific IgM antibody response in experimental animals following PFOA exposure

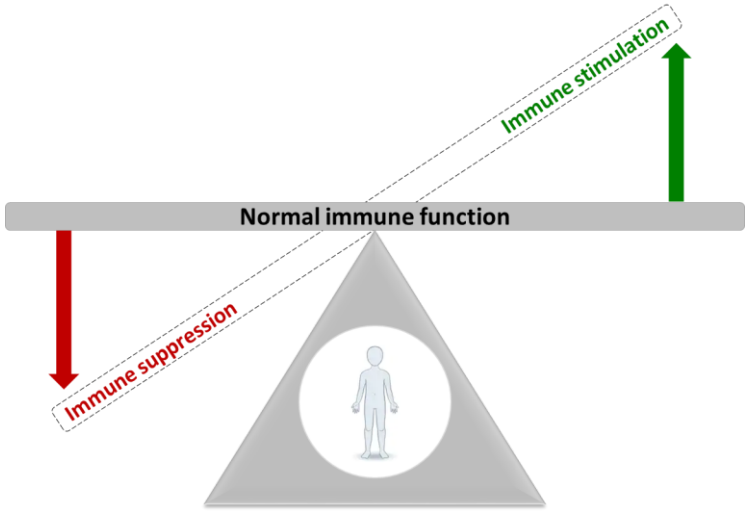
Figure D8. Antigen-specific IgM antibody response in experimental animals following PFOS exposure



Red circles in figures = suppressed antigen-specific antibody response in rodent models orally exposed to PFOA (left) or PFOS (right).
Consistency across studies, durations of exposure, and strains.

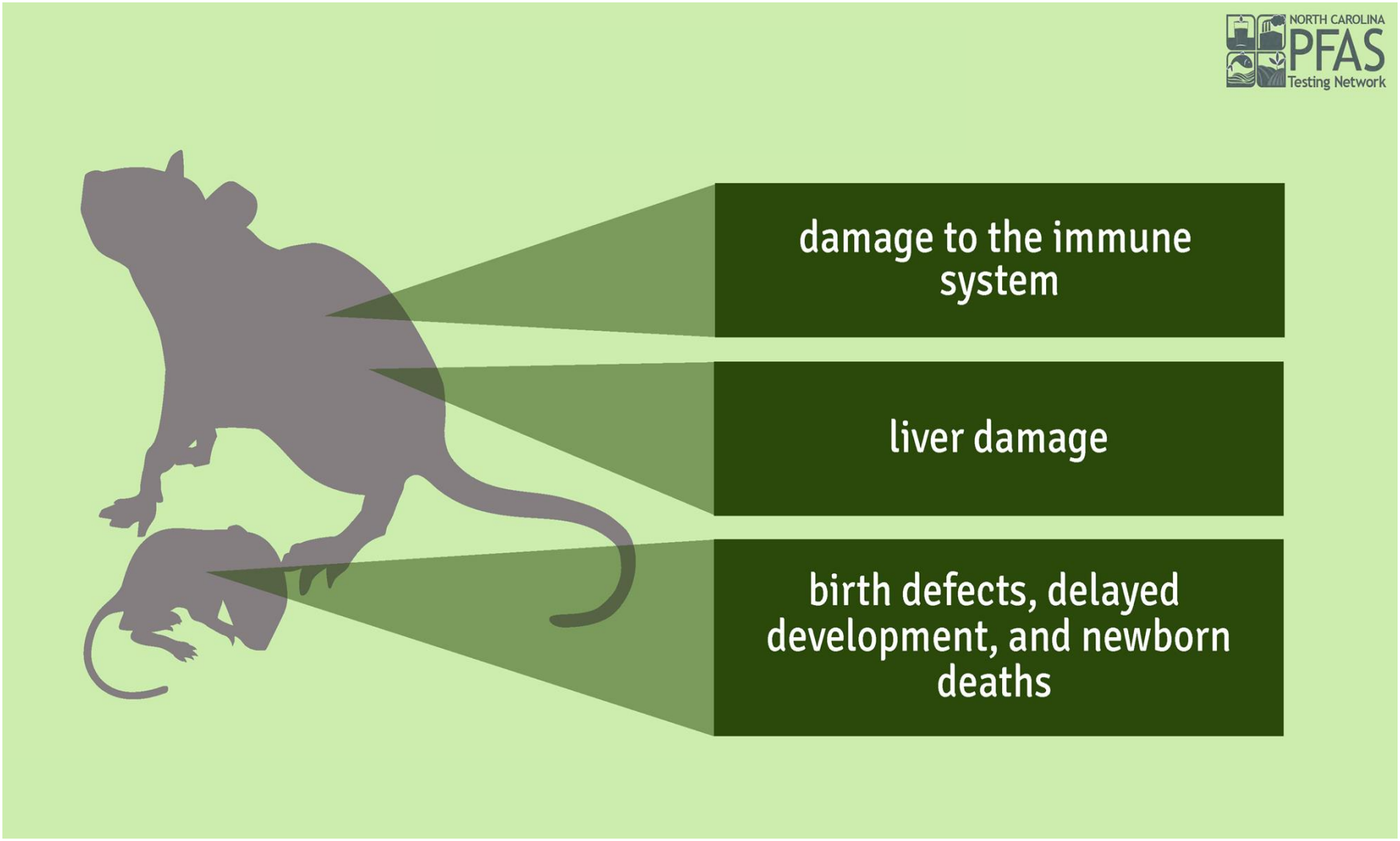
Immunotoxicological outcomes used to protect public health

- States of New Jersey and Michigan maximum contaminant levels for PFOS in drinking water are based on immunotoxicity:
  - NJ PFOS MCL: 13 ng/L (parts per trillion)
  - MI PFOS MCL: 16 ng/L (parts per trillion)
- European Food Safety Authority (EFSA) tolerable weekly intake is based on immunotoxicity:
  - EFSA TWI: 4.4 ng/kg/bw (for PFOS, PFOS, PFNA, and PFHxS)

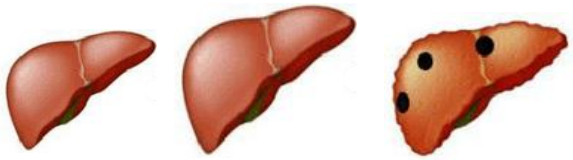


Risks from PFAS exposure on the immune system are supported by toxicological evidence.

Toxicological evidence



Signs of liver damage in experimental models



Liver enlargement, liver damage, and/or liver adenomas are *well known* effects of long-chain PFAS in animal models (rodents and non-human primates). Studies of emerging PFAS confirm that short-chain PFAS can also target the liver.

Signs of liver toxicity include:

- Monotonic dose-dependent increases in liver weight
- Hepatocellular hypertrophy associated with vacuole formation
- Peroxisome proliferation and increases in other liver enzymes
- Proliferation, necrosis, and apoptosis
- Disrupted hepatic metabolism/steatosis

The liver is a target of exposure from many of the PFAS that have been studied.

Putative mechanisms by which liver damage occurs in rodents

Nuclear receptor activation
PPARα, PPARγ, PPARβ/δ, CAR, PXR, LXRα and Erα.

Interference with protein binding
Binding with albumin, liver fatty acid binding protein, transthyretin, and others

Mitochondrial dysfunction
Dysfunctions observed in carbohydrate, lipid and amino acid metabolism as well as oxidative stress

Direct cytotoxicity
Partitioning into lipid bilayers, altered calcium homeostasis, and other interactions

PFAS interact with biological molecules in the liver to produce pathological changes that can progress to tumors in rodent livers.

PPARα activation in rodents

One example

Livers of male mice exposed to PFOA for 14 days demonstrating dose-responsive increases in relative weight and a marker of PPARα activation

(from Loveless et al., 2006 and summarized in NJ DWQI Health-based maximum contaminant level support document on PFOA).

Activation of PPARα that leads to liver tumors in rodents is not thought to be an operable pathway to liver tumors in humans *but other outcomes of PPARα (and other receptors) activation do appear to be operable in humans.*

Liver outcomes used to protect public health

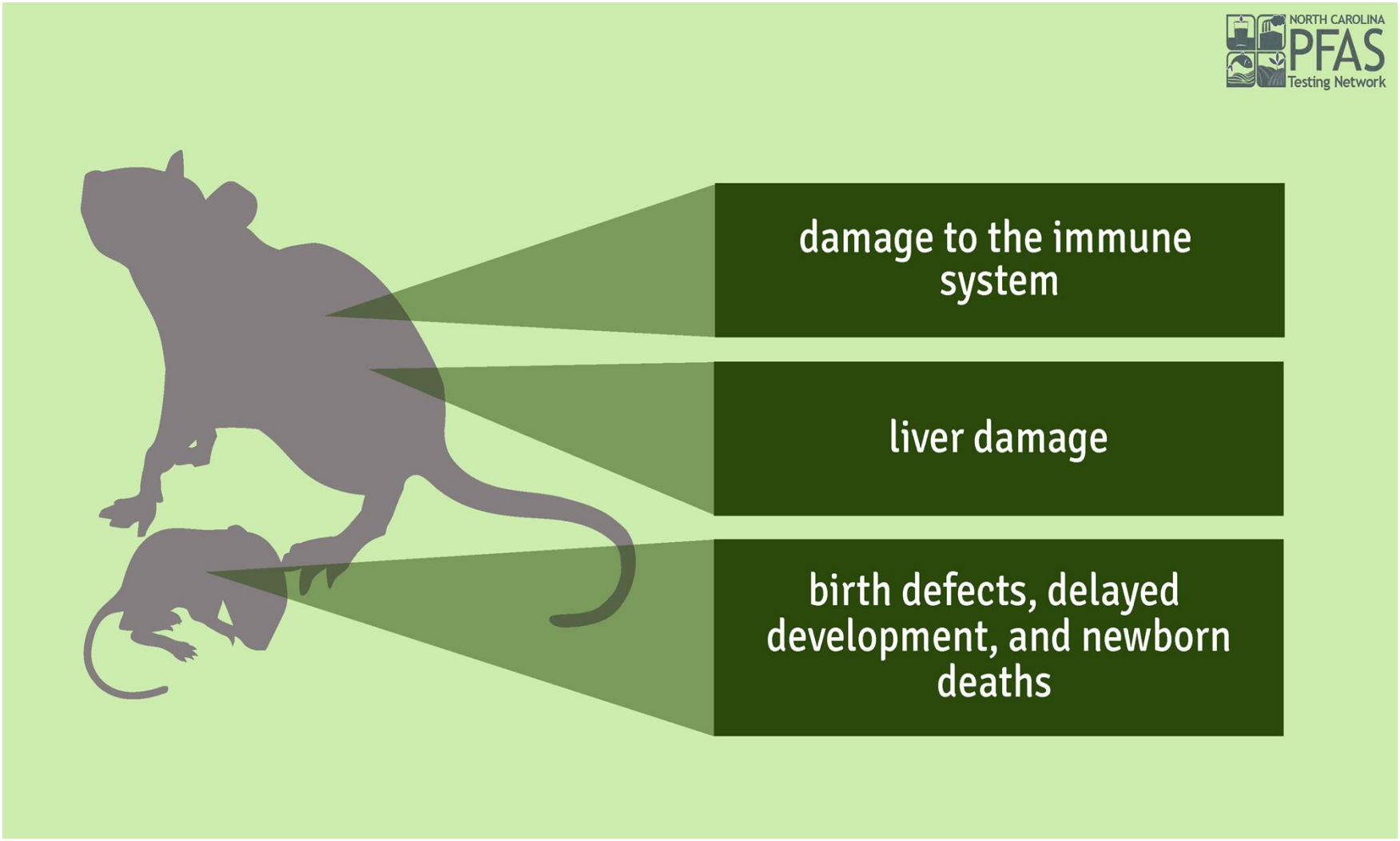
- States of New Jersey, New Hampshire, and New York reference doses (RfDs) for PFOA in drinking water are based on increased liver weight:
  - NJ: 2 ng/kg/day
  - NH: 6.1 ng/kg/day
  - NY: 1.5 ng/kg/day

"The critical endpoint for 3 state Reference Doses is increased relative liver weight, *a well-established and sensitive effect of PFOA* that follows a monotonic dose response, with effect increasing with dose"
(data, quote and table from Post, 2020)

	NJ	NH	NY
Critical effect	Increased liver weight		
Species			
Study	Loveless et al. (2006)		Macon et al. (2011)
Serum PFOA metric	Measured		
Point of departure[a] (ng/mL)	4350 (BMDL)		1060 (BMDL)
Uncertainty factor			
Intraspecies[b]			
Interspecies[c]			
Shorter-than-chronic			
LOAEL-to-NOAEL	1		
Database[e]	10	3	3
Total[f]	300	100	100
Clearance factor[g]			
Reference Dose (ng/kg/d)	2	6.1	1.5

Risks from PFAS exposure on the liver are supported by toxicological evidence.

Toxicological evidence



U.S. EPA drinking water Health Advisory Level for PFOA and PFOS

FACT SHEET

PFOA & PFOS Drinking Water Health Advisories

EPA's 2016 Lifetime Health Advisories, continued

To provide Americans, including the most sensitive populations, with a margin of protection from a lifetime of exposure to PFOA and PFOS from drinking water, EPA established the health advisory levels at 70 parts per trillion. When both PFOA and PFOS are found in drinking water, the combined concentrations of PFOA and PFOS should be compared with the 70 parts per trillion health advisory level. This health advisory level offers a margin of protection for all Americans throughout their life from adverse health effects resulting from exposure to PFOA and PFOS in drinking water.

EPA's health advisory levels were calculated to offer a margin of protection against adverse health effects to the most sensitive populations: fetuses during pregnancy and breastfed infants. The health advisory levels are calculated based on the drinking water intake of lactating women, who drink more water than other people and can pass these chemicals along to nursing infants through breastmilk.

EPA conclusion: Developing organisms need to be protected from PFOA and PFOS present in drinking water.

Developmental effects

PFOA
Accelerated puberty in males and reduced ossification of proximal phalanges (mice)

PFOS
Decreased offspring body weight (rats)

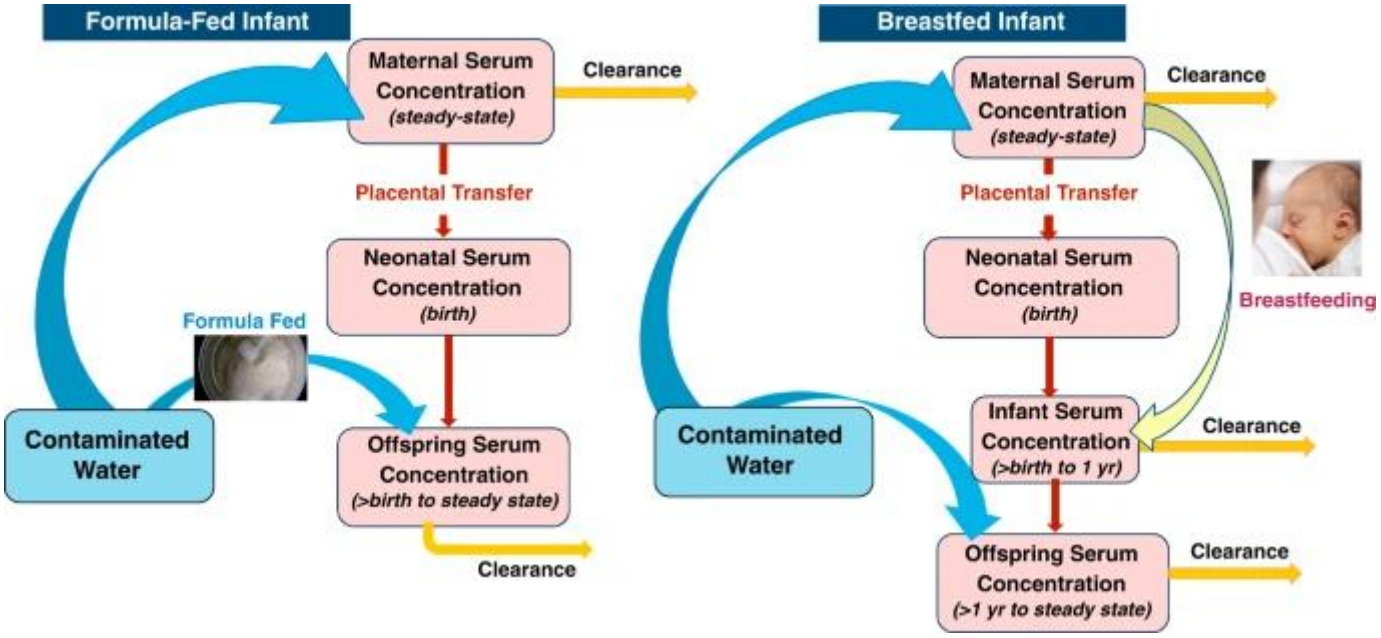
Other PFAS – PFDA, PFNA, PFHxS, GenX, Nafion Byproduct 2 – have been reported to produce changes in developmentally-exposed rodents

Effects observed on litter size, weight, and survival, changes in behavior, delayed mammary gland development, lactational impairment, placental dysfunction, changes of markers in glucose and lipid metabolism and placental health.

Emerging data indicate that mixtures may be dose-additive.

Morbidity and mortality are observed at high doses of legacy PFAS and growth deficits and delays are observed at lower doses (from Fenton et al., 2020).

Minnesota's transgenerational toxicokinetic model



Transgenerational body burden was calculated from mother's serum levels using a placental transfer factor and exposure was simulated from breastmilk or formula reconstituted with contaminated water (from Goeden et al. 2019).

MN PFOA water guidance was derived from model results for protection of breastfed infants.

Developmental outcomes used to protect public health

- States of Massachusetts, Michigan, Minnesota, Vermont, and Washington reference doses (RfDs) for PFOA or PFOS in drinking water are based on developmental toxicity:
  - MA: 5 ng/kg/day (PFOA)
  - MI: 3.9 ng/kg/day (PFOA) and 5 ng/kg/day (PFOS)
  - MN: 18 ng/kg/day (PFOA)
  - VT: 20 ng/kg/day (PFOA) and 20 ng/kg/day (PFOS)
  - WA: 3 ng/kg/day (PFOA)

Some states have included additional uncertainty factors in their RfDs to account for low-dose developmental effects, even if the point of departure for the RfD was not based on developmental toxicity endpoints (from Post, 2020).

Risks from PFAS exposure on development are supported by toxicological evidence.

From models to people

Epidemiological findings
Liver toxicity
Immunotoxicity
Developmental/reproductive toxicity

Supportive animal studies
Liver toxicity
Immunotoxicity
Developmental/reproductive toxicity

Consistency in observed health effects between studies of people and experimental models *increases our confidence in the strength of the link between exposure and these health effects.*

Other toxicological effects have been reported

- Tumor formation (i.e., the "tumor triad" of liver, pancreas, and testicular tumors in rodents)
- Endocrine disruption
- Neurotoxicological outcomes

And knowledge is growing

- Sex differences matter
- Species differences matter
- The presence of comorbid diseases matters
- Mixtures effects matter

Translational research teams will be critical to enhance strategies for informing risk assessment of PFAS (from Fenton et al., 2020).

Summary points

A. PFAS are not inert. The PFAS that have been studied interact with biological molecules in living organisms. These interactions perturb physiology and lead to adverse health outcomes.
B. Mechanisms of action leading to adverse health outcomes may be tissue-specific (i.e., receptor modulation) but modes of action (i.e., mitochondrial dysfunction) may be common across several tissues.
C. Several adverse health outcomes that occur in experimental models following exposure to individual PFAS have been observed in epidemiological studies of people from myriad populations.
D. Exposure to PFAS is not to individual compounds but to mixtures. Emerging data indicate dose-additivity, which suggests broad approaches to PFAS management that move away from a "one-by-one" approach.

Thank you for listening.

I welcome your questions and reflections.

References

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