

Introduction to the Draft NTP Monograph on Immunotoxicity Associated with Exposure to PFOA or PFOS

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Peer Review Meeting
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Exposure to PFOA and PFOS

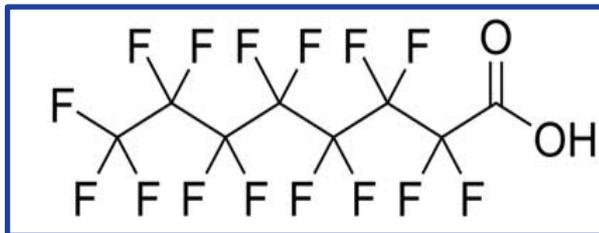
- **Perfluoroalkyl acids including PFOA and PFOS**

- Used extensively in commercial/industrial applications last 50 years
 - food packaging
 - water-resistant coatings
 - lubricants
 - fire-retarding foams

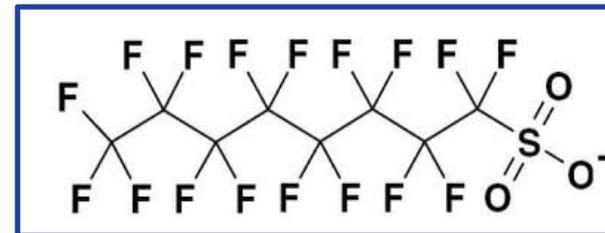


- **PFOA and PFOS**

- US production eliminated; use and emissions reduced in US and much of Europe through voluntary agreements
- Not expected to degrade under typical environmental conditions
- Not metabolized
- Slower human elimination rates
 - Half-lives (2-8 years) humans vs. days or weeks in other animals



PFOA



PFOS



Why Evaluate PFOA, PFOS Immunotoxicity?

- PFOA and PFOS are the most commonly detected perfluoroalkyl acids in environment and human serum

Geometric mean serum concentrations ($\mu\text{g/L}$) for US population

Survey years	PFOA	PFOS
1999-2000	5.21 (4.72-5.74)	30.4 (27.1-33.9)
2005-2006	3.92 (3.48-4.42)	17.1 (16.0-18.2)
2011-2012	2.08 (1.95-2.22)	6.31 (5.84-6.82)

- Reported immune effects of both PFOA and PFOS
 - Effects on antibody response in animals at some of lowest doses
 - Recent studies reporting similar antibody effects in humans
 - PFOA and PFOS appeared to share some effects and differ for others
- OHAT Approach to Systematic Review and Evidence Integration
 - A portion of PFOA and PFOS immunotoxicity dataset used as a case study
 - NTP received multiple requests to complete the case study as a full review



Reported Immune Effects of PFOA and PFOS

- **Studies in animals**

- Experimental studies

- PFOA- and PFOS-associated changes in multiple immune measures
- **Immunosuppression:** reduced antibody response, disease resistance, etc.
- **Hypersensitivity:** increased airway hypersensitivity

- Wildlife studies



- **Studies in humans**

- PFOA- and PFOS-associated measures of immune function or disease

- **Immunosuppression:** reduced antibody response to vaccines
- **Hypersensitivity:** increased asthma in children
- **Autoimmunity:** increased incidence of ulcerative colitis





NTP Conducted A Systematic Review

- To develop NTP hazard identification conclusions on the association between exposure to PFOA or PFOS (or their salts) and immunotoxicity
- Conclusions for each chemical were reached by integrating evidence from human and animal studies with consideration of the degree of support from mechanistic data



Steps in Systematic Review and Evidence Integration

– Problem Formulation and Protocol

- Concept and detailed systematic review protocol
- Protocol peer-reviewed, posted

– Identify Relevant Evidence

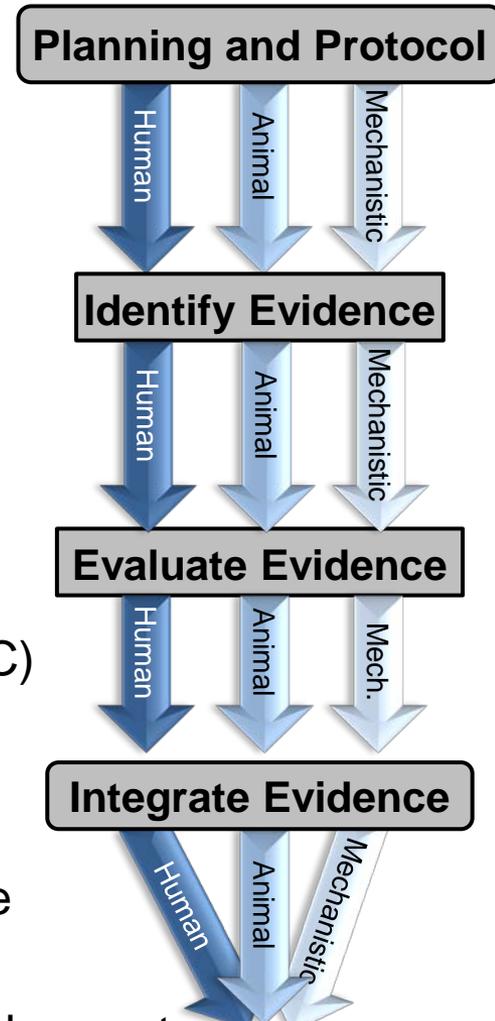
- Literature search
- Select studies
- Extract data into HAWC (<https://hawcproject.org/assessment/57/>)

– Evaluate the Evidence

- Assess individual study quality/risk of bias (also in HAWC)

– Integrate the Evidence

- Bodies of evidence: studies grouped by outcome
- Confidence ratings: developed for each body of evidence
- Levels of evidence: translation from confidence ratings
- Hazard identification conclusions: from integration of evidence streams





Group Results by Same or Related Outcomes

- **Main categories of immune response**

- Immunosuppression
- Hypersensitivity-related effects
- Autoimmunity

- **Focus on primary outcomes**

- Direct health outcomes or endpoints considered to have greater predictive value for overall immunotoxicity
 - Immune-related diseases or disease resistance assays
 - Measures of immune function

- **Secondary outcomes**

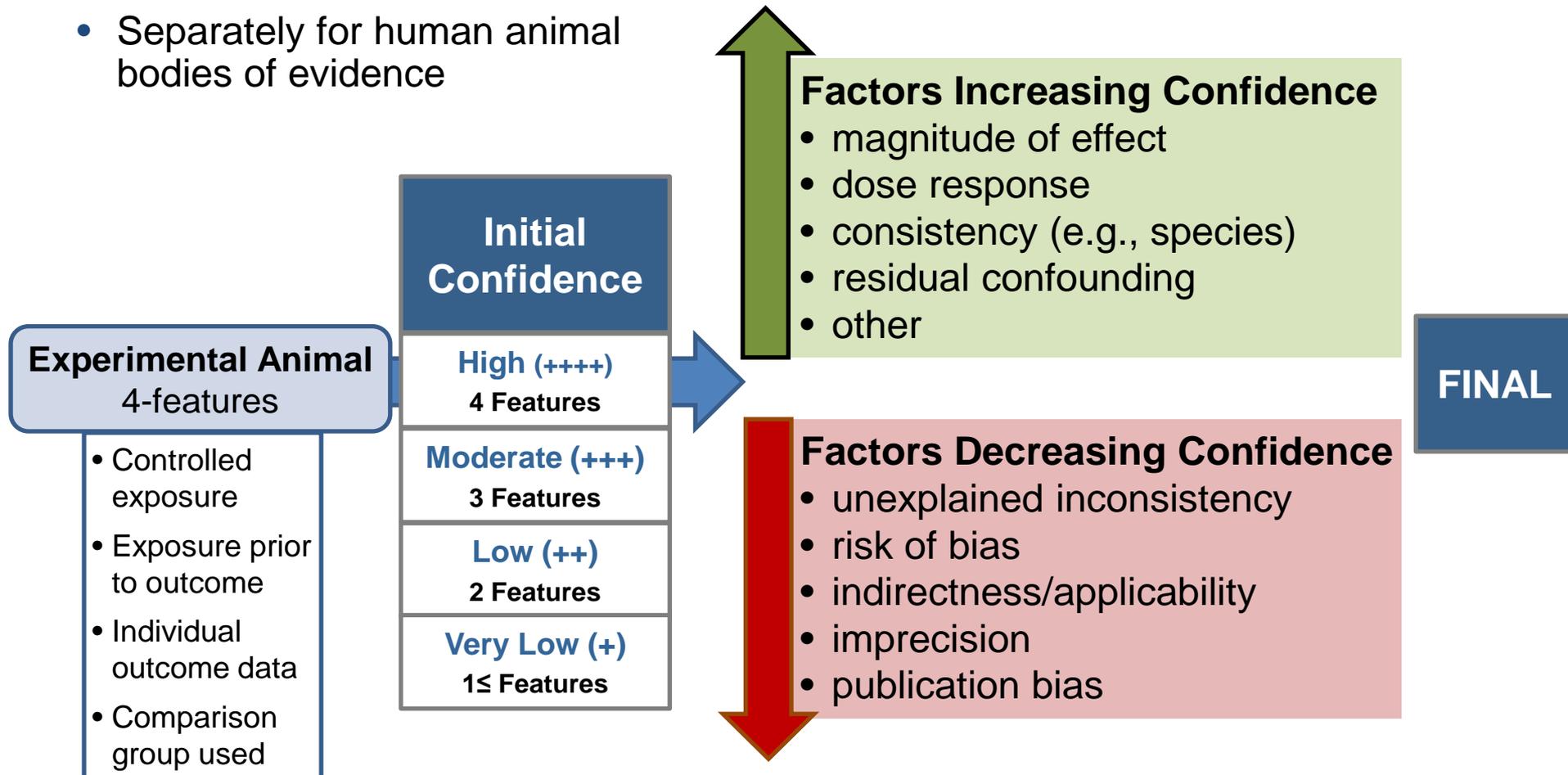
- Used to examine biological plausibility
- Indirect data related to health outcomes
 - Lymphoid organ weights, lymphocyte counts, etc.



Steps to Integrate Evidence

Rating Confidence in Bodies of Evidence

- A measure of the certainty that findings from a group of studies reflect the true relationship between exposure to a substance and effect
- Separately for human animal bodies of evidence

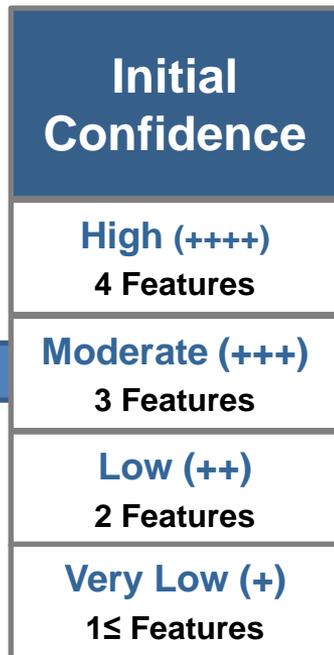




Steps to Integrate Evidence

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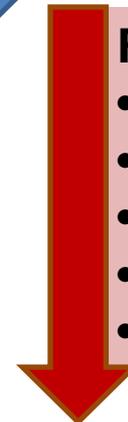
Human Cohort
3-features

- Controlled exposure
- Exposure prior to outcome
- Individual outcome data
- Comparison group used



Factors Increasing Confidence

- magnitude of effect
- dose response
- consistency (e.g., species)
- residual confounding
- other



Factors Decreasing Confidence

- unexplained inconsistency
- risk of bias
- indirectness/applicability
- imprecision
- publication bias

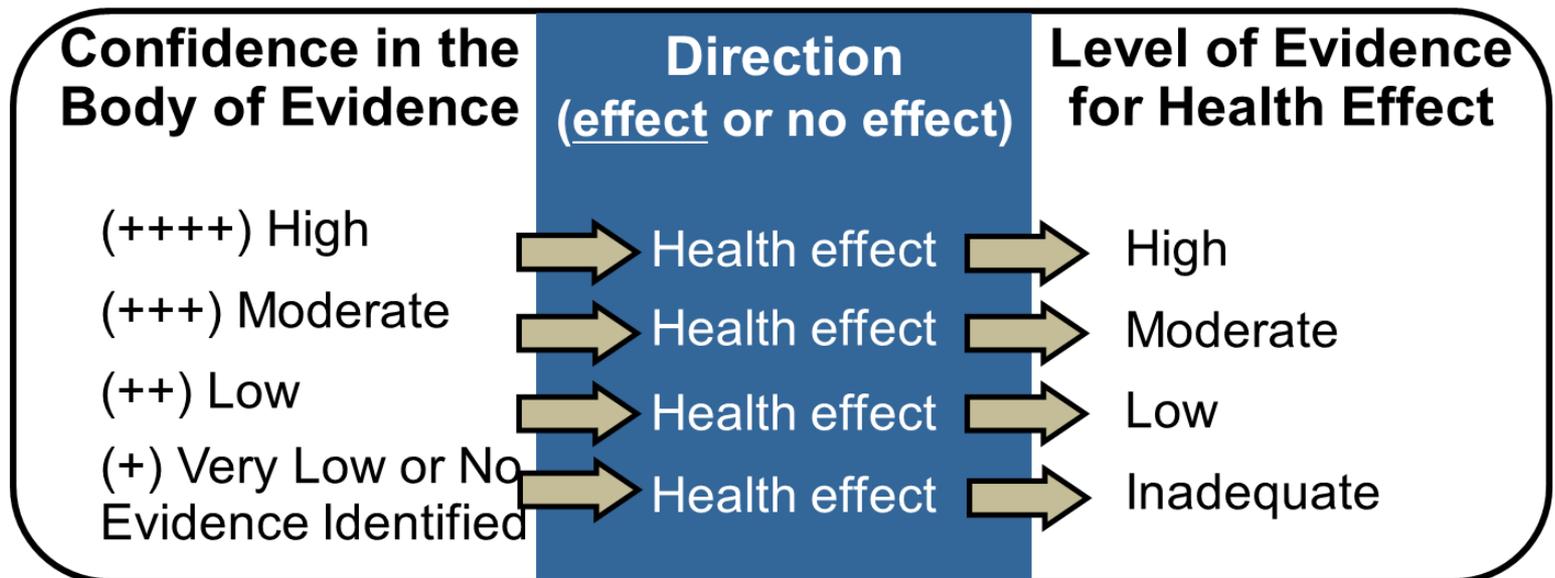
FINAL



Steps to Integrate Evidence

Translate Confidence Into Level of Evidence

- Level of Evidence Considers:
 - Confidence rating in body of evidence from previous step
 - The direction of the outcome (health effect or no effect)
 - Human and animal bodies of evidence still separate at this point





Final Step to Integrate Evidence

Develop Hazard ID

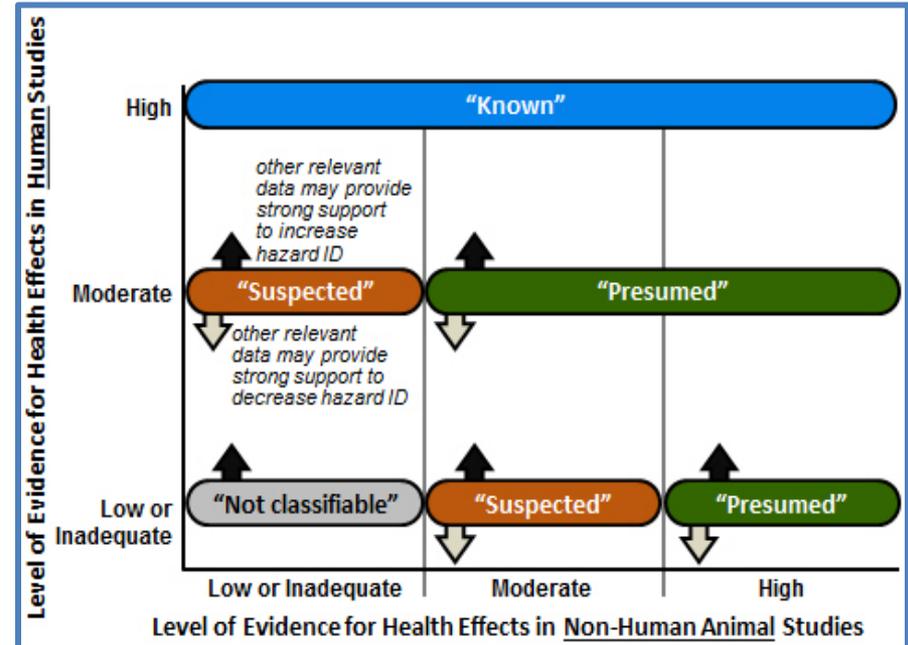
(1) Initial Hazard Conclusion

Consider human and animal evidence together

(2) Final Hazard Conclusion

Consider impact of mechanistic data and biological plausibility of effect

- *In vitro/in vivo* data or upstream indicators
- Data to inform biological plausibility
 - Strong support to increase hazard ID
 - Strong opposition to decrease hazard ID
 - Or may not impact the hazard conclusion



Biological Plausibility

- Are there data showing chemical-associated disruption of early events in the process leading to an observed health effect?
- Were changes at same or lower concentrations as the observed effect?
- **Examples:** Key cell populations, cell signaling, cell activation



The Peer Review Panel's Charge is to:

- Determine whether the scientific information cited in the draft monograph is technically correct and clearly stated, and whether NTP has objectively presented and assessed the scientific evidence.
- Determine whether the scientific evidence presented in the draft NTP monograph supports the NTP's conclusions regarding whether immunotoxicity is associated with exposure to PFOA or PFOS.



Questions?



PFOA



NTP Conclusions on PFOA Immunotoxicity

- NTP conclusions are based on the highest level-of-evidence conclusions for immune effects on an outcome basis
- PFOA is **presumed to be an immune** hazard to humans based on two separate lines of evidence:
 - **(1) PFOA suppressed the antibody response**
 - Animal studies: High level of evidence
 - Human studies: Moderate level of evidence
 - No change in conclusions after considering mechanistic data
 - (2) PFOA increased hypersensitivity-related outcomes
 - Animal studies: High level of evidence
 - Human studies: Low level of evidence
 - No change in conclusions after considering mechanistic data

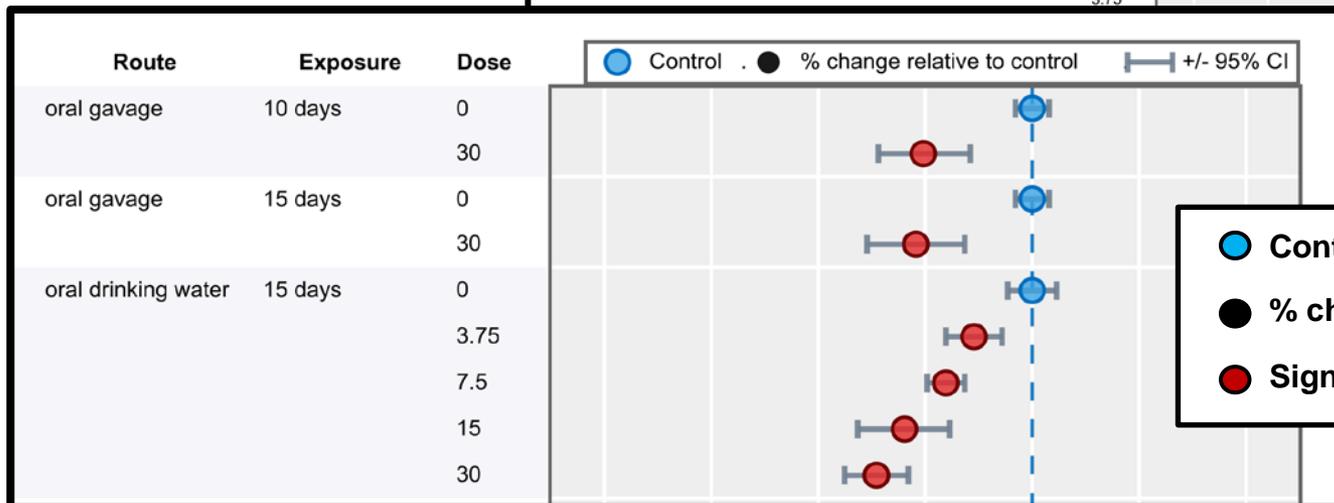
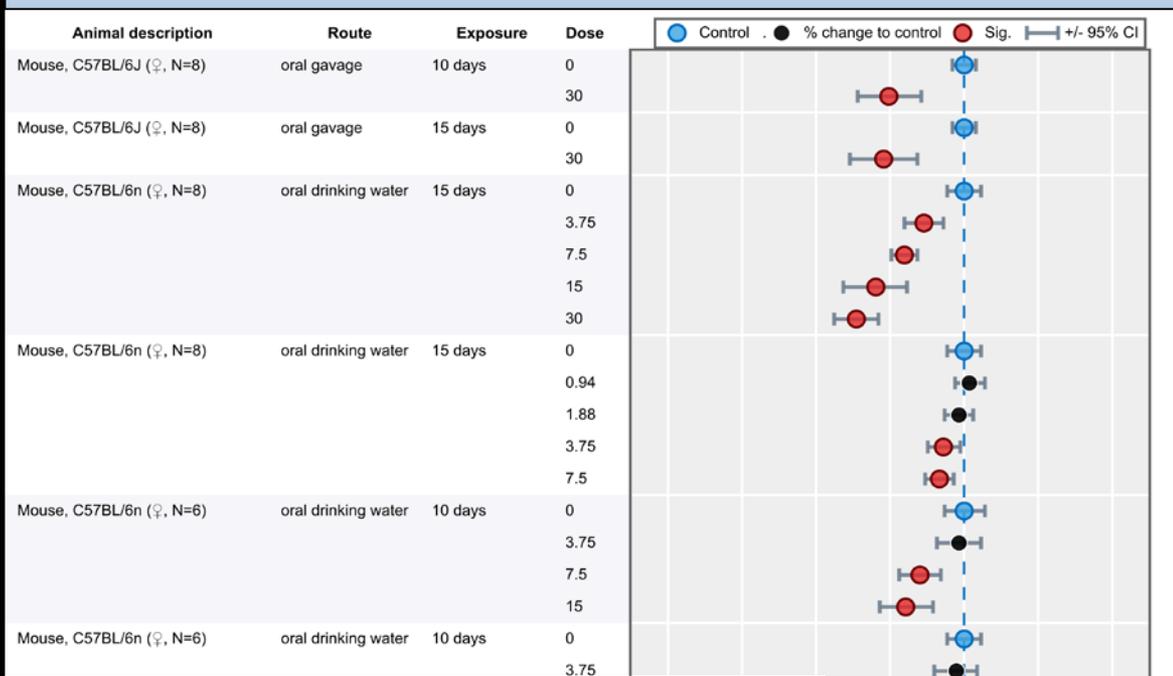


PFOA: Antibody Response

Animal Data

- 7 experimental studies in mammals
- Consistent suppression of primary antibody response (IgM) in mice

Figure D6. Antigen-specific IgM antibody response in experimental animals - PFOA



● Control
● % change relative to control
● Significantly different



Risk of Bias Considerations

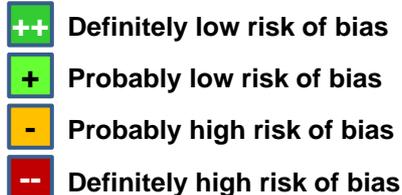
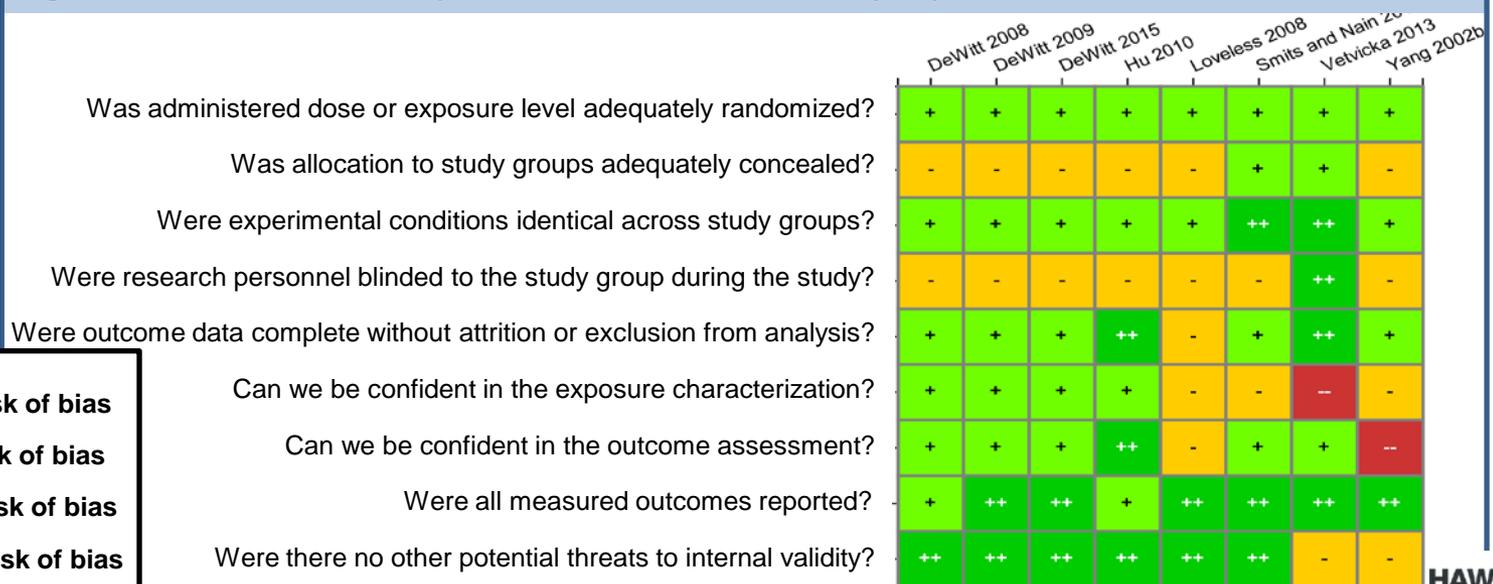
• Key Questions

- Randomization, Outcome Assessment: probably low for most studies
- Exposure Characterization: probably or definitely high for half studies due to use of PFOA with purity <98% and no independent confirmation of purity

• Other Questions

- Allocation concealment: probably high for most studies – not reported (NR)
- Researcher blinding during study: probably high for most studies – NR

Figure D13. Risk of bias heatmap for PFOA studies of the antibody response in animals





PFOA: Antibody Response

Antibody Response Evidence Profile for PFOA

INITIAL CONFIDENCE 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	
PFOA										
<i>Animal</i>										
Initial High (7 mammal studies)	↓	---	---	---	---	---	↑	---	---	High

- **High confidence** that exposure to PFOA is associated with suppression of the antibody response
- Consistent suppression of the primary antibody response in mice
- Heterogeneity in findings may be attributed to differences by
 - Species – rats less susceptible
 - Outcome measure – primary vs secondary antibody response

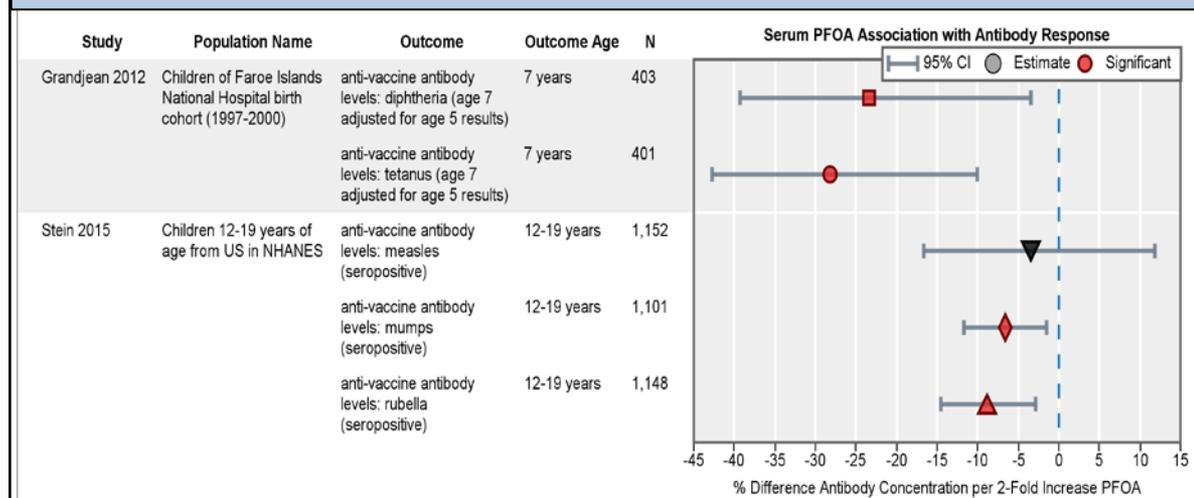


PFOA: Antibody Response

• Human Data

- 4 prospective, 2 cross-sectional studies
- suppression in one or more measure of anti-vaccine antibody response associated with prenatal, childhood, and adult exposures

Subset of Figure D3. Antibody response in children relative to PFOA levels in children



■* Significantly different

Anti-vaccine antibodies

- diphtheria
- ▼ measles
- ◆ mumps
- ▲ rubella
- tetanus



Risk of Bias Considerations

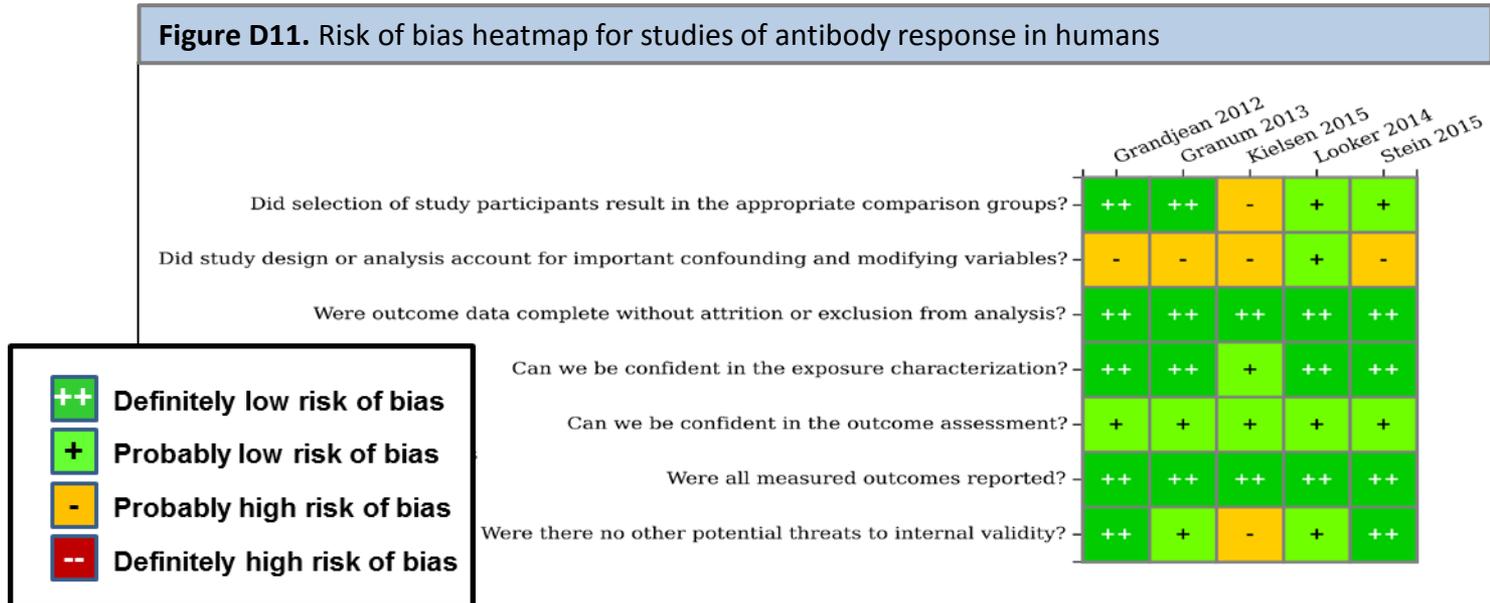
- **Key Questions**

- Exposure Characterization: probably or definitely low for all studies
- Outcome Assessment: probably low for all studies
- Confounding or Modifying: probably high for most studies due to inability to distinguish effects of PFOA from other PFAAs (effects in same direction and more likely to be effect modifier than true confounder)

- **Other Questions**

- Probably low and definitely low for most studies

Figure D11. Risk of bias heatmap for studies of antibody response in humans





PFOA: Antibody Response

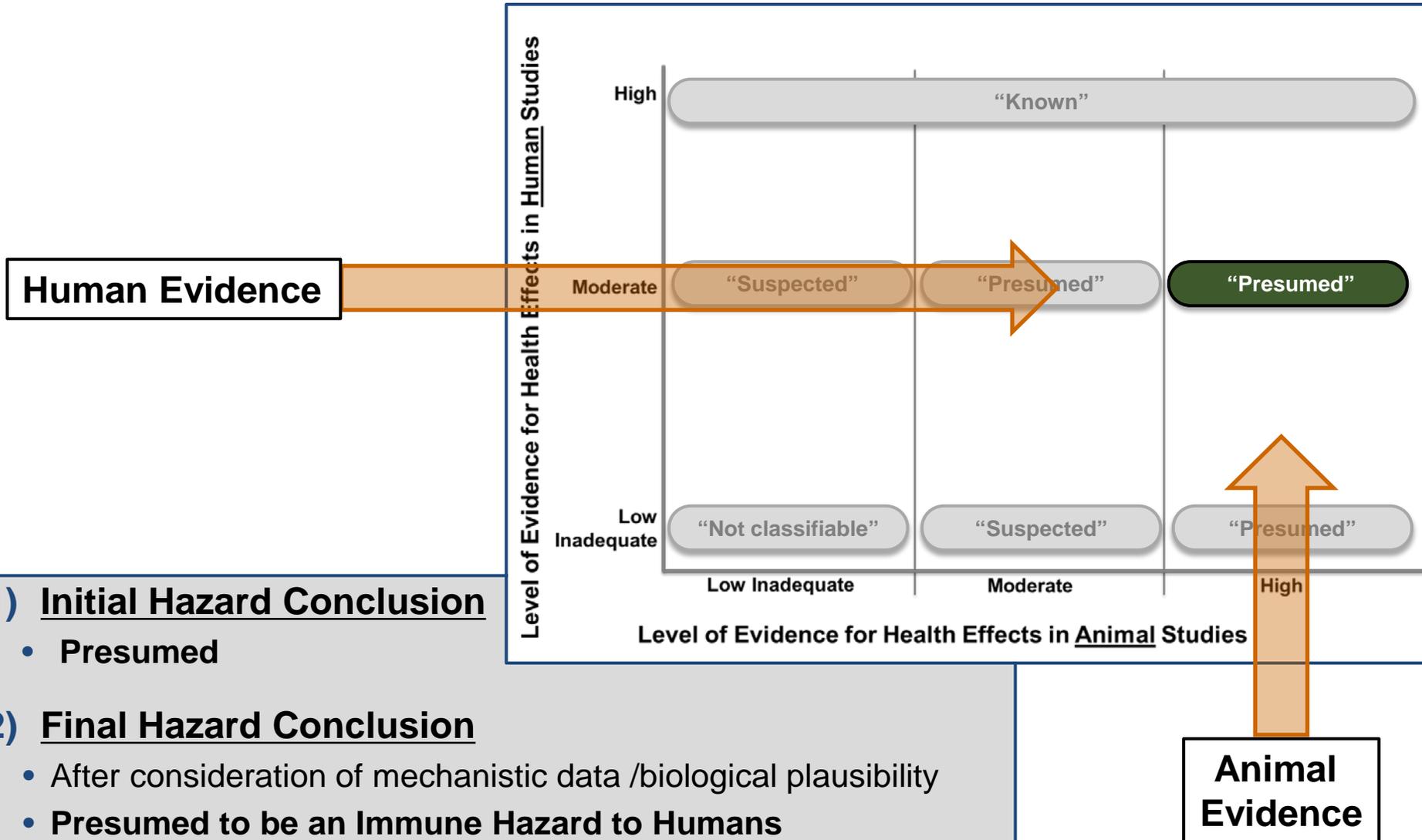
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PFOA										
<i>Human</i>										
Initial Moderate (4 prospective studies)	---	---	---	---	---	---	---	---	---	Moderate
Initial Low (2 cross-sectional studies)	---	---	---	---	---	---	---	---	---	Low
Confidence Across Human Bodies of Evidence	No change for considering across study designs									Moderate

- **Moderate confidence** that exposure to PFOA is associated with suppression of the antibody response in humans
- PFOA-associated suppression in one or more measure of anti-vaccine antibody response across multiple studies with prenatal, childhood, and adult exposures
- Heterogeneity in response may be attributed to different vaccines, measures
 - Limited ability to compare across studies (different vaccines, timing, antibody measures)
 - Strength of antibody response to different vaccines expected



Evidence Integration: Develop Hazard ID

PFOA: Antibody Response





Antibody response levels of evidence

- PFOA is **presumed to be an immune hazard** to humans based on two separate lines of evidence:
 - **(1) PFOA suppressed the antibody response**
 - **Animal studies: High level of evidence**
 - Human studies: Moderate level of evidence
 - No change in conclusions after considering mechanistic data
 - **(2) PFOA increased hypersensitivity-related outcomes**
 - Animal studies: High level of evidence
 - Human studies: Low level of evidence
 - No change in conclusions after considering mechanistic data



Action-Human Level of Evidence

Antibody response levels of evidence

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Questions?



NTP Conclusions on PFOA Immunotoxicity

- NTP conclusions are based on the highest level-of-evidence conclusions for immune effects on an outcome basis
- PFOA is **presumed to be an immune** hazard to humans based on two separate lines of evidence:
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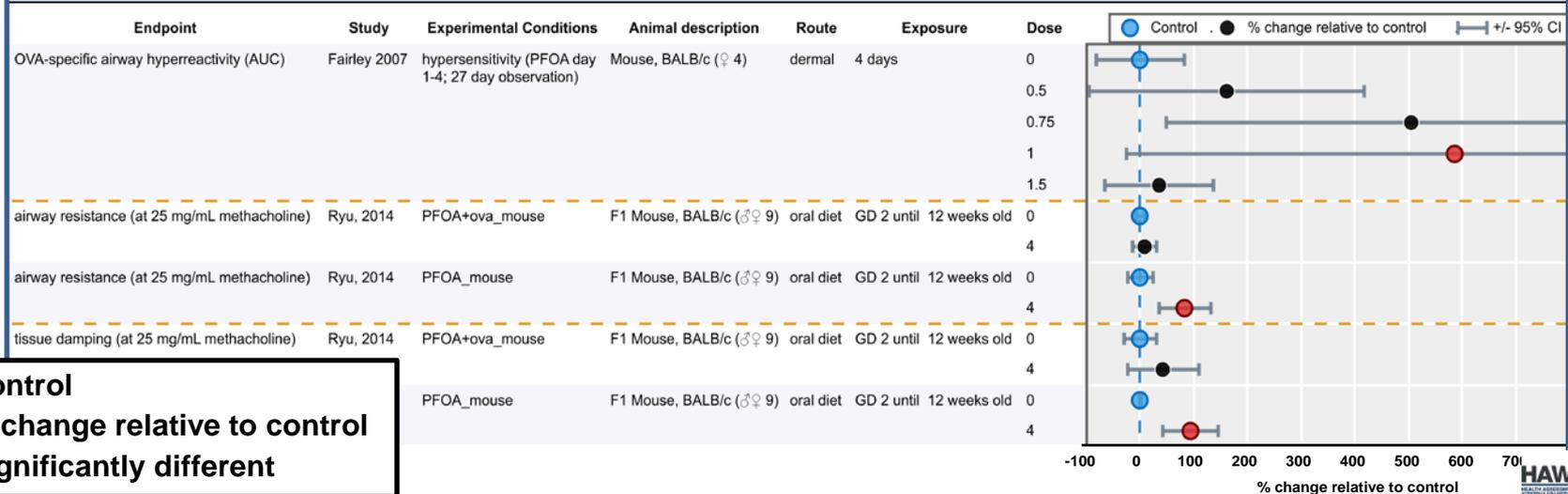


PFOA: Hypersensitivity-related Outcomes

• Animal Data

- 3 experimental studies in mammals (2 studies of airway outcomes)
- Increased hypersensitivity in mice across multiple measures
 - Short-term dermal (Fairly 2007)
 - Increased antigen[OVA]-specific airway hyperreactivity, total IgE, OVA-IgE
 - Oral developmental (Ryu 2014)
 - Increased airway hyperreactivity, lung macrophages
 - Short-term dermal or IP (Singh 2012)
 - Increased serum histamine, and IgE-dependent passive cutaneous anaphylaxis

Figure D29. Airway hypersensitivity in animals IgM antibody response in experimental animals - PFOA





PFOA: Hypersensitivity-related Outcomes

Risk of Bias Considerations

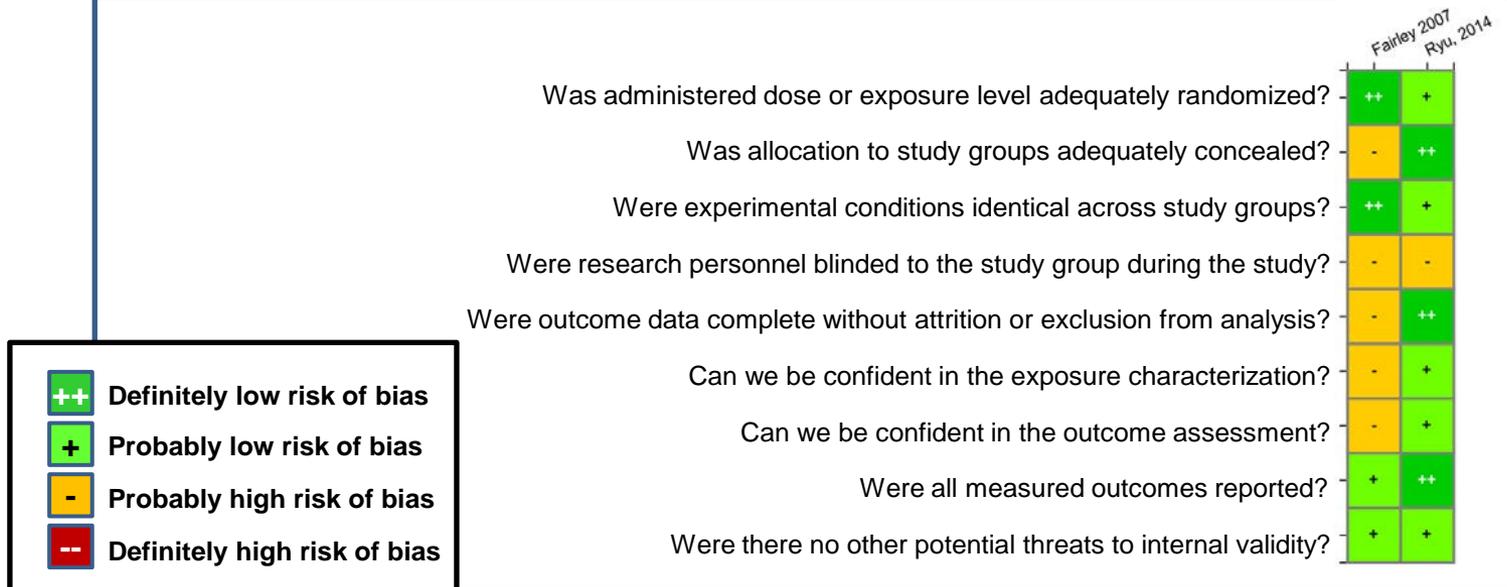
- **Key Questions**

- Randomization: probably low for both studies
- Exposure and Outcome: probably low for one study probably high for other due to use of PFOA <98% purity without independent confirmation and lack of blinding of outcome assessors

- **Other Questions**

- Researcher blinding during study: probably high for both studies

Figure D36. Risk of bias heatmap for studies of airway hypersensitivity-related outcomes in animals - PFOA





PFOA: Hypersensitivity-related Outcomes

Hypersensitivity-related Outcomes Evidence Profile for PFOA

INITIAL CONFIDENCE 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		
PFOA											
<i>Animal</i>											
Initial High (7 mammal studies)	---	---	---	---	---	---	---	---	---	---	High

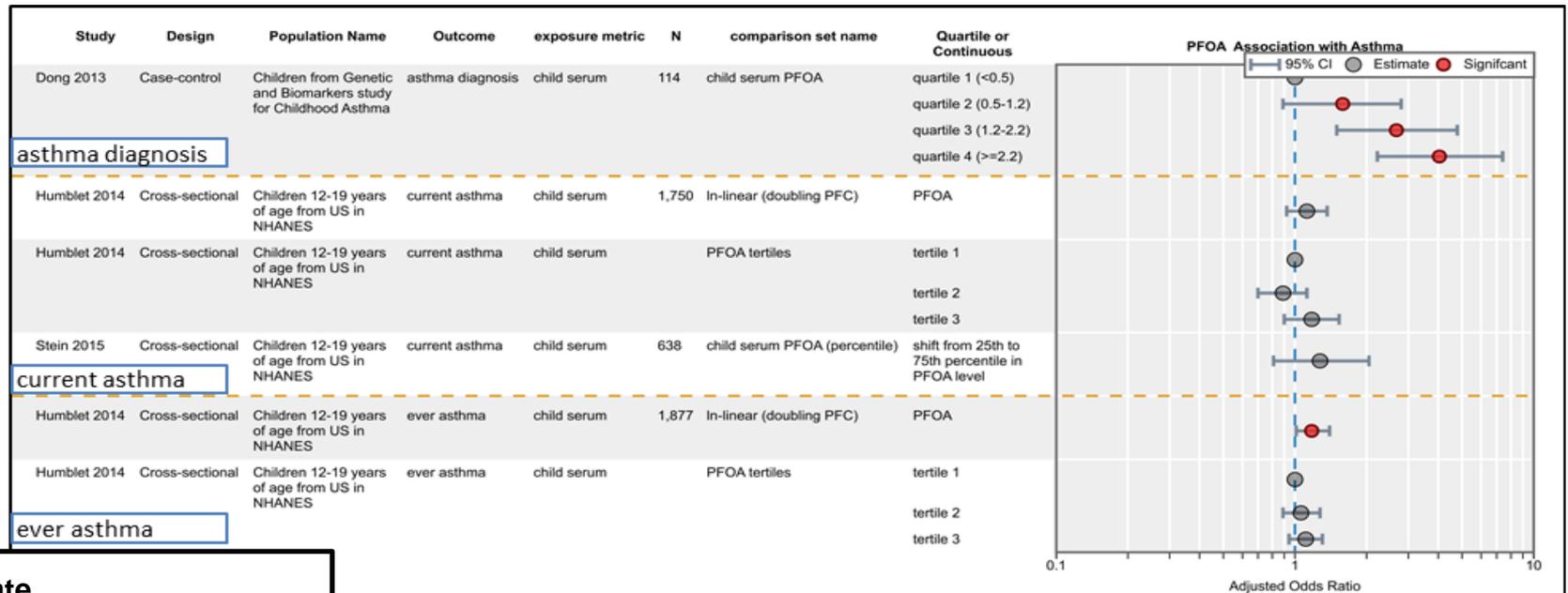
- **High confidence** that exposure to PFOA is associated with increased hypersensitivity-related outcomes
- Consistent enhancement of airway hypersensitivity-related endpoints in mice and clear involvement of IgE where studied
- Heterogeneity in findings may be attributed to differences by
 - Route and duration of exposure



PFOA: Hypersensitivity-related Outcomes

Human Data (children with current exposure levels)

- 2 cross-sectional studies based on NHANES data on children age 12-19
 - Higher odds of ever diagnosis of asthma (Humblet 2014), current rhinitis (Stein 2015)
- Case-control asthma study in children age 10-15 in Taiwan
 - Higher odds of doctor diagnosis of asthma (Dong 2013, Zhu 2016)
 - Increased total serum IgE, eosinophil count and eosinophilic cationic protein concentration among asthmatics



- Estimate
- Significantly different



PFOA: Hypersensitivity-related Outcomes

Risk of Bias Considerations

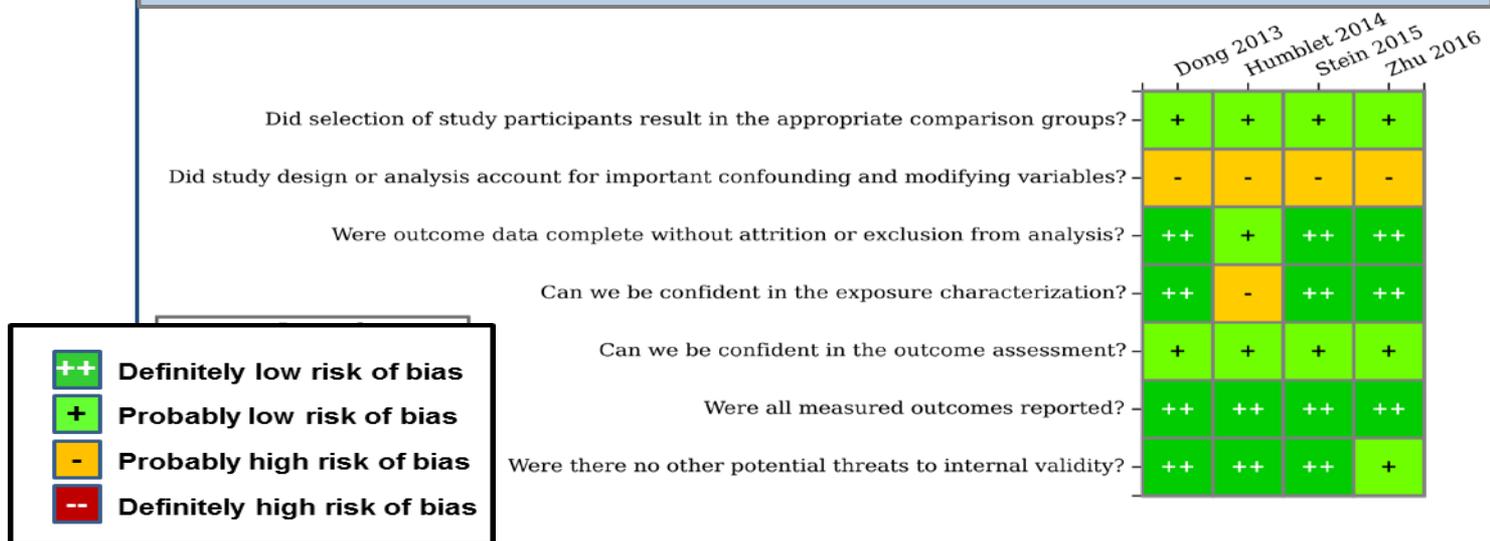
- **Key Questions**

- Exposure Characterization: definitely low for three of the four studies
- Outcome Assessment: probably low for all studies
- Confounding or Modifying: probably high for all studies due to inability to distinguish effects of PFOA from other PFAAs (effects in same direction and may be effect modifier, rather than true confounder)

- **Other Questions**

- Probably low and definitely low for most studies

Figure D33. Risk of bias heatmap for studies of asthma in children with current PFOA levels





PFOA: Hypersensitivity-related Outcomes

Hypersensitivity-related Outcomes Evidence Profile for PFOA

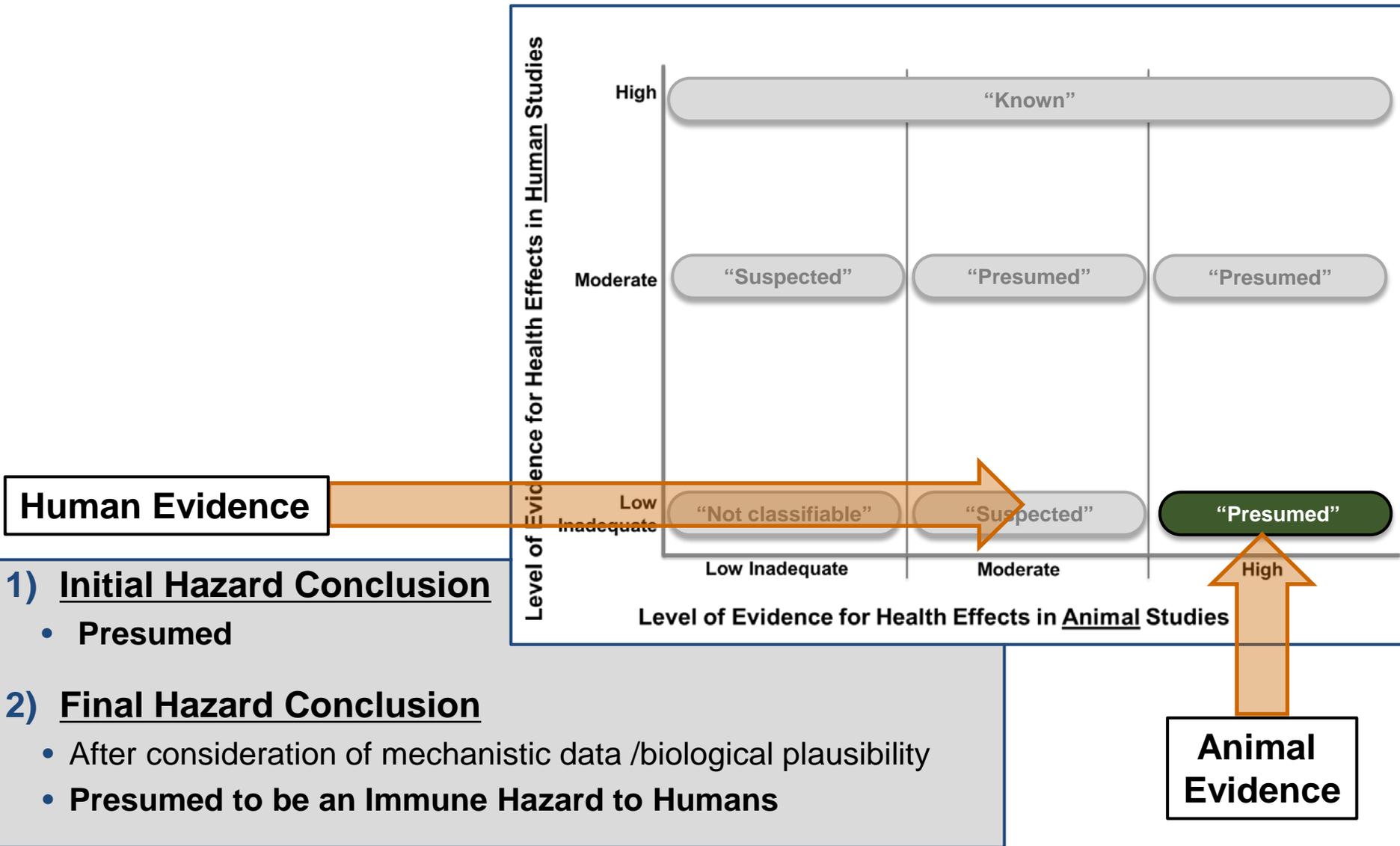
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PFOA										
<i>Human</i>										
Initial Low (3 cross-sectional child exposure studies) [4 publications]	---	---	---	---	---	---	---	---	---	Low

- **Low confidence** that exposure to PFOA is associated with increased hypersensitivity-related outcomes in humans
- Increased diagnosis of asthma, increased IgE and several hypersensitivity-related endpoints in children with higher current serum PFOA concentrations across several cross-sectional studies
- Heterogeneity in response may be attributed to
 - Timing of exposure measure (no evidence with prenatal exposure)



Evidence Integration: Develop Hazard ID

PFOA: Hypersensitivity-related Outcomes





Hypersensitivity-related outcomes levels of evidence

- PFOA is **presumed to be an immune** hazard to humans based on two separate lines of evidence:
 - (1) PFOA suppressed the antibody response
 - Animal studies: High level of evidence
 - Human studies: Moderate level of evidence
 - No change in conclusions after considering mechanistic data
 - **(2) PFOA increased hypersensitivity-related outcomes**
 - **Animal studies: High level of evidence**
 - Human studies: Low level of evidence
 - No change in conclusions after considering mechanistic data



Action-Human Level of Evidence

Hypersensitivity-related outcomes levels of evidence

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Hypersensitivity-related outcomes levels of evidence

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 - No change in conclusions after considering mechanistic data
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 - Animal studies: High level of evidence
 - Human studies: Low level of evidence
 - **No change in conclusions after considering mechanistic data**



Questions?



Other Outcomes that Did Not Reach Hazard Conclusions

- Immunosuppression: Disease Resistance
 - **Animal studies:** Inadequate level of evidence (no exper. studies)
 - **Human studies:** Low level of evidence (low confidence due to lack of consistency in human body of evidence)
- Immunosuppression: NK Cell Activity
 - **Animal studies:** Inadequate level of evidence (single dose study)
 - **Human studies:** Inadequate level of evidence (no studies)
- Autoimmunity-related Effects
 - **Animal studies:** Inadequate level of evidence (no studies)
 - **Human studies:** Low level of evidence - low confidence
 - Two C8 studies report PFOA-associated increases in ulcerative colitis
 - Low confidence because studies are from the same population
 - First analysis: workers plus residents (Steenland 2013)
 - Second analysis: workers only (Steenland 2015)



Questions?



Action: NTP Conclusions for PFOA

Table 7. PFOA Main Immune Effects Summary Table

Category of Immune Response	Immune Outcomes	Confidence Ratings in the Body of Evidence		Level of Evidence in the Body of Evidence		Hazard Conclusion
		Human	Animal	Human	Animal	
Immunosuppression	Antibody response	Moderate	High	Moderate	High	<u>Presumed</u> to be an Immune Hazard to Humans
Hypersensitivity	Asthma and other hypersensitivity-related outcomes	Low	High	Low	High	<u>Presumed</u> to be an Immune Hazard to Humans

PFOA is **presumed to be an immune hazard to humans** based on:

- Suppressed antibody response
 - **Animal studies:** High level of evidence
 - **Human studies:** Moderate level of evidence
 - No change in conclusions after considering mechanistic data
- Increased hypersensitivity-related outcomes
 - **Animal studies:** High level of evidence
 - **Human studies:** Low level of evidence
 - No change in conclusions after considering mechanistic data



Questions?



PFOS



NTP Conclusions on PFOS Immunotoxicity

- NTP conclusions are based on the highest level-of-evidence conclusions for immune effects on an outcome basis
- PFOS is **presumed to be an immune** hazard to humans based on:
 - **(1) PFOS suppressed the antibody response**
 - Animal studies: High level of evidence
 - Human studies: Moderate level of evidence
 - No change in conclusions after considering mechanistic data

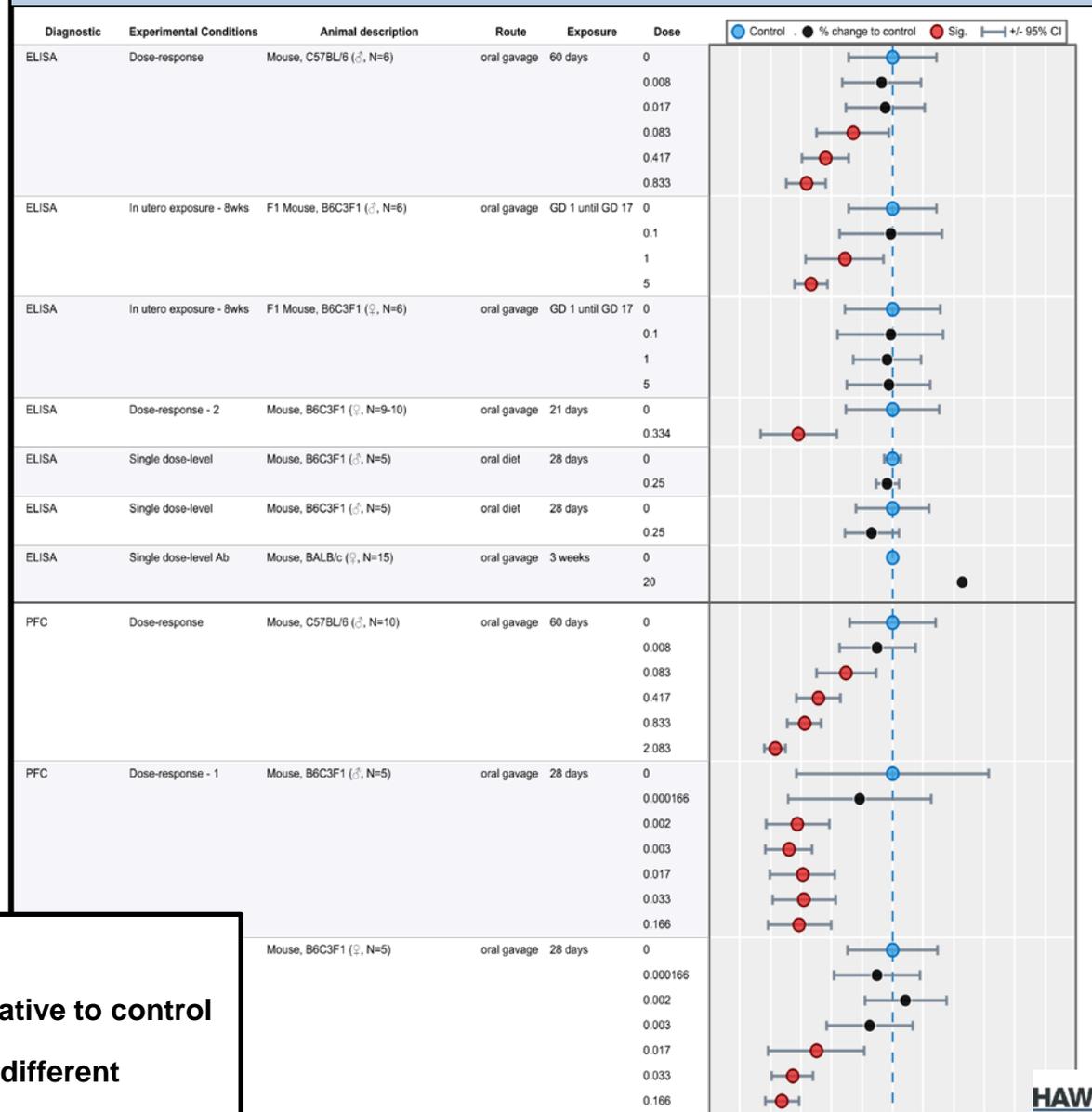


PFOS: Antibody Response

Animal Data

- 8 experimental studies in mammals
- Consistent suppression of primary antibody response (IgM) in mice

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



- Control
- % change relative to control
- Significantly different



Risk of Bias Considerations

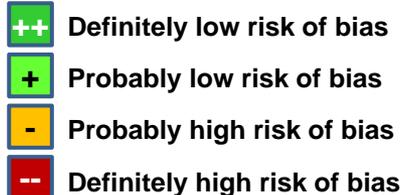
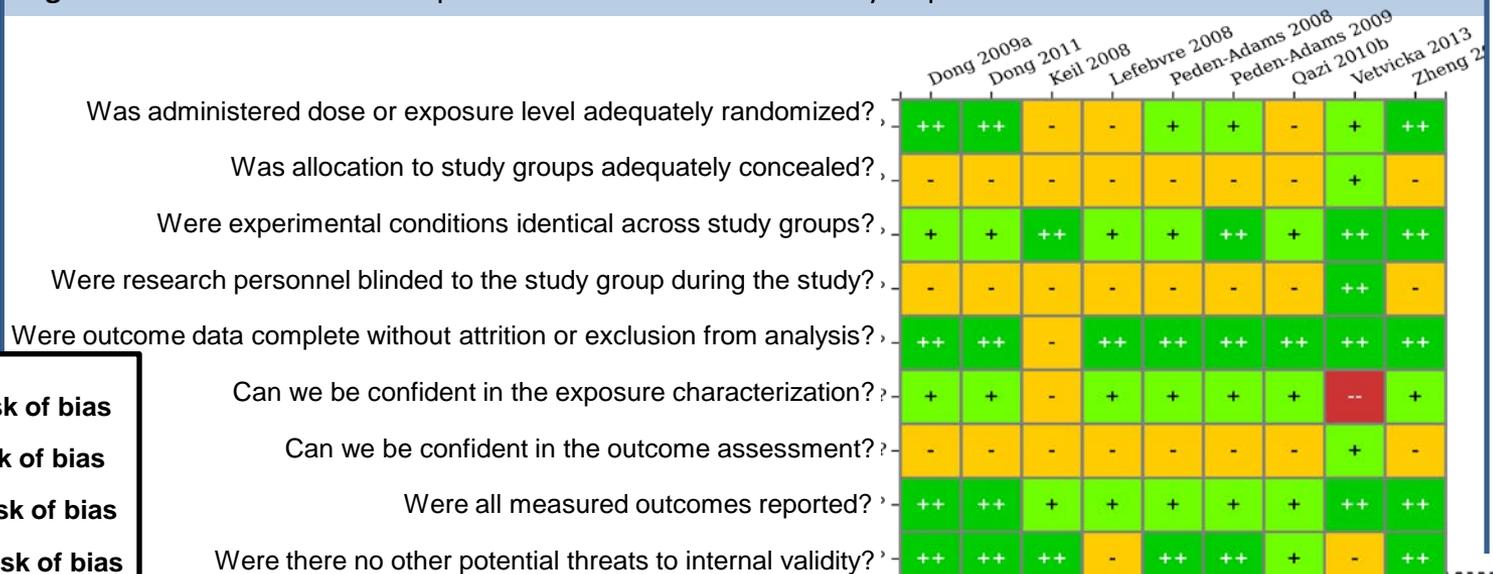
- **Key Questions**

- Exposure Characterization, Randomization: probably low for most studies
- Outcome Assessment: probably high for most studies due to lack of blinding of outcome assessors

- **Other Questions**

- Allocation concealment: probably high for most studies - not reported (NR)
- Researcher blinding during study: probably high for most studies - NR

Figure D13. Risk of bias heatmap for PFOS studies of the antibody response in animals





PFOS: Antibody Response

Antibody Response Evidence Profile for PFOS

INITIAL CONFIDENCE 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	

PFOS

Animal

Initial High (8 mammal studies)	↓	---	---	---	---	---	↑	---	---	High
------------------------------------	---	-----	-----	-----	-----	-----	---	-----	-----	------

- **High confidence** that exposure to PFOS is associated with suppression of the antibody response
- Consistent suppression of the primary antibody response in mice
- Heterogeneity in findings may be attributed to differences by
 - Species – rats less susceptible
 - Outcome measure – primary vs secondary antibody response

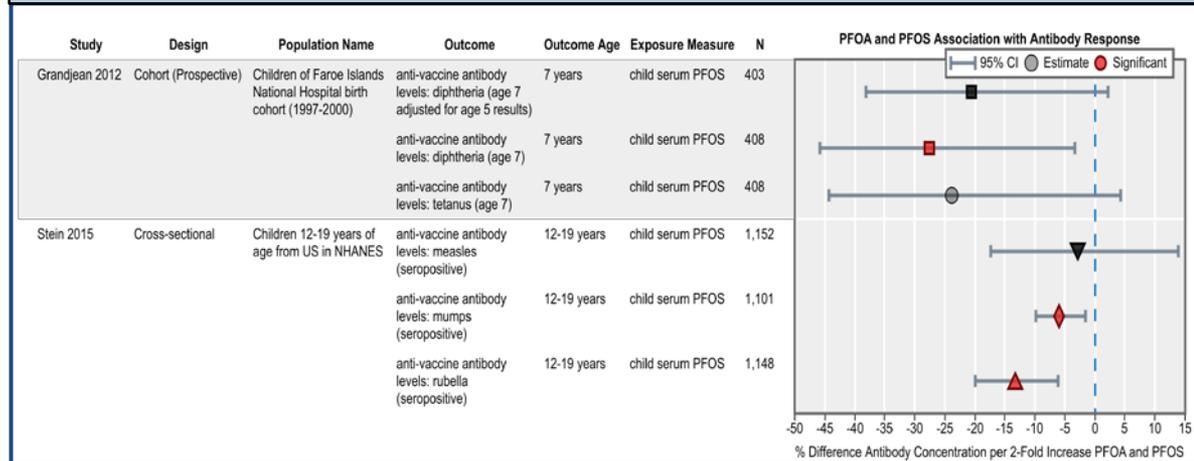


PFOS: Antibody Response

• Human Data

- 4 prospective, 2 cross-sectional studies
- suppression in one or more measure of anti-vaccine antibody response associated with prenatal, childhood, and adult exposures

Subset of Figure D3. Antibody response in children relative to PFOS levels in children



■* Significantly different

Anti-vaccine antibodies

- diphtheria
- ▼ measles
- ◆ mumps
- ▲ rubella
- tetanus



Risk of Bias Considerations

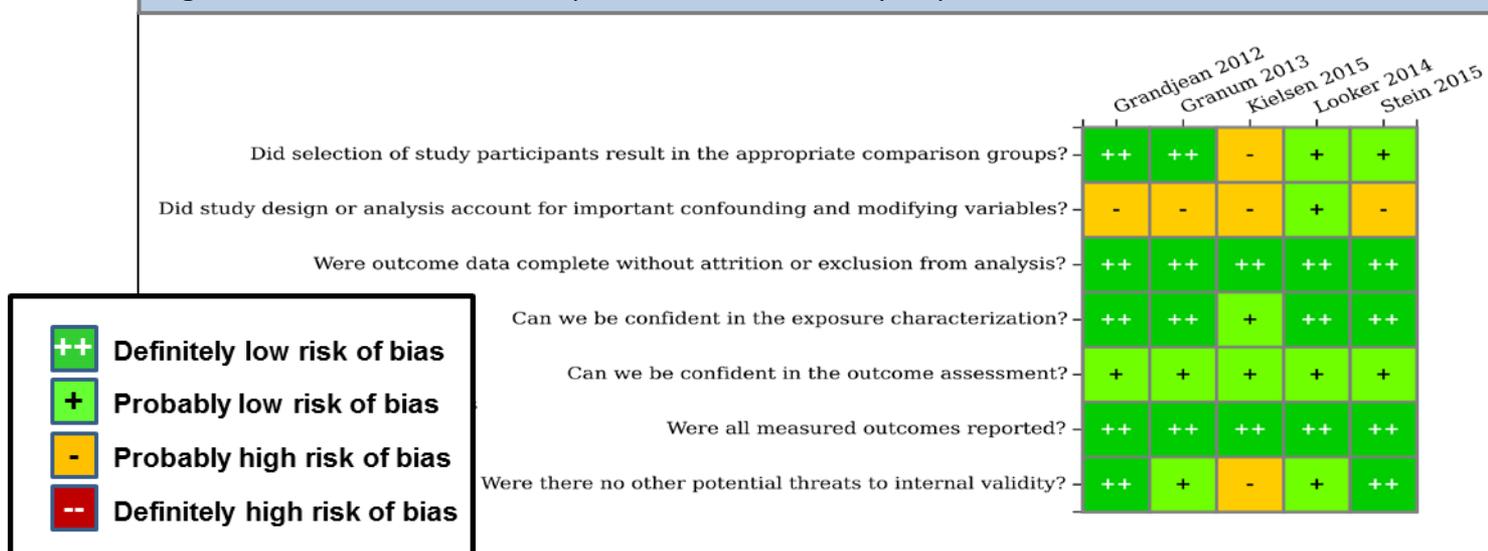
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- **Other Questions**

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Figure D11. Risk of bias heatmap for studies of antibody response in humans





PFOS: Antibody Response

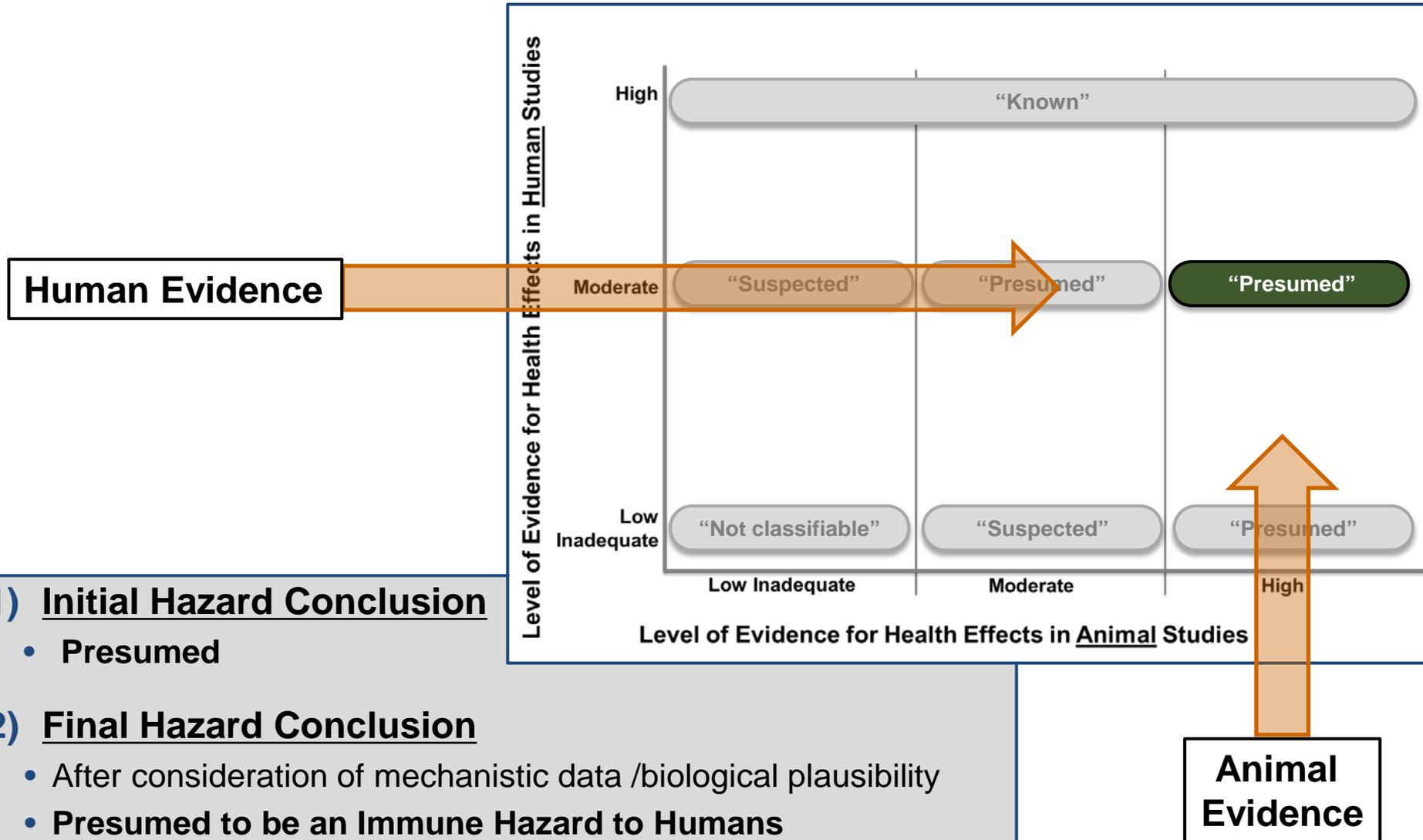
Antibody Response Evidence Profile for PFOS										
INITIAL CONFIDENCE each body of evidence (# of studies)	Factors decreasing confidence “---” if no concern; “↓” if serious concern to downgrade confidence					Factors increasing confidence “---” if not present; “↑” if sufficient to upgrade confidence				FINAL CONFIDENCE RATING
	Risk of Bias	Unexplained Inconsistency	Indirectness	Imprecision	Publication Bias	Large Magnitude	Dose Response	Residual Confounding	Consistency Species/Model	
PFOS										
<i>Human</i>										
Initial Moderate (4 prospective studies)	---	---	---	---	---	---	---	---	---	Moderate
Initial Low (2 cross-sectional studies)	---	---	---	---	---	---	---	---	---	Low
Confidence Across Human Bodies of Evidence	No change for considering across study designs									Moderate

- **Moderate confidence** that exposure to PFOS is associated with suppression of the antibody response in humans
- PFOS-associated suppression in one or more measure of anti-vaccine antibody response across multiple studies with prenatal, childhood, and adult exposures
- Heterogeneity in response may be attributed to different vaccines, measures
 - Limited ability to compare across studies (different vaccines, timing, antibody measures)
 - Strength of antibody response to different vaccines expected



Evidence Integration: Develop Hazard ID

PFOS: Antibody Response





Antibody response levels of evidence

- PFOS is presumed to be an immune hazard to humans based on:
 - **(1) PFOS suppressed the antibody response**
 - **Animal studies: High level of evidence**
 - Human studies: Moderate level of evidence
 - No change in conclusions after considering mechanistic data



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 - Human studies: Moderate level of evidence
 - **No change in conclusions after considering mechanistic data**



Questions?



- **Immunosuppression: Disease Resistance**
 - **Animal studies:** Moderate level of evidence based on single study of reduced resistance to influenza A virus, dose-response, risk of bias concerns (outcome assessor blinding, allocation, and researcher blinding)
 - **Human studies:** Low level of evidence due to inconsistent evidence and few specific diseases examined
 - No change in conclusions after considering mechanistic data

- **Immunosuppression: Natural Killer (NK) Cell Activity**
 - **Animal studies:** Moderate level of evidence based on consistent evidence for suppression of NK cell activity in mice but risk of bias concerns (outcome assessor blinding, allocation, and researcher blinding)
 - **Human studies:** Inadequate level of evidence (no studies)
 - No change in conclusions after considering mechanistic data



Questions?



Other Outcomes that Did Not Reach Hazard Conclusions

- Hypersensitivity-related Outcomes
 - **Animal studies:** Low level of evidence due to inconsistent evidence within a single study of airway hypersensitivity
 - **Human studies:** Very low level of evidence due to inconsistent evidence from several cross-sectional studies

- Autoimmunity-related Effects
 - **Animal studies:** Inadequate level of evidence (no studies)
 - **Human studies:** Inadequate level of evidence (single pilot study on autoantibodies to several neural antigens)



Action: NTP Conclusions for PFOS

Category of Immune Response	Immune Outcomes	Confidence Ratings in the Body of Evidence		Level of Evidence in the Body of Evidence		Hazard Conclusion
		Human	Animal	Human	Animal	
Immunosuppression	Antibody response	Moderate	High	Moderate	High	<u>Presumed</u> to be an Immune Hazard to Humans

PFOS is *presumed to be an immune hazard to humans* based on:

- Suppressed antibody response
 - **Animal studies:** High level of evidence
 - **Human studies:** Moderate level of evidence
 - No change in conclusions after considering mechanistic data



Thank you