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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• **Perfluoroalkyl acids including PFOA and PFOS**
  - Used extensively in commercial/industrial applications last 50 years
    - food packaging
    - lubricants
    - water-resistant coatings
    - 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

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Exposure to PFOA and PFOS

![PFOA and PFOS structures](images)
Why Evaluate PFOA, PFOS Immunotoxicity?

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

**Geometric mean serum concentrations (μg/L) for US population**

<table>
<thead>
<tr>
<th>Survey years</th>
<th>PFOA</th>
<th>PFOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999-2000</td>
<td>5.21 (4.72-5.74)</td>
<td>30.4 (27.1-33.9)</td>
</tr>
<tr>
<td>2005-2006</td>
<td>3.92 (3.48-4.42)</td>
<td>17.1 (16.0-18.2)</td>
</tr>
<tr>
<td>2011-2012</td>
<td>2.08 (1.95-2.22)</td>
<td>6.31 (5.84-6.82)</td>
</tr>
</tbody>
</table>

- 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/](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
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.
Factors Decreasing Confidence
- unexplained inconsistency
- risk of bias
- indirectness/applicability
- imprecision
- publication bias

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

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 and animal bodies of evidence

Steps to Integrate Evidence

<table>
<thead>
<tr>
<th>Experimental Animal</th>
<th>Initial Confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High (++++)</td>
</tr>
<tr>
<td></td>
<td>Moderate (+++)</td>
</tr>
<tr>
<td></td>
<td>Low (+)</td>
</tr>
<tr>
<td></td>
<td>Very Low (+)</td>
</tr>
</tbody>
</table>

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

Factors Decreasing Confidence
- unexplained inconsistency
- risk of bias
- indirectness/applicability
- imprecision
- publication bias
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

**Initial Confidence**

<table>
<thead>
<tr>
<th>Level</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>High (++++)</td>
<td>4 Features</td>
</tr>
<tr>
<td>Moderate (++)</td>
<td>3 Features</td>
</tr>
<tr>
<td>Low (++)</td>
<td>2 Features</td>
</tr>
<tr>
<td>Very Low (+)</td>
<td>1≤ Features</td>
</tr>
</tbody>
</table>

**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
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

<table>
<thead>
<tr>
<th>Confidence in the Body of Evidence</th>
<th>Direction (effect or no effect)</th>
<th>Level of Evidence for Health Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>(++++) High</td>
<td>Health effect</td>
<td>High</td>
</tr>
<tr>
<td>(+++) Moderate</td>
<td>Health effect</td>
<td>Moderate</td>
</tr>
<tr>
<td>(++) Low</td>
<td>Health effect</td>
<td>Low</td>
</tr>
<tr>
<td>(+) Very Low or No Evidence Identified</td>
<td>Health effect</td>
<td>Inadequate</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Animal description</th>
<th>Route</th>
<th>Exposure</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse, C57BL/6J (♂, N=8)</td>
<td>oral gavage</td>
<td>10 days</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Mouse, C57BL/6J (♂, N=8)</td>
<td>oral gavage</td>
<td>15 days</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Mouse, C57BL/6n (♀, N=8)</td>
<td>oral drinking water</td>
<td>15 days</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.75</td>
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<td></td>
<td></td>
<td></td>
<td>7.5</td>
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<td></td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Mouse, C57BL/6n (♀, N=8)</td>
<td>oral drinking water</td>
<td>15 days</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.94</td>
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<td>1.88</td>
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<td></td>
<td></td>
<td></td>
<td>7.5</td>
</tr>
<tr>
<td>Mouse, C57BL/6n (♀, N=6)</td>
<td>oral drinking water</td>
<td>10 days</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Mouse, C57BL/6n (♀, N=6)</td>
<td>oral drinking water</td>
<td>10 days</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.75</td>
</tr>
</tbody>
</table>

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

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

- **Definitely low risk of bias**
- **Probably low risk of bias**
- **Probably high risk of bias**
- **Definitely high risk of bias**
### Antibody Response Evidence Profile for PFOA

<table>
<thead>
<tr>
<th>INITIAL CONFIDENCE each body of evidence (# of studies)</th>
<th>Factors decreasing confidence “---” if no concern; “↓” if serious concern to downgrade confidence</th>
<th>Factors increasing confidence “---” if not present; “↑” if sufficient to upgrade confidence</th>
<th>FINAL CONFIDENCE RATING</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk of Bias</td>
<td>Unexplained Inconsistency</td>
<td>Indirectness</td>
<td>Imprecision</td>
</tr>
<tr>
<td>PFOA</td>
<td>Animal</td>
<td>Initial High (7 mammal studies)</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High</td>
</tr>
</tbody>
</table>

- **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

**Anti-vaccine antibodies**
- diphtheria
- measles
- mumps
- rubella
- tetanus

* Significantly different

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

<table>
<thead>
<tr>
<th>Study</th>
<th>Population Name</th>
<th>Outcome</th>
<th>Outcome Age</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grandjean 2012</td>
<td>Children of Faroe Islands National Hospital birth cohort (1997-2000)</td>
<td>anti-vaccine antibody levels: diphtheria (age 7 adjusted for age 5 results)</td>
<td>7 years</td>
<td>403</td>
</tr>
<tr>
<td></td>
<td></td>
<td>anti-vaccine antibody levels: tetanus (age 7 adjusted for age 5 results)</td>
<td>7 years</td>
<td>401</td>
</tr>
<tr>
<td>Stein 2015</td>
<td>Children 12-19 years of age from US in NHANES</td>
<td>anti-vaccine antibody levels: measles (seropositive)</td>
<td>12-19 years</td>
<td>1,152</td>
</tr>
<tr>
<td></td>
<td></td>
<td>anti-vaccine antibody levels: mumps (seropositive)</td>
<td>12-19 years</td>
<td>1,101</td>
</tr>
<tr>
<td></td>
<td></td>
<td>anti-vaccine antibody levels: rubella (seropositive)</td>
<td>12-19 years</td>
<td>1,148</td>
</tr>
</tbody>
</table>

Serum PFOA Association with Antibody Response

% Difference Antibody Concentration per 2-Fold Increase PFOA

- [Graph showing antibody response vs. PFOA levels]
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

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

- **Grøndahl 2012**: ++
- **Granum 2013**: ++
- **Kloeden 2015**: ++
- **Looke 2014**: ++
- **Stein 2015**: ++

Legend:

- **++**: Definitely low risk of bias
- **+**: Probably low risk of bias
- **-**: Probably high risk of bias
- **==**: Definitely high risk of bias
### Antibody Response Evidence Profile for PFOA

<table>
<thead>
<tr>
<th>INITIAL CONFIDENCE each body of evidence (# of studies)</th>
<th>Factors decreasing confidence “---” if no concern; “↓” if serious concern to downgrade confidence</th>
<th>Factors increasing confidence “---” if not present; “↑” if sufficient to upgrade confidence</th>
<th>FINAL CONFIDENCE RATING</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFOA</td>
<td>Risk of Bias</td>
<td>Unexplained Inconsistency</td>
<td>Indirectness</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial Moderate (4 prospective studies)</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Initial Low (2 cross-sectional studies)</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Confidence Across Human Bodies of Evidence</td>
<td>No change for considering across study designs</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **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.
1) Initial Hazard Conclusion  
   • Presumed

2) Final Hazard Conclusion  
   • After consideration of mechanistic data /biological plausibility  
   • Presumed to be an Immune Hazard to Humans

Evidence Integration: Develop Hazard ID

PFOA: Antibody Response

Level of Evidence for Health Effects in Human Studies

- High
- “Known”
- Moderate
  - “Suspected”
  - “Presumed”
- Low
  - “Not classifiable”
  - “Suspected”
  - “Presumed”

Level of Evidence for Health Effects in Animal Studies

- Low Inadequate
- Moderate
- High

Animal 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
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
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
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:
  - (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
• **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
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

- **Was administered dose or exposure level adequately randomized?**
  - Definitely low risk of bias
- **Was allocation to study groups adequately concealed?**
  - Definitely low risk of bias
- **Were experimental conditions identical across study groups?**
  - Definitely low risk of bias
- **Were research personnel blinded to the study group during the study?**
  - Definitely low risk of bias
- **Were outcome data complete without attrition or exclusion from analysis?**
  - Definitely low risk of bias
- **Can we be confident in the exposure characterization?**
  - Definitely low risk of bias
- **Can we be confident in the outcome assessment?**
  - Definitely low risk of bias
- **Were all measured outcomes reported?**
  - Definitely low risk of bias
- **Were there no other potential threats to internal validity?**
  - Definitely low risk of bias
PFOA: Hypersensitivity-related Outcomes

Hypersensitivity-related Outcomes Evidence Profile for PFOA

<table>
<thead>
<tr>
<th>INITIAL CONFIDENCE each body of evidence (# of studies)</th>
<th>Factors decreasing confidence “---” if no concern; “↓” if serious concern to downgrade confidence</th>
<th>Factors increasing confidence “---” if not present; “↑” if sufficient to upgrade confidence</th>
<th>FINAL CONFIDENCE RATING</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk of Bias</td>
<td>Unexplained Inconsistency</td>
<td>Indirectness</td>
<td>Imprecision</td>
</tr>
<tr>
<td>Animal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial High (7 mammal studies)</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

- **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
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

- Did selection of study participants result in the appropriate comparison groups?
  - + + + +

- Did study design or analysis account for important confounding and modifying variables?
  - - - -

- Were outcome data complete without attrition or exclusion from analysis?
  - ++ + ++ ++

- Can we be confident in the exposure characterization?
  - ++ - ++ ++

- Can we be confident in the outcome assessment?
  - + + + +

- Were all measured outcomes reported?
  - ++ ++ ++ ++

- Were there no other potential threats to internal validity?
  - ++ ++ ++ +
## PFOA: Hypersensitivity-related Outcomes

### Hypersensitivity-related Outcomes Evidence Profile for PFOA

<table>
<thead>
<tr>
<th>INITIAL CONFIDENCE</th>
<th>Factors decreasing confidence</th>
<th>Factors increasing confidence</th>
<th>FINAL CONFIDENCE RATING</th>
</tr>
</thead>
<tbody>
<tr>
<td>each body of evidence (# of studies)</td>
<td>“---” if no concern; “↓” if serious concern to downgrade confidence</td>
<td>“---” if not present; “↑” if sufficient to upgrade confidence</td>
<td></td>
</tr>
<tr>
<td>Risk of Bias</td>
<td>Unexplained Inconsistency</td>
<td>Indirectness</td>
<td>Imprecision</td>
</tr>
<tr>
<td>Low</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

- **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

1) Initial Hazard Conclusion
   - Presumed

2) Final Hazard Conclusion
   - After consideration of mechanistic data /biological plausibility
   - Presumed to be an Immune Hazard to Humans
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 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 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
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?
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**

<table>
<thead>
<tr>
<th>Diagnostic</th>
<th>Experimental Conditions</th>
<th>Animal description</th>
<th>Route</th>
<th>Exposure</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>Dose-response</td>
<td>Mouse, C57BL/6 (♂, N=9)</td>
<td>oral gavage</td>
<td>60 days</td>
<td>0.008</td>
</tr>
<tr>
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<td></td>
<td>0.017</td>
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<td>0.033</td>
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<td>0.417</td>
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<td></td>
<td></td>
<td></td>
<td>0.833</td>
</tr>
<tr>
<td>ELISA</td>
<td>In utero exposure - F1</td>
<td>F1 Mouse, B6C3F1 (♂, N=9)</td>
<td>oral gavage</td>
<td>GD 1 until GD 17</td>
<td>0</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>0.1</td>
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<tr>
<td>ELISA</td>
<td>In utero exposure - F1</td>
<td>F1 Mouse, B6C3F1 (♂, N=9)</td>
<td>oral gavage</td>
<td>GD 1 until GD 17</td>
<td>0</td>
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<td>5</td>
</tr>
<tr>
<td>ELISA</td>
<td>Dose-response - 2</td>
<td>Mouse, B6C3F1 (♂, N=9-10)</td>
<td>oral gavage</td>
<td>21 days</td>
<td>0.334</td>
</tr>
<tr>
<td>ELISA</td>
<td>Single dose-100</td>
<td>Mouse, B6C3F1 (♂, N=5)</td>
<td>oral diet</td>
<td>21 days</td>
<td>0.25</td>
</tr>
<tr>
<td>ELISA</td>
<td>Single dose-100</td>
<td>Mouse, B6C3F1 (♂, N=5)</td>
<td>oral diet</td>
<td>21 days</td>
<td>0.25</td>
</tr>
<tr>
<td>ELISA</td>
<td>Single dose-100</td>
<td>Mouse, BALB/c (♂, N=15)</td>
<td>oral gavage</td>
<td>3 weeks</td>
<td>0</td>
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<td>20</td>
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<tr>
<td>PFC</td>
<td>Dose-response</td>
<td>Mouse, C57BL/6 (♂, N=10)</td>
<td>oral gavage</td>
<td>60 days</td>
<td>0.008</td>
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<td></td>
<td>0.833</td>
</tr>
<tr>
<td>PFC</td>
<td>Dose-response - 1</td>
<td>Mouse, B6C3F1 (♂, N=5)</td>
<td>oral gavage</td>
<td>21 days</td>
<td>0</td>
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<td>0.00168</td>
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<td>0.017</td>
</tr>
</tbody>
</table>

**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

- **Was administered dose or exposure level adequately randomized?**
- **Was allocation to study groups adequately concealed?**
- **Were experimental conditions identical across study groups?**
- **Were research personnel blinded to the study group during the study?**
- **Were outcome data complete without attrition or exclusion from analysis?**
- **Can we be confident in the exposure characterization?**
- **Can we be confident in the outcome assessment?**
- **Were all measured outcomes reported?**
- **Were there no other potential threats to internal validity?**

- **Definitely low risk of bias**
- **Probably low risk of bias**
- **Probably high risk of bias**
- **Definitely high risk of bias**
### Antibody Response Evidence Profile for PFOS

<table>
<thead>
<tr>
<th>INITIAL CONFIDENCE</th>
<th>Factors decreasing confidence</th>
<th>Factors increasing confidence</th>
<th>FINAL CONFIDENCE RATING</th>
</tr>
</thead>
<tbody>
<tr>
<td>each body of evidence (# of studies)</td>
<td>“---” if no concern; “↓” if serious concern to downgrade confidence</td>
<td>“---” if not present; “↑” if sufficient to upgrade confidence</td>
<td>High</td>
</tr>
<tr>
<td>Risk of Bias</td>
<td>Unexplained Inconsistency</td>
<td>Indirectness</td>
<td>Imprecision</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>PFOS Animal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial High (8 mammal studies)</td>
<td>↓</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

- **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
**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**

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Population Name</th>
<th>Outcome</th>
<th>Outcome Age</th>
<th>Exposure Measure</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grandjean 2012</td>
<td>Cohort (Prospective)</td>
<td>Children of Ferox Islands National Hospital birth cohort (1997-2000)</td>
<td>anti-vaccine antibody levels: diphtheria (age 7 years adjusted for age 5 results)</td>
<td>7 years</td>
<td>child serum PFOS</td>
<td>403</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>anti-vaccine antibody levels: diphtheria (age 7)</td>
<td>7 years</td>
<td>child serum PFOS</td>
<td>408</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>anti-vaccine antibody levels: mumps (age 7)</td>
<td>7 years</td>
<td>child serum PFOS</td>
<td>408</td>
</tr>
<tr>
<td>Stein 2015</td>
<td>Cross-sectional</td>
<td>Children 12-19 years of age from US in NHANES</td>
<td>anti-vaccine antibody levels: measles (seropositive)</td>
<td>12-19 years</td>
<td>child serum PFOS</td>
<td>1,152</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>anti-vaccine antibody levels: mumps (seropositive)</td>
<td>12-19 years</td>
<td>child serum PFOS</td>
<td>1,101</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>anti-vaccine antibody levels: rubella (seropositive)</td>
<td>12-19 years</td>
<td>child serum PFOS</td>
<td>1,148</td>
</tr>
</tbody>
</table>

* Significantly different

**Anti-vaccine antibodies**
- □ diphtheria
- ▼ measles
- ◆ mumps
- ▲ rubella
- ○ tetanus
PFOS: Antibody Response

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 PFOS 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](image)
## PFOS: Antibody Response

### Antibody Response Evidence Profile for PFOS

<table>
<thead>
<tr>
<th>INITIAL CONFIDENCE each body of evidence (# of studies)</th>
<th>Risk of Bias</th>
<th>Unexplained Inconsistency</th>
<th>Indirectness</th>
<th>Imprecision</th>
<th>Publication Bias</th>
<th>Large Magnitude</th>
<th>Dose Response</th>
<th>Residual Confounding</th>
<th>Consistency Species/Model</th>
<th>FINAL CONFIDENCE RATING</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PFOS</strong></td>
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<td></td>
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</tr>
<tr>
<td><strong>Human</strong></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial Moderate (4 prospective studies)</td>
<td>---</td>
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<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>Moderate</td>
</tr>
<tr>
<td>Initial Low (2 cross-sectional studies)</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
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<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>Low</td>
</tr>
<tr>
<td>Confidence Across Human Bodies of Evidence</td>
<td>No change for considering across study designs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Moderate</td>
</tr>
</tbody>
</table>

- **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.
1) Initial Hazard Conclusion
   • Presumed

2) Final Hazard Conclusion
   • After consideration of mechanistic data / biological plausibility
   • Presumed to be an Immune Hazard to Humans
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 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
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
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)
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

### Table 9. PFOS Main Immune Effects Summary Table

<table>
<thead>
<tr>
<th>Category of Immune Response</th>
<th>Immune Outcomes</th>
<th>Confidence Ratings in the Body of Evidence</th>
<th>Level of Evidence in the Body of Evidence</th>
<th>Hazard Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Human</td>
<td>Animal</td>
<td>Human</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>Antibody response</td>
<td>Moderate</td>
<td>High</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

**Action: NTP Conclusions for PFOS**
Thank you