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

Peer Review Report

Peer Review of the Draft NTP Monograph on Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid (PFOA) or Perfluorooctane Sulfonate (PFOS)

July 19, 2016

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I. Attendees

Peer Review Panel

Weihsueh Chiu, Texas A&M University (Chair)
Joseph Braun, Brown University (via WebEx)
Emanuela Corsini, Università degli Studi di Milano (via WebEx)
Berit Granum, Norwegian Institute of Public Health (via WebEx)
Deborah Keil, Montana State University (via WebEx)
Michael Woolhiser, The Dow Chemical Company (via WebEx)

NTP Board of Scientific Counselors Liaison

Paul Brandt-Rauf, Columbia University (via Webcast)

National Institute of Environmental Health Sciences (NIEHS) Staff

Chad Blystone
Windy Boyd
Abee Boyles
John Bucher
Sue Fenton
Dori Germolec
Jean Harry
Kembra Howdeshell
Gloria Jahnke
Beruk Kiros
Grace Kissling
Kelly Lenox
Ruth Lunn
Robin Mackar
Katie Pelch
Andrew Rooney
Andy Shapiro
Kris Thayer
Nigel Walker
Vicki Walker
Mary Wolfe
Yun Xie (Designated Federal Official)

NIEHS Contract Support Staff

Rachel McIntosh-Kastrinsky, Kelly Services

Registered Public Attendee

Glenn Simon, Solvay USA

Public Attendee

Bevin Blake, University of North Carolina at Chapel Hill
Ernie Hood, Bridport Services, LLC
Elena Perkins, Yale University
Lior Vered, University of North Carolina at Chapel Hill

Webcast

Attendance was available via webcast. There were 68 total unique connections to the webcast during the meeting.

II. Welcome and Introductions

The National Toxicology Program (NTP) convened a peer review meeting for the draft NTP monograph on *Systematic Review of Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid (PFOA) or Perfluorooctane Sulfonate (PFOS)* on July 19, 2016, in Rodbell Auditorium, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. Weihsueh Chiu served as chair in person. The other peer review panel members, who attended via WebEx, were Drs. Joseph Braun, Emanuela Corsini, Berit Granum, Deborah Keil, and Michael Woolhiser. Dr. Paul Brandt-Rauf attended via webcast as the NTP Board of Scientific Counselors liaison and did not participate in the discussion.

Dr. Chiu and NTP Associate Director Dr. John Bucher welcomed everyone to the meeting. Dr. Chiu asked all attendees to introduce themselves for the record. Designated Federal Official Dr. Yun Xie, Office of Liaison, Policy, and Review, read the conflict of interest policy statement. Dr. Chiu briefly summarized the meeting format.

III. Introduction to the Draft NTP Monograph

Project leader Dr. Andrew Rooney, Deputy Director of the Office of Health Assessment and Translation (OHAT), presented an introduction to the draft NTP monograph, providing background information about PFOA and PFOS and the rationale for NTP having undertaken a systematic review of the literature associated with these compounds, with a focus on potential immunotoxicity associated with exposure.

PFOA and PFOS are perfluoroalkyl acids that have been used for commercial and industrial applications for over 50 years. Exposure to PFOA and PFOS is widespread. Although they are no longer in commercial production in the United States, they are very persistent in the environment. PFOA and PFOS are the most commonly detected perfluoroalkyl acids in environment and human serum. PFOA and PFOS have been reported to have immune effects in animals and humans. Previously, a portion of PFOA and PFOS immunotoxicity datasets were used as a case study, when the OHAT Approach to Systematic Review and Evidence Integration was being developed. Upon receiving multiple requests to complete the case study, NTP conducted a full review and produced this draft monograph.

Dr. Rooney briefly summarized the reported immune effects of PFOA and PFOS in studies in animals and in humans. The objective of the systematic review was to develop NTP hazard identification conclusions on the association between PFOA and 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. Dr. Rooney described the methods used in the

evaluation and the steps employed to integrate the evidence. The systematic review protocol was peer reviewed by subject matter experts and posted before completing the evaluation. Ultimately, an initial hazard conclusion was developed by considering the human and animal levels of evidence, followed by a final hazard conclusion that accounted for the impact of mechanistic data and biological plausibility.

Dr. Rooney stated the peer review panel's charge:

- 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 and PFOS.

Dr. Corsini asked how written public comments would be addressed. Dr. Bucher noted that public comments are provided to the panel for their consideration and deliberation during the peer review meeting. For example, panel members may raise questions from the written public comments at the meeting for NTP's response.

Dr. Woolhiser asked how factors were considered for the overall evidence. He thought that not all factors were considered equal. Positive and negative factors sometimes canceled each other out in the process of evaluating confidence in the body of evidence, when in fact the negative factor questioned the data and completely negated the positive one. Dr. Rooney said that all of the factors that could increase or decrease confidence in the body of evidence were considered in developing the confidence ratings for each body of evidence. Although these factors are considered separately, sometimes the issues could be addressed in more than one factor. NTP then considers how to address the issue without double counting. For some factors, such as the risk of bias considerations, a conservative approach was taken.

IV. Public Comments

NTP received seven written public comments on the draft monograph. Dr. Chiu noted that the comments were distributed to the panel, and the panelists were asked to consider written public comments in preparing for the peer review meeting. The written public comments were also posted to the meeting Web page and distributed to NTP staff working on this project. Dr. Chiu acknowledged the following seven written comments:

1. Dr. Avery August on behalf of the 3M Company
2. Dr. Ellen Chang of Exponent, Inc. on behalf of the 3M Company
3. Gradient on behalf of the 3M Company

4. Dr. Carol Kwiatkowski and Ms. Ashley L. Bolden of the Endocrine Disruption Exchange
5. Dr. Carol Ley and Dr. Sue Chang of the 3M Company
6. Dr. Michael Osterholm of the University of Minnesota
7. Dr. John Newsted of Natural Resource Technology, Inc., on behalf of the 3M Company

No one registered to provide oral public comments, and no one asked to provide ad hoc oral public comments during the meeting.

V. PFOA

Dr. Rooney began the presentation on PFOA by outlining the draft NTP conclusions on PFOA immunotoxicity:

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

A. Antibody Response

1. Presentation

Dr. Rooney presented the animal data related to antibody response from PFOA exposure. He noted there were seven experimental studies in mammals. There was consistent suppression of the primary antibody response (Immunoglobulin M) in mice in these studies. He delineated the risk of bias considerations associated with the studies, along with the evidence profile depicting initial confidence and factors that decreased or increased confidence in the body of evidence. The evidence profile reflected high initial confidence, decreased confidence for risk of bias concerns and increased confidence for evidence of a dose response. Ultimately, there was high confidence that exposure to PFOA is associated with suppression of the antibody response based on animal studies.

For human data related to antibody response from PFOA exposure, Dr. Rooney described four prospective and two cross-sectional studies in humans. There was

suppression in one or more measures of anti-vaccine antibody response associated with prenatal, childhood, or adult exposures to PFOA. He showed the risk of bias considerations associated with the studies, along with the evidence profile depicting the moderate initial confidence rating and factors decreasing or increasing confidence. There were no changes for factors that may decrease or increase confidence in the body of evidence. There was moderate confidence that exposure to PFOA is associated with suppression of the antibody response in humans.

Dr. Rooney showed the integration of the evidence regarding the antibody response. In the initial hazard conclusion, PFOA was presumed to be an immune hazard to humans. The final hazard conclusion, after consideration of mechanistic data and biological plausibility, was that **PFOA is presumed to be an immune hazard to humans.**

2. Questions for Clarification

Dr. Chiu asked about the distinction between "key questions" and "other questions" in the risk of bias assessment. Dr. Rooney said that the key questions (randomization, confidence in the outcome assessment, and confidence in the exposure characterization) are risk of bias questions considered to have more potential impact on the magnitude or direction of the results based on empirical evidence. Other risk of bias questions were considered separately. The distinction is presented in the protocol and monograph. Overall consideration of risk of bias for the body of evidence was considered in potentially downgrading the level of evidence.

Dr. Woolhiser questioned how much weight the different factors carried in arriving at the hazard conclusion. From his perspective, the risk of bias, confounding circumstances, the heterogeneity of some of the data, and understanding the lack of exposure knowledge would lower the degree of confidence in the evidence to be below moderate. Dr. Chiu said that these concerns would be addressed as part of the discussions of specific data sets such as the human studies.

3. Animal Studies – Peer Review Comments and Panel Discussion

Dr. Keil, the first reviewer, stated that the approach as described in the monograph was objectively applied to interpreting the antibody response data. She agreed with the conclusions and interpretation of the data, and with the level of evidence conclusion. She added that it would be important to emphasize that there were no changes in body weight reported, indicating no evidence of overt or systemic toxicity at the low doses where Immunoglobulin M (IgM) suppression was noted. She concluded that the lack of evidence of overt or systemic toxicity adds strength to the interpretation of the data.

Dr. Woolhiser, the second reviewer, agreed that the evidence of no overt toxicity is important to note. He stated that the summaries of the approach, methods, and results

in animals were clear and objectively presented. He agreed with the antibody response results, the degree of confidence, and level of evidence conclusion.

Dr. Rooney thanked the reviewers for their comments, and agreed to look for an opportunity to add clarification to the monograph on the changes in body weight and overt toxicity.

Dr. Corsini asked about the lack of data regarding Immunoglobulin G (IgG) response in the draft monograph. She noted that one of the main comments from the public was that the animal studies focused on IgM and the primary immune response, while the human studies focused on IgG and the secondary immune response. She was more concerned with the effects of PFOA on the secondary immune response, because it is more difficult to suppress. Dr. Rooney said that most of the studies considered in the body of evidence focused on the IgM response. The studies that considered IgG response looked at primary IgG response and are less relevant to IgG levels in humans, which reflect secondary response. There were fewer studies that reported IgG data; thus, making conclusions based on IgG is more difficult. He agreed with Dr. Corsini that IgG data might lead to stronger conclusions because the IgG immune response is more difficult to suppress. The guideline studies suggested focusing on IgM response, as it is a good indicator of immunotoxicity. He concluded that while additional studies could result in more informative data, the data available from IgM studies support evidence of suppression of the primary immune response.

Dr. Chiu asked if the IgG response would be considered more severe. Dr. Rooney said that it would and reiterated that the primary IgM response is considered adverse and applicable to the human condition. Dr. Keil mentioned Dr. Michael Luster's 1993 work on risk assessment in immunotoxicology and his paper on sensitivity and predictability of immune tests, because these publications identify the strength of the IgM assay.

Dr. Chiu asked the panel for comments on weighing the results from papers in the draft monograph, noting that some of the written public comments had contended that the downgrade should outweigh the potential upgrade. Dr. Braun said that the considerations are not a binary yes or no decision; there is a continuum of bias. He said that any confounding factor would have to be sufficient to obliterate a relative risk; thus, it would need to be a very strong confounder in the case of a high relative risk. It would be necessary to judge the potential bias posed by confounders against the exposure and response relationship. Dr. Rooney said that within the OHAT method, there is also the ability to exclude a study in the case of severe risk of bias concerns; however, that was not the case for the vast majority of studies in this evaluation.

Dr. Chiu asked the panel members to consider in their final vote the use of downgrading for risk of bias and subsequent upgrading for dose response, and whether NTP properly balanced these considerations in their evaluation.

4. Action: Panel Vote on Antibody Response, Animal Studies

Dr. Keil moved to accept the conclusion of a high level of evidence for antibody response from animal studies as written. Dr. Corsini seconded. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to pass the motion.[1]

5. Human Studies – Peer Review Comments and Panel Discussion

Dr. Braun, the first reviewer, said the authors had applied the methods objectively and clearly. He reiterated his concerns about the balance of upgrading and downgrading confounding factors versus relative risk. He added comments about how the cumulative effects from other perfluorinated substances may confound interpretation of the dose response. He asked if any of the studies had compared their unadjusted and confounder-adjusted results to see if they differed. He noted that the cumulative effects of exposure should be considered because the effects of PFOA and PFOS do not occur in isolation. Given the recent U.S. Environmental Protection Agency (EPA) drinking water health advisory on both PFOA and PFOS, the combined concentrations should be discussed. He mentioned the potential for negative confounding, particularly with regard to breastfeeding, which is a major determinant of exposure to perfluorinated substances in children, and likely to be immune-protective. The studies considered in the monograph had not adjusted for breastfeeding, which could be a negative confounder and cause something to be missed in the studies. Responding to a question from Dr. Corsini, Dr. Braun elaborated on his point about breastfeeding, citing a study showing that breastfeeding in the first three years of life was a major determinant of perfluorinated substance exposure and continued to be a determinant until later in life. Dr. Granum noted the citation of a paper that had adjusted for breastfeeding and that her group had a paper accepted showing that breastfeeding is an important determinant of PFOA and PFOS levels in three-year-old children.

Dr. Granum, the second reviewer, agreed with the conclusion of a moderate level of evidence. She proposed that the conclusions were supported because several of the shared immunosuppression findings point to the same direction for the response. She mentioned that even though some of the decrease in antibody response was subclinical and might not have a true adverse effect on an individual level, on a population level such a reduction is an important marker of immunosuppression.

Dr. Rooney agreed with Dr. Granum's comments. He noted several studies in the monograph where the authors examined potential confounders with and without

[1] The chair only votes in the event of a tie.

adjustment. He said it was challenging to assess cumulative exposures, including what exposure timing (developmental, childhood, recent) would be most important for the antibody response. There was an attempt in the Grandjean study to adjust for the other perfluorinated compounds. While such adjustment decreased the significance of individual exposures, there was still a significant association for PFOA and suppression of the antibody response.

Dr. Chiu added that the need was not necessarily to achieve statistical significance. The need may be to examine changes in magnitude. Dr. Braun agreed that it would be important to focus on the change in magnitude. He said that disentangling the importance of various windows of exposure would be difficult, because of the high correlation of perfluorinated compound levels between a mother and her offspring.

Dr. Woolhiser questioned the degree of evidence combined with the risk of bias and whether it should be moderate or low for human antibody response. Dr. Chiu said it was his understanding that the initial rating was based simply on study design, with the various factors then used to upgrade or downgrade confidence. Dr. Chiu suggested it might be helpful to look at the OHAT framework to see how the demarcations of low, moderate, or high are described. Dr. Bucher noted that the framework is mainly a mechanism to allow transparency about how decisions are reached; scientific judgment remains the key to judging the datasets. Dr. Braun was not concerned about confounding raising a risk of declaring a false positive; he was worried about potential biases that could lead judgment astray (i.e., a false negative), such as exposure misclassification, poor assessment, or noise in the outcome measurements.

Dr. Chiu summarized the public comments and concluded that the panel discussed all of the comments.

6. Action: Panel Vote on Antibody Response, Human Studies

Dr. Corsini moved to accept the draft conclusion of a moderate level of evidence for antibody response from human studies as written. Dr. Braun seconded. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to pass the motion.

7. Mechanistic Studies – Peer Review Comments and Panel Discussion

Dr. Corsini, the lead reviewer, remarked that the NTP did a great job in presenting all of the evidence. There are no specific *in vitro* studies on the effects of PFOA and PFOS on antibody production. The *in vitro* studies show that PFOA and PFOS affect the immune system and can therefore be considered immunotoxic. She noted that the mechanisms of action of PFOA and PFOS are not completely unknown. The only element that could support an effect on antibody response would be decreasing cytokine production by T cells, which may be more relevant to PFOS than to PFOA. For *in vitro* studies, high concentrations are needed, perhaps making those results only relevant for occupational

settings. She agreed that the current data neither support nor contradict the conclusion regarding the mechanistic studies. Dr. Rooney said NTP could more explicitly describe in the monograph that there is incomplete information regarding mechanisms.

Dr. Woolhiser observed a gap in understanding of the most likely cell types or mechanism involved. He asked if there was something missing from the monograph or whether there is an overall interpretation that could be added, or whether collectively the field does not have a good understanding of the mechanism.

Dr. Corsini reiterated that overall there is not a complete understanding, because most *in vitro* studies have had different endpoints and did not directly address *in vivo* effects of exposure. She agreed that there is a gap in understanding of mechanisms.

Dr. Keil noted that her group has preliminary mechanistic data on PFOS, which shows that PFOS suppression of the antibody response is not PPAR-α dependent. Dr. Corsini said that data support the evidence of PPAR-α activation by PFOA at high *in vitro* doses in humans.

8. Action: Panel Vote on Antibody Response, Mechanistic Studies

Dr. Woolhiser moved to accept the draft conclusion for antibody response from mechanistic studies as written. Dr. Keil seconded. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to pass the motion.

B. Hypersensitivity-Related Outcomes

1. Presentation

Dr. Rooney presented the background information for PFOA and hypersensitivity-related outcomes. There were three experimental studies in mammals; two were studies of airway outcomes. The animal data showed increased hypersensitivity-related outcomes across multiple measures. Dr. Rooney went over the risk of bias considerations, including the key questions and other questions involved. The initial confidence rating in the body of evidence was high based on the experimental animal studies. There were no changes for factors that may decrease or increase confidence in the body of evidence. The evidence profile presented the conclusion that there is high confidence that exposure to PFOA is associated with increased hypersensitivity-related outcomes in animals.

Human data encompassed two cross-sectional studies based on National Health and Nutrition Examination Survey (NHANES) data in children aged 12 to 19, and two publications from a case-control asthma study of children aged 10 to 15 in Taiwan. Dr. Rooney summarized the risk of bias considerations applied to the studies. There was low initial confidence in the body of evidence based on the cross-sectional studies. There were no changes for factors that may decrease or increase confidence in the

body of evidence. The evidence profile showed the conclusion that there is low confidence that exposure to PFOA is associated with increased hypersensitivity in humans.

Dr. Rooney presented the integration of the evidence regarding hypersensitivity-related outcomes leading to hazard identification. In the initial hazard conclusion, PFOA was presumed to be an immune hazard to humans. The final hazard conclusion, after consideration of mechanistic data and biological plausibility, was that **PFOA is presumed to be an immune hazard to humans.**

2. Questions for Clarification

Dr. Woolhiser asked if the process for considering mechanistic data and biological plausibility was the same for hypersensitivity as it had been for antibody response. Dr. Rooney said all of the relevant data received due consideration.

Dr. Woolhiser asked Dr. Rooney to more specifically define "hypersensitivity-related outcomes." Dr. Rooney said there was no evidence that PFOA is a specific sensitizer (i.e., there was no evidence for PFOA-specific anti-Immunoglobulin E). Exposure to the compound influenced the response to other antigens or contributed to an overall hyper reactivity response, so PFOA is more of an immunomodulator. The evidence suggests an increase in a range of hypersensitivity-related outcomes. Dr. Woolhiser asked if any thought had been given to the reaction being a divergent response from IgM primary immune suppression. Dr. Corsini mentioned *in vitro* data that showed a pro-inflammatory response in mast cells, as well as other evidence suggesting that PFOA is not a sensitizer and has immunomodulatory effects. Dr. Rooney said that it is unlikely that there is a single effect on the immune system. The data support multiple effects that can be considered as immunomodulatory. PFOA may increase hypersensitivity while suppressing the specific antibody response.

3. Animal Studies – Peer Review Comments and Panel Discussion

Dr. Woolhiser, the first reviewer, said the evidence is limited. The immunomodulation appears to take place via a nontraditional mechanism. He questioned the conclusion of a high level of evidence, based on the limited number of studies and the divergent response seen in these studies. He instead suggested the data support the conclusion of a moderate level of evidence.

Dr. Keil, the second reviewer, also questioned the high level of evidence because it is based on only two studies. She felt that the OHAT approach was objectively applied; however, she did support a moderate level of evidence conclusion.

Dr. Rooney asked the reviewers to reiterate the basis for their conclusion of moderate evidence, noting that conclusions are not based on counting the number of studies. Dr.

Woolhiser said he focused on the protocol, where levels of evidence may be upgraded or downgraded, as opposed to looking at the evidence holistically using an overall interpretation. In looking at the three studies, based on the low number of studies and taking into consideration the hypersensitivity properties of the molecule, he proposed there is insufficient evidence to support a high degree of evidence that PFOA exposure results in hypersensitivity-related effects. Dr. Keil was uncomfortable with the word "known" to support a high level of evidence. Dr. Rooney explained that the level of evidence conclusion should be approached separately from the conclusion of "known." He described each of the three studies involved and how the evidence of hypersensitivity in each study supports the conclusion of immunomodulation. He concluded that all studies are consistent with one another and with a conclusion of immunomodulation.

Dr. Corsini agreed that there is insufficient evidence to support a high level of evidence conclusion due to the limited number of studies. She supported a downgrade to a moderate level of evidence. Dr. Braun noted that with the limited studies available, he was comfortable with a moderate level of evidence, particularly given the possibility of publication bias. Dr. Granum agreed that the conclusion should be lowered to moderate level of evidence.

Dr. Keil asked if a change in the title from "hypersensitivity-related outcomes" to "airway hyperactivity" might address some of the panel members' concerns and lead to retaining the "high level of evidence" conclusion. Dr. Woolhiser said that would not change his impression of moderate versus high level of evidence because of the low number of studies and the divergent response seen in the studies.

4. Action: Panel Vote on Hypersensitivity-Related Outcomes, Animal Studies

Dr. Woolhiser moved to modify the conclusion from "high level of evidence" to "moderate level of evidence" for hypersensitivity-related outcomes from animal studies. Dr. Corsini seconded. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to pass the motion.

5. Human Studies – Peer Review Comments and Panel Discussion

Dr. Granum, the first reviewer, noted that several triggers, including infection, could cause wheezing and asthma; therefore, immunosuppression leading to increased risk of airway infections may in turn trigger wheeze or asthma. Thus, it is possible that immunosuppression may have caused the increased risk of asthma identified in some of the cross-sectional studies cited in the draft monograph. She proposed that the other outcomes did not have a pattern. Overall, she supported the low level of evidence conclusion for human studies.

Dr. Braun, the second reviewer, agreed that the data are inconsistent. He noted as stated in the monograph that the studies are cross-sectional and no high-quality prospective studies have been conducted. He noted that NTP applied the methods objectively and clearly. He mentioned that the participants in the C8 studies were plaintiffs in a class-action lawsuit, which presents a potential source of bias. He said that there is just one mention of that potential bias in the draft monograph and that it should be noted in other sections of the monograph. He had the same comments about confounders as his comments in the antibody response discussion.

Dr. Rooney agreed that the cross-sectional nature of the studies is one of the limitations. He said that the C8 study reference is where it is in the draft monograph because the outcomes for asthma are self-reported and not based on objective measurements as in the antibody response data discussed earlier. Additionally, the potential bias as plaintiffs/participants in the C8 study is more important in the related section than for objective antibody measurements discussed previously.

Dr. Chiu mentioned that the panel addressed all the issues raised in the public comments.

6. Action: Panel Vote on Hypersensitivity-Related Outcomes, Human Studies

Dr. Braun moved to accept the draft conclusion of low level of evidence for hypersensitivity-related outcomes from human studies as written. Dr. Granum seconded.

Dr. Woolhiser asked about a note in the monograph regarding risk of bias in the human hypersensitivity studies. He wondered if there were other confounding factors in some of the studies, such as family history, that could impact whether or not the observation is relevant. Dr. Braun did not believe that family history was explored in the NHANES (Humblet) study. Dr. Woolhiser noted genetic background could have an impact on hypersensitivity and allergy; however, he did not have an issue with the “low level of evidence” conclusion. Dr. Rooney added that although several factors were considered in the Humblet study, family history was not one of them. Dr. Braun said he was more concerned about the potential influence of socioeconomic factors stemming from family history than the genetic influence. He added that the race confounder could capture some of this.

The panel voted unanimously (5 yes, 0 no, 0 abstentions) to pass the motion.

7. Mechanistic Studies – Peer Review Comments and Panel Discussion

Dr. Corsini, the lead reviewer, said there is no consistent evidence that can either support or explain the *in vivo* evidence. The other concern is that it is difficult to determine which concentrations of PFOA used in *in vitro* studies would be relevant for

exposure in children. Ultimately, she concluded that the mechanistic data presented did not support changing the conclusions that had been reached.

8. Action: Panel Vote on Hypersensitivity-Related Outcomes, Mechanistic Studies
Dr. Woolhiser moved to accept the draft conclusion for hypersensitivity-related outcomes from mechanistic studies as written. Dr. Keil seconded.

The panel voted unanimously (5 yes, 0 no, 0 abstentions) to pass the motion.

C. Other Outcomes that Did Not Reach Hazard Conclusions

1. Presentation
Dr. Rooney presented the outcomes that had been considered and did not contribute to the hazard identification conclusions. They include human and animal studies of immunosuppression outcomes (disease resistance and natural killer (NK) cell activity) and autoimmunity-related effects. For disease resistance, the animal studies had an inadequate level of evidence, with only wildlife studies and no experimental studies. The human studies had a low level of evidence, due to a lack of consistency. For NK cell activity, both animal and human studies had inadequate level of evidence because there were no human studies and only a single animal study that tested a single dose level. For autoimmunity-related effects, the animal studies had an inadequate level of evidence because there were no studies. Human studies had a low level of evidence because both studies were from the same population. For all of these bodies of evidence, the conclusion was lower than for the antibody response and hypersensitivity-related outcomes; therefore, they did not contribute to the hazard conclusion.

2. Other Outcomes – Panel Discussion
Dr. Woolhiser noted that some of the text on Dr. Rooney's slide was not consistent with that presented in the monograph. Dr. Rooney said he would double-check for inconsistencies.

Dr. Chiu asked the other panel members if they had comments. Drs. Braun, Corsini, Keil, and Granum said they agreed with the conclusions and interpretation written in the Other Outcomes section of the draft monograph.

D. PFOA Overall Conclusions

1. Conclusions
Dr. Chiu called for the revised conclusions to be projected, based on the peer review panel's proceedings. The revised conclusion, with prior reference to hypersensitivity-related outcomes deleted, was:

> PFOA is **presumed to be an immune hazard** to humans based on the following:
> A. Suppressed antibody response.

I. Animal studies: High level of evidence
II. Human studies: Moderate level of evidence
III. No change in conclusions after considering mechanistic data

2. Panel Discussion

Dr. Woolhiser asked how, given the changes that were made, the conclusions still added up to "presumed" rather than "suspected." He suggested this did not follow the previously shown matrix for reaching the conclusion. Dr. Rooney replied that the conclusions still followed the matrix. Before the change, there were two lines of evidence supporting the conclusion of "presumed to be an immune hazard": the antibody response data and the hypersensitivity data. He noted that the antibody response data alone would support the conclusions. With the change in the animal studies from "high" to "moderate," the hypersensitivity evidence would now support the conclusion of "suspected to be an immune hazard," a lower call. Because the method calls for using the best data and highest level of evidence, the hypersensitivity evidence no longer factors into the final conclusion. The hazard conclusion after the change is still "presumed to be an immune hazard," based only on the antibody response data. Dr. Chiu made an analogy to the situation for a potential carcinogen. Dr. Rooney clarified that only evidence that directly contradicts the original conclusion would be used to downgrade the conclusion, not a lower level of evidence for a different effect.

3. Action: Panel Vote on PFOA, Overall Conclusions

Dr. Chiu called for a motion to accept the revised conclusion as currently written. Dr. Keil so moved. Dr. Woolhiser seconded. The panel voted unanimously (5 yes, 0 no, 0 abstentions) in favor of the motion.

VI. PFOS

Dr. Rooney began the section on PFOS by outlining the draft NTP conclusions on PFOS immunotoxicity:

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

A. Antibody Response

1. Presentation

Dr. Rooney presented the animal data related to antibody response from PFOS exposure. He noted that there were eight experimental studies in mammals, with

consistent evidence of suppression of the primary antibody response (IgM) in mice. He delineated the risk of bias considerations associated with the studies, along with the evidence profile depicting initial confidence and factors decreasing or increasing confidence in the body of evidence. The evidence profile reflected high initial confidence, decreased confidence for risk of bias concerns, and increased confidence for evidence of a dose response. Ultimately, there was high confidence that exposure to PFOS is associated with suppression of the antibody response based on animal studies.

Dr. Rooney presented the human data related to antibody response from PFOS exposure. He described four prospective and two cross-sectional studies in humans. The studies showed suppression in one or more measures of anti-vaccine antibody response associated with prenatal, childhood, and adult exposures. He showed the risk of bias considerations associated with the studies, along with the evidence profile depicting the moderate initial confidence rating and factors decreasing or increasing confidence. There were no changes for factors that may decrease or increase confidence in the body of evidence. There was moderate confidence that exposure to PFOS is associated with suppression of the antibody response in humans.

Dr. Rooney showed a graphic depicting the integration of the evidence regarding the antibody response, leading to hazard identification. In the initial hazard conclusion, PFOS was presumed to be an immune hazard to humans. The final hazard conclusion, after consideration of mechanistic data and biological plausibility, was that **PFOS is presumed to be an immune hazard to humans.**

2. Questions for Clarification

Dr. Braun asked about the degree of bias introduced by the lack of blinding of the investigators; in the animal studies, the outcome assessors were not blinded as to the treatment. Dr. Rooney said there is some empirical evidence suggesting that the effect size is changed when the outcome assessors are not blinded, with more of an impact for more subjective measures. Even for some objective measures, the possibility of increasing effect size due to bias exists. He noted the studies that lacked blinded outcome assessors tend to have an increased effect size; however, the exact size of the effect is unknown. Dr. Thayer added that when judging studies, there is consideration of how qualitative, quantitative, or subjective a measure is. This consideration is captured in the rationale following guidance developed at a project-specific level in the protocol.

3. Human Studies – Peer Review Comments and Panel Discussion

Dr. Braun, the first reviewer, said the team did an excellent job of assessing and summarizing the literature. He was mainly concerned about assessing the magnitude of the bias from potential confounders and comparing unadjusted with adjusted estimates. He was also concerned about the cumulative effects of PFOA and PFOS exposures,

given the EPA Drinking Water Health Advisory action levels. He expressed concern about considering bias and dose response together, specifically referring to the discussion on page 31 of the draft monograph, with potential "double counting" of potential bias. He recommended assessing the magnitude and precision of the dose response relationship both before and after adjustment for other perfluoroalkyl acids. He said that in some cases, it was unclear how the initial confidence ratings were determined. He suggested it would be helpful to add a column to the table about each study denoting the features of a study that gave them a particular confidence rating.

Dr. Granum, the second reviewer, noted that the studies all pointed in the same direction regarding immunosuppression, whether for antibody responses or infectious diseases. She added that chance findings could be ruled out because the effects were in the same direction. She agreed that the lack of adjustment for other perfluoroalkyl acids is a weakness in some of the studies. She added that because PFOS is present at much higher concentrations than other perfluoroalkyl acids, she was not sure that considering the sum of different perfluoroalkyl acids is the correct thing to do either.

Dr. Rooney agreed with Dr. Braun's suggestion about adding information describing initial study designs. Regarding potential double counting, he said that the decision had been made to not *downgrade* for multiple perfluoroalkyl acids and to not *upgrade* for dose response (with the potential adjustment being in opposite directions). Thus, not doing either action counted just once, not double, as he explained.

Dr. Woolhiser reiterated his comments from PFOA regarding his uncertainty about the level of evidence being moderate versus low because of potential inconsistencies, potential confounders, and risk of bias. Despite his misgivings, he could support a moderate level of evidence.

Dr. Corsini noted that there was more relevant evidence for PFOS from the *in vitro* studies, because the doses used were more relevant to human exposure levels. Thus, the *in vitro* data partially support the *in vivo* evidence, and she agreed with the moderate level of evidence in humans.

Dr. Keil also agreed with the moderate level of evidence in humans.

Dr. Chiu mentioned that the public comments were addressed during the discussion.

4. Action: Panel Vote on PFOS Antibody Response, Human Studies

Dr. Braun moved to accept the draft conclusion of a moderate level of evidence for antibody response from human studies as written. Dr. Keil seconded. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to pass the motion.

5. Animal Studies – Peer Review Comments and Panel Discussion

Dr. Keil, the first reviewer, said the approach was objectively applied to interpreting the antibody response data, and she agreed with the level of evidence conclusion and interpretations of the data. She noted that there was no weight change greater than 10%, and there were no signs of overt toxicity at lower doses of PFOS.

Dr. Woolhiser, the second reviewer, said that the summaries of the data, methods, and results were clearly and objectively presented. He found the figures helpful and useful. He agreed with the conclusion that there was a high level of evidence for antibody suppression, particularly IgM, following animals' exposure to PFOS.

Dr. Rooney agreed it would be useful to add to the draft monograph that there were no overt signs of toxicity at the levels of PFOS where antibody suppression was observed, particularly at the lower levels.

Dr. Chiu summarized the public comments and concluded that the panel addressed the comments during the discussion.

6. Action: Panel Vote on PFOS Antibody Response, Animal Studies

Dr. Keil moved to accept the draft conclusion of a high level of evidence for antibody response from animal studies as written. Dr. Woolhiser seconded. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to pass the motion.

7. Mechanistic Studies – Peer Review Comments and Panel Discussion

Dr. Corsini, the lead reviewer, said that the mechanistic studies were clear and well presented in the monograph. She agreed that none of the mechanistic data would change the animal or human conclusions. She noted that there are no specific *in vitro* studies that address the effects of PFOS on B cells, or studies that identify the specific cell types involved or link the cytokine changes to suppression of the antibody response. Nevertheless, some of the *in vitro* studies show that exposure to PFOS decreased cytokine production, and that PFOS seems to be more potent *in vitro* compared to PFOA. She concluded that while the data do not sufficiently address the details, there is some indication of immunosuppression *in vitro*, which supports the results of the animal and human studies.

Dr. Rooney noted that there was some evidence of changes in cytokines at a much more relevant concentration compared to PFOA or the changes seen in *in vivo* studies. However, the evidence was not sufficient to change the overall conclusion, either up or down.

Dr. Braun suggested using the NHANES biomonitoring data to justify the relevance of the exposures, particularly for the *in vitro* studies. Dr. Corsini said that while the doses

used in *in vitro* studies are higher than those in NHANES, these doses are relevant for occupational exposure. Dr. Rooney agreed.

8. Action: Panel Vote on PFOS Antibody Response, Mechanistic Studies

Dr. Corsini moved to accept the draft conclusion for antibody response from mechanistic studies as written. Dr. Woolhiser seconded. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to pass the motion.

B. Other Supporting Evidence

1. Presentation

Dr. Rooney presented conclusions from other supporting evidence related to PFOS exposures: immunosuppression resulting in reduced disease resistance and immunosuppression resulting in suppression of NK cell activity.

There was a moderate level of evidence for disease resistance for the animal studies based on a single study of reduced resistance to the influenza A virus. Human studies had a low level of evidence due to inconsistent evidence and few specific diseases examined. There was no change in conclusions after consideration of mechanistic data.

There was a moderate level of evidence for suppression of NK cell activity for the animal studies based on consistent evidence for suppression of NK cell activity in mice; however, there were risk of bias concerns. There were no human studies, resulting in a conclusion of an inadequate level of evidence. There was no change in conclusions after consideration of mechanistic data.

Because neither disease resistance nor NK cell activity reached a hazard identification conclusion that would support “presumed” to be a human hazard, they were not used in the hazard identification conclusion. They were used as supporting evidence because the effects were related to suppression of the antibody response.

2. Other Supporting Evidence – Panel Discussion

Dr. Woolhiser asked about potential overt toxicity observed concurrent with decreased NK cell activity in animals. He said the observed effects on body weight are not accounted for in the NTP interpretation. Dr. Rooney said that at the highest doses tested, there was suppression of NK response and some evidence of overt toxicity. However, lower doses of PFOS were also associated with NK activity suppression, and there were no changes in parameters such as body weight or other evidence of overt toxicity at lower doses. Thus, it was not considered to be a factor in drawing the conclusion of moderate evidence for suppression of NK cell activity across multiple studies. Dr. Chiu asked Dr. Rooney about the issue of cellularity in the spleen. Dr. Rooney replied that there were no changes at the lower dose levels associated with reduced NK cell activity; therefore, changes in cellularity in the spleen were not

supported as a potential mechanism. Dr. Keil agreed that changes in NK cell activity were not associated with overt toxicity.

Dr. Chiu asked for a roll call of the panel to agree or disagree with the conclusions as presented. The panel unanimously agreed with the conclusions as presented.

C. Other Outcomes that Did Not Reach Hazard Conclusions

1. Presentation

Dr. Rooney presented information on other PFOS outcomes that did not reach hazard conclusions.

He noted that for hypersensitivity-related outcomes, the animal studies had a low level of evidence because of inconsistent evidence, and the human studies had a very low level of evidence because of inconsistent evidence. For autoimmunity-related effects, the animal studies had an inadequate level of evidence because there were no studies. The human studies also had an inadequate level of evidence, because there was a single pilot study with data on autoantibodies, which is not a reliable indicator or autoimmunity without further supporting data.

2. Panel Discussion

Dr. Woolhiser commented that the airway hypersensitivity results for animals in PFOS were even lower than those presented for PFOA. He questioned whether that had any practical impact on the overall assessment. He accepted the low level of evidence conclusion.

Dr. Chiu asked for a roll call of the panel to agree or disagree with the conclusions as presented. The panel unanimously agreed with the conclusions as presented.

D. PFOS Overall Conclusions

1. Conclusions

Dr. Chiu called for the PFOS conclusions to be projected. The overall conclusions were:

- PFOS is **presumed to be an immune hazard to humans** based on the following:
    A. Suppressed antibody response.
        I. Animal studies: High level of evidence
        II. Human studies: Moderate level of evidence
        III. No change in conclusions after considering mechanistic data

    B. Other supporting evidence: Suppressed disease resistance and suppressed NK cell activity

2. Panel Discussion

Dr. Woolhiser supported the conclusions regarding antibody response. However, he suggested that the conclusion referring to other supporting evidence did not add anything significant or necessary to the hazard identification because all of the level of evidence conclusions are low except for moderate level of evidence for animal studies of disease resistance. Dr. Rooney noted that the level of evidence was also moderate for NK cell activity. Dr. Keil said that there was a host resistance assay, which constitutes supporting evidence for suppressed antibody response.

Dr. Chiu said the question on the table was whether point B in the conclusions is necessary or supported. He said that point A alone would support the "presumed" conclusion, while perhaps point B would only support a "suspected" conclusion. Dr. Rooney explained that the elements cited in point B potentially support the immunosuppression conclusion; thus, they are included in the overall conclusions. Dr. Chiu suggested a potential reorganization of the conclusions.

Dr. Corsini said that since the outcomes in point B support immunosuppression, she was fine with the conclusions as written. Dr. Woolhiser agreed.

3. Action: Panel Vote on PFOS, Overall Conclusions

Dr. Corsini moved to accept the overall conclusions for PFOS as written. Dr. Keil seconded the motion. The panel voted unanimously (5 yes, 0 no, 0 abstentions) to agree with the conclusions as written.

VII. Concluding Remarks

Dr. Chiu congratulated the peer review panel for the successful completion of the review.

Dr. Thayer thanked the panel, and said that NTP was pleased with the content of the comments and the efficiency of the discussions. She appreciated the positive comments about the clarity of the draft monograph, citing Dr. Rooney's hard work in preparing it.

Dr. Rooney thanked the panel members for the time and effort they had put in, including their consideration of the extensive public comments that were received.

Dr. Bucher thanked the panel for its outstanding job and Dr. Chiu for his efforts chairing the panel.

Dr. Chiu added his thanks to everyone and adjourned the meeting at 1:30pm, July 19, 2016.

VIII. Approval of the Peer Review Report by the Chair of the Peer Review Panel

This peer review report has been read and approved by the Chair of the July 19, 2016, National Toxicology Program Peer Review Panel for the Draft NTP Monograph on Immunotoxicity Associated with Exposure to PFOA or PFOS

[Signature Redacted]

Weihsueh A. Chiu, Ph.D.

Chair, NTP Peer Review Panel

Date: 9/28/2016