

## OVERALL SUMMARY

- i) Suppression by PFOA and PFOS of the IgM TDAR in mouse studies is deemed as supportive of human data from epidemiology studies showing an association between PFOA and PFOS exposure and a decrease in antibody titers to vaccines. The IgM TDAR is a primary antibody response, whereas, vaccine titers are mainly of the IgG antibody isotype. In addition, in the few animal studies where a bona fide memory response was evaluated, antigen-specific IgG was not suppressed by PFOA and PFOS. The final hazard conclusion for immunosuppression should be **downgraded**.
- ii) The NTPs conclusion “there is **high confidence** that exposure to PFOA is associated with increased hypersensitivity responses based on the available animal data”, should be downgraded. Two animal studies were primarily deemed as supportive of this conclusion, Fairley et al. and Ryu et al. (Fair et al., 2013; Ryu et al., 2014). By definition, hypersensitivity is an exaggerated immune response to an exogenous antigen. Importantly, Ryu et al. found that PFOA induced AHR in the absence of exposure to an allergen (Ova) and also PFOA did not potentiate the AHR response to Ova sensitization and challenge. Therefore the Ryu study does **not** support the conclusion that PFOA-induced AHR is due to a hypersensitivity response. By contrast, Fairley et al. showed an increase in AHR, which corresponded with an increase in serum anti-Ova IgE levels, which they concluded could be involved in enhanced AHR by PFOA. A common finding in both studies that deserves greater attention is the increase in airway associated inflammatory cells in PFOA treated mice, which could be involved in the underlying cause of AHR in a hypersensitivity-independent manner.
- iii) The NTP concluded that “there is **moderate confidence** that exposure to PFOS is associated with suppression of NK cell activity in animals”. The level of confidence should be downgraded to “**low confidence**”, based on the fact that impairment of NK cell activity in the majority of studies cited occurred at doses well above those that are relevant to human exposure. Moreover, in several of the studies there were indications that doses producing a suppression of NK activity also induced overt toxicity as suggested by an elevation in corticosterone, decreased body and lymphoid organ weights and decreased lymphoid tissue cellularity (Dong et al., 2009; Zheng et al., 2009).
- iv) The NTP final hazard conclusion based on the body of evidence for infection disease resistance is “**Suspected to be a Immune Hazard to Humans**”. Collectively, there does not appear to be sufficient supporting evidence in either humans or animals to support the NTP conclusion. The NTP should seriously consider down grading the final hazard conclusion for infectious disease resistance to something less than “**Suspected to be an Immune Hazard to Humans**”.

## DETAILED COMMENTS

The NTP categorized the health effects of PFOA and PFOS on the immune system into three categories: (a) immune suppression; (b) hypersensitivity-related outcomes and (c) autoimmunity. For each of these categories, the NTP gave the greatest weight to primary outcomes (e.g., for immune suppression, suppression of antibody responses) and less weight to secondary endpoints (e.g., decrease in spleen weight, changes in cytokine production). Evidence related to secondary outcomes was used only as supportive evidence since the NTP felt that there was sufficient primary outcome data to draw conclusions. In addition, evidence for animal data was used to support human health outcomes in order to draw a final human hazard conclusion. This review of the NTP Systematic Review of Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid (PFOA) or Perfluorooctane Sulfonate (PFOS) will address each of the three health categories individually, for PFOA and PFOS, with a primary focus on whether the animal data supports the the NTP conclusions for human health outcomes.

### PFOA Immune Evidence

A. **Immune Suppression:** Within the category, ‘Immune Suppression’, the NTP identified published studies in three subcategories antibody response, natural killer NK cell activity, and infection disease resistance based on the rationale that different cell types can be involved in each of these three responses.

- 1) **Antibody Response:** The NTP concluded that “there is **moderate confidence** that exposure to PFOA is associated with suppression of the antibody response in human based studies”. Evidence for this conclusion comes from retrospective, cross-sectional and prospective epidemiological studies in which antibody titers to vaccinations were quantified in combination with measurements of serum PFOA levels coupled with supportive animal studies. The strengths and weaknesses of the epidemiological studies have been extensively reviewed by the NTP and by Chang and co-workers (Chang et al., 2016) and therefore will only be discussed within the context of animal data.

Animal data supporting the NTP conclusion “there is moderate confidence that exposure to PFOA is associated with suppression of the antibody response in humans” is based on the observation that PFOA administration to mice suppressed the antigen specific (sRBC or hRBC) T cell-dependent IgM antibody response (TDAR) (DeWitt et al., 2009; Dewitt et al., 2008; Loveless et al., 2008; Yang et al., 2002). Results from the TDAR were viewed as especially important by the NTP for several reasons. The first being that the TDAR is viewed as one of the most sensitive immunotoxicological assays for identifying immune modulating agents. The rationale being that the TDAR requires the involvement of numerous immune cell types including B cells as effector cells (antibody secreting plasma cells), as well as T cells and macrophages as accessory cells for cytokine

secretion as well as antigen processing and presentation. The response also requires cell activation, proliferation and differentiation by B and T cells. Hence, the TDAR has many critical components and if one or more of these components is altered, it will affect the magnitude of the TDAR. Second, suppression of antibody titers to a number of different vaccines was observed in association with PFOA exposure in epidemiological studies. The NTP viewed suppression of humoral immune responses by PFOA in mice as being evidence of “high confidence” and supportive of human evidence deemed to be of “moderate confidence”. The animal and human data collectively led the NTP to the final hazard conclusion for the antibody response: **“Presumed to be an Immune Hazard in Humans”**. The critical humoral immune response data from animal studies is briefly summarized and discussed below.

Yang and coworkers administered PFOA in feed (0.02% w/w) for 10 consecutive days and a single sensitization with horse RBC followed by measurement of antigen specific IgM and IgG (IgG1, IgG2, and IgG3) using a plaque assay (enumerates the number of antibody secreting B cells) and also by ELISA. Suppression of both the IgM and IgG response was observed. Importantly, although antigen specific IgG was quantified, the measurements were to a single sensitization on day 6, which is not a secondary response to hRBC. Moreover, the actual PFOA serum concentrations were not determined as in other PFOA mouse immunotoxicology studies. Interestingly, Yang and coworkers demonstrated that removal of PFOA containing feed resulted in a rapid recovery from humoral immune suppression, which is difficult to explain based on the relatively long half-life of PFOA in mice. Yang et al. also suggested activation of the peroxisome proliferator activator receptor alpha (PPAR $\alpha$ ) as a putative mechanism for PFOA-induced suppression of the TDAR. Dewitt and coworkers showed similar sensitivity of the TDAR to suppression by PFOA in PPAR $\alpha$  knockout and wild type mice, ruling out the involvement of PPAR $\alpha$ . in suppression of the IgM TDAR.

Dewitt and coworkers (Dewitt et al., 2008) attempted to reproduce the Yang et al. studies. At high doses (15 and 30 mg/kg/day) suppression of the sRBC IgM TDAR was observed which coincided with a loss in body weight as well as spleen and thymus weight, suggesting PFOA at the doses used, induced overt toxicity. Using lower doses administered either by oral gavage or in drinking water, suppression of the sRBC IgM TDAR was observed at doses as low as 3.75 mg/kg/day, which occurred in the absence of decreased body weight or lymphoid organ weights. A second group of mice were also sensitized a second time with sRBC to assess the IgG response (memory response). In contrast to Yang et al., the IgG response was not suppressed by PFOA. At all doses with the exception of 30 mg/kg/day, the IgG response was enhanced by PFOA. Antigen specific IgM and IgG responses were determined ELISA.

Loveless and co-workers also evaluated the effects of PFOA on humoral immune responses in CD1 mice and CD(SD)IGS BR rats. Using the IgM TDAR, Loveless et al

observed suppression of the anti-sRBC response in mice at 10 and 30 mg/kg/day with a corresponding decrease in spleen and thymus weight as well as an increase in corticosterone levels. No suppression of the anti-sRBC IgM response was observed in the rat even at 30 mg/kg/day. In both the mouse and rat study, the anti-sRBC IgM TDAR was measured by ELISA. The authors speculated that suppression of the IgM TDAR in mice was putatively through release of corticosterone due to the high doses of PFOA used in the study. In a subsequent study, DeWitt and coworkers ruled out the involvement of corticosterone as the mechanism for PFOA-mediated IgM suppression using adrenalectomized mice, which exhibited similar sensitivity to PFOA as sham control mice in the IgM TDAR (DeWitt et al., 2009).

In spite of the importance placed on the evidence for suppression of humoral immune responses in mice (“high confidence”), which is viewed by the NTP as supportive evidence for suppression of humoral immune response in humans from epidemiology studies (“moderate confidence”), there exists a major incongruence in how the NTP reached its conclusions. The humoral immune response to vaccinations, as measured in the human epidemiology studies, is mainly a secondary IgG memory response. By contrast, the anti-sRBC/hRBC TDAR measured in mice is a primary, or IgM response. Virgin B cells (B cells never having been activated by an antigen) when activated by an antigen undergo clonal expansion and differentiate either into short lived IgM secreting plasma cells or long-lived memory cells. Clearly, suppression of the IgM response by PFOA was demonstrated by at least three independent laboratories, albeit in several studies at doses that also induced signs of overt toxicity (i.e., reductions in body and lymphoid organ weight). Only in one mouse study by DeWitt and coworkers, was the IgG memory response correctly assessed such that mice received a second sensitization with antigen (sRBC) after induction of the primary IgM response (DeWitt et al., 2008). Yang and coworkers reported a decrease in the IgG response (IgG1, IgG2, and IgG3) but the response was not measured correctly, as mice only received a single antigenic sensitization, by i.v. injection. By contrast, when a bona fide secondary response was assessed in mice using two antigenic sensitizations with sRBC, PFOA treated mice demonstrated an enhanced IgG response (DeWitt et al., 2008).

It is difficult to interpret why the primary IgM response was suppressed in mice by PFOA and yet the secondary response was either not affected or enhanced. As discussed above virgin B cells after antigenic stimulation undergo numerous rounds of proliferation and then undergo commitment to become either an IgM secreting plasma cell or memory cell. Since the memory response in mice was either unaffected or enhanced, as determined by the IgG response, these data suggests that there is no impairment of memory B cell formation and in their capability to respond to antigenic stimulation to secrete IgG. This is in contrast to those epidemiologic studies suggesting suppression by PFOA of antibody titers to vaccinations, which is mainly an IgG response by memory B cells. The mouse

studies also suggest that, either: (1) PFOA suppresses B cell to IgM plasma cell differentiation; or (2) the same number of plasma cells are formed during the primary IgM response, in the absence and presence of PFOA, but the capacity of the plasma cells to secrete large quantities of IgM is partially impaired by PFOA. **Regardless of the mechanism responsible for suppression of the mouse IgM TDAR, it is mechanistically distinct from suppression by PFOA of antibody titers to vaccines reported in the human studies.**

It is also important to emphasize that with the exception of Yang and coworkers (Yang et al., 2002), the effect of PFOA on antibody responses in mice were quantified by ELISA. Although there are a number of methods to quantify humoral immune responses, either by enumerating antibody-secreting cells or quantifying secreted antibody, with both approaches being widely accepted and used, each provides different mechanistic information. As discussed above, suppression of antibody levels by a xenobiotic can be due to: (a) a decrease in the amount of antibody being secreted by each differentiated plasma cell with no effect on the total number of plasma cells; or (b) a decrease in the total number of B cells that have differentiated into plasma cells with no effect on the amount of IgM being secreted per plasma cell.

Finally, it is unclear mechanistically from either the animal or human studies, why PFOA decreased antibody titers to one vaccine in human subjects but not for another vaccine, even when the vaccinations were related (e.g., suppression to influenza type B but not type A/H1N1 or A/H3N3) (Looker et al., 2014).

Collectively, human and animal bodies of evidence for antibody response are divergent. Mouse studies show suppression of the IgM response with no impairment of the secondary antigen specific IgG response. By contrast, epidemiology studies suggest suppression by PFOA of antibody titers to vaccinations, which are mainly a memory IgG response.

- 2) Infectious Disease Resistance: The NTP concluded that “there is **low confidence** that exposure to PFOA is associated with suppression of infectious disease resistance in human based studies”. The basis for this conclusion is a lack of data due to few infectious disease endpoints having been measured in humans. The NTP also concluded that “there is **very low confidence** that exposure to PFOA is associated with a change in the ability of animals to respond to infectious disease because there are no experimental studies on disease resistance endpoints in mammals and wildlife studies have serious risk of bias”. The conclusions by the NTP are appropriate for the effects of PFOA on infectious disease resistance.

3) **Natural Killer Cell Activity:** NTP identified no data on the effects of PFOA on human NK cell activity. The NTP also concluded that “there is **very low confidence** that exposure to PFOA is associated with suppression of NK cell activity in animals”. Presently there is only one published study in mice in which the effects PFOA were evaluated on NK cell activity (Vetvicka and Vetvickova, 2013). Vetvicka and coworker used a single (20 mg/kg/day) dose of PFOA administered for 7 days, which suppressed NK cell activity. The study was viewed as having significant bias. In addition, there is one study in wildlife that showed no correlation between PFOA serum levels and NK cell activity in bottlenose dolphins (Fair et al., 2013). Based on the lack of data in combination with negative data, the NTP did not develop an evidence synthesis for PFOA and NK cell activity. Appropriately, NK cell activity was not considered by the NTP for hazard identification conclusions.

B. **Hypersensitivity-related Effects and Outcomes:** The NTP concluded “there is **low confidence** that exposure to PFOA during childhood is associated with increased hypersensitivity responses based on available human data”. Evidence for this conclusion comes from retrospective, cross-sectional and prospective epidemiological studies of clinical measures and/or biomarkers of hypersensitive (e.g., asthma, rhinitis, skin disorders, serum IgE). The strengths and weaknesses of the epidemiological studies have been extensively reviewed by the NTP and by Chang and co-workers (Chang et al., 2016) and therefore will only be discussed within the context of animal data.

The NTPs conclusion “there is **high confidence** that exposure to PFOA is associated with increased hypersensitivity responses based on the available animal data”, is based primarily on two studies both of which evaluated the effects of PFOA on airway hyperresponsiveness (AHR) in mice (Fair et al., 2013; Ryu et al., 2014). In addition, a study by Singh et al. (2012) is cited which showed that PFOA treatment in mice enhanced the IgE-dependent local allergic reaction in mice dosed dermally with 10 and 50 mg/kg/day PFOA for four days. In this same study i.p. injection of 1 and 5 mg/kg of PFOA increased histamine release (Singh et al., 2012).

In the Fairley study, PFOA was administered dermally in acetone for 4 consecutive days (0, 0.25, 2.5, 6.25, 12.5, 18.75, 25 and 50 mg/kg/day). Mice were then sensitized (i.p.) and challenged (pharyngeal aspiration) with ovalbumin (OVA) followed by measurement of airway hypersensitivity and AHR. At the 50 mg/kg/day dose a significant decrease in body weight, spleen weight, thymus weight, spleen cellularity and thymic cellularity were observed, all suggestive of overt toxicity. Mice treated with 25 mg/kg/day PFOA exhibited an increase in Ova-specific serum IgE and at doses of 18.75, 25 and 50 mg/kg/day an increase in total serum IgE. The increase in IgE serum antibodies was viewed as important since IgE is involved in type I hypersensitivity reactions by facilitating release of mast cell-derived mediators (e.g., histamine, prostaglandins, leukotrienes). As a measure of pulmonary function, penH values were determined in response to methacholine (MCH) challenge. Mice exhibited a trend toward increasing antigen-specific AHR with increasing concentration

of PFOA up to 50 mg/kg/day. Histopathology also showed a dose-dependent increase in airway associated inflammatory cells. Fairley et al. concluded that PFOA exposure increased IgE and AHR to Ova in mice that were concurrently exposed to Ova and PFOA. Overall the Fairley studies were of good technical quality and the study conclusions were consistent with results reported. It is also noteworthy that although serum PFOA levels were not determined, adverse PFOA related effects were observed primarily at high doses with the highest dose likely inducing overt toxicity.

Ryu coworkers (Ryu et al., 2014) also assessed the effects of PFOA on airway hypersensitivity and AHR but used a very different PFOA exposure paradigm compare to the Fairley study. Specifically, PFOA exposure was initiated in pregnant dams on gestation day 2 and continued through week 12 after birth by mixing 4 mg of PFOA/kg of diet with an estimated exposure level equivalent of 1 mg/kg oral gavage dose for 63 day (~105 mg/kg cumulative dose). Ryu et al also reported that PFOA exposure induced AHR but occurred in the absence of exposure to an allergen (i.e., Ova). Moreover, AHR induced by MCH challenge in mice sensitized and challenge with Ova was not enhanced by PFOA. These results suggest that PFOA does not appear to augment allergen-induced AHR. Interestingly, Ryu and co-workers also found that mice treated with PFOA only (i.e., no Ova treatment) exhibited an increase in inflammatory cells as assessed by bronchoalveolar lavage. The increase was primarily due to an increase in infiltrating macrophages. Serum level determinations showed that 12 week-old mice possessed  $4,800 \pm 1,100$  ng/ml, which is significantly higher than what is observed in the general public (0.5 -12 ng/ml). The Ryu study was of good technical quality and the conclusions reached by the authors are supported by the study results.

Although both Fairley and Ryu reported that PFOA exposure induced AHR, only the Fairley study results support the NTP conclusion that PFOA AHR is mediated by a hypersensitivity response. By definition, hypersensitivity is an exaggerated immune response to an exogenous antigen. In the Ryu study, PFOA induced AHR in the absence of exposure to an allergen (Ova) and also did not potentiate the AHR response to Ova sensitization and challenge. It is noteworthy that although the PFOA daily dose in the Ryu study was significantly less than in the Fairley study, the overall cumulative dose in the Ryu study was at least an order of magnitude greater due to the duration of the exposure period. The mechanism for AHR by PFOA in the Ryu study is unclear but may be due, in part, to the marked increase in airway associated inflammatory cells, which was also identified by histopathology in the Fairley study. The NTP considered results by Singh and coworkers showing an enhanced IgE-dependent local allergic reaction in mice dosed dermally with 10 and 50 mg/kg/day PFOA and histamine release by i.p. injection of 1 and 5 mg/kg of PFOA as additional supportive evidence that PFOA induces hypersensitivity in mice. Importantly, in the Singh study it appears that histamine release by mast cells both *in vitro*, after direct addition of PFOA to cultured cells, and *in vivo*, after i.p. administration of PFOA, was due to

spontaneous release and not IgE mediated, as in a type 1 hypersensitivity response. In summary, the NTP considered both the both Fairley and Ryu studies as evidence for hypersensitivity related outcomes with “high confidence”. **Both the Fairley and Ryu studies support the conclusion that PFOA at high doses can induce AHR in mice, but only the Fairley study supports hypersensitivity as a putative mode of action for AHR.**

Collectively, based on the human body of evidence, which was deemed by the NTP as “Low Confidence” and animal body of evidence as “High Confidence”, the final NTP hazard conclusion based on hypersensitivity-related evidence was that PFOA is “Presumed to be an Immune Hazard in Humans”.

- C. **Autoimmunity:** The NTP concluded that “there is **low confidence** that exposure to PFOA is associated with ulcerative colitis and rheumatoid arthritis in humans based on epidemiological studies. The strengths and weaknesses of the epidemiological studies have been extensively reviewed by the NTP and by Chang and co-workers (Chang et al., 2016). No animal studies were identified by the NTP on potential associations between PFOA and autoimmunity.

## **PFOS Immune Evidence**

- A. **Immune Suppression:** Within the category ‘Immune Suppression’, the NTP identified published studies in four subcategories antibody response, natural killer NK cell activity, and infection disease resistance based on the rationale that different cell types can be involved in each of these three responses.

- 1) **Antibody Response:** The NTP concluded that “there is **moderate confidence** that exposure to PFOS is associated with suppression of the antibody response in human based studies”. Evidence for this conclusion comes from epidemiological studies in which antibody titers to vaccinations were quantified in combination with measurements of serum PFOS levels coupled with supportive animal studies. The strengths and weaknesses of the epidemiological studies have been extensively reviewed by the NTP and by Chang and co-workers (Chang et al., 2016) and therefore will only be discussed within the context of animal data.

The NTP concluded that based on animal studies “there is a **high confidence** that exposure to PFOS is associated with suppression of the antibody response”. The conclusion that PFOS suppresses antibody responses in mice is supported by a number of studies which show that exposure to PFOS at various life stages can suppress the IgM TDAR (Dong et al., 2011; Keil et al., 2008; Peden-Adams et al., 2008). Suppression of the IgM TDAR occurred at doses significantly lower with PFOS than PFOA. In several studies male mice exhibited greater sensitivity to suppression of the IgM TDAR than female mice (Keil et al., 2008) (Peden-Adams et al., 2008). In another study, Quazi and



coworkers showed that PFOS administered at 250 µg/kg/day over 28 days with a total administered dose of 7 mg/kg did not suppress the IgM TDAR (Qazi et al., 2010). Studies also show that PFOS does not suppress IgG after a single sensitization with antigen and, in fact, modestly enhanced the IgG response at a dose of 50 mg/kg/day (Dong et al., 2011).

As with PFOA, the NTP concluded that suppression of the IgM response in animal studies is supportive evidence of human data showing an association between PFOS exposure and decreased vaccine titers. As discussed above, antibody titers to vaccinations are primarily of the IgG antibody isotype and the animal studies demonstrating suppression of the primary antibody response, as measured in mice by the TDAR, is of the IgM isotype. It is also important to emphasize that the secondary IgG response was not appropriately induced to elicit a bona fide memory response as only a single antigen sensitization was used in the mouse studies (Dong et al., 2011; Qazi et al., 2010). In addition, one study was identified in white longhorn chickens in which the secondary IgG (IgY) response was assessed after a secondary sRBC sensitization (Peden-Adams et al., 2009). These studies showed no suppression of the IgG response at PFOS doses of 1, 2.5 and 5 mg/kg egg weight, compared to control, although the IgM and combined IgM and IgY response was suppressed.

Based on the aforementioned studies the NTP concluded with respect to suppression of antibody responses, the human body of evidence being of “Moderate Confidence” and the animal body of evidence being of “High Confidence” with the Final hazard conclusion “Presumed to be an Immune Hazard to Humans”.

- 2) Infectious Disease Resistance: The NTP concluded that “there is **low confidence** that exposure to PFOS is associated with suppression of infection disease resistance in human based studies”. The basis for this conclusion is limited data due to few infection disease endpoints having been measured in humans, weak or no association with PFOS exposure, and bias in experimental design. The NTP also concluded that “there is **moderate confidence** that exposure to PFOS is associated with reduced ability of animals to respond to infectious disease”, which is based on one study in female mice (Guruge et al., 2009) and two wildlife studies (Kannan et al., 2006; Kannan et al., 2010).

Guruge et al. assessed the effect of PFOS on resistance to influenza virus A/PR/8/34 (H1N1) in B6C3F1 mice. In the Guruge and coworker study two doses of PFOS were employed, 5 or 25 µg/kg/day for 21 days yielding serum PFOS concentrations of 189 and 670 ng/ml, respectively. Mice exposed to PFOS at 25 µg/kg/day exhibited a significant decrease in survival (~15%) compared to control (~50%). The study appears to be of good technical quality.

In addition two wild life studies, one on sea otters found freshly dead on the California coast (Kannan et al., 2006) and a second in brown bats with white nose syndrome

(Kannan et al., 2010), were considered by the NTP. It is difficult to judge the conclusion from the wild life studies as there were many potential confounding factors. For example, in the sea otter study, the investigators categorized dead otters into one of three groups based on presumed cause of death, nondisease, emaciated, or diseased. It is not clear how there can be certainty on whether the cause of death was infectious disease-based. The investigators attempted to correlate PFOA/PFOS tissue levels to one of the three causes.

The NTP final hazard conclusion based on the body of evidence for infectious disease resistance is **“Suspected to be a Immune Hazard to Humans”**. There does not appear to be sufficient supporting evidence in either humans or animals to support the NTP conclusion. The NTP should seriously consider down grading the final hazard conclusion for infection disease resistance to something less than **“Suspected to be a Immune Hazard to Humans”**.

- 3) NK Cell Activity: The NTP identified no human data on the potential association between PFOS and NK cell activity. The NTP also concluded that “there is **moderate confidence** that exposure to PFOS is associated with suppression of NK cell activity in animals”.

The NTP conclusion that “there is **moderate confidence** that exposure to PFOS is associated with suppression of NK cell activity in animals”, is based on several studies in which NK cell activity was impaired in mice at dose from 0.833 to 40 mg/kg/day PFOS (Keil et al., 2008) (Dong et al., 2009; Vetvicka and Vetvickova, 2013; Zheng et al., 2009). Based on the studies cited, suppression NK cell activity by PFOS exposure appears to be a high dose phenomenon, which in at least one studies was also correlated with increased corticosterone serum levels (Dong et al., 2009), a biomarker of overt toxicity and known immunosuppressive factor. Specifically, Dong et al. showed increased NK cell activity at 5 mg/kg total administered dose (TAD) and suppression at 50 and 125 mg/kg (TAD), notably high PFOS doses. Peden-Adams showed increased NK cell activity at PFOS dose of 0.5, 1 and 5 mg/kg (TAD). Vetvicka showed NK cell activity was decrease after 20 mg/kg/day administration for 7 days; a high PFOS dose. Final Keil et al., showed suppressed NK cell activity at 8 weeks post gestational exposure but not at 4 weeks, which the authors stated was an “unusual observation”. The above studies suggest that PFOS impairs NK cell activity at very high doses which may be mediated in part by overt toxicity as suggested by increased corticosterone serum levels, decreased body and lymphoid organ weights and decreased lymphoid tissue cellularity (Dong et al., 2009; Zheng et al., 2009).

The animal studies do not support the NTP conclusion that there is a **“Moderate Level of Evidence”** that PFOS suppresses NK cell activity in the absence of overt toxicity.

- B. **Hypersensitivity-related Effects and Outcomes:** The NTP concluded “there is **very low confidence** that exposure to PFOS is associated with increased hypersensitivity responses based on available human data”. Evidence for this conclusion comes from epidemiological studies of clinical measures and/or biomarkers of hypersensitive (e.g., asthma, rhinitis, skin disorders, serum IgE). The strengths and weaknesses of the epidemiological studies have been extensively reviewed by the NTP and by Chang and co-workers (Chang et al., 2016) and therefore will only be discussed within the context of animal data.

The NTP concluded “there is **low confidence** that exposure to PFOs is associated with increased hypersensitivity responses based on the available animal data”. The conclusion is based primarily on limited data and inconsistencies within the relevant animals studies.

Based on the above, the NTP did not develop an evidence profile or detailed discussions of the evidence for PFOS and hypersensitivity related outcomes.

- C. **Autoimmunity:** The NTP appropriately concluded that “there is **very low confidence** that exposure to PFOS is associated with autoimmunity due to very limited data in this area. No animal studies were identified by the NTP on potential associations between PFOA and autoimmunity. The NTP concluded that there is an inadequate level of evidence to draw conclusions on whether exposure to PFOS is associated with autoimmunity.

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