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

NTP Monograph

Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid or Perfluorooctane Sulfonate

September 2016

hazard identification conclusion of *presumed to be an immune hazard to humans* or PFOS exposure is presumed to suppress the antibody response in humans.

- **Human body of evidence:** Moderate Confidence = Moderate Level of Evidence
- **Animal body of evidence:** High Confidence = High Level of Evidence
- **Initial hazard conclusion (Moderate x High)** = Presumed to be an Immune Hazard to Humans
- **Final hazard conclusion (after consideration of biological plausibility)** = Presumed to be an Immune Hazard to Humans

Taken together, the human and animal bodies of evidence present a consistent pattern of findings that higher prenatal, childhood, or adult serum concentrations of PFOS are associated with suppression of the antibody response. Mechanistic data from *in vitro* or *in vivo* studies can then be used to examine the biological plausibility of PFOS-associated suppression of the antibody response to develop the final hazard identification conclusion.

The antibody response begins with B-cell surface antibody recognizing a specific antigen. Then, for a T-cell dependent antibody response (e.g., most of the experimental animal data on SRBC-specific antibody response), T-cells must also recognize the specific antigen involved (generally after processing by a macrophage, dendritic cell, or other antigen presenting cell). When B- and T-cells both recognize the same antigen, the T-cell activates the B-cell and releases cytokines to help the B-cell multiply and mature into an antibody secreting plasma cells that produces the antigen-specific antibody response. Therefore, relevant mechanistic data would include effects of PFOS at relevant concentrations on key cell populations (B-cells, T-cells, or macrophages as antigen presenting cells), antigen processing and cell activation, or cytokines important for cell signaling during the antibody response.

PFOS-related decrease in B-cell or T-cell numbers would present a possible mechanism for reduced antibody response if it was observed at the same or lower concentrations at which reduced antibody response was observed. However, there is inconsistent evidence of reduced B-cell number (see B cells (B220)) at higher exposure levels for PFOS (≥2 mg/kg) and no change in B-cell or T-cell (CD4 or CD8 subpopulations) numbers observed at lower doses PFOS (0.00166 to 0.08 mg/kg) associated with decreased antibody levels (see cell phenotyping data, spleen and thymus cellularity in **Appendix 5**) (Dong *et al.* 2009a, Zheng *et al.* 2009, Fair *et al.* 2011). Similarly, at lower exposure levels of PFOS, there are no changes in percentage or cell numbers of macrophages or other antigen presenting cells (Qazi *et al.* 2009a, Fair *et al.* 2011, Dong *et al.* 2012). Overall changes in leukocyte numbers and cellularity in the spleen and thymus are also not affected at lower doses of PFOS. There is no evidence that PFOS-induced changes in cell populations could explain the reduced antibody response at lower doses.

Cytokine release of interleukin-4 (IL-4), IL-5, and IL-6 by T-cells are important for T-cell dependent antibody response (e.g., to SRBC). In studies designed to examine these cell signaling pathways in mice, PFOS exposure *in vivo* resulted in increased IL-6 secretion from B-cells under culture conditions designed to test cytokine communication necessary for the antibody response (Fair *et al.* 2011) (i.e., including stimulation of the CD40 cell surface protein critical to IL-6 stimulation of IgM secretion) (Baccam *et al.* 2003, Bishop and Hostager 2003). Under these same conditions, PFOS did not affect IL-4, IL-5, or IL-6 secretion by T-cells in mice (Fair *et al.* 2011) (see cytokine data in **Appendix 5**). In a separate set of studies, PFOS exposure was associated with increased secretion of IL-4 and IL-6 in mixed cultures of splenocytes from mice exposed to higher doses (0.833 to 20 mg/kg/day), doses that are at or above PFOS doses associated with decreased antibody response (Dong *et al.* 2011, Mollenhauer *et al.* 2011, Zheng *et al.* 2011). Similarly, PFOS exposure was associated with increased IL-6 secretion in cultures of peritoneal macrophages from mice (Qazi *et al.* 2009a, Mollenhauer *et al.* 2011, Dong *et al.* 2012). As