CRIS RESPONSE TO EFSA: RE-EVALUATION OF BPA SUBMITTED RESPONSE

Background

In the 2021 "Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuff", the EFSA CEP concluded that the "immune system was identified as the most sensitive health outcome category to BPA exposure. Specifically, an increase of Th17… in the development of allergic lung inflammation. ***(Line 23-25)***". The CEP has attempted to link an increase in Th17 cells due to BPA exposure in rodent studies to epidemiology studies claiming a positive association between BPA exposure and atopic allergic respiratory disorders (e.g., wheezing, rhinitis and asthma). In reviewing the EFSA opinion on BPA we have dissenting opinions that fall into four general categories. We have focused on the key papers cited by the CEP.

Submission limitations

Due to the format required by the EFSA, responses were limited to specific character counts. For the full response, including citations, please visit https://go.msu.edu/CRIS_BPA_Full_Response.

To read the EFSA's draft opinion on BPA, please visit https://go.msu.edu/EFSA_Opinion_Draft.

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About CRIS

The Center for Research on Ingredient Safety at Michigan State University is a collaborative initiative between academia, government, non-governmental organizations, and private organizations to provide research-based information to the global community.

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(Epidemiology studies – Lines 2862-2983)

The CEP concluded that a significant positive association between BPA exposure during pregnancy and allergy is ALAN, since a similar number of cited studies found no association as found a positive association. Notably, the epidemiology studies are weak on determining the level of BPA exposure and the presented assessment of inflammatory respiratory disease is equally lacking. For example, Zhou et. al. (RefID 9013) utilized single time point exposure data for mother and child for assessing BPA exposure. Such data cannot be extrapolated to emulate average human exposure over time. Furthermore, the assessment of allergy was by questionnaire six months after birth, which is subjective and unreliable, to determine the incidence of eczema or wheezing. Also wheezing is common in many respiratory etiologies. Beyond these points, Zhou et al. did not reach significance in the incidence of either eczema or wheezing alone, which were the only outcomes of allergic disease measured. Another study cited by the CEP, supporting an association between BPA exposure and "development of allergic lung inflammation", is Gascon et. al. (RefID 2206). Here children were evaluated for association of prenatal BPA exposure on lung parameters. The data show an association between BPA and wheeze, chest infections, and bronchitis but the associations are weak, with relative risk of 1.2, 1.15, and 1.18%, respectively. The CEP has drawn parallels from epidemiology data and animal data, calling for causality between the Th17 cells, IL-17, and the development of "allergic lung inflammation". Gascon et. al. found no association between prenatal exposure to BPA and IgE levels or the development of asthma, as expected in "allergic lung inflammation". Furthermore, literature shows the protective nature of IL-17 in the clearance of lung infections, which were found to be associated with BPA exposure by Gascon et al.

Immunotoxicology

The premise that Th17 cells drive the development of atopic airway disease, as stated by the CEP, is not substantiated by the current science. Although Th17 cells have been implicated in a host of respiratory disease processes, including acute lung injury, inflammation associated with cystic fibrosis, hypersensitivity pneumonitis, and lung fibrosis, none of these are IgE mediated diseases. Th17 cells have also been implicated in "severe" chronic asthma, but Th2 driven disease is the primary endotype with early onset. These findings are supported by animal studies showing involvement of Th17 cells in mice in an allergic immune response but only in aged mice. There is no scientific literature that we are aware of, and importantly, none found cited in the EFSA opinion, implicating the role of Th17 cells in the "development" of asthma or other atopic allergic respiratory diseases.

The presented report argues that production of IL-17 by Th17 cells is mechanistically responsible for the development of atopic lung inflammation. The role played by Th17 cells is complicated and context dependent, ranging from damaging to the clearance of pathogens and maintenance of epithelial homeostasis. Furthermore, IL-17 has been shown to be produced by a variety of cells including CD8 T cells, NKT cells, neutrophils, and ILCs. One cannot simply conclude that quantifiable IL-17 is due to the presence of Th17 cells. In a recent pediatric study, no correlation was observed between Th17 or IL-17A in children with asthma. Moreover, in clinical randomized, double blinded placebo-controlled studies evaluating a monoclonal blocking antibody directed against the IL-17 receptor did not produce a treatment effect in subjects with severe asthma. These data suggest that IL-17 does not play a critical role, even in subjects with severe asthma (DOI: 10.1164/rccm.201212-2318OC.)(DOI: 10.1089/ped.2021.0067).

(Developmental exposure – Line 3383-3417)

O'Brien (Tier 1) utilized an allergic asthma model in mice. In male mice BPA decreased airway inflammation at the highest dose and produced no inflammation in all other groups. Contrary to the CEB conclusion, BPA produced a suppression of IL-17 in the lungs in both sexes at every dose. Given the inconsistencies in immune parameters in the lungs and the spleen, we question whether the effects are indicative of the model rather than the biology (i.e., increased IgE in the lungs with no pulmonary inflammation). Also, there was no increase in BALF-associated neutrophils, characteristic of IL-17-mediated airway inflammation. The authors did not assess whether the immunologic changes by BPA exposure produced any adverse effects on pulmonary function.

Malaise (Tier 1) performed a perinatal study in mice. It is unclear why 0.1% ethanol in corn oil was the vehicle, when BPA is water soluble. Only a single dose of BPA was used, limiting the ability to demonstrate cause-and-effect. Malaise claimed the dose to be relevant to human exposure, which was 10-fold higher than the TDI and administered as a bolus, which will result in higher systemic concentrations than experienced by humans. Malaise claims BPA-induced inflammation with no histopathology. Analyses were conducted on cells ex vivo with no demonstration of "inflammation". Three different tissues were assessed for Th17 cells and IL-17 ex vivo, none of which were airways. Although difficult to determine T cell recovery from tissues, only 2% of the splenic T cells were activated by CD3/CD28 after 72h, suggesting unsuccessful T cell activation, making cytokine analysis uninterpretable. Consistent with poor T cell activation, changes in IL-17 between control and BPA were 1 to 3.2 ng/ml, 0.06 to 0.08 ng/ml, 0.4 – 0.6 ng/ml for lamina propria, MLN, and spleen, respectively. The reported BPA produced changes are miniscule, with no attempts to evaluate pulmonary function.

(Cellular immunity – Line 3453-3498)

CEP cites Luo (RefID 4679) exposed pregnant dams with BPA in drinking water, GD0 through PND 21. The CEP cites Luo et al. multiple times as evidence for BPA promoting the expansion of Th17 cells. The report states "the panel assigned the likelihood level of Likely to the cellular immunity effect" to BPA and that is why Th17 cells were brought to the BMD analysis. The identification of what is being defined as Th17 cells was not in the lungs but rather in the spleen. Typically, Th17 cells are defined phenotypically as $CD4^+$, $IL\text{-}17^+$, and $ROR\gamma t^+$; however, Luo only utilized $CD4^+$ and $IL\text{-}17^+$. The investigators then quantify RORγt by PCR on bulk RNA isolated from the entire spleen. The approach is flawed as RORγt is expressed by multiple cell types within the immune system. Beyond this, the authors reported a change in RORγt from PCR experiments due to BPA exposure from 1.3-1.8 in males on PND21 and 1.3 -2.2 in females on PND21, which wanes on PND42. Because the y-axis in figure 4 of the manuscript lacks units, it is unclear what is being presented. What is clear is that the changes are unremarkable as they are less than modest. Also, the increase in Th17 cells in spleen was identified by ex vivo stimulation of the isolated cells with PMA/Ionomycin, a nonphysiologically relevant, nonspecific leukocyte activator. In spite of this questionable approach, the increase in Th17 cells due to BPA hardly rises above background. Specifically, in females at 21 days at the highest dose Th17 cells increased from ~1.3% to 3.3% in the spleen. At 42 days, in females, the percentage of Th17 cells increased with BPA treatment, from ~1.2% to 2.5% in the spleen, waning over time. Again, no increase in the percentage of Th17 cells was reported in the lungs, and these miniscule changes in the periphery are of questionable biological relevance.

Conclusion

Only a few cited epidemiology studies show a positive association between BPA exposure and the "development of allergic lung inflammation". These are not compelling due to poor study design, poor BPA exposure data, and/or fail to demonstrate allergy. The closest Zhou et al. come to demonstrating allergic lung inflammation is parent-reported accounts of wheezing in infants by questionnaire. Gascon et al. found no association between BPA exposure and IgE levels or asthma, which are indicative of allergic airway disease. Many of the epidemiology studies cited found no positive association between BPA exposure and "allergic lung inflammation". The lack of compelling epidemiology data is paralleled by a dearth of science linking Th17 cells/IL-17 to "development" of atopic airway allergy. Key animal studies cited have flawed experimental design, presentation, and interpretation yet deemed by the CEP as tier 1 quality. Importantly, these studies failed to even assess pulmonary function. Further, the current science is not congruent with the stated conclusions, specifically that Th17 cells/IL17 are responsible for the "development of allergic lung inflammation". Even the CEP questions their conclusion as evidenced by: "Even if mechanisms along which the immune system is affected by BPA are not clear, it is clear from the studies shedding some light on these mechanisms, that effects may be

on non-specific cells, such as APCs and epithelial cells, that through presentation of antigens to T lymphocytes or release of mediators influence the regulatory homeostasis of the immune system. (Line 3662-3665)". This acknowledgement is extraordinary and contrasts the assertion by the CEP of a BPA-mediated "… increase of Th17 cells was identified as the critical effect; these cells are pivotal in cellular immune mechanisms and involved in the development of allergic lung inflammation. (Line 23-25)"