



CRIS RESPONSE TO EFSA: RE-EVALUATION OF BPA FULL 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. This report includes CRIS’s full response and corresponding citations. For the submitted response, please visit https://go.msu.edu/CRIS_BPA_EFSA_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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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.

(Epidemiology studies – Lines 2862-2983)

First, the CEP concluded that the evidence for a significant positive association between BPA exposure during pregnancy and allergy is ALAN, as there are a similar number of studies that found no association as reported a positive association. Also, the epidemiology studies are weak on determining the level of BPA exposure. Moreover, the presented assessment of inflammatory respiratory disease is equally lacking. For example, Zhou et. al. (RefID 9013) utilized single time point data for both mother and child for the assessment of BPA in blood and urine. Such data cannot be extrapolated to emulate average human exposure over time. Furthermore, the assessment of wheeze by questionnaire six months after birth is overtly subjective, as a wheeze is common in many etiologies. Additionally, parental-reporting of wheeze after a six-month period will result in highly variable data. Beyond these points, Zhou and colleagues did not reach significance in the incidence of either eczema or wheezing alone, which were the only outcomes of allergic disease.

Another epidemiology study that has been cited by the CEP, supporting an association between BPA exposure and “development of allergic lung inflammation”, is Gascon et. al. (RefID 2206). In these studies, children were evaluated for association of prenatal BPA exposure on lung parameters. The data demonstrated an association between BPA and wheeze, chest infections, and bronchitis; but the associations between BPA exposure and wheeze, chest infections, bronchitis 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. [1-4].



(Th17 and IgE – Line 118-124)

Second, 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 [5-8]. Th17 cells have also been implicated in “severe” chronic asthma, but Th2 driven disease is the primary endotype with early onset [7, 9]. These findings are supported by animal studies showing involvement of Th17 cells in mice in an allergic immune response but only in aged mice [10]. 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.

(Conclusion on hazard identification for immunotoxicity of BPA – Line 3648-3691)

Third, 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 [1-4, 11]. Furthermore, IL-17 has been shown to be produced by a variety of cells including CD8 T cells, NKT cells, neutrophils, and ILCs [12, 13]. 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 [14]. 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 [15]. These data suggest that IL-17 does not play a critical role, even in subjects with severe asthma.

Fourth, we will address serious confounding factors and/or results that are contrary to the conclusion reached by the CEB in papers deemed of high import by the committee (ranked as tier 1), specifically Luo et al 2016 (RefID 4679), Maliase et al. 2018 (RefID 11172) and O’Brien et al. 2014 (RedID 5462).

(Developmental exposure (pre-natal and/or post-natal until weaning) – Line 3383-3417)

O’Brien et al. utilized ovalbumin as the sensitizing antigen in an allergic asthma model in mice. In male mice BPA decreased airway inflammation at the highest dose and produced no enhancement of inflammation at all other doses, in either sex. Contrary to the CEB conclusion, BPA produced a suppression of IL-17 in the lungs in both sexes at every dose. Given the inconsistencies between immunological parameters in the lungs and the periphery, we question whether the effects are indicative of the model rather than the biology, as evidenced by a three-fold increase in IgE in the lungs with no pulmonary inflammation. Also, there was no increase in BALF-associated neutrophils, characteristic of



IL-17-mediated airway inflammation. Notably, the authors did not assess whether the immunologic changes by BPA exposure produced any adverse effects on pulmonary function, which is commonly evaluated.

(Cellular immunity - Line 3453-3498)

Luo et al. 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-17⁺, and ROR γ t⁺; however, Luo only utilized CD4⁺ and IL-17⁺. The investigators then quantify ROR γ t by PCR on bulk RNA isolated from the entire spleen. The approach is flawed as ROR γ t is expressed by multiple cell types within the immune system [16, 17]. Beyond this, the authors reported a change in ROR γ t 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.

Malaise et al. performed a perinatal study in mice treated daily by oral gavage, from the 15th day of gravidity to weaning of pups, with 50 μ g/kg/day of BPA. It is unclear why the investigators used 0.1% ethanol in corn oil as the vehicle for BPA, when BPA is soluble in aqueous solutions. A major shortcoming of this study was that only a single dose of BPA was used, limiting the ability to demonstrate a cause-and-effect relationship. Although the authors claim that the dose is relevant to human exposure, the dose was 10-fold higher than the TDI and was administered as a bolus dose, which will likely result in higher systemic concentrations than experienced by humans through consumption of BPA containing foodstuffs. The authors claim that BPA treatment induced inflammation; however, it is noteworthy that no histopathology was performed, and all measurements were conducted on cells ex vivo. Hence there is no evidence presented that demonstrates “inflammation”. In this study three different tissues were assessed for Th17 cells and IL-17 production ex vivo, after CD3/CD28 activation (lamina propria, spleen and mesenteric lymph nodes). Although it is difficult to determine the overall recovery of T cells from the lamina propria and MLN, it is concerning that only 2% of the splenic T cells were activated by CD3/CD28 stimulation after 72 hours.



This suggested that the authors were unsuccessful in activating T cells and their subsequent cytokine analysis is uninterpretable in terms of quantification or determining differences between treatment groups. Consistent with the poor T cell activation, the changes in IL-17 between control versus BPA treatment groups 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. Again, the reported BPA produced changes are arguably miniscule. Also no attempt was made to evaluate the effects of BPA on pulmonary function in this study.

In conclusion, the few epidemiology studies that claim to have identified a positive association between BPA exposure and the “development of allergic lung inflammation”, are not compelling due to questionable study design, insufficient measures of BPA exposure, and/or having failed to demonstrate actual allergic lung inflammation. For example, in the study by Zhou et al., the closest the investigators come to demonstrating allergic lung inflammation is parent-reported accounts of wheezing in infants six months after birth. In another study, Gascon et al. found no association between BPA exposure and IgE levels or asthma, which would have been indicative of allergic airway disease. Moreover, many of the epidemiology studies cited by the CEP found no positive association between exposure to BPA in humans that resulted in “development of allergic lung inflammation”. The lack of compelling epidemiology data may be due to a similar dearth of compelling literature linking Th17 cells/IL-17 to the “development” of atopic allergy in the lung. Studies using in vivo BPA exposure with questionable experimental design, presentation, and interpretation were cited and deemed to be of tier 1 quality, to make a causal link between toxicant and outcome (i.e., BPA-induced Th17 cells/IL-17 driven atopic lung inflammation). Importantly, these studies failed to demonstrate any change in pulmonary function. Moreover, the current state-of-the-science is not congruent with the conclusions put forward by the CEP, specifically that Th17 cells/IL17 are responsible for the “development of allergic lung inflammation”. In fact, even the CEP appears to question their own conclusion as evidenced in the following statement: *“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 by the CEP is extraordinary in that the CEP neither knows what cell type(s) or mechanism(s) may be involved in what are claimed to be the cause of the putative, BPA-mediated “development of allergic lung inflammation”. The aforementioned statement is in contrast to the previous assertion in the abstract of the report that the *“immune system was identified as the most sensitive health outcome category to BPA exposure. Specifically, an 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)”



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