Bisphenol A, \( \text{T}_\text{H} \text{17 cells}, \) and allergy: A commentary

Ian Kimber\(^a, \* \) Nicole Woelffen\(^b, \* \) and Kevin Sondenheimer\(^b, \* \)

\(^a\)Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK; \(^b\)Covestro Deutschland AG, Leverkusen, Germany

**ABSTRACT**

There is a continuing interest in whether Bisphenol A (BPA) is able to cause adverse health effects through interaction with elements of the immune system. That interest has been fuelled further by the recent publication of a draft opinion on BPA prepared by the European Food Safety Authority (EFSA) Panel on Food Contact Materials, Enzymes and Processing Aids (CEP). This draft opinion judged effects on the immune system to be the most sensitive health outcome, and identified BPA-induced changes in the frequency of T-helper (\( \text{T}_\text{H} \text{17} \)) cells in the spleens of mice as being the critical effect based on an association of these cells with inflammation. Based on these evaluations the CEP Panel recommended that a revised Tolerable Daily Intake (TDI) for BPA of 0.04 ng/kg bw/day should be adopted; representing a very substantial reduction (100,000-fold) compared with the existing TDI. The purpose of this commentary is to summarize briefly the role of \( \text{T}_\text{H} \text{17} \) cells in immune responses, and to review relevant literature regarding the influence of BPA on these cells, and on inflammatory responses in the lung and respiratory allergy. The conclusion drawn is that based on uncertainties about the effects of BPA on \( \text{T}_\text{H} \text{17} \) cells and lung inflammation in mice, the absence of consistent or persuasive evidence from human studies that exposure of BPA is associated with inflammation or allergy, and unresolved questions regarding the species selectivity of immune effects induced by BPA, it is inappropriate to adopt the revised TDI. Additional research is required to explore further the influence of BPA on the immune system and immune responses.

**Introduction and background**

Bisphenol A [BPA; 4,4’-\( \text{-(propane-2,2-diyl)-diphenol} \)] is a high production volume chemical that is mainly used to produce polycarbonates and epoxy resins that are used for a wide variety of applications. (Geens et al. 2012; Michalowicz 2014). It is known that BPA has estrogenic activity and that under some circumstances it is able to interact with a variety of receptors. Various diseases and adverse health effects have been attributed to BPA, including metabolic, neurobehavioral, and reproductive and developmental effects (Krishnan et al. 1993; Welshons et al. 2006; Kabir et al. 2015; Matuszczak et al. 2019). Given the continuing interest in BPA, and the range of biological mechanisms and health effects that have been ascribed to it (Rochester 2013; Michalowicz 2014; Murata and Kang 2018), there is no surprise that attention has been paid to the possibility that BPA is also associated with perturbation of the immune system and/or immunotoxicity (Rogers et al. 2013; Del Rio Araiza et al. 2021).

The spectrum of immunological effects that have been associated with BPA (based upon human studies, animal experiments or *in vitro* investigations) is broad, and much of the data available at the time were reviewed in a commentary published in 2017 (Kimber 2017). That commentary included a consideration of a comprehensive review of risks to public health associated with the presence of BPA in foodstuffs published by the European Food Safety Authority (EFSA) Panel on Food Contact Materials, Enzymes, Flavorings and Processing Aids (CEF) (European Food Safety Authority (EFSA) 2015). Based on the data reviewed, the EFSA report concluded that there was some uncertainty about the relationship between BPA and adverse immunological effects. One conclusion drawn by EFSA was that ‘potential immunotoxicity therefore currently presents an uncertainty area in BPA risk assessment’ (European Food Safety Authority (EFSA) 2015).

The previous commentary (Kimber 2017) also considered in detail two papers published by Ménard, Guzylack-Piriou, Lencina et al. (2014a); Menard, Guzylack-Piriou, Leveque, et al. (2014b) that were not available for the 2015 EFSA review. These papers described experimental studies in rats that were interpreted by the authors as indicating that perinatal exposure to BPA compromises the development of immunological tolerance, and thereby potentially encourages the development of food intolerance in later life. In 2016, EFSA was asked by the Dutch Ministry of Health, Welfare and Sport to consider the results of those papers. The CEF Panel identified various limitations in the studies reported by Ménard, Guzylack-Piriou, Lencina et al. (2014a); Menard, Guzylack-Piriou, Leveque, et al. (2014b) and concluded that such limitations served to ‘confound interpretation of the results and prevent the assessment of the relevance to human health’ (EFSA. 2016). The commentary published in 2017 (Kimber 2017) drew the conclusion that: ‘Although the possibility that BPA may have subtle effects on the immune system that have not yet been disclosed cannot be ruled out, it can be
concluded that presently there is no persuasive evidence that BPA has the potential to cause immunotoxicity.

This current commentary was triggered by the recent publication by the EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) of a new draft opinion on BPA: 'Re-evaluation of the Risks to Public Health Related to the Presence of Bisphenol A (BPA) in Foodstuffs' (EFSA, 2021). In this new draft opinion the immune system was identified as being the most sensitive health effect category for BPA. The abstract of the draft opinion states that: ‘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.’ Based on this effect, a Tolerable Daily Intake (TDI) of 0.04 ng BPA/kg bw/day was proposed, the implication of which would be that a health concern exists for all age groups resulting from dietary exposure to BPA (EFSA, 2021).

The purpose of this new commentary is to consider the scientific evidence on which concerns about increases in TH17 cells, and a predisposition to allergic lung inflammation, are based. As a prelude to that it is relevant to summarize very briefly the role of Th17 cells in immune and allergic responses.

\section*{TH17 cells: a brief summary}

It is now well established that there exists considerable heterogeneity among T-lymphocytes. There are a number of well-recognized functional sub-populations of CD4\(^+\) T-helper (TH) cells. This operational diversity among TH cells enables the adaptive immune system to tailor and regulate responses to meet the wide variety of antigenic challenges to which the host may be exposed. Among the most fully characterized TH sub-populations are the TH1 cells, TH2 cells, TH17 cells, and regulatory-T (T\(_{\text{reg}}\)) cells. The focus here is on TH17 cells.

In the immune system TH17 cells have diverse roles. They were first described as cells that produce the cytokine interleukin (IL)-17, and that express high levels of the transcriptional regulator retinoic acid-related orphan receptor-yt (ROR\(\gamma\)t) (Harrington et al. 2005). It is now known that IL-17 plays a critical role in the recruitment of neutrophils and macrophages and thereby orchestrates inflammatory responses (Kolaczkowska and Kubes 2013). In addition, TH17 cells can secrete other cytokines, including IL-21 and IL-22 that promote inflammatory responses (Zhang et al. 2021). In the context of considering TH17 cells as a toxic endpoint it is important to appreciate that the differentiation and function of TH17 cells can be subject to changes driven by external biochemical influences, such as perturbations in metabolism (Wang et al. 2021; Zhang et al. 2021). Furthermore, it is known that TH17 cells, in common with other TH1 cell subsets, show some plasticity of phenotype, and in fact according to the cytokine milieu TH17 cells can undergo conversion to other effector cell phenotypes (Wan 2010; Cerboni et al. 2021).

There is a certain yin/yang relationship between TH17 cells and T\(_{\text{reg}}\) cells. The latter are a defined lineage of T-lymphocytes the function of which is to regulate and contain immune responses, and to provide homeostatic control of the immune system. The most important properties of T\(_{\text{reg}}\) cells are to maintain tolerance and to prevent excessive immune responses that can result in tissue damage (Holm et al. 2004; Sakaguchi 2004). A defining characteristic of T\(_{\text{reg}}\) cells is their expression of the forkhead box protein P3 (FOXP3) that is their master transcription factor and regulator of their functional activity. T\(_{\text{reg}}\) cells produce anti-inflammatory cytokines such as IL-10 and transforming growth factor (TGF)-\(\beta\)1 (Santamaria et al. 2021).

Thus, TH17 cells and T\(_{\text{reg}}\) cells are two separate phenotypes of T-lymphocytes that have reciprocal developmental pathways, and exhibit completely different, and opposing functions (Bettelli et al. 2006). It follows that the balance between TH17 and T\(_{\text{reg}}\) cells and their respective cytokine products will have an important, and in some cases possibly decisive, influence on the nature and outcome of adaptive immune responses. Under circumstances where the influence of TH17 cells outweighs that of T\(_{\text{reg}}\) cells then autoimmune responses and inflammatory reactions may be favored.

Given the potentially important influence of TH17 cells it is appropriate to consider the evidence for induced increases in numbers of these cells, and changes in related immunological metrics, cited by the EFSA CEP in the recent draft opinion on BPA (EFSA, 2021).

\section*{BPA and TH17 cells}

In addressing this issue, it is appropriate to start with a consideration of the paper by Luo et al. (2016) that reported that gestational and lactational exposure of mice to low doses of BPA was associated with an increase in TH17 cells in offspring. It was the results described in this particular paper that were relied upon by the EFSA CEP Panel to recommend a very significant reduction in the TDI for BPA to 0.04 ng BPA/kg bw/day (a reduction of 100,000-fold compared with the current TDI of 4\(\mu\)g BPA/kg bw/day) (EFSA, 2021).

Luo et al. (2016) reported that developmental exposure of mice to BPA caused an increase in the percentage of TH17 cells in the spleens of offspring. The increases in splenic TH17 cell numbers (based on cytoplasmic expression of IL-17) were consistently statistically significant in the offspring of mice treated with the top dose of BPA (1000 nM, equivalent to 47.5 \(\mu\)g/kg bw/day), but were nevertheless modest, ranging from \(\approx\) 1.2 to 2.2\% of total CD4\(^+\) splenocytes. Increases in the percentage of TH17 cells were observed at postnatal days (PND) 21 and 42, but this effect had attenuated by the latter age. Consistent with changes in the frequency of TH17 cells, there was also an elevated expression of ROR\(\gamma\)t mRNA in the spleen, and of IL-21 cytokine levels in the serum. In both cases the increases were modest at PND42. T\(_{\text{reg}}\) cell numbers were not measured (Luo et al. 2016).

There is no evidence from the studies reported by Luo et al. (2016) that the small increases in splenic TH17 cells were associated with any physiological changes or adverse health effects. Nor is there information about whether the changes in TH17 cell numbers were sustained as - in fact - they were already declining by PND42. It is uncertain whether such modest, and possibly transient, changes in TH17 cell numbers in the spleen would translate into enhanced inflammatory activity, or a predisposition to mount allergic responses in the lung. However, before drawing any conclusions regarding the importance or otherwise of changes in the frequency of TH17 cell numbers it is necessary to consider other relevant data.

The EFSA draft opinion (2021) cited three other studies (e.g. Bodin et al. 2014; Malaise et al. 2017; Malaise et al. 2018) and asserted that the results of the investigations described in these papers ‘support the findings (of Luo et al. 2016) showing an effect in the same direction.’ Bodin et al. (2014) used non-obese diabetic mice to explore the influence of pre- and peri-natal exposure to BPA on the development of Type 1 diabetes. As part of those investigations exposure to the top dose of BPA was found to be associated with a significant increase in the level of
IL-17 produced by stimulation of isolated spleen cells with bacterial lipopolysaccharide (LPS). This was not, however, an IL-17 specific effect as significant increases in the production of other unrelated cytokines (i.e. IL-2 and IL-10) were also reported.

Malaisé et al. (2017) examined gut dysbiosis and immune system homeostasis in mice exposed perinatally to BPA (50 µg/kg bw/day). Among a variety of endpoints examined, the frequencies of T<sub>H17</sub> cells found in the lamina propria and in the spleen were measured at PND45 and PND170. In the lamina propria T<sub>H17</sub> cells were significantly reduced in number at PND45 compared with vehicle-treated controls, but by PND 170 there were no significant differences between treated and control animals. A small, but statistically significant, increase in T<sub>H17</sub> cells was found in BPA exposed mice at PND45, but there was no significant difference in the frequency of splenic T<sub>H17</sub> cells between treated and control mice by PND170.

Malaise et al. (2018) investigated the effects of perinatal exposure of mice to BPA (50 µg/kg bw/day) on a wide variety of endpoints in adult female offspring at PND50. There was a statistically significant, but small (from ≈ 2% to less than 3% of total T-lymphocytes) increase in the percentage of splenic T<sub>H17</sub> cells. In this study, an increase in the number of T<sub>H17</sub> cells in the lamina propria at PND50 was reported.

The data available from the three papers cited above do not directly support the findings of Luo et al. (2016), or even show effects 'in the same direction.' Moreover, on no instance are any of the effects described linked with changes in T<sub>H17</sub> cell numbers. However, since the deliberations of the CEP Panel that resulted in the publication of the EFSA draft opinion in 2021, there have become available other papers that are relevant to a consideration of the potential impact of BPA on T<sub>H17</sub> cells.

Consistent with the report by Malaise et al. (2018) cited above, Malaise, Lencina, et al. (2020) found that perinatal exposure of mice to BPA (50 µg/kg bw/day) was associated with an increase in both splenic and lamina propria T<sub>H17</sub> cells at PND70. In the same analyses, treatment with BPA at this dose also resulted in a small but statistically significant decrease in T<sub>reg</sub> cells in both tissue compartments. A similar differential effect of perinatal exposure to BPA on T<sub>H17</sub> and T<sub>reg</sub> cell numbers in the mouse spleen has also been reported by Gao et al. (2020). At PND51 there was a significant increase in splenic T<sub>H17</sub> cells (associated with elevated expression of ROR<sub>γt</sub>), and a significant decrease in T<sub>reg</sub> cells (associated with decreased expression of FOXP3).

A paper that was not considered by the EFSA CEP was published by Dong et al. (2020). In that work, pregnant mice were exposed to BPA from gestational day 6 until the end of lactation. In female offspring treatment was associated with a small increase in the percentage of T<sub>H17</sub> cells in the spleen, and a small decrease in splenic T<sub>reg</sub> cells.

Of some interest are recent papers that have explored the impact of Vitamin D supplementation on BPA-induced changes in T<sub>H17</sub> cell numbers in mice. Wang et al. (2020) exposed pregnant mice to BPA (at same concentration used by Luo et al. [2016]), with or without Vitamin D supplementation. It was found that Vitamin D was associated with a dose-dependent attenuation of BPA-induced increases in splenic T<sub>H17</sub> cells numbers and IL-17 expression in offspring at both PND21 and PND42. The conclusion drawn was that changes induced by activation of the Vitamin D receptor (VDR) can dampen or ameliorate completely changes in T<sub>H17</sub> cells induced by BPA, possibly via inhibition of ROR<sub>γt</sub> mRNA expression (Wang et al. 2020). It has more recently been shown that Vitamin D can inhibit T<sub>H17</sub> cell differentiation induced by intrauterine inflammation (Zhang et al. 2022).

However, it is important to note that with respect to T<sub>H17</sub> cells and the impact of BPA there may be significant species differences. Malaise, Le Montec et al. (2020) examined the impact of BPA (and other bisphenols, i.e. BPS and BPF) on the production of IL-17 by mouse and human T-lymphocytes in vitro. The authors reported that BPA, at low and environmentally-relevant concentrations, increased significantly the production of IL-17 by mouse T-lymphocytes, but not by human T-lymphocytes. It is perhaps relevant also that the authors point out that a study by Tuomela et al. (2016) has described differences between mouse and human T<sub>H17</sub> cells. These data probably require further confirmation by other investigators. However, the results suggest that there may exist important species differences in the potential influence of BPA on immuno-logical parameters, and in particular on T<sub>H17</sub> cells and IL-17 expression.

One possibility is that there are species differences in Vitamin D and/or Vitamin D-mediated regulation of ROR<sub>γt</sub>. Indeed, there is some evidence that there are differences between humans and mice with respect to the regulation and composition of the VDR gene and its expression (Marcinkowska 2020). In this context it is of some interest that a recent review article entitled ‘Are rats more human than mice?’ (Wildner 2019) has made the case that in many instances, the similarities between the rat and human immune systems are greater than between the mouse and human immune systems.

Taken together, the data available on the influence of perinatal exposure to BPA on T<sub>H17</sub> cells in the mouse is rather fragmentary. The papers cited above do not provide evidence that modest increases in the percentage of T<sub>H17</sub> cells among total splenic T-lymphocytes are associated with adverse health effects. Nor is it clear whether induced changes in T<sub>H17</sub> cell numbers are systemic, or are sustained. It also needs to be ascertained whether there exist species differences in the effects on immune function induced by BPA, and in particular whether, under similar conditions, exposure of humans to BPA would result in changes to T<sub>H17</sub> and/or T<sub>reg</sub> cells.

In the context of possible species differences in the impact of BPA on immune function in general, and on T<sub>H17</sub> cells number and function in particular, it is relevant to consider studies conducted in rats. Three exhaustive and high quality rat studies were reviewed by the EFSA CEP Panel (Li, et al. 2018a, 2018b; Camacho et al. 2019). Each of these studies formed part of the Consortium Linking Academic and Regulatory Insights on Toxicity of BPA (CLARITY-BPA). Li et al. (2018a) reported studies in which the effects of BPA exposure (from 2.5 to 25,000 µg/kg bw/day, for up to one year) on the cellular composition of the spleen and thymus of rats were investigated. Very comprehensive analyses found remarkably few changes resulting from exposure to BPA (only 10 of 530 measurements made), and these were transient in nature. In a second study, and using the same dosing schedule, Li et al. (2018b) focused on the impact of BPA exposure of rats on responses by spleen cells. Few significant changes compared with controls were recorded, and these were described as being moderate in magnitude, not dose-dependent, and displaying no discernible trend. The authors concluded that ‘the observed BPA-mediated changes observed in this study are unlikely to alter immune competence in adult rats.’ Finally, Camacho et al. (2019) described the results of a two year toxicology study in rats with various dose groups of up to 25,000 µg/kg bw/day BPA. Few effects were observed, and none that were indicative of an adverse effect on the immune
system. Although it must be acknowledged that these three studies in rats did not include a specific analysis of T_h17 cells, it can nevertheless be concluded that the immune system of the rat appears to be unaffected by exposure to BPA. Taken together the data reviewed above suggests that there might be important species differences with respect to the potential impact of BPA on the immune system. This is an area that now requires further exploration.

A premise of the EFSA CEP draft opinion (2021) is that there is an association between small increases in splenic T_h17 cells and lung inflammation. It is relevant therefore to address the question whether under certain conditions of exposure BPA is able to exacerbate lung inflammation and respiratory allergy, and additionally whether any such changes have been associated alterations in T_h17 cell numbers. Both human and animal data are available.

**BPA and lung inflammation/respiratory allergy**

There is no consistent evidence from longitudinal human studies (studies both of exposure during pregnancy, and exposure during childhood) that there is an association between exposure to BPA and allergy or asthma. In many of the studies reviewed by the EFSA CEP Panel there were no statistically significant effects of exposure to BPA on a range of endpoints considered relevant for asthma and other respiratory effects.

The EFSA CEP Panel concluded that the evidence for a positive association between BPA exposure during pregnancy and allergy is ALAN [As Likely As Not; defined as there being a low confidence in the body of evidence for an association between exposure to the substance and health effect(s)]. The EFSA CEP Panel further concluded that the evidence for a positive association between BPA exposure during childhood and allergy is also ALAN (EFSA 2021).

In one cross-sectional study cited by the EFSA CEP Panel (Ashley-Martin et al. 2015) it was found that pre-natal exposure to BPA was not associated with increased levels of IgE antibody in cord blood. The relevance of this observation is that IgE is the class of antibodies that induce allergic reactions and inflammation. This and other cross-sectional studies reviewed led the EFSA CEP Panel to conclude that the data supported the findings from the longitudinal studies (ALAN) (EFSA 2021).

The conclusion drawn from human (longitudinal and cross-sectional) studies reviewed by the EFSA CEP Panel is that exposure to BPA during pregnancy or childhood is not associated with consistent changes in airway inflammation or allergy, or with changes associated with allergy. There are four other papers that have been published since the deliberations of the EFSA CEP Panel, and that have some relevance, though only two of these papers consider BPA in relation to lung function/asthma. One paper described a case controlled study conducted in Turkey which comprised 140 children with allergic rhinitis, and 140 healthy children as the control group. Levels of BPA were found to be higher in the children with rhinitis than in the control group (Nalbantoglu et al. 2021). A somewhat different approach was taken by Abellan et al. (2022) who undertook a prospective meta-analysis of eight European birth cohorts. In this case, maternal exposure to BPA was measured in spot urine samples during pregnancy. It was found that in utero exposure to BPA was associated with an increased odds ratio (of 1.13) of current asthma and an increased odds ratio (of 1.4) of wheeze among girls, but not in boys. Moreover, there was no overall association between in utero exposure to BPA and lung function as measured by spirometry.

A third paper reported a population-based birth cohort study that sought to examine the impact of prenatal exposure to BPA on IgE levels, cytokines and infant lung function. A significant association between BPA concentration and the level of IgE in cord blood was found, but this correlation was no longer present in serum at one year and beyond. Moreover, there were no associations between cord blood levels of BPA and allergic symptoms (eczema, wheezing or rhino-conjunctivitis) or lung function (Liao et al. 2020). A fourth paper described a nested prospective cohort study conducted in China (Li et al. 2021). The aim was to examine the impact of exposure to BPA (and also to BPF and BPS) during pregnancy and infantile eczema. A correlation between BPA and an increased risk of infantile eczema was found, the speculation being that the development of eczema might be associated with down-regulation of FOXP3 expression in cord blood (Li et al. 2021).

Although, in the papers summarized above, there might be evidence for some association between BPA levels and eczema and rhinitis in certain cohorts, there is a need for confirmatory studies. Taken together, investigations that have sought to explore whether in humans exposure to BPA drives an increased risk of allergy or lung inflammation are inconclusive, and there is a paucity of persuasive evidence. Against this background it is of interest to consider animal studies that have addressed the same or similar questions. Unless otherwise stated, the studies cited below do not include analyses of T_h17 cell numbers.

A paper published by O’Brien et al. (2014), and cited in the EFSA CEP draft opinion (2021), describes a study which examined the effects of gestational and lactational exposure of mice to various concentrations of BPA on the development of allergic sensitization and pulmonary inflammation in adult offspring. At the higher concentrations of BPA there was increased sensitization to a model allergen (ovalbumin [OVA]), measured as a function of IgE antibody production. However, exposure to BPA had no influence on any parameters of pulmonary inflammation. The authors of the paper drew the following conclusion: ‘While these data suggest that perinatal BPA exposure beginning before gestation enhances allergen sensitization by increasing serum IgE and splenocyte cytokine production, a substantial impact of BPA on OVA-induced pulmonary inflammation in adulthood was not observed,’ and also ‘Pulmonary inflammation as indicated by total and differential leukocyte counts, cytokines and pulmonary histopathology inflammatory scores was, however, not different, or was reduced, in offspring exposed to BPA’ (O’Brien et al. 2014). In addition, the authors suggested that ‘differences in mouse strains or BPA exposure routes may influence the levels of OVA-specific IgE which have been demonstrated to be variable between different strains of mice and rats’ (O’Brien et al. 2014). The findings of this paper suggest that gestational and lactational exposure of mice to BPA, under conditions where treatment-related effects are observed in the offspring, does not result in evidence of lung inflammation.

Other studies in mice have reported somewhat variable results that may, in part at least, reflect differences in dosimetry and/or exposure metrics. A report by Nakajima et al. (2012) found that exposure of mice to BPA in utero, but not exposure to BPA postnatally from breast milk, displayed airway hyperreactivity following challenge with methacholine. A more recent study reported increased IgE antibody levels following exposure to BPA in the diet. At the top dose of BPA (equivalent to 9.01 μg/kg/day) tested, there was an increase in IgE anti-OVA anti-
bodies together with an increase in IgG\_1 anti-OVA antibodies (Yanagisawa et al. 2019). In a separate study, the same authors measured IgE and IgG\_1 anti-OVA antibodies after intratracheal exposure of juvenile mice to BPA for 6 wk. Increases in both IgE and IgG\_1 antibodies in BPA-treated mice were observed, but in no instances were these increases statistically significant (Koike et al. 2018). In both studies airway inflammation was reported. However, as in the case of O’Brien et al. (2014), and other studies cited below, enhanced airway inflammation has not always been found.

It has also been reported that oral exposure of adult mice to BPA resulted in enhanced airway inflammation following sensitization and challenge with toluene diisocyanate (Tajiki-Nishino et al. 2018). It was found that the top dose of BPA caused a significant increase in eosinophils in the bronchoalveolar lavage fluid (BALF) of mice challenged with toluene diisocyanate. There was, however, no significant increase in total levels of IgE immunoglobulin. Moreover, there were comparable increases in the levels in BALF of IL-4 and interferon (IFN)-\(\gamma\) cytokines that have opposing effects on IgE antibody production. Employing a mouse model of allergic rhinitis to OVA, Wang et al. (2020) reported that BPA exposure resulted in increased nasal symptoms and elevated levels of anti-OVA IgE antibodies.

Several other studies have yielded different outcomes. Thus, studies in mice reported by Bauer et al. (2012) found that developmental exposure to BPA did not cause any significant changes in overall airway inflammation, the conclusion being that early life exposure to BPA does not exacerbate allergic lung inflammation into adulthood. Another independent investigation found that the influence of BPA on airway reactions in mice was variable. Of note, it was found that while lifelong exposure of mice to BPA apparently enhanced airway inflammation, exposure to BPA during pregnancy and lactation had no effect on respiratory allergic reactions in offspring. Also of interest was the fact that exposure of mice to BPA during the period of sensitization in fact inhibited the development of airway inflammation (Petzold et al. 2014). In that study variable effects on IgE anti-OVA antibody responses were found, and in fact exposure to BPA during sensitization was associated with a significant reduction in IgE antibody levels.

Finally, a study reported very recently has explored the effects of oral exposure to BPA on a variety of immune parameters in mice (Misme-Aucouturier et al. 2022). Although these investigations focused on food allergy, rather than lung inflammation or respiratory allergy, the data are relevant because they indicate a possible link in mice between T\(\text{H}17\) cells and allergy in mice. Groups of mice were exposed orally to BPA at doses of 0.4, 4, or 40 \(\mu\)g/kg bw/day from PND14 to PND49. Using a model of wheat allergy it was found that there was an increase in the levels of specific (anti-wheat) IgE antibodies in sensitized mice treated with all doses of BPA compared with sensitized controls. In addition, sensitized and challenged mice treated with the top two doses of BPA displayed increased serum levels of mouse mast cell protease-1 (mMCP-1) which was used as a marker of food allergy. In addition, a number of immune parameters (both humoral and cellular) were measured. Of greatest relevance in the context of this commentary is the fact that the frequency of T\(\text{H}17\) cells in mesenteric lymph nodes was measured as a function of ROR\(\gamma\)t expression. In wheat-sensitized mice, exposure to 4 and 40 \(\mu\)g BPA/kg bw/day increased the number of T\(\text{H}17\) cells compared with controls, whereas in the non-sensitized mice T\(\text{H}17\) cells were only increased relative to controls in animals exposed to 40 \(\mu\)g BPA/kg bw/day. In some instances interpretation the changes in immune parameters described in Misme-Aucouturier et al. (2022) are somewhat challenging. In the case of T\(\text{H}17\) cells, for instance, sensitization alone caused a significant increase in the number of T\(\text{H}17\) cells in mesenteric lymph nodes, and there was then a further increase associated with exposure to the higher concentrations of BPA. Nevertheless, one important observation was that, in the absence of sensitization, BPA caused an increase in T\(\text{H}17\) cells only at the highest BPA dose (40 \(\mu\)g/kg bw/day). This is comparable with the studies of Luo et al. (2016) in which a consistent increase in splenic T\(\text{H}17\) cells was associated with exposure to 47.5 \(\mu\)g BPA/kg bw/day.

Although the above data indicate that oral exposure to BPA under the experimental conditions used by the investigators is associated with an increase in lymph node T\(\text{H}17\) cells and an exacerbation of food allergy based on elevated serum levels of mMCP-1, it is premature to conclude that changes in T\(\text{H}17\) cell numbers in lymphoid tissue is the pivotal event that will drive augmented allergic responses. At very least it would be helpful to have independent confirmation of these data, possibly using alternative models of allergic reactions in mice, together with a more systematic assessment of T\(\text{H}17\) cell number in different lymphoid tissue compartments. Certainly, it is premature to propose, as suggested by Misme-Aucouturier et al. (2022), that their results suggest that ‘the 2015 European Food Safety Authority (EFSA) TDI should be reviewed to consider the immunotoxicity of BPA.’

**Concluding comments**

A starting point for the present commentary was the report of the EFSA CEP Panel (2021) indicating that they identified the immune system as being the most sensitive health outcome category to BPA exposure, and specifically that an increase in spleen Th17 cells in mice associated with exposure to BPA was the critical effect. It was the effect of BPA on T\(\text{H}17\) cell numbers in the spleens of mice that was described by Luo et al. (2016) which drove the proposal for a revised TDI for BPA of 0.04 ng/kg bw/day.

The data reviewed in this article show that in some experimental systems, and at certain levels of exposure, BPA can indeed cause a (usually modest) increase in T\(\text{H}17\) cell numbers, in some lymphoid compartments, in mice. However, before consideration is given to changes in the TDI for BPA based on the data currently available, it is suggested that the following issues are reflected upon:

- Little is known of the influence of BPA on murine T\(\text{H}17\) cells with respect to which lymphoid compartments are affected, and how persistent are the observed changes in cell frequency. Moreover, it is still not clear whether modest changes in T\(\text{H}17\) cell numbers induced in mice by exposure to BPA will necessarily cause exacerbation of inflammation or allergy;
- Comprehensive studies in rats have failed to identify important immunological changes, or to provide evidence of immunotoxicity, associated with exposure to BPA;
- Human data are inconclusive and sometimes contradictory. There is an absence of persuasive evidence that exposure to BPA is associated with lung inflammation or respiratory allergy in humans; and,
- There are indications of relevant species differences in T\(\text{H}17\) cells and their regulation, and/or in Vitamin D biology, that
underpin differences between humans and mice with respect to interaction of BPA with elements of the immune system.

Given these important uncertainties it would be premature to adopt a revised TDI for BPA until there is a more complete understanding of the relevance of relatively modest changes in the number of T_{H17} cells in lymphoid compartments, and an appreciation of how species differences may confound extrapolation of changes observed in mice to humans. At present there is no compelling evidence that small changes in immune parameters associated with exposure of mice to BPA result in adverse health effects.

Acknowledgements

The authors would like to acknowledge the support of the BPA PlasticsEurope Group and the American Chemistry Council in preparation of this article.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

Ian Kimber was supported by funding from the American Chemistry Council.

ORCID

Ian Kimber http://orcid.org/0000-0002-3538-8581

References

Abellan A, Mensink-Bout S, Garcia-Esteban R, Beneito A, Chatzi L, Duarte-Salles T, Fernandez M, Garcia-Aymerich J, Granum B, Iniguez C, et al. 2022. In utero exposure to bisphenols and asthma, wheeze and lung function in school-age children: A prospective meta-analysis of eight European Birth Cohorts. Environ Int. 162:106178.

Ashley-Martin J, Dodds L, Levy A, Platt R, Marshall J, Arbuckle T. 2015. Prenatal exposure to phthalates, bisphenol A, and perfluorooalkyl substances and cord blood levels of IgE, TSLP and IL-33. Environ Res. 140: 360–368.

Bauer S, Roy A, Emo J, Chapman T, Georas S, Paige Lawrence B. 2012. The Bodin J, Bolling A, Becher R, Kuper F, Lovik M, Nygaard U. 2014. Translocation of changes observed in mice to humans. At present there is no appreciation of how species differences may confound extrapolation of this article.

Cerboni S, Gehrmann U, Preite S, Mitra S. 2021. Cytokine-regulated TH17 differentiation of regulatory T-cells and TH17 cells induced by perinatal bisphenol A exposure in female offspring mice. Mol Cell Toxicol. 16(2): 167–174.

EFSA. 2016. A statement on the developmental immunotoxicity of bisphenol A (BPA): answer to the question from the Dutch Ministry of Health, Welfare and Sport. EFSA J. 14:4580.

EFSA. 2021. Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA J. (unassigned volume and page numbers).

European Food Safety Authority (EFSA). 2015. Panel on food contact materials, enzymes, flavorings and processing aids (CEF) scientific opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA J. 13:3978.

Gao L, Dong Y, Lin R, Meng Y, Wu F, Jia L. 2020. The imbalance of T_{effector} T_{H17} cells induced by perinatal bisphenol A is associated with activation of the P13K/Akt/mTOR signalling pathway in male offspring mice. Food Chem Toxicol. 137:111177.

Geens T, Aerts D, Berthot C, Bourguignon J-P, Goeyens L, Leconte P, Maghuin-Roger P, Pironnet A-M, Pussiemer L, Scippo M-L, et al. 2012. A review of dietary and non-dietary exposure to Bisphenol A. Food Chem Toxicol. 50(10):3725–3740.

Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT. 2005. Interleukin-17 producing CD4\(^{+}\) effector T-cells develop via a lineage distinct from T-helper type 1 and 2 lineages. Nat Immunol. 6(11):1123–1132.

Holm T, Nielsen J, Claesson M. 2004. CD4\(^{+}\)CD25\(^{+}\) regulatory T-cells: 1. Phenotype and physiology. APMIS. 112(10):629–641.

Kabir E, Rahman M, Rahman I. 2015. A review on endocrine disruptors and their possible impacts on human health. Environ Toxicol Pharmacol. 40(1):241–258.

Kimber I. 2017. Bisphenol A and immunotoxic potential: a commentary. Regul Toxicol Pharm. 90:358–363.

Kolke E, Yanagisawa R, Win-Shwe T, Takano H. 2018. Exposure to low-dose bisphenol A during the juvenile period of development disrupts the immune system and aggravates allergic airway inflammation in mice. Int J Immunopathol Pharmacol. 32:1–14.

Kolaczkowska E, Kubes P. 2013. Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol. 13(3):159–175.

Krishnan A, Stathis P, Permutt S, Tokes L, Feldman D. 1993. Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology. 123(6):2279–2286.

Li J, Bach A, Crawford RB, Phadnis-Moghe AS, Chen W, D’Ingillo S, Kovalova N, Suarez-Martinez JE, Zhou J, Kaplan BLF, et al. 2018a. CLARITY-BPA: Effects of chronic bisphenol A exposure on the immune system: part 1. Quantification of the relative number and proportion of leukocyte populations in the spleen and thymus. Toxicology. 396-397: 46–53.

Li J, Bach A, Crawford RB, Phadnis-Moghe AS, Chen W, D’Ingillo S, Kovalova N, Suarez-Martinez JE, Zhou J, Kaplan BLF, et al. 2018b. CLARITY-BPA: effects of chronic bisphenol A exposure on the immune system: Part 2. Characterization of lymphoproliferative and immune effector responses by splenic T-lymphocytes. Toxicology. 396-397:54–67.

Li X-N, Wu D, Liu Y, Zhang S-S, Tian F-L, Sun Q, Wei W, Cao X, Jia L-H. 2021. Prenatal exposure to bisphenols, immune responses in cord blood, and infantile eczema: a nested prospective cohort study in China. Environ Health. 22(112987):112987.

Liu S, Chen L, Tsai M, Hua M, Yao T, Su K, Yeh K, Chiu C, Lai S, Huang J. 2020. Prenatal exposure to bisphenol-A is associated with dysregulated perinatal innate cytokine response and elevated cord IgE level: a population-based birth cohort study. Environ Res. 191:111023.

Luo S, Li Y, Li Y, Zhu Q, Jiang J, Wu C, Shen T. 2016. Gestational and lactational exposure to low-dose bisphenol A increases T_{H17} cells in mice offspring. Environ Toxicol Pharmacol. 47:149–158.

Malaise Y, Le Mentec H, Sparfel L, Guzlyack-Piriou L. 2020. Differential influences of the BPA, BPS, and BPF on in vitro IL-17 secretion by mouse and human T-cells. Toxic in Vitro. 69:104993.

Malaise Y, Lencina C, Cartier C, Olier M, Menard S, Guzlyack-Piriou L. 2020. Perinatal oral exposure to low doses of bisphenol A, S or F impairs immune functions at intestinal and systemic levels in female offspring mice. Environ Health. 19(1). doi:10.1186/s12940-020-00614-w

Malaise Y, Menard S, Cartier G, Gauthier E, Lasserre F, Lencina C, Harkat C, Geoffre N, Lakhal L, Castan I, et al. 2017. Gut dysbiosis and impairment of immune system homeostasis in perinatally-exposed mice to bisphenol A. A precede obese phenotype development. Sci Rep. 7(1):1.

Malkise Y, Menard S, Cartier C, Lencina C, Sommer C, Gauthier E, Houdeau E, Guzlyack-Piriou L. 2018. Consequence of Bisphenol A perinatal exposure on immune responses and gut barrier function in mice. Arch Toxicol. 92(2):347–358.

Marcinkowska E. 2020. The vitamin D system in humans and mice: similar but not the same. Reports. 3(1):1.
Matuszczak E, Komarowska M, Debek W, Hermanowicz A. 2019. The impact of bisphenol A on fertility, reproductive system, and development: a review of the literature.Intl J Endocrinol. 2019:1–8.

Ménard S, Guzyłack-Piriou L, Lencina C, Leveque M, Naturel M, Sekkal S, Harkat C, Gaultier E, Olier M, Garcia-Villar R, et al. 2014a. Perinatal exposure to a low dose of bisphenol A impaired systemic cellular immune response and predisposes young rats to intestinal parasitic infection. PLOS One. 9(11):e112752.

Menard S, Guzylack-Piriou L, Leveque M, Braniste V, Lencina C, Naturel M, Moussa L, Sekkal S, Harkat C, Gaultier E, et al. 2014b. Food intolerance at adulthood after perinatal exposure to the endocrine disruptor Bisphenol A. Faseb J. 28(11):4893–4900.

Michalowicz J. 2014. Bisphenol A – sources, toxicity and biotransformation. Environ Toxicol Pharmacol. 37:738–758.

Misme-Aucouturier B, De Carvalho M, Delage E, Dijoux E, Klein M, Brosseau C, Bodinier M, Guzyłack-Piriou L, Bouchaud G. 2022. Oral exposure to Bisphenol A exacerbates allergic inflammation in a mouse model of food allergy. Toxicology. 472:153188.

Murata M, Kang J. 2018. Bisphenol A (BPA) and cell signalling pathways. Biotechnol Adv. 36(1):311–327.

Nakajima Y, Goldblum RM, Midoro-Horiuti T. 2012. Fetal exposure to Bisphenol A as a risk factor for the development of childhood asthma: An animal model study. Environ Health. 11:8.

Nalbantoglu A, Celikkol A, Samanci N, Gunaydin NC, Nalbantoglu B. 2021. Bisphenol A as a risk factor for allergic rhinitis in children. Human Exp Toxicol. 30(1):103–111.

O’Brien E, Bergin IL, Dolinoy DC, Zaslona Z, Little RJA, Tao Y, Peters-Golden M, Mancuso P. 2014. Perinatal bisphenol A exposure beginning before gestation enhances allergen sensitization, but not pulmonary inflammation, in adult mice. J Dev Orig Health Dis. 5(2):121–131.

Petzold S, Averbek M, Simon J, Lehmann I, Polte T. 2014. Lifetime-dependent effects of Bisphenol A on asthma development in an experimental mouse model. PLOS One. 9(6):e100468.

Rochester J. 2013. Bisphenol A and human health: a review of the literature. Reprod Toxicol. 42:132–155.

Rogers JA, Metz L, Yong VW. 2013. Review: endocrine disrupting chemicals and immune responses: A focus on bisphenol-A and its potential mechanisms. Mol Immunol. 53(4):421–430.

Sakaguchi S. 2004. Naturally arising CD4+ regulatory T-cells for immunological self-tolerance and negative control of immune responses. Annu Rev Immunol. 22:531–562.

Santamaría J, Borelli A, Iria M. 2021. Regulatory T-cell heterogeneity in the thymus: impact on their functional activities. Front Immunol. doi:10.3389/fimmu.2021.678355.