Meeting

# Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs - Immunotoxicity

**This is a paper for discussion.**

**This does not represent the views of the Committee and should not be cited.**

PDF
View Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs- Immunotoxicity as PDF (377.43 KB)

Please note this PDF is not in an accessible format, please refer to the below page for accessible content.

## Immunotoxicity

Please note - this paper should be read in conjunction with the introductory paper which sets out how the evaluation was done.

## Epidemiological studies

1. A total of 9 studies were appraised for the health outcome category (HOC) Immunotoxicity. Details of the appraisal are in Annex B.

2. The following Cluster (C) asthma/allergy and exposure periods (Exp) childhood and pregnancy were brought forward for weight of evidence (WoE) analysis. The main information extracted from the studies are summarised in Annex C (EFSA, 2021), the outcome of the WoE is described below and in Table format in Annex D (EFSA, 2021).

## Asthma/allergy

3. In total the European Food Safety Authority (EFSA) assessed 8 longitudinal studies; the studies assessed different exposure windows, however all used spot urine samples. The observed heterogeneity for the endpoint definitions was considerable, including asthma (n=3), wheezing (n=3), chest infection (n=1), allergic disease (n=1), atrophy (n=1), rhinitis (n=1), atopic dermatitis (n=1), forced respiratory volume in first second (FEV1; n=1), polycarbonate 20 (PC20; n=1), immunoglobulin (Ig)E (n=1), fraction of exhaled nitric oxide (FENO; n=1). Statistically significant associations in more than one study were seen for asthma and wheezing.

## Exposure during pregnancy

4. Seven cohort studies, including four with a European-descent population, assessed the association between bisphenol A (BPA) exposure measured in pregnancy and allergy-related endpoints (with a cumulative sample size of 2836 participants). The populations under study were comparable in size but varied in their characteristics.

5. EFSA noted that the currently available longitudinal epidemiological evidence was characterised by a small number of studies, suboptimal exposure assessment and considerable heterogeneity in the assessed populations, exposure levels and endpoints.

6. Donohue et al. (2013) used a birth cohort to investigate the association between pre-natal BPA exposure and wheeze, asthma and increased fraction of FENO in African American and Dominican children in the United States (USA) followed up until the age of 11 years old (n = 568). Maternal spot urine samples were collected during the third trimester (n=375), children’s spot urine samples were collected at ages 3 (n=408), 5 (n=401) and 7 (n=318) years from 2001 to 2010. No statistically significant associations were observed.

7. Spanier et al. (2014b) assessed pre-natal BPA exposure in a population of European ancestry (Health Outcomes and Measures of the Environment (HOME) study, n = 398) and parent-reported wheeze (5-year follow-up) and FEV1 at age 4 and 5 years. Statistically significant associations were observed for BPA exposure at 16 weeks gestation and for FEV1 at 4 years and wheeze at 5 years [Odds Ratio (OR), 95% Confidence Interval (CI); 1.79, 1.16–2.78], but not for BPA exposure at 26 weeks gestation or mean gestational BPA exposure and wheeze at 5 years or FEV1 at 5 years.

8. Gascon et al. (2015) in the INMA (INfancia y Medio Ambiente (Environment and Childhood) Project)) birth cohort (n = 657) evaluated whether pre-natal exposure to BPA and phthalates increases the risk of respiratory and allergic outcomes (chest infections, bronchitis, wheeze, eczema, asthma, IgE) in children (7-year follow-up). Samples were taken between week 23 and 29 of gestation. Pre-natal BPA levels were statistically significantly associated with wheeze [Relative Risk (RR), 1.20; 95% CI, 1.03–1.40], chest infections (RR, 1.15; 95% 2913 CI, 1.00–1.32) and bronchitis (RR, 1.18; 95% CI, 1.01–1.37).

9. Vernet et al. (2017) reporting on the EDEN birth cohort (n = 587) assessed the association between pre-natal BPA exposure (among 9 other phenols and 11 other phthalates) and respiratory outcomes related to allergy (FEV1, asthma, bronchitis, wheezing). Follow ups were conducted at 5 years of age. No statistically significant associations were observed.

10. Zhou et al. (2017) evaluated the association between BPA concentrations collected at delivery and eczema and wheeze in infants at age 6 months (n = 412). Follow ups were conducted at 6 months and BPA was associated with an increased risk of allergic diseases (OR, 1.21; 95% CI, 1.02–1.47).

11. Buckley et al. (2018) in the Mount Sinai Children's Environmental Health Study evaluated the pre-natal exposure to BPA (among other phenol and phthalate biomarkers) and its association to asthma, wheeze and skin atopy at ages 6 and 7 years (n = 164). For asthma, a statistically significant association for boys was observed (diagnosis, OR 3.04, 95% CI, 1.38–6.68; emergency room visits, OR 3.28, 95% CI 1.15–9.34), no significant change for girls or any other endpoint considered.

12. Liao et al. (2016) in a birth cohort conducted in Taiwan (n = 250) addressed the association between pre-natal BPA exposure (cord blood) and production of tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and IL-10 after stimulating mononuclear cells with Toll-like receptor ligands (TLR1–4 and 7/8), bacteria from nasopharyngeal specimens and the incidence of infections. Children were followed up until 1 year of age. Significant associations were identified for TLR3-stimulated and TLR4-stimulated TNF-α response as well as for TLR7/8-stimulated IL-6 response, but not for infection or bacterial colonisation during the first year of life. (Not able to find this information in Annex C)

13. Based on the above, EFSA concluded that the association between BPA exposure during pregnancy and allergy is as likely as not (ALAN).

## Exposure during childhood

14. Four cohort studies, including one with European-decent population USA; n= 398), assessed the association between BPA exposure measured in childhood and allergy-related endpoints (cumulative n=1546). The populations were of comparable size but varied in characteristics. BPA was measured via a single spot urine sample but varied between studies; for individuals of European descent the total maternal BPA geometric mean (µg/g of creatinine) was 2.4 (95% CI, 2.1-2.7; range 0.5-294). Hence, EFSA concluded that the current evidence is characterised by a small number of studies, suboptimal exposure assessment and considerable heterogeneity in the assessed populations, exposure levels and endpoints.

15. Donohue et al. (2013) implemented a birth cohort to investigate the association between BPA exposure (3, 5 and 7 years) and wheeze, asthma and increased FENO in African American and Dominican children in the USA followed up until the age of 11 years old (n = 568). No statistically significant associations were observed. (The ages provided in Annex C differ slightly)

16. Spanier et al. (2014b) was the only study including a population of European ancestry (HOME study, n = 398). Urine samples were collected annually, and parent-reported wheeze was assessed every 6 months for 5 years along with FEV1 at age 4 and 5 years. No statistically significant associations were observed.

17. The study by Kim et al. (2014) included older children (n = 127, age range 7–8, 9-10 and 11-12 years) in Korea which were assessed three times every 2 years. The association between BPA concentration at 7–8 years of age and wheezing, asthma and PC20 at ages up to 11–12 years were examined. A statistically significant association was observed at 11-12 years of age for wheezing (OR 2.48; 95% CI 1.15–5.31), asthma (HR 2.13; 95% CI 2958 1.51–3.00) and PC20 (beta 22.33; P = 0.02).

18. Wang et al. (2016) evaluated 453 children in Taiwan (Childhood Environment and Allergic Diseases Study) with a follow up age of 6 years. Urinary BPA-G levels at age 3 were statistically significantly associated with asthma at age 6 (OR, 95% CI; 1.27, 1.04–1.55) and IgE.

19. EFSA concluded that the evidence for a positive association between BPA exposure during childhood and allergy was ALAN.

Overall conclusion

20. EFSA concluded that the evidence for a positive association between BPA exposure and asthma/allergy was ALAN.

### Cross-sectional studies

21. Three cross-sectional studies investigated the relationship between BPA exposure (maternal and foetal serum, placenta) and immunity-related endpoints. Ashley-Martin et al (2015), Savastano et al. (2015) and Ferguson et al. (2016a) assessed immunity biomarkers, while Spanier et al. (2014b), Lin et al. (2018) and Youssef et al. (2018) examined asthma-related endpoints, including lung function. The immunity biomarkers assessed included IgE, thymic stromal lymphopoietin (TSLP), IL-33, IL1Beta, IL-6, IL-10, TNF- α, plasma monocyte chemoattractant protein 1 and C-reactive protein (CRP).

22. Statistically significant results were observed for IL-6 in populations of European ancestry (n=76, Savastano et al. 2015; n=482, Ferguson et al., 2016a) and TNF-alpha (Savastano et al., 2015, n=76).

23. All three studies that assessed allergic disorders of the lung included children and yielded statistically significant results, supporting the findings from the longitudinal studies.

## Animal studies

24. A total of 42 studies were appraised by EFSA, details can be found in Annex E (internal and external validity).

25. The endpoints for each study identified as relevant can be found in Annex F (EFSA), including endpoints identified as key in the uncertainty analysis in 2015. The exception being “inflammation of the uterus” (pyometra and macrophage infiltration) and serum parameters (histamine and beta-hexosamidase), as no new data were available (Annex A (EFSA), Section 2.5).

26. The key endpoints in the uncertainty analysis in 2015 were the effects on serum parameters (IgE), lung effects following a) intraperitoneal sensitisation and inhalatory challenge to ovalbumin (OVA) (airway hyperactivity, eosinophiles in lavage), b) mucosal sensitisation and inhalatory challenge to OVA (inflammation severity, eosinophiles in lavage, neutrophiles, lymphocytes and T lymphocytes in lavage), c) mucosal sensitisation and inhalatory challenge to OVA (inflammation severity, eosinophiles in lavage, neutrophiles, lymphocytes and T lymphocytes in lavage), d) mucosal sensitisation and inhalation challenge to OVA and

lipopolysaccharides (LPS) (inflammation severity, eosinophiles in lavage, neutrophiles, lymphocytes and T-lymphocytes in lavage) and e) intraperitoneal sensitisation and inhalation challenge to OVA and alum (eosinophiles in lavage, neutrophiles and lymphocytes in lavage, airway resistance, airway elastance).

## Identification of clusters of relevant endpoints

27. Based on the available studies and the nature of the immune system, the relevant immune outcomes identified were: innate immunity, cellular immunity, humoral immunity, inflammation and allergic lung inflammation.

Innate immunity

28. The cells of the innate immune system, i.e., monocytes/macrophages, natural killer cells, antigen-presenting dendritic cells, and their products (such as antimicrobial molecule lysozyme produced by macrophages) were considered relevant by EFSA. Reduced cell numbers/activity may result in reduced resistance to pathogens/induction of acquired immunity and should therefore be regarded as adverse.

29. The specific effects of BPA on the innate immunity were lysozyme activity, mast cells (cysteinyl leukotriene (CysLT), prostaglandin D2 (PGD2), TNF- α, IL-13) monocytes/macrophages, major histocompatibility complex (MHC) class II+ cells, natural killer (NK) cells, natural killer T (NKT) cells and dendritic cells, granulocyte-colony stimulating factor (G-CSF).

Cellular immunity

30. The specific endpoints that were included for assessing the effects on BPA on cellular immunity were spleen weight, spleen histology, spleen proliferation, spleen total cell number, T-cell proliferation, Th17 cells, regulatory T (Treg) cells, Th1 cells, Th cells (CD4+ cells), Tc cells (CD8+ cells), CD25+ T cells, interferon- γ (IFN-γ; not in lung), IL-4 (not in lung), IL-5 (not in lung), IL-13 (not in lung), IL-17 (not in lung), IL-21 (not in lung), IL-23 (not in lung).

31. EFSA noted that the spleen contains basically all immune cells, including B lymphocyte cells (B cells), T lymphocyte cells (T cells), natural killer (NK) cells, macrophages, etc. Therefore, in reality, effects on the spleen could indicate effects on the cellular immunity (T cells), humoral immunity (B cells and antibody production) and innate immunity (NK cells and macrophages).

Humoral immunity

32. The specific endpoints included for the effects of BPA were IgA+ cells, IgA, IgM production and B-cell proliferation of spleen cells (e.g., using LPS or Pokeweed mitogen (PWM) as mitogen).

33. IgE is one of the isotypes produced and potentially affected by BPA exposure, but typically this antibody is associated with allergic reactions. Therefore, it is included in the allergic lung inflammation.

Inflammation

34. The specific endpoints that were included for the effects of BPA were IL-1, IL-6, IL-12p70, IL-22, IL-31, TNF- α, vascular endothelial growth factor (VEGF), lung stromal cell-derived factor 1 (SDF1), neutrophils, eosinophils and colonic inflammation score.

Allergic lung inflammation

35. Allergic lung inflammation may be brought about by serum IgE levels; the production of IgE is regulated by an array of cytokines, released from T-lymphocytes as well as other cell types. The specific endpoints included were lung IL-4, IL-13, IL-17, IL-33, lung TNF- α, lung (serum anti-OVA) IgE, lung inflammatory score, lung CysLT, lung Kupffer cells (KC), lung RANTES (Regulated upon activation, normal T cell expressed and secreted), lung cellularity in bronchoalveolar lavage (BAL) (total cells, macrophages, neutrophils, eosinophils, lymphocytes).

## Weight of Evidence of relevant endpoints

36. The main information extracted by EFSA from the studies addressing the relevant endpoints are summarised in Annex G.

37. Please note, that EFSA has split the endpoints/results of the studies by clusters (innate immunity, cellular immunity, humoral immunity, inflammation, and allergic lung inflammation), rather than study.

38. While the Secretariat has aimed to provide the relevant endpoints per section, they would like to make Members aware that, for ease of reading the study details and results/endpoints have been added in more detail the first time each study is mentioned by EFSA.

39. The outcome of the WoE is described in brief below and presented by EFSA in tabulated format in Annex H (EFSA).

40. EFSA noted that as a general approach for the WoE and selection of endpoints to be taken forward for risk characterisation, not all parameters in a cluster/subcluster needed to show effects in the same direction, to still prove an effect. Whereas all intermediate endpoints may shed light on the likelihood of a cluster to be affected by BPA, many are not specific for the adverse outcome, or not even predictive. Hence, a (sub)cluster which identified a likely effect, apical endpoint parameters within the cluster were taken forward for benchmark dose (BMD) analysis rather than these intermediate ones.

## Innate immunity

41. Six studies were identified in mice, five of which provided exposure during development, one during adulthood; four studies in rats were identified, two of which provided exposures during development, three during development and adulthood, one during growth phase/young age.

42. EFSA noted that some studies cover different stages of life, i.e., exposure and endpoint measurement timing.

Developmental exposure (pre-natal and/or post-natal until weaning)

43. Studies in mice by Malaise et al. (2018) and Rogers et al. (2017) and both studies in rats by Camacho et al. (2019; National Toxicology Program (NTP) Clarity Report, 2018) and Li et al. (2018a; part of the NTP Clarity Study) were allocated to **Tier 1**.

44. Malaise et al. (2017;2018) exposed C3H/HeN mice and female offspring to BPA (orally) at 50 µg/kg bw per day from gestation day 15 (GD15) to post-natal day 21 (PND21). Measurements were taken on PND50 (F1) for Malaise et al. (2018) and reported increased IFN-y (colon, lamina propria, mesenteric lymph nodes (MLN)), Th1 cells (spleen) and Th17 (lamina propria, MLN, spleen) and a decrease in lysosome activity (intestine), IgA concentration (faecal samples), IgA plasma cells (lamina propria), IgA cells (colon), activated T cells (lamina propria), Th cells (MLN), Treg cells (lamina propria, spleen) and MLN dendritic cells. No changes were reported for any of the other investigated parameters.

45. Rogers et al. (2017) exposed C57BL/6 mice and offspring to BPA at a concentration of 1000-3000 µg/kg bw per day from mating to birth by oral gavage. Measurements were taken at 8-10 weeks (F1) and no changes in body weights (bw) of male and female offspring was reported.

46. Camacho et al. (2019) exposed NCTR Spraque-Dawley rats, pregnant dams (F0; GD6-PND0) and female/male offspring (F1, PND1 to PND21 (stop-dose), 1 year (continuous dose), 2 years) to BPA at 2, 5, 25, 250, 2,500, 25,000 (only dams) µg/kg bw per day by oral gavage. Measurements were taken for F1 at PND90 (females) and at 1 and 2 years. An increased trend in spleen pigmentation in 250 µg/kg bw per day in stop-dose males and haematopoietic cell proliferation in the spleen in continuous dose in males at 1 year were reported. Furthermore, increased haematopoietic cell proliferation in the spleen in continuous dose in males at 25,000 µg/kg bw per day (1 year), lymphoid hyperplasia in spleen in 250 and 2500 µg/kg bw per day in stop dose in males (2 years; trend), thymus atrophy in 25 and 250 and 2500 µg/kg bw per day stop dose females at 1 year and 2 years, respectively and increases in monocytes (trend) in females in continuous dose were reported. Decreased spleen weight (absolute) at 25 µg/kg bw per day in stop-dose females and eosinophiles in 250 µg/kg bw per day continuous-dose group at 1 year were also reported. No changes were reported for all other parameters.

47. Li et al. (2018a) exposed Sprague-Dawley rats and offspring to BPA at a concentration of 2.5, 25, 250, 2500 or 25000 µg/kg bw per day by oral gavage on GD6-parturition (F0) and PND1-euthanasia (F1). Measurements were taken for F1 on PND21 (thymus and spleen) and PND90, 6 months and 1 year (spleen) and reported increased total cell number in the spleen (females 25 µg/kg bw per day), CD8 cells and NKT cells in splenocytes (males, 1 year, 25000 µg/kg bw per year) and DC (CD11b, CD11c) in spleen (males, PND90, 2.5 µg/kg bw per day). Decreases were seen in macrophages and mature dendritic cells in spleen in males at 1 year (2500 µg/kg) and 6 months (2.5 and 2500 µg/kg), respectively. No changes were reported for any other parameters.

48. Studies in mice by Malaise et al. (2017) and Patel et al. (2015a) were allocated to **Tier 2.**

49. Measurements were taken at PND45 and PND170 (F1) by Malaise et al. (2017) and reported increases in IL-17 and TNF- α in the liver, Th1 cells, IFN-y cells and Th17 cells in the spleen (PND45), increases in IL-22 in the liver, gonadal M1 macrophages, gonadal IL-17 and Treg cells in lamina propria (PND170) and increases in IL-17 in the spleen and decrease in Th1 cells in lamina propria on both days. Furthermore, a decrease in Th17 cells in the lamina propria on PND45 and Th1 cells in spleen and lysosome activity were reported on PND170. All other parameters investigated did not show any changes.

50. Patel et al. (2015a) exposed C57BL/6N mice (dams and male offspring) to 25 ng BPA per mL drinking water (~ 5 µg/kg bw per day) from GD11.5 until birth, and male pups until 3 and 4 months of age. No changes (spleen weight, monocytes, macrophages, dendritic cells) were reported.

51. The study by Bodin et al. (2014) in mice was allocated to **Tier 3**. Non-diabetic NOD/ShiltJ mice and female offspring were exposed to BPA at a concentration of 0.1, 1 and 10 mg/L in drinking water from mating to PND21. Measurements were taken at 7 weeks (F1, cytokine levels and release from splenocytes) and 7 and 11 weeks (F1, phenotyping cells from pancreatic lymph node). Increases in IL-17 release from splenocytes after LPS stimulation was reported at 7 weeks at exposures of 10 mg/L. No changes were reported for any of the other parameters.

52. EFSA concluded that except for the decrease in production of lysozyme, none of the other endpoints provided in these studies showed a consistent effect following BPA exposure. However, the effect was demonstrated in two publications from the same authors and may have been from the same study, with only one exposure concentration.

53. Therefore, EFSA concluded that the effects of BPA during the developmental exposure period was as likely as not and none of the endpoints were taken forward for BMD analysis. The uncertainty analysis can be found in Appendix D.

Developmental and adult exposure (pre-natal and post-natal in pups until adulthood

54. All three studies in rats were allocated to **Tier 1**.

55. Camacho et al. (2019) and Li et al. (2018a) have been described previously.

56. In the study by Li et al. (2018b) measurements were taken for F1 on PND90, 6 months and 1 year and reported increased spleen cell proliferation after LPS at 1 years in females at 2500 µg/kg and in males at 2.5, 2500 and 25000 µg/kg. Increased spleen cell proliferation was also reported after PWM stimulations in females at 6 months (25 and 2500 µg/kg) and males at 1 year (all concentrations except 250 µg/kg). Increased intracellular IgM was reported after LPS and PWM stimulations in females at PND90 at 2500 and 25000 µg/kg, respectively. Furthermore, the study reported increased CD25+ cells after anti-CD3/CD28 stimulation (2.5, 2500, 25000 µg/kg), CD80+ NK-cells after 24 hours ( 2.5 µg/kg ) and CD86+ NK-cells after 24 hours (25000 µg/kg) in males at 6

months, CD86+ NK-cells after 24 hours (25000 µg/kg ) and CD86+ NK-cells after 48 hours (25 µg/kg) in males at 1 year and MHC II+ cells within CD11b/c+ cells after 48 hours in females at PND90 (250, 25000 µg/kg). A decrease in spleen cell proliferation after PWM stimulation in females (PND90, 25000 µg/kg) and males (6 months, 2.5, 250 and 2500 µg/kg) and spleen cell proliferation after anti-CD3/CD28 stimulation in females (1 year, 2.5 µg/kg) was reported. A decrease in intracellular IgM after LPS stimulation in males (PND90, 25 µg/kg), CD80+ NK cells in females (after 24 hours, at 6 months, 25000 µg/kg), CD86 cells and MHCII cells within CD172α cells in males (after 48 hours, 6 months, 2500 µg/kg) were also reported. No changes in any of the other parameters were reported.

57. Overall, EFSA concluded that no consistent effects were shown/identified and therefore the effects were considered not likely and none of the endpoints were taken forward for BMD analysis.

58. Ogo et al. (2018) exposed male Wistar rats to BPA at a concentration of 20 or 200 µg/kg bw per day at PND36-66 via oral gavage. Measurements were taken on PND67 and increased numbers of neutrophiles (200 µg/kg) and labelled cells for IL-6 (both doses) in caput/corpus and cauda epididymis were reported. No other changes were reported.

59. EFSA allocated this study to **Tier 2**. As no effects on macrophages were observed, EFSA considered the effects to be not likely and did not take the endpoints forward for BMD analysis.

Adult exposure (after puberty)

60. Cetkovic-Cyrlje et al. (2017) orally exposed C57BL/6 mice to 1 and 10 mg/L (corresponding to 160 and 1600 µg/kg bw per day) from 4 - 16 weeks of age; in male mice diabetes was induced at 9 weeks of age with streptozotocin (STZ) over 5 consecutive days. Measurements were taken 11 and 50 days after STZ (74 and 113 days old). Increased T cell proliferation was reported at age 74 days (1000 µg/L) and 113 days (10000 µg/L). Decreased absolute number of Th cells and decreased percentages of Th, Tc, T and NK cells were reported at 74 days of age at 1000 µg/L. TNF- α and IFN-y in splenocytes were also decreased at 10000 µg/L at age 74 days. No other changes were reported.

61. EFSA allocated this study to **Tier 2**. Some statistical differences were shown on macrophages and natural killer cells, however without a clear dose-response relationship. Therefore, EFSA concluded that overall, no changes could be shown and judged the effect as not likely. None of the endpoints were taken

forward for BMD analysis.

Indirect (germline) exposure

62. No studies were available.

Overall selection of endpoints/studies for BMD analysis

63. Overall EFSA concluded that innate immunity effects of BPA were as likely as not across all exposure periods and therefore none of the endpoints were taken forward for BMD analysis.

## Cellular immunity

64. Ten studies in mice were identified by EFSA, six of which focused on exposure during development, one during development and adulthood and three during adulthood. An additional eight studies in rats were identified, five of which focused on exposure during development, three during development and adulthood and one during adult phase.

65. EFSA noted that some studies covered different stages of life.

Developmental exposure (pre-natal and/or post-natal until weaning)

66. Studies in mice by Luo et al. (2016) and Rogers et al. (2017) Malaise et al. (2018) and O'Brien et al. (2014a) and studies in rats by Lejonklou et al. (2017), Camacho et al. (2019; NTP Clarity Report, 2018), Dunder et al. (2018) and Li et al. (2018a) were allocated to **Tier 1**.

67. Luo et al. (2016) exposed ICR mice and offspring to BPA at concentrations of 10, 100 and 1000 nM (corresponding to 0.48, 4.75 and 48 µg/kg bw per day) via drinking water on GD0-PND21. Measurements were taken on PND21 and PND42 (F1) and increased Th17 cells in spleen in male (1000nM) and females (100 and 1000 nM) were reported at both sampling days. Increased IL-17, IL-21, IL-6 and IL-23 in serum was reported in males and females at PND21 (100 and 1000nM) and PND42 (1000nM). No other changes were reported for any of the other parameters.

68. O'Brien et al. (2014a) exposed BALB/c mice and offspring to BPA at concentrations of 0.05, 50 and 50,000 µg/kg diet (corresponding to 0.0075, 7.5 and 7500 µg/kg bw per day) from 2 weeks before mating until PND21. Measurements were taken at 12 weeks of age (F1). Increased Anti-OVA IgE in serum was reported in females at every dose and in males at 50 and 50,000

µg/kg. Increased IL-13 in splenocytes (50 and 50,000 µg/kg), IFN-y in splenocytes (every dose group), % macrophages in BAL in females (50,000 µg/kg) % lymphocytes in BAL in males and females (0.05 µg/kg) and RANTES in lung in females (0.005 µg/kg) were also reported. Decreased total cells in BAL were shown for females (50,000 µg/kg) and males (0.05 and 50,000 µg/kg), eosinophils in females (50000 µg/kg), eosinophils and macrophages in males (0.05 µg/kg), neutrophils in males (every dose group), lymphocytes in males (50000 µg/kg), % eosinophils in (females in 50000 µg/kg). Furthermore, a decrease in IL-4, IL-13 and TNF- α in lung in females (0.05 and 50000 µg/kg), IL-17 in lung in males and females (every dose group), lung CysLT in males and females (50000 µg/kg) and lung inflammatory score in males (50000 µg/kg bw) was reported.

69. Lejonklou et al. (2017) exposed pregnant female F344/DuCrl rats and offspring to BPA at a concentration of 0.5 or 50 µg/kg bw per day via drinking water at GD3.5-PND22. Measurements were taken at PND35 (F1) and no changes in spleen weight were reported (absolute) in any dosing group.

70. Dunder et al. (2018) exposed pregnant F344/DuCrl rats and offspring to BPA at a concentration of 0.5 and 50 µg.kg bw per day via drinking water at GD3.5 to PND22. Measurements were taken at 52 weeks of age (F1) and no changes in spleen weight (absolute) were reported in either males or females.

71. Studies in mice by Malaise et al. (2017) and Patel et al. (2015a) were allocated to **Tier 2**.

72. A study in mice by Bodin et al. (2014) and a study in rats by Tarapore et al. (2017) were allocated to **Tier 3**.

73. Tarapore et al. (2017) exposed Sprague-Dawley rats and male offspring to BPA at a concentration of 25 µg/kg per day via the diet. Exposure occurred 1 week before mating until the end of pregnancy and measurements were taken on PND210 or PND549 (F1). No change in spleen weight (absolute) was reported (data not shown).

74. Overall, a consistent dose-related increase in Th17 cells and associated cytokines (i.e., IL-17, IL-21 and IL-23) was observed at doses as low as 100 nM drinking water (equivalent to 4.75 µg/kg bw per day) in the study by Luo et al. (2016). These findings were supported by three other studies (Malaise et al., 2017; 2018 and Bodin et al., 2014), showing effects in the same direction. Other parameters including IFN-y, IL-13 and other T-cell subpopulations were not consistently affected (Bodin et al., 2014; Malaise et al., 2017; O’Brien et al.,

2014a). However, EFSA noted that this should not be considered an inconsistency as under cellular immunity many cells with different functions are included.

75. EFSA considered cellular immunity effects to be likely during the developmental exposure period and took the increase in Th17 cells (Luo et al., 2016) forward for BMD analysis (and uncertainty analysis). The endpoints Il-17, IL-21 and IL-23, although likely, were not taken forward as these parameters can be triggered by different stimuli and not considered very close to the apical endpoint (allergic lung inflammation).

Developmental and adult exposure (pre-natal and post-natal in pubs until adulthood)

76. Studies by Li et al. (2018b), Li et al. (2018) and Camacho et al. (2019) were allocated into **Tier 1**.

77. Li et al (2018b) exposed Sprague-Dawley rats and offspring to BPA at a concentration of 2.5, 25, 250, 2500 or 25,000 µg/kg bw per day via oral gavage on GD6 to parturition (G0) and PND1 to euthanasia (F1). Measurements were taken at PND90, 6 months and 1 year (F1) and reported increased spleen cell proliferation after LPS stimulation in females (2500 µg/kg bw per day) and males (2.5, 2500 and 25000 µg/kg bw per day) after 1 year. Increased spleen cell proliferation after PWM stimulation was reported in females at 6 months (25 and 2500 µg/kg bw per day) and males at 1 year (2.5, 25, 2500 and 25000 µg/kg bw per day). Increased intracellular IgM after LPS and PWM stimulation in females at PND90 at 2500 and 25000 µg/kg bw per day, respectively was reported, as was an increase in CD25+ cells after anti-CD3/CD28 stimulation in males at 6 months (2.5, 2500 or 25000 µg/kg bw per day), CD80 NK-cells after 24 hours in males at 6 months (2.5 µg/kg bw per day), CD86+ NK-cells after 24 hours and 1 year (25000 µg/kg bw per day), CD86+ NK-cells after 48 hours in males at 1 year (25 µg/kg bw per day) and MHC II+ cells within D11b/c+ cells after 48 hours in females at PND90 (250 and 25000 µg/kg bw per day). A decrease in spleen cell proliferation was reported after PWM stimulation in females at PND90 (25000 µg/kg bw per day), in males at 6 months (2.5, 250 and 2500 µg/kg bw per day) and after anti-CD3/CD28 stimulation in females at 1 years (2.5 µg/kg bw per day). Decreases in intracellular IgM after LPS stimulation in males at PND90 (2.5 µg/kg bw per day), CD80+ NK-cells after 24 h in females at 6 months (25000 µg/kg bw per day), CD86+ cells within CD172α+ cells after 48 h in males at 6 months (2500 µg/kg bw per day) and MHCII+ cells within CD172α+ cells after 48 h in males at 6 months in 2500 µg/kg bw per day) were also demonstrated. No changes for any of the other parameters were reported.

78. A study in mice by Gear and Belcher (2017) was allocated to **Tier 3**.

79. Gear and Belcher (2017) exposed female and male CD-1 mice and offspring to BPA at a concentration of 4, 40, 400, 4000 and 40,000 µg/kg bw per day via the diet from conception to 12 weeks of age. Measurements were taken at 12 weeks and increased spleen weight (absolute) was reported in male and females at 4000 µg/kg bw per day. No changes were reported at any of the other doses.

80. EFSA concluded that while some effects were identified, most of these alterations were transient and not dose dependent (Th17 cells were not evaluated in this exposure period). Overall, no consistent effects were observed in any of the studies and EFSA considered the effects to be not likely and therefore did not take any of the endpoints forward for BMD analysis.

Growth phase/young age exposure

81. No studies were available for this exposure period.

Adult exposure (after puberty)

82. Studies in mice by Dong et al. (2013) and DeLuca et al. (2018) were allocated into **Tier 1**.

83. Dong et al. (2013) exposed C57BL/6 mice to BPA at a concentration of 1 or 10 µg/mL in drinking water (corresponding to 225 and 2250 µg/kg bw per day) at 5 weeks to 9 weeks. Measurements were taken at 9 weeks and no changes in spleen weight (absolute, relative) were reported.

84. DeLuca et al. (2018) exposed ovariectomised female C57BL/6 mice to BPA at a concentration of 50 µg/kg bw per day via oral gavage; in one treatment group colitis was induced with dextran sodium sulfate (DSS). Exposure occurred at age 84-99 days and measurements were taken at day 15 of treatment. Increased inflammation score in the middle colon region I was reported in the BPA+DSS group. In the same group increased colon IL-1α, IL-13, IL-31 and IL-12p70 were reported. A decrease in colon VEGF was also seen in the BPA and BPA+DSS group. No changes on any other parameters were observed.

85. A study in mice by Cetkovic-Cyrlje et al. (2017) was allocated into **Tier 2**.

86. A study in mice by Özaydın et al. (2018a) was allocated to **Tier 3**.

87. Özaydın et al. (2018) orally exposed male Wister albino rats to BPA at a concentration of 5, 50 and 500 µg/kg bw per day at 8 weeks to 16 weeks.

Measurements were taken at 16 weeks old and increased IL-4 and IL-6 in plasma at 50 and 500 µg/kg bw per day and TNF- α in plasma at all doses were reported. Increased CD8+ lymphocytes in spleen (5 and 50 µg/kg bw per day), CD8+ lymphocytes in ileum (every dose group) and decreased CD4+ lymphocytes in spleen and ileum in every dose group were also reported. No changes were reported for any of the other parameters investigated.

Indirect (germline) exposure

88. No studies were available for this exposure period.

Overall selection of endpoints/studies for BMD analysis

89. Overall, EFSA considered cellular immunity effects of BPA to be likely across all exposure periods. The evidence from the available studies showed a likely effect of BPA on Th17 cells (Luo et al., 2016) during the developmental exposure period and EFSA therefore took this endpoint forward for BMD analysis.

## Humoral immunity

90. In total two studies were identified by EFSA, one study by Li et al. (2018b, NTP Clarity Study) investigating the effects of BPA during development in rats and one study by Malaise et al. (2018) investigating the effects of BPA during development and adulthood in mice.

Developmental exposure (pre-natal and/or post-natal until weaning)

91. For local IgA parameters the study by Malaise et al. (2018) was allocated to **Tier 3**; for systemic IgA parameters the study was allocated to **Tier 1**.

92. The study results demonstrated a consistent decrease in IgA endpoints at PND50. However, EFSA noted that the endpoints were all in one study and are not independent from each other, and only one dose was applied.

93. EFSA therefore concluded, humoral effects of BPA to be as likely as not during development and did not take any of the endpoints forward for BMD analysis; they were however considered for the uncertainty analysis (Annex D).

Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

94. The study by Li et al. (2018b) was allocated to **Tier 1**.

95. No consistent effects on LPS-induced proliferation were observed, an increase was observed in females at 2500 µg/kg bw per day at PND90, while a decrease was observed in males at 2.5 µg/kg bw per day. No effects were observed at 6 months or 1 year. EFSA concluded that the effects were transient with no dose-response and therefore considered not likely.

96. Statistically significant values of IgM production were found but inconsistent between sexes and without a clear dose-response. EFSA therefore considered these chance findings and decided the study did not show a likely effect for IgM production nor cell proliferation.

97. Overall, EFSA concluded that the effects of BPA on humoral immunity during developmental and adult exposure were not likely and did not take the endpoints forward for BMD analysis.

Growth phase/young age

98. No studies were available for this exposure period.

Adult exposure (after puberty)

99. No studies were available for this exposure period.

Indirect (germline) exposure

100. No studies were available for this exposure period.

Overall selection of endpoints/studies for BMD analysis

101. Overall, EFSA considered humoral immunity effects of BPA to be as likely as not across all exposure periods. Therefore, none of the endpoints were taken forward for BMD analysis.

## Inflammation

102. EFSA identified five studies, two studies in rats focused on developmental and adult exposure, one study on exposure during the growth phase and four studies in mice, rabbits and rats on adult exposure.

Developmental exposure (pre-natal and/or post-natal until weaning)

103. Studies in mice by Luo et al. (2014) and Luo et al. (2016) were allocated to **Tier 1**.

104. Luo et al. (2014) exposed CD-1 mice and female offspring to BPA at a concentration of 50,000 µg/kg diet (corresponding to 7500 µg/kg bw per day) 2 weeks before mating to weaning. Measurements were taken on PND24 (F1) and an increase in IL-6 and TNF- α in the prefrontal cortex were reported.

105. Mouse studies by Malaise et al. (2017) and Bodin et al. (2014) were allocated to **Tiers 2** and **3**, respectively.

106. A rat study by Camacho et al. (2019; NTP Clarity Report, 2018) was allocated to **Tier 1**.

107. As there was insufficient evidence to support an effect, having only a trend in a decrease in neutrophils, EFSA concluded that it was not likely for BPA to have an inflammation adverse effect during this exposure period and did not take the endpoint forward for BMD modelling.

Developmental and adult exposure (pre-natal and post-natal in pups until adulthood)

108. The two rat studies by Camacho et al (2019; NTP Clarity Report, 2018) and Ben-Jonathan et al. (2018; NTP Grantee study) were allocated into **Tier 1**.

109. Ben-Jonathan et al. (2018) exposed NCTR Sprague-Dawley rats and offspring to BPA at a concentration of 2.5, 25, 250, 2500 or 25,000 µg/kg bw per day via oral gavage. Exposures occurred on GD6 to PND- (dams) and PND1 to PND90 or PND1 to PND180 (F1), measurements were taken on PND90 and 180 (F1). No change in serum IL-6 was reported, in any dose group. EFSA noted that no statistics were performed since a number of the values were below the limit of detection (LOD).

110. While Camacho et al. (2019) detected a decrease in blood eosinophils at 250 µg/kg bw in both males and females at interim sacrifice, the effect was without a dose-response and no effects were reported at any other endpoints, including neutrophils. No changes in IL-6 were observed in the study by Ben-Jonathan et al. (2018).

111. Hence, EFSA concluded that effects on inflammation by BPA in this exposure period were not likely and did not take these endpoints forward for BMD analysis.

Growth phase/young age exposure

112. One rat study by Ogo et al. (2018) was allocated to **Tier 1**. The study reported inflammation at tissue level in the epididymis, characterised by an increase in IL-6 and neutrophils.

113. EFSA concluded that it was likely for BPA to have had an effect on inflammation in the growth phase/young exposure age. As the effect on IL-6 can be triggered by different stimuli (including physiological stimuli) it was not considered very close to the apical endpoint and EFSA decided not to use IL-6 for BMDL analysis. Instead EFSA took the increase in neutrophils forward for BMD analysis.

Adult exposure (after puberty)

114. One study in rabbits by Fang et al. (2014) was allocated to **Tier 1**.

115. Fang et al. (2014) exposed WHHL rabbits to BPA at a concentration of 400 µg/kg bw per day via oral gavage at age 14-16 weeks. Measurements were taken at 14 and 28 weeks of age and increases in TNF- α and IL-6 were observed at 26 weeks. No changes were observed prior to that.

116. Two studies in mice by Cetkovic-Cyrlje et al. (2017) and DeLuca et al. (2018) and one study in rats by Özaydın et al. (2018a) were allocated to **Tier 2**.

117. The only dose-related effect was reported by Özaydın et al. (2018a) on IL-6 in male rats at 50 and 500 µg/kg bw per day. Overall, there were inconsistencies between species, models and strains used.

118. Due to insufficient evidence, including studies with only one dose and Tier 2 studies showing no effect, EFSA considered the effect of BPA on inflammation to be not likely during the adult exposure period. Therefore, the endpoints were not taken forward for BMD analysis.

Indirect (germline) exposure

119. No studies were available in this exposure period.

Overall selection of endpoints/studies for BMD analysis

120. Overall, EFSA concluded that the evidence from the studies available showed a likely effect of BPA on inflammation across all exposure periods and took the effect of BPA on neutrophils (Ogo et al., 2018) forward for BMD analysis.

## Allergic lung inflammation

121. Four studies were identified by EFSA, two focused on exposure during development and one on exposure during adulthood.

Developmental exposure (pre-natal and/or post-natal until weaning)

122. Two mice studies by O'Brien et al. (2014a; 2014b) were allocated into **Tier 2**.

123. O'Brien et al. (2014b) exposed c57BL/6 and C3H/HeJ mice and offspring to BPA at a concentration of 0.05, 50 or 50,000 µg/kg diet (corresponding to 0.0075, 7.5 or 7500 µg/kg bw per day) from 2 weeks before mating until PND21. Measurements were taken at 6 months of age (F1) and increases in mast cell CYsLT and TNF- α were observed in every dose group while increases in mast cell PGD2 and IL-13 were observed at 50,000 µg/kg.

124. As the effects on ovalbumin specific IgE were dose-related in both sexes (O'Brien et al., 2014a), EFSA considered this to be a very likely effect of BPA and decided to take the endpoint forward for BMD analysis.

125. The effects on mast cell PGD2, lung CysLT and lung IL-17, while also considered likely effects, can also be triggered by other stimuli and are not considered close to the apical endpoint. Therefore, these endpoints were not taken forward for BMD analysis, but they are considered in the uncertainty analysis in Annex D.

126. As the effects on lung cellularity, IL-13, IL-4, TNF- α and lung inflammation score were either affected in one sex or without a clear dose-response, EFSA concluded these effects to be as likely as not. EFSA furthermore considered the effects on RANTES as not likely, as the only changes observed were at the lowest dose, in females only.

Developmental and adult exposure (pre-natal and post-natal pups until adulthood)

127. No studies were available for this exposure period.

Growth phase/young age exposure

128. No studies were available in this exposure period.

Adult exposure (after puberty)

129. One study in mice by Tajiki-Nishino et al. (2018) was allocated to **Tier 1**.

130. The study exposed male BALB/CanN mice to BPA at a concentration of 60 or 200 µg/kg bw as a single dose administered orally three times at 7 weeks of age (48, 24 and 2 hrs before toluene-2,4-diisocyanate (TDI) administration). Measurements were taken 24 hours after TDI challenge. Increased eosinophil infiltration in the allergic airway inflammation model was observed at 200 µg/kg bw. Furthermore, increased Th cell infiltration in the lymph node was observed in the airway allergic inflammation model at 60 µg/kg bw, increased IL-4 in lymph node in the allergic dermatitis inflammation model and in lung in the allergic inflammation model at 200 µg/kg bw and increased IL-33 in lung in the allergic inflammation model in both dose groups. Decreases in IL-4 in lymph node in the allergic dermatitis test were observed, as well as decreases in IL-1β and TNF- α in ear auricle in the allergic dermatitis model in both dose groups and IL-4 at 60 µg/kg bw. No changes were observed in any of the other parameters investigated.

131. EFSA considered the dose-dependent increment in Il-4 and IL-33 observed in the lung as likely effects. Since the effect is likely for eosinophils in the bronchioalveolar lavage and this endpoint represents a very close adverse atypical endpoint, this effect was taken forward for BMD analysis.

132. The cytokine endpoints were also considered very likely or likely effects by EFSA, however, as these endpoints can be triggered by other stimuli and are not considered very close apical endpoints, they were not taken forward for BMD analysis. However, they were considered in the uncertainty analysis in Annex D.

Indirect (germline) exposure

133. No studies were available for this exposure period.

Overall selection of the endpoints/studies for BMD analysis

134. Overall, EFSA concluded that an effect of BPA on allergic lung inflammation is likely. The evidence from the studies showed a very likely effect of BPA in the developmental exposure period for serum OVA specific IgE (O'Brien et al., 2014a) and a likely effect in adult exposure for eosinophils in BAL (Tajiki-Nishino et al., 2018). Therefore, EFSA took these two endpoints forward for BMD analysis.

## Integration of likelihoods from human and animal studies

135. The following table (Table 9 in the EFSA opinion) presents the overall likelihood for the human and animal stream separately (for each cluster) and the integration of the likelihoods for immunotoxicity.

| Human stream | | Animal stream | | Integrated likelihood |
|---|---|---|---|---|
| **Cluster: Asthma/ allergy** | | **Cluster: Allergic lung inflammation** | | |
| Exposure during Pregnancy | ALAN | Developmental exposure (pre-natal and/or post-natal until weaning) | Likely | |
| Exposure during Childhood | ALAN | Adult exposure (after puberty) | Likely | |
| Overall likelihood: | ALAN | Overall likelihood: | Likely | Likely |
| **Cluster: Cellular immunity** | | **Cluster: Cellular immunity** | | |
| Not applicable | | Developmental exposure (pre-natal and/or post-natal until weaning) | Likely | |
| | | Developmental and adult exposure (pre-natal and/or post-natal in pups until adulthood) | Not Likely | |
| | | Adult exposure (after puberty) | Not Likely | |
| | | Overall likelihood: | Likely | Likely |

| | | | |
|---|---|---|---|
| **Cluster: Humoral immunity** | **Cluster: Humoral immunity** | | |
| Not applicable | Developmental exposure (pre-natal and/or post-natal until weaning) | ALAN | |
| | Developmental and adult exposure (pre-natal and/or post-natal in pups until adulthood) | Not Likely | |
| | Overall likelihood: | ALAN | ALAN |
| **Cluster: Innate immunity** | **Cluster: Innate immunity** | | |
| Not applicable | Developmental exposure (pre-natal and/or post-natal until weaning) | ALAN | |
| | Developmental and adult exposure (pre-natal and/or post-natal in pups until adulthood) | Not Likely | |
| | Growth phase/young age exposure | Not Likely | |
| | Adult exposure (after puberty) | Not Likely | |
| | Overall likelihood: | ALAN | ALAN |

| **Cluster: Inflammation** | **Cluster: Inflammation** | | |
|---|---|---|---|
| Not applicable | Developmental exposure (pre-natal and/or post-natal until weaning) | Not Likely | |
| | Developmental and adult exposure (pre-natal and/or post-natal in pups until adulthood) | Not Likely | |
| | Growth phase/young age exposure | Likely | |
| | Adult exposure (after puberty) | Not Likely | |
| | Overall likelihood: | Likely | Likely |

# In vitro and mechanistic studies

## Cellular immunity

136. The mechanisms along which BPA influences the immune system are largely unknown.

137. Since there was no direct human evidence available for cellular immunity, the overall likelihood of an effect of BPA was scored based on animal evidence. Exposure to low dose BPA (4.75 µg/kg bw per day) during gestation and lactation lead to a sustained sex-specific and dose-dependent increase in Th17 cells in the offspring mice. This effect was mediated by specific alterations of the transcription factors and regulatory cytokines (IL-17, IL-21, IL-23) as shown by Luo et al. (2016). EFSA noted that the deficient dendritic cell maturation in the lamina propria (intestine and spleen) after exposure to 50 µg/kg bw per day may be the basis for the effects on regulatory T cells in the lamina propria and subsequent effects on Th17 cells systemically. EFSA further noted that direct effects of BPA on

lymphocytes may play a role. Cipelli et al. (2014) (Reference not included in Tables/Annexes) reported effects of *in vitro* exposure at 100 nM on proliferation and adenosine triphosphate (ATP) content of human leukaemia T-cell lymphoblasts, in which oestrogen receptor 2 (ER2) and oestrogen-related receptor-alpha (ERRA) appear to be involved.

138. EFSA noted that the concentrations may still be quite high compared to actual internal exposures in humans and mechanisms identified by *in vitro* studies may therefore not necessarily be operational.

139. Overall, EFSA considered the effect of BPA on cellular immunity to be likely during the developmental exposure period.

## Humoral Immunity

140. Since there was no direct human evidence available for humoral immunity, the overall likelihood of an effect of BPA was scored based on animal evidence.

141. As mentioned previously, EFSA did not include reagins (i.e., antibodies involved in allergic reactions) in this section but under allergic lung inflammation, as IgE is a crucial component of the latter. IgE is of course also a component of humoral immunity, under strict regulation of T cells, hence under regulation of the cellular immune system. However, here EFSA only considered the humoral aspects other than IgE and IgG1.

142. The only effect of BPA exposure on IgA was found during development, however, only in one study and not supported by other parameters. EFSA further acknowledged that IgA is also regulated by cellular immune components and even if the effect on IgA was not sufficiently convincing, effects on both IgE and IgA do indicate dysregulation of humoral immunity. Malaise et al. (2018) suggested that an effect of 50 µg/kg bw per day on IgA may be associated with influence of BPA on the intestinal barrier functions.

143. Chailurkit et al. (2016) (Reference not included in Tables/Annexes) investigated the mechanisms underlying humoral immunity in humans by looking at the association of BPA exposure with autoantibodies and found a significant trend associated with antithyroglobulin and antithyroperoxidase. Their findings may lend further support to an effect on humoral immunity, brought about by the suppression of T-cell regulation and subsequent enhancement of Th17 mechanisms.

144. However, overall EFSA did not consider the effect of BPA on humoral immunity to be likely during any exposure period, based on the available animal evidence.

## Allergic lung inflammation

145. Based on the integration of human and animal evidence EFSA considered the effects of BPA on allergic lung inflammation to be likely.

146. More specifically EFSA considered the evidence from animal studies show a very likely effect of BPA on the serum OVA-specific IgE during the developmental period and likely on eosinophils in BAL during the adult exposure period. Even if the effect of exposure in the humoral immunity were judged as likely as not, the effects are in line with the effects on allergic lung inflammation, especially during development. This supports the susceptibility of the developing immune system.

147. A study by Koike et al. (2018) further supported these findings. Male C3H/HeJJc1 mice were exposed to BPA at 0.0623, 1.25 or 25 pmol (BPA/animal 25 g/week, with and without OVA) from 6-12 weeks. Measurements were taken at 12 weeks of age. Results showed an increase in total cells and macrophages in BAL at 1.25 pmol, without OVA. In combination with OVA increased neutrophils in BAL, IL-4 in lung at 0.0625 pmol were reported, as well as increased IL-13 and IL-33 in lung at every dose compared to the vehicle and at 1.25 pmol compared to OVA, increased KC and RANTES in lung at every dose compared to vehicle and increased RANTES compared to OVA at 0.0625 pmol. Furthermore, increased OVA IgE and total dendritic cells in MLN were reported in every dose group, MHC class II+ dendritic cells and CD86+ MHC class II+ dendritic cells in MLN (0.0625 and 1.25 pmol, increased IL-4 in MLN in 0.0625 pmol and after restimulation with OVA and increased IFN-γ in MLN in 0.0625 and 25 pmol after restimulation with OVA. Decreases in total cells in spleen (1.25 and 25 pmol) and cell proliferation in spleen after restimulation with OVA in 25 pmol group were also observed. EFSA concluded that after an intratracheal installation of BPA an exacerbated OVA-induced lung inflammation and specific IgE responses could be seen, by enhancing Th2 responses via disruption of the immune system.

148. A possible hypothesis was put forward by Tajiki-Nishino et al. (2018) suggesting bronchial epithelial cells and TSLP may activate antigen presenting cells (APCs), resulting in stimulation of Th cells and subsequent exacerbation of local cytokine level (found after IgE production). Cross-linking of IgE on mast cells triggers these cells to release their mediators, with O’Brien et al. (2014b)

demonstrating that perinatal BPA exposure to BPA (7.5 ng/kg bw per day) displayed a long-term influence on mast-cell mediated production of pro-inflammatory mediators associated with asthma and global DNA methylation levels. Thus, supporting the role of mast cells in pulmonary inflammation associated with allergic airway disease into adulthood.

149. Direct inflammation effects of mediators from bronchial fibroblasts may also play a role in an eventual lung inflammation. Mahemuti et al. (2016) (Reference could not be found in Tables/Annexes) exposed human lung fibroblasts (HLFL) to 100 µM BPA *in vitro*, resulting in the release of growth differentiation factor-15 (GDF15), endothelin-1 (ET-1), IL-6 and interferon y-induced protein 10 (IP-10) and phosphorylation of nuclear factor kappa B (NF-kB). However, EFSA noted that these effects were seen at concentrations higher than the cut-off for inclusion here and may not be relevant to humans.

150. Throughout the studies EFSA noted a potential age dependency, i.e., more pronounced effects after exposure during development. This is in accordance with findings by Petzold et al. (2014), who observed an asthma-promoting effect after life-long exposure, including during pregnancy and breastfeeding (0.9 µg/kg bw per day). BPA exposure of adult mice during sensitisation with ovalbumin led to reduced allergic response, which could be reverted using the glucocorticoid receptor (GR) antagonist RU486.

151. In more detail, Petzold et al. (2014) exposed BALB/cByJ mice and offspring to BPA at a concentration of 5 µg/mL in drinking water (0.45 µg/kg bw per day) from 1 week before mating until delivery (pre-natal exposure), PND21 (perinatal exposure), lifetime exposure and from 1 week before sensitisation until the end of protocol (adult exposure). At 6 weeks old, offspring were immunised intraperitoneally with OVA. Results showed an increase in IL-13 in lymph node (perinatal), lung cellularity in BAL (eosinophils; lifelong), lung inflammation (lifelong) and increased OVA-specific IgE (lung; lifelong). Results also showed a decrease in lung cellularity (eosinophils and macrophages), lung inflammation, OVA-specific IgE (lung) and IL-4 and IL-13 in lymph node in the adult exposure group. No changes were reported for any of the other parameters.

## Innate immunity

152. Since there was no direct human evidence available for innate immunity, the overall likelihood of an effect of BPA was scored based on animal evidence. EFSA concluded that the evidence available from animal studies showed overall as likely as not effects.

153. Malaise et al. (2017; 2018) showed a reduction in the functional parameter lysozyme, while an increase in plasma G-CSF was reported by Rogers et al. (2017) in an experimental model of multiple sclerosis. The increase in G-CSF was associated with an increased number of circulating neutrophils; blocking G-CSF by a monoclonal antibody resulted in a decreased incidence and severity of Experimental Allergic Encephalomyelitis. This suggested that the mechanism by which gestational BPA exposure increases the risk for autoimmunity could be through priming macrophages that produce G-CSF after activation and subsequent mobilisation of neutrophils by G-CSF. Following adult exposure (intratracheal exposure), an increased number of antigen-presenting (dendritic) cells were observed by Koike et al. (2018), which EFSA noted may be in line with the effects on allergic lung.

154. *In vitro* studies were conducted using both primary cells from humans and rodents or cell lines (e.g., human monocytic cells (THP-1)). Results indicated that BPA can directly act on innate immune cells modulating cytokine production, showing increased pro-inflammatory cytokines and decreased anti-inflammatory cytokines at a concentration of 10 and 100 nM (Liu et al., 2014; Couleau et al., 2015; Chen et al., 2018) (Reference not included in Tables/Annexes. Liu et al. 2014 is mentioned in the Annex/Tables but this specific reference is not included. The references are for *in vivo* studies.) as well as decreased phagocytosis at a concentration of 1 nM and 100 µM (Couleau et al., 2015; Berntsen et al., 2018). Increased ROS were observed by Michalowicz et al. (2015) (Reference not included in the Annex/Tables) but only at concentrations higher than 0.3 µM.

155. Berntsen et al. (2018) exposed NOD mice and female offspring to BPA at a concentration of 5 mg/L via drinking water (corresponding to 15 µg per day) from mating to lifetime female offspring. Measurements were taken at 12 weeks (F1) and a decreased number of phagocytic peritoneal cavity macrophages and F4/80 positive macrophages were seen.

156. EFSA noted that the concentrations used for *in vitro* studies may be quite high compared with the actual internal exposure in humans and mechanisms identified may therefore not necessarily all be operational.

157. Overall, the mechanistic elucidation of the BPA effect was minimal. However, several studies provide evidence for these effects to be induced by modulation of the extracellular signal-related kinase (ERK)/NF-kB signalling pathway at a concentration of 1 nM and 100 nM (Herz et al., 2017; O'Brien et al., 2014c (these references are not included in the Annexes/Tables); Couleau et al., 2015; Liu et al., 2014), potentially mediated via ERs (ERa/b and the membrane

receptor GPER (G protein-coupled oestrogen receptor) or GPR30). Furthermore, Michalowicz et al. (2015) and Neri et al. (2015) suggested that BPA is capable of damaging innate immune cells through oxidative stress and DNA damage leading to apoptosis and necrosis at concentrations ranging from 6.57 nM to 0.3 µM. Finally, O'Brien et al. (2014c) studied the effects of BPA *in vitro*, demonstrating that BPA could enhance mast cell release at concentrations of 100 nM and that this could be mediated partly through the ERK pathway and extracellular Ca2+ concentrations, but independent of an ER-mediated mechanism. Thus, supporting the role for mast cells in pulmonary inflammation associated with allergic airway diseases (O'Brien et al., 2014b; Petzold et al., 2014; Koike et al., 2018; Tajiki-Nishino et al., 2018).

158. Liao et al. (2016) investigated the effect of pre-natal exposure to BPA on TLR-induced cytokine responses in neonates (human birth cohort) from Taiwan. Male and female (N=250) children were followed up to 1 year of age and production of TNF-α, IL-6 and IL-10 was evaluated after stimulating mononuclear cells with TLR ligands (TLR1–4 and TLR7/8). Although the study did not yield a statistically significant result for the epidemiological risk of infection during early infancy, an association between cord blood BPA concentration and supressed TLR3-stimulated and TLR4-stimulated TNF-α response and TLR7/8-stimulated IL-6 response were reported.

159. Overall, the studies on innate immunity cells support *in vivo* evidence indicating an immune de-regulation and possibly increased susceptibility to develop inflammatory reactions.

## Inflammation

160. Since there was no human evidence available for inflammation, the overall likelihood of an effect of BPA was scored based on animal evidence. EFSA considered the effects of BPA on inflammation overall to be likely.

161. EFSA noted that some endpoints, e.g., pro-inflammatory cytokines (produced by innate immune cells), have been discussed under innate immunity.

162. Effects following growth phase/young age were shown in the epididymis by Ogo et al. (2018), as a clear indication of increased IL-6 and neutrophils at 20 µg/kg bw per day.

163. EFSA concluded that overall, the findings supported an increased production of pro-inflammatory cytokines and are consistent with *in vitro* results.

However, results were inconsistent or obtained from a single-dose study. At adulthood, an increase in IL-6 and TNF- α was observed in two studies (Özaydin et al., 2018a; Fang et al., 2014), with no effect and a decrease seen in another study, respectively (Cetkovic-Cvrlje et al., 2017). Increased IL-1a, IL-12p70, IL-31, VEGF were reported in a single-dose study (DeLuca et al., 2018) and in SDF1a (Koike et al., 2018).

164. Two mechanistic studies in humans reported inflammation markers in Asian populations.

165. Song et al. (2017) supplemented an *in vitro* study with evidence from epidemiological studies and investigated the association between urinary BPA levels and well-known inflammation-related markers including white blood cells (WBC), CRP, IL-10, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyl transpeptidase (γ-GTP). Significant positive associations between BPA level and WBC, ALT and γ-GTP levels were found.

166. Yang et al. (2016) (Reference not included in Annexes/Tables.) also supplemented their *in vivo* and *in vitro* investigations with evidence from epidemiological studies on ALT, γ-GT and high-sensitivity (hs-)CRP, leptin and TNF-α. BPA was associated with inflammation markers (leptin, TNF-α) in lean subjects but not in overweight/obese subjects and stratified analyses suggested a possible attenuation by sex and body mass index (BMI).

167. In addition, there are studies indicating modulation of signalling pathways at concentrations equal to or below 100 nM (e.g. ERK, c-Jun N-terminal kinase (JNK), NF-kB) (Couleau et al., 2015; Liu et al., 2014;) at 10 nM and 100 nM (Song et al., 2017) and at 10 nM (Zhu et al., 2015) (Reference not included in Annexes/Tables) as well as modulation of cytokine gene expression and secretion at 0.1 nM (Camarca et al., 2016) (Reference not included in Annexes/Tables), 1 nM (Tajiki-Nishino et al., 2018), at 8.76 nM (Li et al., 2018) at 10 nM (Liu YZ et al., 2014), at 100 nM (Couleau et al., 2015; Zhu et al., 2015; Chen Y et al., 2018). There are also studies indicating modulation of histone methylation at 8.76 nM (Li et al., 2018) and modulation of ER signalling pathways, where effects were reversed by ERa/b antagonists at 100 nM (Couleau et al., 2015; Chen et al., 2018) and at 10 nM (Liu et al., 2014). In contrast, Chakhtoura et al. (2017) (Reference not included in Annexes/Tables) found no effects on cytokine expression and no influence on dendritic cell maturation at concentrations as low as 0.05 nM.

168. EFSA again noted that concentrations of 100 nM in *in vitro* studies may be quite high compared with the actual internal exposure in humans and

mechanisms identified may therefore not necessarily all be operational.

169. Taking all results together, EFSA concluded that many of the studies reviewed highlighted a possible role of BPA in inflammatory processes. Modulation of ERK1/2 phosphorylation, NF-kB activation, modulation of the ERs, GR and androgen receptor (AR), as well as cytokine/chemokine secretion, are relatively common hypothesised mechanisms for the effects observed.

### Concluding remarks

170. EFSA concluded that effects of BPA may be on non-specific cells of the immune system or influencing the immune system, such as APCs and epithelial cells, through antigens to T lymphocytes or release of mediators, influence the homeostasis, supressing T regulatory cells and stimulating Th17 cells, leading to enhanced production of IgE. IgE in turn, after cross-linking at the surface of mast cells, may lead to the release of inflammatory mediators, that together with other inflammatory mediators from other cell types, may lead to inflammatory reactions, including in the respiratory tract. Inflammation in the epididymis have also been observed after BPA exposure and may follow the same mechanism.

171. However, EFSA noted that it is currently not clear how BPA interacts with the various cells comprising the immune system or cells such as epithelial cells or fibroblasts. A role for GR, ER2 and the ERRA and subsequent activation of transcription factors may be plausible.

172. EFSA further noted that the response to BPA may differ according to the experimental conditions and that such conditions will also impact the effect on humans.

## Conclusion on hazard identification for Immunotoxicity of BPA

173. While human and animal studies indicated immunological effects of BPA, these effects were not taken forward by EFSA in 2015 due to short comings in the data. A subsequent evaluation of two additional studies in 2016 confirmed EFSAs initial statement that the data was not sufficiently robust for a risk characterisation.

174. The current evaluation is in line with these earlier observations but according to EFSA now indicated a more firm hazard with respect to an adverse

outcome on the immune system, notable effects on cellular immunity and parameters indicating allergic lung inflammation. Even if the mechanisms for the effect of BPA on the immune system are not clear, the studies shed some light on factors involved (as summarised in the concluding remarks above). Whereas the parameters investigated mainly comprise intermediate endpoints (e.g., interleukins, mast cell mediators, specific antibodies related to allergy), and while indications for inflammation were noted, the disease endpoints were only investigated in a small number of studies. EFSA considered these studies to indicate an effect, however, did not judge the studies to be of high quality.

175. An inflammatory effect was also seen in the epididymis, which may be brought about partly by similar mechanisms. While the effect on innate immunity was considered as likely as not by EFSA, the increased number of antigen-presenting (dendritic) cells observed, underscored the effect of BPA on homeostasis of the immune system. The mechanism of how BPA interacts with various cells is currently not clear, however EFSA considered a role of GR, ER2 and ERRA and subsequent activation of transcription factors to be plausible.

176. Overall, EFSA considered a hazard to exist for adverse effects of BPA on the immune system in their current evaluation. Exposure may result, depending on the dose, most likely in inflammatory reactions. Whereas the developing immune system is generally considered more vulnerable, effects were noted both after exposure during development and at adulthood. Hence, EFSA concluded the hazard to exist throughout the different life stages.

177. Using a WoE approach, EFSA considered BPA induced effects on Th17 cells, neutrophils in epididymis, eosinophils in BAL likely and effects on serum-OVA-specific IgE very likely. Therefore, these endpoints were brought forward for BMD analysis.

# References

Ashley-Martin J, Dodds L, Levy AR, Platt RW, Marshall JS, Arbuckle TE (2015). Prenatal exposure to 12504 phthalates, bisphenol A and perfluoroalkyl substances and cord blood levels of IgE, TSLP and IL-33. Environmental Research, 140: 360–368. https://doi.org/10.1016/j.envres.2015.04.010

Ben-Jonathan N (2018/19). Bisphenol A and the metabolic syndrome: Focus on adipose tissue functions. CLARITY-BPA: Obesity/Adipose tissue, Grantee study results. https://doi.org/10.22427/NTP-DATA-018-00005-0001-000-5

Berntsen HF, Bølling AK, Bjørklund CG, Zimmer K, Ropstad E, Zienolddiny S, Becher R, Holme JA, Dirven H, Nygaard UC, Bodin J (2018). Decreased macrophage phagocytic function due to xenobiotic exposures in vitro, difference in sensitivity between various macrophage models. Food and Chemical Toxicology, 112: 86–96. https://doi.org/10.1016/j.fct.2017.12.024

Bodin J, Bølling AK, Becher R, Kuper F, Løvik M, Nygaard UC, 2014. Transmaternal bisphenol A exposure accelerates diabetes Type 1 development in NOD mice. Toxicological Sciences, 137(2): 311–323. https://doi.org/10.1093/toxsci/kft242

Buckley JP, Quirós-Alcalá L, Teitelbaum SL, Calafat AM, Wolff MS, Engel SM (2018). Associations of prenatal environmental phenol and phthalate biomarkers with respiratory and allergic diseases among children aged 6 and 7 years. Environment International, 115: 79–88. https://doi.org/10.1016/j.envint.2018.03.016

Camacho L, Lewis SM, Vanlandingham MM, Olson GR, Davis KJ, Patton R, Twaddle NC, Doerge DR, Churchwell MI, Bryant MS, Mclellen FM, Woodling K, Felton RP, Maisha MP, Juliar BE, Gamboa da Costa G, Delclos KB (2019). A two-year toxicology study of bisphenol A (BPA) in Sprague-Dawley rats. NTP CLARITY-BPA core study results (2019). Food and Chemical Toxicology, 132: 110728. https://doi.org/10.1016/j.fct.2019.110728

Camarca A, Gianfrani C, Ariemma F, Cimmino I, Bruzzese D, Scerbo R, Picascia S, D'Esposito V, Beguinot F, Formisano P, Valentino R (2016). Human peripheral blood mononuclear cell function and dendritic cell differentiation are affected by bisphenol A exposure. PLoS ONE, 11(8): e0161122 https://doi.org/10.1371/journal.pone.0161122

Cetkovic-Cvrlje M, Thinamany S, Bruner KA (2017). Bisphenol A (BPA) aggravates multiple low-dose 12784 streptozotocin-induced Type 1 diabetes in C57BL/6 mice. Journal of Immunotoxicology, 14(1): 160–168. https://doi.org/10.1080/1547691X.2017.1334722

Chailurkit LO, Aekplakorn W, Ongphiphadhanakul B (2016). The association of serum bisphenol A with thyroid autoimmunity. International Journal of Environmental Research and Public Health, 13(11): 1153. https://doi.org/10.3390/ijerph13111153

Chakhtoura M, Sriram U, Heayn M, Wonsidler J, Doyle C, Dinnall JA, Gallucci S, Roberts RA (2017). Bisphenol A does not mimic estrogen in the promotion of the in vitro response of murine dendritic cells to toll-like receptor ligands. Mediators

of Inflammation, 2017, 2034348. https://doi.org/10.1155/2017/2034348

Chen L, Zhao Y, Li L, Xie L, Chen X, Liu J, Li X, Jin L, Li X, Ge RS (2018a). Bisphenol A stimulates differentiation of rat stem Leydig cells in vivo and in vitro. Molecular and Cellular Endocrinology, 474: 158–167. https://doi.org/10.1016/j.mce.2018.03.003

Chen Y, Xu HS, Guo TL (2018). Modulation of cytokine/chemokine production in human macrophages by bisphenol A: A comparison to analogues and interactions with genistein. Journal of Immunotoxicology, 15(1): 96–103. https://doi.org/10.1080/1547691X.2018.1476629

Cipelli R, Harries L, Okuda K, Yoshihara S, Melzer D, Galloway T (2014). Bisphenol A modulates the metabolic regulator oestrogen-related receptor-alpha in T-cells. Reproduction, 147(4): 419–426. https://doi.org/10.1530/rep-13-0423

Couleau N, Falla J, Beillerot A, Battaglia E, D'Innocenzo M, Plançon S, Laval-Gilly P, Bennasroune A (2015). Effects of endocrine disruptor compounds, alone or in combination, on human macrophage-like THP-1 cell response. PLoS ONE, 10(7): e0131428. https://doi.org/10.1371/journal.pone.0131428

DeLuca JA, Allred KF, Menon R, Riordan R, Weeks BR, Jayaraman A, Allred CD (2018). Bisphenol-A alters microbiota metabolites derived from aromatic amino acids and worsens disease activity during colitis. Experimental Biology and Medicine, 243(10): 864–875. https://doi.org/10.1177%2F1535370218782139

Dong YD, Zhai LL, Zhang L, Jia LH, Wang XF (2013). Bisphenol A impairs mitochondrial function in spleens of mice via oxidative stress. Molecular and Cellular Toxicology, 9(4): 401–406. http://dx.doi.org/10.1007%2Fs13273-013-0049-5

Donohue KM, Miller RL, Perzanowski MS, Just AC, Hoepner LA, Arunajadai S, Canfield S, Resnick D, Calafat AM, Perera FP, Whyatt RM (2013). Prenatal and postnatal bisphenol A exposure and asthma development among inner-city children. Journal of Allergy and Clinical Immunology, 131(3): 736-742. https://doi.org/10.1016/j.jaci.2012.12.1573

Dunder L, Halin Lejonklou M, Lind L, Risérus U, Lind PM (2018). Low-dose developmental bisphenol A exposure alters fatty acid metabolism in Fischer 344 rat offspring. Environmental Research, 166, 117–129. https://doi.org/10.1016/j.envres.2018.05.023

Fang C, Ning B, Waqar AB, Niimi M, Li S, Satoh K, Shiomi M, Ye T, Dong SJ, Fan JL (2014). Bisphenol A exposure enhances atherosclerosis in WHHL rabbits. PLoS ONE, 9(10): e110977.https://doi.org/10.1371/journal.pone.0110977

Ferguson KK, Cantonwine DE, McElrath TF, Mukherjee B, Meeker JD (2016a). Repeated measures 13168 analysis of associations between urinary bisphenol-A concentrations and biomarkers of inflammation 13169 and oxidative stress in pregnancy. Reproductive Toxicology, 66: 93–98. https://doi.org/10.1016/j.reprotox.2016.10.002

Gascon M, Casas M, Morales E, Valvi D, Ballesteros-Gómez A, Luque N, Rubio S, Monfort N, Ventura R, 13237 Martínez D, Sunyer J, Vrijheid M (2015). Prenatal exposure to bisphenol A and phthalates and childhood respiratory tract infections and allergy. Journal of Allergy and Clinical Immunology, 135(2): 370–378. https://doi.org/10.1016/j.jaci.2014.09.030

Gear RB and Belcher SM (2017). Impacts of bisphenol A and ethinyl estradiol on male and female CD-1 mouse spleen. Scientific Reports, 7(1): 856. https://doi.org/10.1038/s41598-017-00961-8

Herz C, Tran HTT, Schlotz N, Michels K, Lamy E (2017). Low-dose levels of bisphenol A inhibit telomerase via ER/GPR30-ERK signalling, impair DNA integrity and reduce cell proliferation in primary PBMC. Scientific Reports, 7(1): 16631. https://doi.org/10.1038/s41598-017-15978-2

Kim KN, Kim JH, Kwon HJ, Hong SJ, Kim BJ, Lee SY, Hong YC, Bae S (2014). Bisphenol A exposure and asthma development in school-age children: A longitudinal study. PLoS ONE, 9(10): e111383. https://doi.org/10.1371/journal.pone.0111383

Koike E, Yanagisawa R, Win-Shwe TT, 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. International Journal of Immunopathology and Pharmacology, 32: 2058738418774897. https://doi.org/10.1177/2058738418774897

Lejonklou MH, Dunder L, Bladin E, Pettersson V, Rönn M, Lind L, Waldén TB, Lind PM (2017). Effects of low-dose developmental bisphenol A exposure on metabolic parameters and gene expression in male and female Fischer 344 rat offspring. Environmental Health Perspectives 125(6): 067018. https://doi.org/10.1289/ehp505

Li J, Bach A, Crawford RB, Phadnis-Moghe AS, Chen W, D'Ingillo S, Kovalova N, Suarez-Martinez JE, Zhou J, Kaplan BLF, Kaminski NE (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. https://doi.org/10.1016/j.tox.2018.01.004

Li J, Bach A, Crawford RB, Phadnis-Moghe AS, Chen W, D'Ingillo S, Kovalova N, Suarez-Martinez JE, Zhou J, Kaplan BLF, Kaminski NE (2018b). CLARITY-BPA: Effects of chronic bisphenol A exposure on the immune system: Part 2 — Characterization of lymphoproliferative and immune effector responses by splenic leukocytes. Toxicology 396–397: 54–67. https://doi.org/10.1016/j.tox.2018.02.004

Liao SL, Tsai MH, Lai SH, Yao TC, Hua MC, Yeh KW, Chiang CH, Huang SY, Huang JL (2016). Prenatal exposure to bisphenol A is associated with toll-like receptor-induced cytokine suppression in neonates. Pediatric Research 79(3): 438–444. https://doi.org/10.1038/pr.2015.234

Lin TJ, Karmaus WJJ, Chen ML, Hsu JC, Wang IJ (2018). Interactions between bisphenol A exposure and GSTP1 polymorphisms in childhood asthma. Allergy, Asthma and Immunology Research 10(2): 172–179. https://doi.org/10.4168/aair.2018.10.2.172

Liu YZ, Mei CF, Liu H, Wang HS, Zeng GQ, Lin JH, Xu MY (2014). Modulation of cytokine expression in human macrophages by endocrine-disrupting chemical bisphenol-A. Biochemical and Biophysical Research Communications 451(4): 592–598. https://doi.org/10.1016/j.bbrc.2014.08.031

Luo GY, Wang SL, Li ZG, Wei RF, Zhang LJ, Liu HH, Wang C, Niu RY, Wang JD (2014). Maternal bisphenol A diet induces anxiety-like behavior in female juvenile with neuroimmune activation. Toxicological Sciences 140(2): 364–373. https://doi.org/10.1093/toxsci/kfu085

Luo SM, Li Y, Li YP, Zhu QX, Jiang JH, Wu CH, Shen T (2016). Gestational and lactational exposure to low-dose bisphenol A increases Th17 cells in mice offspring. Environmental Toxicology and Pharmacology 47: 149–158. https://doi.org/10.1016/j.etap.2016.09.017

Mahemuti L, Chen Q, Coughlan MC, Zhang M, Florian M, Mailloux RJ, Cao XL, Scoggan KA, Willmore WG, Jin XL (2016). Bisphenol A exposure alters release of immune and developmental modulators and expression of estrogen receptors in human fetal lung fibroblasts. Journal of Environmental Sciences, 48: 11–23.

https://doi.org/10.1016/j.jes.2016.02.013

Malaisé Y, Menard S, Cartier C, Gaultier E, Lasserre F, Lencina C, Harkat C, Geoffre N, Lakhal L, Castan I, Olier M, Houdeau E, Guzylack-Piriou L (2017). Gut dysbiosis and impairment of immune system homeostasis in perinatally-exposed mice to bisphenol A precede obese phenotype development. Scientific Reports, 7(1): 14472. https://doi.org/10.1038/s41598-017-15196-w

Malaisé Y, Ménard S, Cartier C, Lencina C, Sommer C, Gaultier E, Houdeau E, Guzylack-Piriou L (2018). Consequences of bisphenol A perinatal exposure on immune responses and gut barrier function in mice. Archives of Toxicology, 92(1): 347–358. https://doi.org/10.1007/s00204-017-2038-2

Michałowicz J, Mokra K, Bąk A (2015). Bisphenol A and its analogs induce morphological and biochemical alterations in human peripheral blood mononuclear cells (in vitro study). Toxicology in Vitro, 29(7): 1464–1472. https://doi.org/10.1016/j.tiv.2015.05.012

Neri M, Virzì GM, Brocca A, Garzotto F, Kim JC, Ramponi F, de Cal M, Lorenzin A, Brendolan A, Nalesso F, Zanella M, Ronco C (2015). In vitro cytotoxicity of bisphenol A in monocytes cell line. Blood Purification, 40(2): 180–186. https://doi.org/10.1159/000437039

NTP CLARITY-BPA report (2018). A two-year toxicology study of bisphenol A (BPA) in Sprague-Dawley rats: CLARITY-BPA core study results. Food and Chemical Toxicology, 132. 12728. https://doi.org/10.1016/j.fct.2019.110728

O'Brien E, Bergin IL, Dolinoy DC, Zaslona Z, Little RJA, Tao Y, Peters-Golden M, Mancuso P (2014a). Perinatal bisphenol A exposure beginning before gestation enhances allergen sensitization, but not pulmonary inflammation, in adult mice. Journal of Developmental Origins of Health and Disease, 5(2): 121–131. https://doi.org/10.1017/s204017441400004x

O'Brien E, Dolinoy DC, Mancuso P (2014b). Perinatal bisphenol A exposures increase production of pro-inflammatory mediators in bone marrow-derived mast cells of adult mice. Journal of Immunotoxicology, 11(3): 205–212. https://doi.org/10.3109/1547691x.2013.822036

O'Brien E, Dolinoy DC, Mancuso P (2014c). Bisphenol A at concentrations relevant to human exposure enhances histamine and cysteinyl leukotriene release from bone marrow-derived mast cells. Journal of Immunotoxicology, 11(1): 84–89. https://doi.org/10.3109/1547691x.2013.800925

Özaydın T, Öznurlu Y, Sur E, Çelik İ, Uluışık D (2018a). The effects of bisphenol A on some plasma cytokine levels and distribution of CD8+ and CD4+ T lymphocytes in spleen, ileal Peyer's patch and bronchus associated lymphoid tissue in rats. Acta Histochemica, 120(8): 728–733. https://doi.org/10.1016/j.acthis.2018.08.002

Ogo FM, de Lion Siervo GEM, Staurengo-Ferrari L, de Oliveira Mendes L, Luchetta NR, Vieira HR, Fattori V, Verri Jr WA, Scarano WR, Fernandes GSA( 2018). Bisphenol A exposure impairs epididymal development during the peripubertal period of rats: Inflammatory profile and tissue changes. Basic and Clinical Pharmacology and Toxicology, 122(2): 262–270. https://doi.org/10.1111/bcpt.12894

Patel BB, Kasneci A, Bolt AM, Di Lalla V, Di Iorio MR, Raad M, Mann KK, Chalifour LE (2015a). Chronic exposure to bisphenol A reduces successful cardiac remodelling after an experimental myocardial infarction in male C57bl/6n mice. Toxicological Sciences, 146(1): 101–115. https://doi.org/10.1093/toxsci/kfv073

Petzold S, Averbeck M, Simon JC, Lehmann I, Polte T (2014). Lifetime-dependent effects of bisphenol A on asthma development in an experimental mouse model. PLoS ONE, 9(6): e100468. https://doi.org/10.1371/journal.pone.0100468

Rogers JA, Mishra MK, Hahn J, Greene CJ, Yates RM, Metz LM, Yong VW (2017). Gestational bisphenol-A exposure lowers the threshold for autoimmunity in a model of multiple sclerosis. Proceedings of the National Academy of Sciences of the United States of America, 114(19): 4999–5004. https://doi.org/10.1073/pnas.1620774114

Savastano S, Tarantino G, D'Esposito V, Passaretti F, Cabaro S, Liotti A, Liguoro D, Perruolo G, Ariemma F, Finelli C, Beguinot F, Formisano P, Valentino R (2015). Bisphenol-A plasma levels are related to inflammatory markers, visceral obesity and insulin-resistance: A cross-sectional study on adult male population. Journal of Translational Medicine, 13: 169. https://doi.org/10.1186/s12967-015-0532-y

Song H, Park J, Bui PTC, Choi K, Gye MC, Hong YC, Kim JH, Lee YJ (2017). Bisphenol A induces COX-2 through the mitogen-activated protein kinase pathway and is associated with levels of inflammation-related markers in elderly populations. Environmental Research, 158: 490–498. https://doi.org/10.1016/j.envres.2017.07.005

Spanier AJ, Kahn RS, Kunselman AR, Schaefer EW, Hornung R, Xu YY, Calafat AM, Lanphear BP (2014b). Bisphenol A exposure and the development of wheeze and

lung function in children through age 5 years. JAMA Pediatrics, 168(12): 1131–1137. https://doi.org/10.1001/jamapediatrics.2014.1397

Tajiki-Nishino R, Makino E, Watanabe Y, Tajima H, Ishimota M, Fukuyama T (2018). Oral administration of bisphenol A directly exacerbates allergic airway inflammation but not allergic skin inflammation in mice. Toxicological Sciences, 165(2): 314–321. https://doi.org/10.1093/toxsci/kfy132

Tarapore P, Hennessy M, Song D, Ying J, Ouyang B, Govindarajah V, Leung YK, Ho SM (2017). High butter-fat diet and bisphenol A additively impair male rat spermatogenesis. Reproductive Toxicology, 68: 191–199. https://doi.org/10.1016/j.reprotox.2016.09.008

Vernet C, Pin I, Giorgis-Allemand L, Philippat C, Benmerad M, Quentin J, Calafat AM, Ye XY, Annesi-Maesano I, Siroux V, Slama R, EDEN Mother–Child Cohort Study Group (2017). In utero exposure to select phenols and phthalates and respiratory health in five-year-old boys: A prospective study. Environmental Health Perspectives, 125(9): 097006. https://doi.org/10.1289/ehp1015

Wang IJ, Chen CY, Bornehag CG (2016). Bisphenol A exposure may increase the risk of development of atopic disorders in children. International Journal of Hygiene and Environmental Health, 219(3): 311–316. https://doi.org/10.1016/j.ijheh.2015.12.001

Yang ML, Chen MP, Wang JQ, Xu M, Sun JC, Ding L, Lv XF, Ma QY, Bi YF, Liu RX, Hong J, Ning G (2016). Bisphenol A promotes adiposity and inflammation in a nonmonotonic dose–response way in 5-week-old male and female C57BL/6J Mice Fed a Low-calorie Diet. Endocrinology, 157(6): 2333–2345. https://doi.org/10.1210/en.2015-1926

Youssef MM, El-Din E, AbuShady MM, El-Baroudy NR, Abd El Hamid TA, Armaneus AF, El Refay AS, Hussein J, Medhat D, Latif YA (2018). Urinary bisphenol A concentrations in relation to asthma in a sample of Egyptian children. Human and Experimental Toxicology, 37(11): 1180-1186. https://doi.org/10.1177/0960327118758150

Zhou AF, Chang HL, Huo WQ, Zhang B, Hu J, Xia W, Chen Z, Xiong C, Zhang YQ, Wang YJ, Xu SQ, Li YY (2017). Prenatal exposure to bisphenol A and risk of allergic diseases in early life. Pediatric Research, 81(6): 851–856. https://doi.org/10.1038/pr.2017.20

Zhu JY, Jiang L, Liu YQ, Qian WY, Liu JL, Zhou J, Gao R, Xiao H, Wang J (2015). MAPK and NF-κB pathways are involved in bisphenol A-induced TNF-α and IL-6 production in BV2 microglial cells. Inflammation, 38(2): 637–648. https://doi.org/10.1007/s10753-014-9971-5

# Abbreviations

| | |
|---|---|
| ALAN | As likely as not |
| ALT | Alanine aminotransferase |
| APCs | Antigen presenting cells |
| AR | Androgen receptor |
| AST | Aspartate aminotransferase |
| ATP | Adenosine triphosphate |
| B cells | B lymphocytes |
| BAL | Bronchoalveolar lavage |
| BMD | Benchmark dose |
| BMI | Body mass index |
| BPA | Bisphenol A |
| BPA-G | Bisphenol-A-glucuronide |
| bw | Body weight |

| | |
|---|---|
| Ca2+ | Calcium |
| CD4+ cells | |
| CD8+ cells | |
| CD25+cells | |
| CD80+ cells | |
| CD86+ cells | |
| CD11b/c cells | |
| CD172α cells | |
| Anti-CD3/CD28 stimulation | |
| CI | Confidence interval |
| (hs-)CRP | (high-sensitivity) C-reactive protein |
| CysLT | Cysteinyl leukotriene |
| DSS | Dextran sodium sulfate |
| EFSA | European Food Safety Authority |
| ERK | Extracellular signal-related kinase |

| | |
|---|---|
| ER | Oestrogen receptor |
| ER2 | Oestrogen receptor 2 |
| ERRA | Oestrogen-related receptor alpha |
| ET-1 | Endothelin-1 |
| Exp | Exposure periods |
| FENO | Fraction of exhaled nitric oxide |
| FEV1 | Forced respiratory volume in first second |
| g | gram |
| G-CSF | Granulocyte-colony stimulating factor |
| GD | Gestation day |
| GDF15 | Growth differentiation factor-15 |
| GPER | G protein-coupled oestrogen receptor |
| GR | Glucocorticoid receptor |
| γ-GTP | Gamma-glutamyl transpeptidase |
| HLFL | Human lung fibroblasts |
| HOC | Health outcome category |

| | |
|---|---|
| HOME | Health Outcomes and Measures of the Environment |
| IFN- γ | Interferon gamma |
| Ig | Immunoglobulin |
| IL | Interleukin |
| INMA | INfancia y Medio Ambiente (Environment and Childhood Project) |
| IP-10 | Interferon y-induced protein 10 |
| JNK | c-Jun N-terminal kinase |
| kg | kilogram |
| LOD | Limit of detection |
| KC | Kupffer cells |
| LPS | Lipopolysaccharides |
| MHC | Major histocompatibility complex |
| MLN | Mesenteric lymph nodes |
| NF-kB | Nuclear factor kappa B |
| ng | nanogram |

| | |
|---|---|
| NK cells | Natural killer cells |
| NKT cells | Natural killer T cells |
| nM | nanomolar |
| NTP | National Toxicology Program |
| OR | Odds ratio |
| OVA | Ovalbumin |
| PC | Polycarbonate |
| PGD2 | Prostaglandin D2 |
| pmol | picomolar |
| PND | Post-natal day |
| PWM | Pokeweed mitogen |
| RANTES | Regulated upon activation, normal T cell expressed and secreted |
| RR | Relative risk |
| SDF1 | Stroma cell-derived factor 1 |
| STZ | Streptozocin |

| | |
|---|---|
| TDI | Toluene-2,4-diisocyanate |
| T cells | T lymphocytes |
| Tc cells | Cytotoxic T cells |
| Th cells | T helper cells |
| THP-1 | Human monocytic cells |
| TLR | Toll-like receptor ligands |
| TNF-α | Tumour necrosis factor alpha |
| Treg cells | Regulatory T cells |
| TSLP | Thymic stromal lymphopoietin |
| µg | microgram |
| µM | micromole |
| USA | United States of America |
| VEGF | Vascular endothelial growth factor |
| WBC | White blood cells |
| WoE | Weight of evidence |