Animal studies -(BPA) in foodstuffs – Reproductive and Developmental Toxicity

In this guide

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Animal studies

31. As part of the HOC a Reproductive and Developmental Toxicity, a total of 153 animal studies were reviewed by the CEP panel. Endpoints for which statistically significant changes were reported were extracted from the available literature and grouped into three clusters:

- Developmental toxicity.
- Female reproductive toxicity.
- Male reproductive toxicity.

These clusters include endpoints that were identified as relevant in the 2015 EFSA opinion and were considered in the uncertainty analysis. These endpoints were endometrial hyperplasia, ovarian cysts and anogenital distance (AGD); the first two of these were also identified as relevant in the newly compiled studies. As it was previously identified as relevant, AGD was also included and considered relevant in the new assessment.

32. The information extracted from these studies is summarised and tabulated (Table G5) in Annex G to the opinion. The weight of evidence is discussed in the main opinion and presented in a tabulated format in Annex H (sheet H5). The clusters considered not likely or ALAN are outlined briefly below, with the clusters considered likely are described in more detail.

Cluster - Developmental toxicity

33. Within the cluster developmental toxicity, there were nine studies in mice; seven studies had exposure during development until weaning, two had exposure during development until adulthood and one had exposure during the growth phase. There were 13 studies on rats: 10 studies had exposure during development until weaning, four had exposure during development until adulthood and in one the exposure was to adults. Some studies assessed multiple exposure periods.

34. The specific endpoints that were included in this cluster were blastocyte outgrowth incidence, embryo development, age/day of first oestrus, AGD, mammary gland histology, mammary gland weight, bone development and body weight of F1/F2/F3 generation, as well as body weight of F0 dams. (Note- the assessment of body weight for each exposure period is described in detail in the Metabolic effects category (Chapter 3.1.4.2).

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

AGD

35. One Tier 2 study (Spörndly-Nees et al., 2018) in rats was considered. There was a not statistically significant increase in AGD at 12 months but not at PND 35. In the absence of a dose -response and without other studies available this endpoint was considered **Not Likely.**

Age at first oestrus

36. No effect was seen in a single Tier 1 study in mice (Tucker et al., 2018) using doses of 500, 5000 and 50000 µg/kg bw per day, twice daily, from GD10.5–17.5. The endpoint was judged **Not Likely**.

Bone development

37. Two Tier 1 studies in rats (Lejonklou et al., 2016; Lind et al., 2017 and one Tier 1 study in mice were available (van Esterik, 2014). The studies in rats showed inconsistent and no effects were observed in mice. The endpoint was judged **ALAN.**

Mammary gland weight

38. One Tier 1 study in rats was identified (Montévil et al., 2020, with mammary gland weight in female rats was determined at PND90. It was noted that the study authors described a Non Monotonic Dose Response (NMDR). However, when using more conventional statistical methods, e.g. modelling the data in PROAST (Hill and Exponential models) or using spline and polynomial fit (without overfitting the data) no dose response could be identified by the CEP Panel. The CEP Panel considered that alternative interpretations of the data were plausible, and the findings were likely explained by random fluctuations and variability in the data. The endpoint was judged **Not Likely**.

Mammary gland histology

39. In males: Out of six rat and one mouse studies, three rat studies (all Tier 1) (Kass et al., 2015; Mandrup et al., 2016; NTP Clarity Report, 2018/Camacho et al., 2019 assessed the mammary gland histology of male pups. There were no neoplastic changes but changes in mammary gland growth were observed in Kass et al. (2015) and Mandrup et al. (2016). The study by Mandrup

et al., (2016) showed a decrease in male and increase in female-like mammary gland structures in male rat pups assessed at PND100 when doses of 5,000 and 50000 µg/kg bw per day were given from GD7 until birth. Assessment of the same dose groups at PND22 did not show any changes. However, lower doses in this study (25 and 250 µg/kg bw per day), which were also assessed at PND22, showed an increase in mammary gland growth at 25 µg/kg bw per day. No effects were reported on mammary glands in males in the NTP Clarity Report (2018)/Camacho et al. (2019).

40. The CEP Panel judged the male mammary gland effects as **ALAN.**

41. In females: Non-neoplastic findings in female rat mammary gland were observed in six Tier 1 studies (Kass et al., 2015; Grassi et al., 2016; NTP Clarity Report, 2018/Camacho et al., 2019; Montévil et al., 2020; Tucker et al., 2018; Mandrup et al., 2016 studies and one Tier 3 study (Leung et al., 2017). The findings included delayed ductal growth, increased branching score and number of terminal ducts (TDs) and terminal end buds (TEB+TDs) a decreased incidence of ductal dilatation in F1 females at 1 year. Montévil et al 2020, who assessed samples from the Clarity study, reported several effects at single doses in the mammary glands of female F1 pups, these included increased gland density, a number of structural difference and an increased average branch length at BPA doses of 250 µg/kg bw per day. All effects with indications of a dose–response relationship for which individual data were available (gland density, Dimension 3D and angles of branches between beginning and end, thickness of epithelium, variation of ductal thickness, aspect ratio) were statistically re-analysed by the CEP Panel. This revealed that a formal dose–response could not be identified by fitting flexible biologically based functions or polynomials that are commonly used to describe biological systems except for ductal thickness and aspect ratio, for which a potential non- monotonic dose–response was identified statistically.

42. Another Tier 1 study (Tucker et al., 2018) reported increased branching density at low doses, increased length of TEBs, mammary gland development score and number of TEBs at mid dose. All effects occurred only at PND20 and not at later time points. These non-neoplastic were not consistent among studies with different study designs. Pre-neoplastic findings in adult females were seen in one Tier 1 study, Mandrup et al. (2016), showing an increase in intraductal hyperplasia. One Tier1 study (NTP Clarity Report, 2018/Camacho et al., 2019 revealed a neoplastic effect, an increase in adenoma and adenocarcinoma in the lowest dose, assessed at terminal sacrifice after 2 years. However, four other Tier 1 studies with shorter timepoints showed no pre-neoplastic effects. One

neoplastic lesion was reported, i.e. stromal polyps, in the NTP Clarity Report, 2018/Camacho et al., 2019. For this endpoint, a decrease (no adverse effect) was seen in this Tier 1 study at the highest dose after 2 years.

43. The CEP Panel concluded that, based on the inconsistent findings of non-neoplastic effects as well as the unconfirmed neoplastic or pre-neoplastic effects, that effects on the female mammary gland are **ALAN.**

44. Overall, the CEP Panel assigned a likelihood level of ALAN to the developmental toxicity effects of BPA in the developmental exposure period based on bone development, mammary gland histology and body weight. Therefore, none of the endpoints were taken forward for BMD analysis. However, the ALAN endpoints were considered in the uncertainty analysis (which is set out in Appendix D to the main opinion).

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

AGD

45. Only one single-dose study (Tier 3) by Patel et al., 2013 was available. It was therefore judged that there is Inadequate evidence for this endpoint.

Embryo development

46. A single Tier 3 study was available (Dobrzynska et al., 2018). It was therefore judged that there is Inadequate evidence for this endpoint.

Bone development:

47. Only one single-dose study (Tier 2) by Auger et al., 2013 is available. It was therefore judged that there is Inadequate evidence for this endpoint.

Mammary gland histology

48. Two Tier 1 studies (Montévil et al., 2020; NTP Clarity Report 2018/Camacho et al., 2019) assessed the mammary glands of female rats. Non-neoplastic effects were only observed at single doses without any dose–response, apart for mammary gland scores, where the data were in line with the definition by the CEP Panel of indications for a NMDR. For Montévil et al. (2020), the CEP Panel re-evaluated the dose–response for gland density by fitting flexible biologically based functions or polynomials that are commonly used to describe

biological systems and concluded that there was no dose–response relationship.

49. Among the non-neoplastic effects identified in this exposure group several changes were only reported in one dose group in the female rats, this was an increase in lobular alveolar budding (Montévil et al., 2020), changes in ductal dilatation which was increased at 1 year but decreased at 2 years (the adversity was noted to be unclear) and a decrease in lobular hyperplasia, (NTP Clarity Report 2018/Camacho et al.2019) was also observed. The BPA-induced decreases were different from the results following oestradiol treatment which resulted in clear increases in duct dilatation and lobular hyperplasia as well in adenocarcinomas. In the same study, an increase in alveolar dilatation was only reported in males at the lowest dose after 2 years while the effect was not significant in females in both studies. Therefore, no dose–response could be established for non-neoplastic effects. For neoplastic effects, the NTP Clarity Report (2018)/Camacho et al. (2019) reported an increase in atypical foci and adenocarcinomas at a dose of 2.5 µg/kg bw per day. In addition, there were non-significant increases in atypical foci in the 25 and 250 µg/kg bw per day dose groups at 1 year. One neoplastic lesion, stromal polyps, was also reported. For this endpoint, however, a significant dose trend towards increased incidence at the higher doses at 1 year was observed, while a negative trend (no adverse effect) was observed at 2 years in this Tier 1 study. The CEP Panel therefore considered this result biologically implausible.

50. Based on the above results, histological effects on mammary gland induced by BPA were judged as **ALAN.**

51. Overall, the CEP Panel assigned a likelihood level of ALAN to the developmental toxicity effects of BPA in the developmental and adult exposure period based on effects on body weight and mammary gland histology. Therefore, none of the endpoints was taken forward for BMD analysis. However, the ALAN endpoints were considered in the uncertainty analysis (see Appendix D to the opinion).

Growth phase/young age

Age at first oestrus

52. In the Tier 1 study by Li et al., (2016) in mice, a decreased age at first oestrus was observed at a BPA dose of 60 µg/kg bw per day, and an increased age at first oestrus at a dose of 600 µg/kg bw per day following dosing three times per day after 5 weeks of exposure starting at PND22.

53. The likelihood of changes in age at first oestrus was judged **ALAN** by the CEP Panel.

54. The CEP Panel assigned a likelihood of ALAN to the cluster of developmental toxicity of BPA in the growth phase/young age exposure period. Therefore, none of the endpoints was taken forward for BMD analysis. However, both body weight and age at first oestrus were considered in the uncertainty analysis (see Appendix D).

Adult exposure (after puberty)

Blastocyst outgrowth and F1 embryo development:

55. It was judged that there was Inadequate Evidence for these endpoints as they were both only assessed in a single dose Tier 1 Study (Martinez et al., 2015).

56. The CEP Panel assigned a likelihood level of Not Likely to the developmental toxicity effects of BPA in the adult exposure period based on body weight. Therefore, none of the endpoints were taken forward for BMD analysis.

Indirect (germline) exposure

Bone development

57. As only one Tier 2 single-dose study (Auger et al., 2013 was available for assessment, it was judged that there was inadequate Evidence for this endpoint.

58. The CEP Panel assigned a likelihood level of **Not Likely** to the developmental toxicity effects of BPA in the indirect germline exposure period based on body weight. Therefore, this endpoint was not taken forward for BMD analysis.

Overall cluster selection for endpoints/studies for BMD for developmental toxicity

59. Overall, the CEP Panel assigned a likelihood level of ALAN, to the developmental toxicity effects of BPA in the exposure periods developmental, developmental and adult and growth phase/young age, and of Not Likely in the adult and indirect (germline) exposure. The overall likelihood across all exposure periods, i.e. the highest likelihood given in the cluster developmental toxicity was ALAN.

60. The CEP Panel considered that the evidence from the studies available showed ALAN effects of BPA for the endpoints bone development, mammary gland histology, body weight (developmental exposure), body weight and mammary gland histology (developmental and adult exposure) as well as body weight and age at first oestrus (growth phase/young age). These endpoints were therefore not brought forward for BMD analysis.