Integration of likelihoods from human and animal studies - (BPA) in foodstuffs – Reproductive and Developmental Toxicity

In this guide

244. Table 13 of the EFSA opinion (and reproduced below) presents the overall likelihood per cluster for the human and animal stream separately, as well as the integration of the likelihoods from the human and animal studies for reproductive toxicity. The integration is described by the panel as below:

245. AGD, bone development and breast development are endpoints assessed in two different clusters: in pubertal/endocrine in the human stream and in the developmental toxicity cluster in the animal stream. The CEP panel concluded that overall, the animal and human clusters were not comparable. It was considered of relevance to analyse the endpoints on which the final conclusion for the likelihood is based, both for animal and human clusters. In case of any similar outcomes the evidence of the respective likelihood would increase.

246. In the human stream, the fetal and post-natal growth cluster contains results for body weight and femoral length which can be compared to body weight effects and effects on bone development in the developmental cluster for animals. While human studies show Not Likely effects, animal studies result in ALAN effects on body weight and bone development.

247. The further likelihood of effects observed in developmental animal studies is based on ALAN effects on mammary gland development and age of first estrus. Neither of these effects have been described as changed nor as references of a similar likelihood in human studies. The pubertal/endocrine cluster of the human stream bases its ALAN evaluation on changes to the AGD. The assessment of the AGD in animals revealed, however, no effects. Sex hormones are included in the human pubertal/endocrine and in the animal Male and Female reproductive toxicity clusters, both without any influence on the overall conclusion on the respective likelihoods. Thus, finally there is no substantiation of the ALAN likelihood for the animal cluster developmental toxicity nor of the ALAN likelihood for the human cluster pubertal/endocrine.

248. In the animal Male and Female reproductive toxicity clusters, there were Likely effects on sperm, follicles and implantation, but not on fertility. There was no clear overlap between the endpoints assessed in human and animal studies that focused on reproductive toxicity in females. As for effects on mammary gland development, ovary and uterus weight and histology, there are no studies available in human to be compared and integrated with the animal evidence.

Integration of likelihoods from the human and animal studies- reproduced from Table 13 of the EFSA draft opinion.

Human Stream	Animal Stream	Integrated likelihood
Cluster: Developmental toxicity	**Cluster: Developmental toxicity**	**Integrated likelihood**
	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) – ALAN.	
Not applicable		ALAN
	Growth phase/young age exposure – ALAN.	
	Adult exposure (after puberty) - Not Likely.	
	Indirect (germline) exposure - Not Likely.	
	Overall likelihood ALAN.	

Cluster: Fetal and Post-natal Growth	**Cluster: Fetal and Post-natal Growth**	**Integrated likelihood**
Exposure during pregnancy	Not applicable.	Overall Likelihood: Not Likely
Cluster: Pubertal Endocrine	**Cluster: Pubertal Endocrine**	**Integrated likelihood**
Exposure during pregnancy – ALAN.	Not applicable.	Overall Likelihood: ALAN – Likely.
Exposure during adulthood – Not likelihood.	Not Applicable.	Overall Likelihood: ALAN – Likely.
Cluster: Female fertility	**Cluster: Female reproductive toxicity**	**Integrated likelihood**

	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) – Likely. Growth phase/young age exposure – Likely. Adult exposure (after puberty) - Likely. Indirect (germline) exposure - Inadequate evidence.	
Exposure during adulthood – ALAN.		Overall likelihood – ALAN – Likely.
Cluster: Male fertility	**Cluster: Male reproductive toxicity**	**Integrated likelihood**

	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) - Likely. Growth phase/young age exposure – Likely. Adult exposure (after puberty) - Likely. Indirect (germline) exposure - Inadequate evidence. Overall likelihood - Likely	
Exposure during adulthood – Not Likely. Overall likelihood – Not Likely.		Overall likelihood –Likely.
Cluster: Prematurity	**Cluster: Prematurity**	**Integrated likelihood**
Exposure during pregnancy Not Likely	Not Applicable.	Not Applicable.
Overall likelihood – Not Likely	Not Applicable.	Not Likely.
Cluster: Pre-eclampsia	**Cluster: Pre-eclampsia**	**Integrated likelihood**
Exposure during adulthood.	Not applicable.	Not Applicable.
Overall likelihood - ALAN	Not applicable.	ALAN

249. The Mode of Action studies for clusters and endpoints judged to be ALAN or Likely are discussed in section 3.1.6.4 of the opinion. These have not been considered in detail here. However, mechanisms including epigenetic changes, enhanced sensitivity to carcinogenic changes, altered proliferation and pathological processes, changes to gene expression and oxidative stress, effects on hormonal signalling pathways are considered in this cluster.

250. For the changes in follicles, the mechanisms discussed are considered below as the finding of Hu et al., (2018) were taken forward for BMD modelling.

251. In animals, Thilagavathi et al. (2017) reported that the number of antral follicles in rats was decreased after treatment with BPA at 10 mg/kg bw per day for 12 weeks, but increased at doses of 50 and 100 mg/kg bw per day. They proposed that BPA-induced oxidative stress leads to antioxidant depletion in the ovary, which leads to elevated level of Thiobarbituric acid reactive substances (TBARS – these are byproducts of lipid oxidation and indicate oxidative damage), which leads to overexpression of endothelial nitric oxide (eNOS), which prevented steroidogenic acute regulatory protein (StAR) transport from the outer to the inner mitochondria membrane, which inhibited CYP11A1, leading to the downregulation of aromatase.

252. Berger et al. (2016) reported that in mice dosed during gestation with 0.5–50 µg/kg bw BPA per day, germ cell nest breakdown was directly inhibited, probably by altering gene expression for apoptosis, oxidative stress and autophagy. Preantral follicle numbers were decreased, potentially by causing death of these follicles at time points earlier than 3 months. They concluded that in utero BPA exposure has different effects on the gene expression of steroidogenic enzymes and these effects depend on the dose of BPA, age and the generation of the mice.

253. Santamaria et al. (2016) reported reduced primordial to primary follicle transition and altered steroidogenesis in adult rats dosed during gestation and lactation with 0.5 or 50 mg/kg bw BPA per day. The androgen receptor (AR) was increased in primordial follicles at 5 mg/kg bw per day and decreased in primary follicles at 50 mg/kg bw per day. They concluded that BPA could affect ovulation through different mechanisms depending on dose but did not speculate on what was causing the altered steroidogenesis.

254. Santamaria et al. (2017) reported reduced ovulatory response to exogenous gonadotrophins in pre-pubertal female rats dosed during gestation

and lactation at 50 mg/kg bw per day. They suggested that different mechanisms might be leading the follicles to atresia and that loss of AR in the later stages of follicular development might be part of a paracrine mechanism that affords protection against premature luteinisation and atresia.

255. Cao et al. (2018) reported that BPA decreased the ovarian reserve in adult mice dosed with 5–500 mg/kg bw per day for 28 days. They hypothesised that BPA may reduce ovarian granulosa cell activity and accelerate its apoptosis, leading to the decreased synthesis of AMH. They did not speculate on upstream mechanisms.

256. Soleimani Mehranjani and Mansoori (2016) reported that adult rats dosed with BPA at 60 mg/kg bw per day for 20 days had reduced antral follicles and increased atretic follicles. Concomitant vitamin C ameliorated these adverse effects. They did not propose a MoA, but antagonism of BPA- induced oxidative stress is a plausible possibility.

257. Ganesan and Keating (2016) reported that in vitro incubation of perinatal rat ovary with very high dose of BPA (440 mM) reduced primary and secondary follicle numbers after 2 days, followed by a reduction in primordial follicle numbers after four days and induced ovarian DNA damage. They concluded that BPA, via biotransformation, may be converted to a DNA alkylating agent. It is noted that the concentration tested was likely many orders of magnitude higher than achievable in vivo levels.

258. Overall, at lower doses, oxidative stress leading to steroid alterations is a plausible mechanism for reduced follicle development. DNA damage is only reported at a much higher dose/concentration.