Male reproductive toxicity - Animal Studies - (BPA) in foodstuffs – Reproductive and Developmental Toxicity

In this guide

Male reproductive toxicity

128. Within the cluster male reproductive toxicity, there were 15 studies in mice, of which six studies included exposure during the period development until weaning, two had exposure during development until adulthood, seven were exposed as adults and two had germline exposure; some studies tested multiple exposure periods). Of the 26 studies on rats, 14 included exposure during development until weaning, four had exposure during development until adulthood, five had exposure during the growth phase, five were exposed as adults. In addition, one study was on sheep, which had exposure during the development until weaning period and one study in monkeys which had exposure during adulthood.

129. The specific endpoints that were included for effects of BPA on the male reproductive toxicity cluster were plasma/serum thyroid hormones, testosterone, epididymis weight and histology, prostate histology, seminal vesicle weight, sperm count/morphology/motility/viability, testis weight and histology.

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

Plasma/serum thyroid hormones:

130. For this exposure period the following studies were identified.

- T3: one Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019
- T4: two Tier 1 rat studies (NTP Clarity Report, 2018/Camacho et al., 2019; Bansal and Zoeller, 2019 and one Tier 1 sheep study (Guignard et al., 2017
- T3/TT4: one Tier 1 sheep study (Guignard et al., 2017).

131. No effect was seen on T3 in the Tier 1 rat study.

132. Apart from a decrease at the highest dose level in the Clarity study, no effects was seen on T4 in either Tier 1 rat study or in the sheep study (Guignard et al., 2017. In addition, in the latter study, no change was seen in the ratio T3/T4.

133. During this developmental exposure (pre-natal and/or post-natal until weaning) period changes in thyroid hormones (T3, T4, rT3/TT4) were judged as Not Likely by the CEP Panel.

Plasma/serum testosterone

134. for this endpoint four Tier 3 studies in rats (Quan et al., 2017; Wang C et al., 2014; Castro et al., 2018; Johnson et al., 2016 - the latter study is related to the Clarity study) and one Tier 3 mouse study (Shi et al., 2018 were identified. Therefore, data were considered Inadequate to judge the likelihood of an effect of BPA on testosterone levels.

Epididymis weight:

135. For this endpoint one Tier 1 (NTP Clarity Report, 2018/Camacho et al., 2019, one Tier 2 study (Spörndly-Nees et al., 2018, one Tier 3 rat study (Tarapore et al., 2017) and one Tier 2 mouse study (Meng Y et al., 2018) were identified.

136. No effect was seen on epididymis weight in any of the studies, and the likelihood for this endpoint was considered Not Likely.

Epididymis histology

137. This endpoint included non-neoplastic, inflammatory changes, inflammation). For this endpoint one Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 and one Tier 2 rat study (Spörndly-Nees et al., 2018 were identified.

138. No effect was seen on epididymis histology in the Tier 1 rat Clarity study (NTP Clarity Report, 2018/Camacho et al., 2019. An increase in inflammatory changes was seen at the top dose (40 µg/kg) bw per day in the other Tier 2 rat study (Spörndly-Nees et al., 2018): in this study animals were exposed via the drinking water to doses of 4 or 40 µg/kg per day from GD3.5–PND22 and necropsied at PND35 or 12 months.

139. As no effects were seen for the endpoint in one Tier 1 study (NTP Clarity Report, 2018/Camacho et al., 2019 and an effect only at the highest dose in a Tier 2 rat study, the likelihood assigned to this endpoint was Not Likely.

Prostate histology

140. For this endpoint four Tier 1 (NTP Clarity Report, 2018/Camacho et al., 2019; Bernardo et al., 2015; Prins et al., 2018; Brandt et al., 2014, one Tier 2 (Hass et al., 2016 and one Tier 3 (Prins et al., 2017) studies in rats were identified. In these studies, several histological effects were examined.

141. No effect was seen on prostate histology (non-neoplastic proliferative lesions, hyperplasia of the ventral prostate, epithelium hyperplasia and inflammatory changes, inflammation, dorsal/lateral prostate histology, suppurative inflammation) in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019.

142. At histological examination, an increase (same effect size) in the incidence of inflammatory changes, pre-neoplastic lesions (atypical hyperplasia), non-neoplastic proliferative lesions (reactive hyperplasia), in the prostate was seen at both doses tested in a Tier 1 rat study (Bernardo et al., 2015). In this study animals were given 25 or 250 µg/kg bw BPA per day from GD10–21 and were necropsied at PND180.

143. No effect was seen on prostate histology (non-neoplastic proliferative lesions, hyperplasia of the ventral prostate and inflammatory changes, inflammation, dorsal/lateral prostate histology, suppurative inflammation) in the Tier 1 rat study (Prins et al., 2018). This study is a component of the Clarity study. In a Tier 1 study by Brandt et al., (2014) rats were administered 25 and 250 µg/kg bw per day from GD10–21. F1 pups/adults were sacrificed on PND21/180. At histological examination on PND21 an increase in proliferation and hyperplasia/dysplasia of the prostate was observed in the lowest dose and of apoptosis in the highest dose. At both doses an increase (effect the same size) of multifocal inflammation in the ventral prostate on PND180 was observed.

144. In the Tier 2 rat study (Hass et al., 2016), no change in prostate histology (interstitial inflammation, proliferation or epithelial atypical hyperplasia) was observed when examined in F1 males at 3 or 8 months; F0 dams were treated at oral doses of 25, 250, 5000 or 50000 µg/kg bw per day GD7–PND22.

145. In the two Tier 1 rat studies (Bernardo et al., 2015; Brandt et al., 2014 from the same laboratory), inflammatory effects and reactive hyperplasia in the prostate were reported at doses of 25 and 250 µg/kg bw per day GD10–GD21. This effect was not confirmed in the two other Tier 1 rat studies or in the Tier 2 rat study (Hass et al., 2016). The likelihood for this endpoint was considered to be ALAN.

Seminal vesicle weight

146. For this endpoint one Tier 1 (NTP Clarity Report, 2018/Camacho et al., 2019 and one Tier 2 study (Spörndly-Nees et al., 2018 in rats and one Tier 3 mouse study (Patel et al., 2013) were identified.

147. No effect was seen in either of the Tier 1 or Tier 2 studies. Therefore, the likelihood assigned to this endpoint is Not Likely.

Sperm count

148. For this endpoint one Tier 1 (NTP Clarity Report, 2018/Camacho et al., 2019) and one Tier 3 study (Hass et al., 2016 in rats and two Tier 3 studies in mice (Rahman et al., 2017; Shi et al., 2018) were identified.

149. No effect was seen on epididymal sperm count and count of testicular sperm heads in the Tier 1 rat studies. The likelihood assigned to this endpoint is Not Likely.

Sperm morphology

150. For this endpoint one Tier 1 study (NTP Clarity Report, 2018/Camacho et al., 2019 and one Tier 2 study (Spörndly-Nees et al., 2018 in rats and one Tier 3 mouse study (Kalb et al., 2016 were identified. No effects on sperm morphology were observed and the likelihood assigned to the endpoint was Not Likely.

Sperm motility

151. For this endpoint, one Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019) and three Tier 3 studies in mice (Shi et al., 2018; Kalb et al., 2016; Rahman et al., 2017) were identified.

152. No effects were observed in the Tier 1 rat study so the likelihood assigned to this endpoint is Not Likely.

Sperm viability

153. For this endpoint one Tier 3 mouse study (Rahman et al., 2017) was identified. Therefore, the data were Inadequate to judge the likelihood of an effect of BPA on sperm viability.

Testis weight

154. For this endpoint two Tier 1 studies (NTP Clarity Report, 2018/Camacho et al., 2019; Cao et al., 2015), and one Tier 3 study (Tarapore et al., 2017 in rats and two Tier 2 studies (Meng Y et al., 2018; Shi et al., 2018) and one Tier 3 study (Patel et al., 2013) in mice were identified. No effect was reported on testis

weight in the Tier 1 rat study Clarity study.

155. However, in a single-dose Tier 1 rat study (Cao et al., 2015), an increase in testis weight was observed on PND 120. In this study the F0 females were administered BPA via drinking water (2 mg BPA/L; equivalent to 100 µg/kg bw per day) and co-treated with soy in the diet from GD1–PND21. No change was observed in the BPA-treated group with a soy-free diet. As noted elsewhere, soy is thought to ameliorate the effects of BPA.

156. No effect was seen on testis weight in a Tier 2 mouse study (Meng Y et al., 2018). In this study animals were exposed via drinking water to doses equivalent to 18 or 180 µg/kg per day from GD6–PND21 and necropsied at PND50.

157. No effect was seen on testis weight in the other Tier 2 mouse study (Shi et al., 2018). In this study, animals were exposed via the drinking water to doses equivalent to 0.5, 20 or 50 µg/kg bw per day from GD11 to birth and necropsied at PND60.

158. As no effect was observed in either the Tier 1 rat study or the two Tier 2 mouse studies, the likelihood was considered to be Not Likely for this endpoint.

Testis histology

159. For this endpoint, one Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019, two rat Tier 2 studies (Quan et al., 2017; Spörndly-Nees et al., 2018), two Tier 2 mouse studies (Shi et al., 2018; Xie et al., 2016) and one Tier 3 mouse study (Rahman et al., 2017) were identified.

160. At histological examination, an increased incidence of testis (and pancreas) polyarteritis was seen in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019) at a dose of 2500 µg/kg bw per day.

161. In the Tier 2 rat study (Quan et al., 2017) an increase was seen in seminiferous tubular changes in the testis, cell-specific and/or stage-specific: degeneration, germ cell, at all dose levels at PND50 in F1 Sprague Dawley rats; this was not dose related. In this study, the F0 animals were dosed with 1000; 10000 or 100000 µg/kg bw BPA by gavage from GD14–21.

162. At histological examination, following 12 months exposure, an increase was seen in inflammatory changes in the testis of the low-dose group (4 µg/kg per day from GD3.5–PND22) in a Tier 2 rat study (Spörndly-Nees et al., 2018). No

such effects were seen at the other dose tested (40 µg/kg per day) or other histological effects in the testis (seminiferous tubular changes, non-specific seminiferous epithelial height and seminiferous tubule diameter) at either dose level.

163. At histological examination of the testis in a Tier 2 mouse study (Shi et al., 2018), a decrease in seminiferous tubular changes, cell and/or stage-specific, in stage VII seminiferous epithelial cells and an increase in stage VIII seminiferous epithelial cells was seen in the mid-dose group (20 µg/kg bw per day) at PND60. On PND12, an increase without dose–response was seen in testicular apoptosis in the mid and high-dose groups. In this study CD- 1 mice were dosed with 0.5, 20 or 50 µg/kg bw per day from GD11 to birth by micropipette.

164. In another Tier 2 mouse study (Xie et al., 2016) a dose-related increase was observed at histological examination of the testis (degeneration, germ cell). In this study male mice were s.c. injected with10, 100 or 5000 µg/kg bw per day BPA (equivalent to oral doses of 2222; 22220 or 1111000 µg/kg bw per day).

165. The Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 reported an increased incidence of polyarteritis at 2500 µg/kg bw per day only (dose range from 2.5– 25000 µg/kg bw per day). The Tier 2 rat studies showed effects on inflammatory changes at only one dose (4 µg/kg bw per day) (Spörndly-Nees et al., 2018), or effects (apoptosis) without a dose response at doses of 1000–100000 µg/kg bw per day (Quan et al., 2017). In the Tier 2 mouse study (Shi et al., 2018) only the mid dose group (20 µg/kg bw per day) showed effects on the testis (decrease in stage VII and decrease in stage VIII seminiferous epithelial cells) and apoptosis in the mid and high dose, 20 or 50 µg/kg bw per day, with no dose–response. In another Tier 2 mouse study (Xie et al., 2016) with s.c. administration dose-related testicular findings (germ cell (apoptosis)) were observed at doses equivalent to oral doses of 2220; 22220 or 1111000 µg/kg bw per day.

166. The likelihood of the histological changes in the testis were considered to be ALAN: During developmental exposure (pre-natal and/or post-natal until weaning), the CEP Panel assigned a likelihood level of ALAN to the cluster male reproductive toxicity of BPA. Hence, none of these endpoints were taken forward for BMD analysis. However, the Likely and ALAN endpoints were considered in the uncertainty analysis.

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

Plasma/serum thyroid hormones

167. For this exposure period only one Tier 1 rat study was identified (NTP Clarity Report, 2018/Camacho et al., 2019. In this study T3 and T4 were measured. No effect was seen on T3 but according to the authors, T4 levels in the serum showed a significant trend, however the panel noted that the nature of the trend was not evident from their inspection of the data. The likelihood of an effect on T4 was therefore considered as Not Likely.

168. During this exposure period the likelihood of changes in thyroid hormones (T3, T4) were considered as Not Likely by the CEP Panel.

Testosterone

169. For this endpoint one Tier 3 rat study (Gonzalez-Cadavid, 2018 – part of the Clarity study) was identified. Therefore, data were Inadequate to judge the likelihood of an effect of BPA on serum testosterone.

Epididymis weight

170. For this exposure period, two Tier 1 rat studies (Dere et al., 2018 NTP Clarity Report, 2018/Camacho et al., 2019) were identified.

171. No change in epididymis weight was seen in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho 9251 et al., 2019). In the other Tier 1 rat study (Dere et al., 2018; this study is part of the Clarity consortium), rats were dosed with 2.5, 25, 250, 2500 or 25000 µg/kg bw per day; F0 dams from GD6 to PND0 and F1 pups from PND1 to PND90. In this study an extra satellite control and 250,000 µg/kg bw per day dose group was added. A decrease in epididymis weight was only seen in the 250000 µg/kg bw per day group when compared with the extra (satellite) control group. This satellite control group showed a higher epididymis weight than the other control group.

172. The likelihood of an effect on epididymis weight was considered as Not Likely.

Epididymis histology

173. For this exposure period the following Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019 was identified.

174. In this study (NTP Clarity Report, 2018/Camacho et al., 2019) an increased change in exfoliated germ cells and inflammation was seen at histological examination of the epididymis in the high-dose group (25,000 µg/kg bw per day) at interim sacrifice (1 year); these effects were not observed at terminal sacrifice (2 years).

175. The likelihood of the changes in epididymis histology (exfoliated germ cells and inflammation) was considered to be Likely, although as effects were only seen in the highest dose group at the interim and not at the terminal sacrifice, the effect was apparently transient.

Prostate histology

176. For this exposure period the following Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019) was identified; this study had two different times of sacrifice. In addition, another set of animals of the same study was examined (Prins et al., 2018).

177. In the Clarity study no change in hyperplasia of the epithelium (non-neoplastic, proliferative lesions) was observed at interim sacrifice and at terminal sacrifice an increase was only observed at the 250 µg/kg bw per day dose. At interim sacrifice, an increase without dose–response in inflammatory changes of the prostate was observed at 2.5, 250, 2500 or 25000 µg/kg bw per day; no change was seen at 25 µg/kg bw per day. At terminal sacrifice an increase in inflammatory changes of the prostate was only seen in the lowest dose group. There was no change in inflammation of the prostate in the Tier 1 rat study by Prins et al., (2018).

178. The likelihood of the changes in prostate histology were considered to be Not Likely as effects were not seen in different sets of animals and were only examined at interim sacrifice.

179. Seminal vesicle weight: For this exposure period one Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019) and one Tier 3 mouse study (Patel et al., 2013) were identified. As no effects on seminal vesicle weight were observed in the Tier 1 study, the likelihood of an effect on seminal vesicle weight was considered as Not Likely.

Sperm count

180. For this endpoint one Tier 1 (NTP Clarity Report, 2018/Camacho et al., 2019 was identified. No effect was seen on epididymal sperm count and count of testicular sperm heads. The likelihood of an effect on sperm count was considered as Not Likely.

Sperm morphology

181. For this endpoint one Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019) and one Tier 3 mouse study (Dobrzynska et al., 2018) were identified. As no effect on sperm morphology was observed, the likelihood of an effect on sperm morphology was considered as Not Likely.

Sperm motility

182. For this endpoint, one Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019) and one Tier 3 mouse study (Dobrzynska et al., 2018) were identified. As no effect was seen on sperm motility in the Tier 1 rat study, the likelihood of an effect on sperm motility was considered as Not Likely.

Testis weight

183. For this exposure period two Tier 1 studies (NTP Clarity Report, 2018/Camacho et al., 2019; Dere et al., 2018) and one Tier 3 mouse study (Patel et al., 2013) were identified. No change in testis weight was seen in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019).

184. In the other Tier 1 rat study (Dere et al., 2018), The doses were the same as those used in the main clarity study, but extra satellite groups, 0 and 250000 µg/kg bw per day were added. A decrease in testis weight was only seen in the 250000 µg/kg bw per day group when compared with the extra control group. However, this extra control group showed a higher testis weight than the other control group.

185. The likelihood of an effect on testis weight was considered as Not Likely.

Testis histology

186. For this exposure period two Tier 1 studies (NTP Clarity Report, 2018/Camacho et al., 2019; Dere et al., 2018) were identified. At histological examination in the testis. No changes were observed in either study after histological examination.

187. The likelihood of an effect on testis histology was considered as Not Likely:

188. During developmental exposure (pre-natal and/or post-natal in pups until adulthood), the CEP Panel assigned a likelihood level of Likely to the cluster male reproductive toxicity of BPA.

189. Since the likelihood level is Likely for the endpoint epididymis histology (exfoliated germ cells and inflammation) in the Tier 1 study (NTP Clarity Report, 2018/Camacho et al., 2019, this study was taken forward for BMD analysis and uncertainty analysis.

Growth phase/young age

Testosterone

190. For this endpoint three Tier 3 rat studies (Ullah et al., 2018a; Ullah et al., 2018b; Gurmeet et al., 2014) were identified (note - the Gurmeet study has different tier ratings depending on the endpoint) As only Tier 3 studies were identified, the data were considered Inadequate to judge the likelihood of an effect on serum testosterone.

Epididymis weight:

191. For this endpoint, two Tier 1 rat studies (Ogo et al., 2018; Gurmeet et al., 2014) and one Tier 3 rat study (Ullah et al., 2018b) were identified. No effect on epididymis weight was seen when rats were dosed with 20 or 200 µg/kg bw BPA per day from PND36–66 (Ogo et al., 2018). In the Tier 1 rat study (Gurmeet et al., 2014) no effects were observed on epididymis weight when rats were dosed with 1000, 5000 or 100000 µg/kg bw per day from PND28–70.

192. The likelihood was considered as Not Likely as no effect was observed in either of two Tier 1 rat studies.

Seminal vesicle weight

193. For this endpoint one Tier 1 rat study (Gurmeet et al., 2014) and one Tier 3 rat study (Ullah et al., 2018b) were identified. No effect on seminal vesicle weight was observed when rats were administered BPA via the drinking water with doses equivalent to oral doses of 1000, 5000 and 100000 µg/kg bw per day from PND28– 70 weeks (Gurmeet et al., 2014).

194. The likelihood was considered as Not Likely as no effect was seen on seminal vesicle weight in one Tier 1 rat study.

Sperm count (testis/epididymis)

195. For this endpoint one Tier 1 rat study (Ogo et al., 2018) and one Tier 3 rat study (testicular count, Ullah et al., 2018b) were identified. No effect on epididymal sperm count was observed in the Tier 1 study when rats were dosed with 20 or 200 µg/kg bw per day from PND36–66 (Ogo et al., 2018).

196. The likelihood was considered as Not Likely as no effect was seen on epididymal sperm count in one Tier 1 rat study.

Sperm motility

197. For this endpoint, only one Tier 3 rat study (Ullah et al., 2018b) was identified. The data were therefore Inadequate to judge the likelihood of an effect of BPA on sperm motility.

Testis weight

198. For this endpoint two Tier 1 rat studies (Ullah et al., 2018a; Gurmeet et al., 2014), one Tier 2 rat study (Brouard et al., 2016) and one Tier 3 rat study (Ullah et al., 2018b) were identified.

199. In the Tier 1 rat study (Ullah et al., 2018a) no effect on testis weight was observed. In this study, rats were dosed for 4 weeks with 5000, 25000 or 50000 µg/kg bw per day from PND70–80. In another Tier 1 rat study (Gurmeet et al., 2014, no effects were observed on testis weight when rats were dosed with 1000, 5000 or 100000 µg/kg bw per day from PND28–70.

200. In a Tier 2 study (Brouard et al., 2016) rats were exposed from PND15–PND30 by s.c. injection with a single dose of 50 µg/kg bw per day (equivalent to an oral dose of 1800 µg/kg bw per day) and testis weight was increased.

201. The likelihood was considered to be Not Likely as no effects on testis weight were seen in two Tier 1 oral (gavage) studies and an effect was only observed in the Tier 2 single-dose rat study via the s.c. route.

Testis histology

202. For this endpoint two Tier 2 rat studies (Gurmeet et al., 2014; Brouard et al., 2016) and two Tier 3 rat studies (Ullah et al., 2018a; Ullah et al., 2018b) were identified.

203. In the Tier 2 rat study by Gurmeet et al., 2014), a decrease of the seminiferous tubule diameter was observed in the high-dose group. In this study, the rats were dosed with 1000, 5000 or 100000 µg/kg bw per day from PND28–PND70.

204. In another Tier 2 study (Brouard et al., 2016), rats were exposed from PND15–PND30 by s.c. injection with a single dose of 50 µg/kg bw per day (equivalent to an oral dose of 1800 µg/kg bw per day), and incidences of seminiferous tubules with lumen, with acrosomal vesicles and acrosome reaction were increased in the BPA-treated group.

205. The likelihood of the effect was considered as Likely as in a Tier 2 rat study (Gurmeet et al., 2014) a decrease in seminiferous tubule diameter at dose level of 100000 µg/kg bw per day was observed. These effects were supported by testicular effects in another Tier 2 rat study (Brouard et al., 2016) at a s.c. dose equivalent to an oral dose of 1800 µg/kg bw per day. In this study, the incidence of seminiferous tubules with lumen, acrosomal vesicles and acrosome reaction was increased. This single-dose study was not taken forward for BMD analysis.

206. During the exposure during the growth phase, the CEP Panel assigned a likelihood level of Likely to the cluster male reproductive toxicity of BPA. Since the likelihood level is Likely for the endpoint testis histology (decrease in seminiferous tubule diameter) in the Tier 2 rat study (Gurmeet et al., 2014); this study was taken forward for BMD analysis and uncertainty analysis.

Adult exposure (after puberty)

Plasma/serum testosterone

207. For this endpoint, four Tier 3 rat studies (Srivastava and Gupta, 2018; Wu et al., 2016; Huang DY et al., 2018; Rashid et al., 2018), three Tier 3 mouse studies (Xu XH et al., 2015; Chouhan et al., 2015; Gao et al., 2018) and one Tier 3 monkey study (Vijaykumar et al., 2017) were identified.

208. As only Tier 3 studies were identified, data were considered Inadequate to judge the likelihood of an effect of BPA on testosterone.

Epididymis weight

209. For this exposure period only one Tier 3 mouse study (Dobrzynska et al., 2014 was identified, data were therefore considered Inadequate to judge the likelihood of an effect on epididymis weight.

Prostate histology

210. For this exposure period one Tier 2 (Olukole et al., 2018) and two Tier 3 studies (Huang DY et al., 2018; Wu et al., 2016) in rats were identified.

211. In the Tier 2 study (Olukole et al., 2018), rats were dosed with 10000 µg/kg bw per day for 14 days. The following observations were increased in the prostate histology of the BPA-treated group: degenerative changes, atrophy (atrophic tubules); non-neoplastic proliferative lesions: functional hyperplasia, proliferative lesions, reduced glandular diameter, hyperplasia reactive (accompanied by inflammation); inflammatory changes: inflammation and vascular digestion; atypical hyperplasia.

212. The likelihood of the effects on prostate histology was judged as Inadequate evidence as the effects on prostate histology were observed in a Tier 2 rat study using only a single dose level (Olukole et al., 2018).

Seminal vesicle weight

213. For this exposure period one Tier 3 mouse study (Chouhan et al., 2015) was identified. The data were therefore considered Inadequate to judge the likelihood of an effect on seminal vesicle weight.

Sperm count

214. For this exposure period one Tier 1 study (Wang HF et al., 2016), two Tier 2 studies (Park et al., 2018; Yin et al., 2017) and three Tier 3 studies (Dobrzynska et al., 2014; Gao et al., 2018; Chouhan et al., 2015) in mice and one Tier 3 rat study (Srivastava and Gupta, 2018) were identified.

215. In the Tier 1 study (Wang HF et al., 2016) mice were dosed for 8 weeks with 10, 50 or 250 µg/kg BPA per day; no effect on sperm count was noted. Similarly, in the Tier 2 mouse study (Yin et al., 2017) no effect was observed on sperm count after 5 weeks dosing with 3000, 30000 or 300000 µg/kg bw per day. In another Tier 2 mouse study (Park et al., 2018) a decrease in sperm count was seen at the only dose tested (10000 µg/kg bw per day) for 12 weeks.

216. The likelihood of the effect on sperm count was judged Not Likely as no effects were seen in either the Tier 1 or Tier 2 study in mice. While a decrease in sperm count was seen in the study by Park et al., (2018a) this used only a single dose level of 10000 µg/kg bw per day.

Sperm motility

217. For this exposure period one Tier 1 study (Wang HF et al., 2016), one Tier 2 study (Park et al., 2018) and one Tier 3 study (Dobrzynska et al., 2014) in mice were identified.

218. In the Tier 1 study (Wang HF et al., 2016) mice were dosed for 8 weeks with 10, 50 or 250 µg/kg bw per day; a dose-related decrease in sperm motility was observed. In the Tier 2 mouse study (Park et al., 2018) a decrease in sperm motility was observed at the only dose tested (10000 µg/kg bw per day) for 12 weeks.

219. The likelihood of the decrease in sperm motility was judged as Likely based on the dose-related decrease in the Tier 1 mouse study (Wang HF et al., 2016) at doses 10, 50 or 250 µg/kg bw per day. This effect was supported by decrease in sperm motility seen in the single dose (10000 µg/kg bw per day) in the Tier 2 study (Park et al., 2018). This single-dose level study was not brought forward for BMD analysis.

Sperm morphology:

220. For this exposure period one Tier 2 study (Park et al., 2018) and one Tier 3 study (Dobrzynska et al., 2014) in mice were identified.

221. In the Tier 2 mouse study (Park et al., 2018) an increase in abnormal sperm was observed at the only dose tested (10000 µg/kg bw per day) for 12 weeks.

222. The likelihood of the increase of abnormal sperm was judged as Inadequate as the increase was observed at a single dose level (10000 µg/kg bw per day) in a Tier 2 mouse study (Park et al., 2018).

Sperm viability

223. For this exposure period one Tier 1 mouse (Wang HF et al., 2016) study was identified. In this, groups of 8 C57BL/6 mice/dose group aged 15-17 weeks were dosed for 8 weeks with 10, 50 or 250 µg/kg bw BPA per day; a significant

dose-related decrease in sperm motility (as assessed by Computer Assisted Sperm Analysis) CASA was observed at the highest dose level and a non statistically significant decrease seen in the mid-dose group. Body weights and testicular weights were unaffected.

224. The likelihood of the decrease in sperm viability was judged as Likely based on this study.

Sperm acrosome reaction

225. For this exposure period one Tier 1 mouse study (Wang HF et al., 2016 was identified (see above for details. A dose-related decrease in acrosome reaction was seen at the two highest dose levels. This was assessed by chlortetracycline staining.

226. The likelihood of the decrease in acrosome reaction was considered to be Likely.

Testis weight

227. For this exposure period one Tier 1 study (Wang HF et al., 2016) and three Tier 3 studies (Dobrzynska et al., 2014, Gao et al., 2018, Chouhan et al., 2015) in mice were identified.

228. In the Tier 1 study; no effect on testis weight was noted.

229. The likelihood for an effect on testis weight was judged as Not Likely.

230. Overall, the CEP Panel assigned a likelihood of Likely to the cluster male reproductive toxicity during adult exposure. As the likelihood level for male reproductive toxicity is Likely for the endpoint sperm motility, viability and acrosome reaction in the Tier 1 mouse study (Wang HF et al., 2016) these data were taken forward for BMD analysis and uncertainty analysis.

Indirect (germline) exposure

231. For this exposure period two Tier 3 mouse studies (Dobrzynska et al., 2015; Dobrzynska et al., 2018), in which epididymis weight, sperm count and sperm motility were measured, were identified. In addition, in the Tier 3 mouse study (Dobrzynska et al., 2015) sperm morphology was examined and in the other Tier 3 study (Dobrzynska et al., 2018) testis weight was measured.

232. However, the CEP Panel noted that as only Tier 3 studies were available, the evidence was considered Inadequate for this exposure period.

Overall cluster selection for endpoints/studies for BMD analysis for male reproductive toxicity

233. Overall, the CEP Panel assigned a likelihood level of:

- ALAN to the male reproductive toxicity cluster in the developmental exposure period.
- Likely in the developmental and adult, growth phase/young age and adult exposure periods.
- Inadequate evidence in the indirect (germline) exposure period.

234. The overall likelihood across all exposure periods, i.e. the highest likelihood given in the cluster male reproductive toxicity was Likely. The CEP Panel considered that the evidence from the studies available showed a Likely effect for epididymis histology (exfoliated germ cells and inflammation) in the Tier 1 rat study (NTP Clarity Report, 2018/Camacho et al., 2019) during the developmental exposure (pre-natal and/or post- natal until adult) period. In addition, with exposure during the growth phase a Likely effect was reported for testis histology (decrease in seminiferous tubule diameter) in a Tier 2 rat study (Gurmeet et al., 2014) and during adult exposure effects on sperm (motility; viability; acrosome reaction) were observed in a Tier 1 mouse study (Wang HF et al., 2016). Therefore, these endpoints were taken forward for BMD analysis.